International Food Research Journal 32(2): 565 - 579 (April 2025)
Journal homepage: *http://www.ifrj.upm.edu.my*

Effect of mild yeast fermentation on aroma compounds and functional components of *Lycium ruthenicum* Murray

[1,2,3]Peng, B., [1,2,3]Fei, L. Y., [1,2,3]Li, Y., [1, 2,3]Wei, S. Y., [1,2,3]Zhang, Q., [1,2,3]Zhao, X. X., [1,2,3]Tang, F. X., [1,2,3]Shan, C. H., [1,2,4]*Zhang, D. S. and [1,2,3]*Cai, W. C.

[1]*Engineering Research Center of Storage and Processing of Xinjiang Characteristic Fruits and Vegetables, Ministry of Education, School of Food Science, Shihezi University, Shihezi, Xinjiang, China*
[2]*Key Laboratory of Processing and Quality and Safety Control of Specialty Agricultural Products (Co-construction by Ministry and Province), Ministry of Agriculture and Rural Affairs, School of Food Science, Shihezi University, Shihezi, Xinjiang, China*
[3]*Key Laboratory for Food Nutrition and Safety Control of Xinjiang Production and Construction Corps, School of Food Science, Shihezi University, Shihezi, Xinjiang, China*
[4]*Office of the Party Committee of Xinjiang Production and Construction Corps, Urumqi, Xinjiang, China*

Article history

Received:
22 July 2024
Received in revised form:
14 April 2025
Accepted:
16 April 2025

Keywords

Lycium ruthenicum Murray, fermentation, functional components, volatile compounds, yeast

Abstract

Mild fermentation of *Lycium ruthenicum* Murray (LR) by yeast facilitates the leaching of nutrients from LR, increases the functional properties of the fermentation broth, and improves its aroma quality. Headspace solid phase microextraction was used to extract the volatile compounds from raw LR juice and fermented LR beverage samples. The volatile compounds were identified using gas chromatography-mass spectrometry (GC-MS), and analysed by relative odour activity value (ROAV). The contents of total flavonoids, polysaccharides, and anthocyanins were also determined. The results showed that the contents and types of volatile compounds changed significantly after fermentation. In total, 24 volatile compounds were identified in raw LR juice, and 39 volatile compounds were identified in fermented LR beverage. The relative content of esters increased from 20.65 to 72.59%, and alcohols increased from 2.05 to 5.72%. However, acids decreased significantly, from 70.76 to 1.74%. A total of seven volatile compounds with ROAV ≥ 0.01 were found in fermented and raw LR juices, most of which belonged to esters and aldehydes, and mainly provided strong fruity and slightly boozy and oily flavours to fermented LR beverages. For functional components, the content of total flavonoids increased significantly from 1.08 to 1.70 g/100 g ($p < 0.05$). However, the total polysaccharide content decreased significantly from 10.88 to 9.99 g/100 g ($p < 0.05$). The content of anthocyanins did not change significantly. The findings could provide theoretical guidance for improving the aroma quality and functional components of fermented LR beverages, and deliver new insights into the process of aroma production in this fermented juice.

DOI
https://doi.org/10.47836/ifrj.32.2.19

Introduction

Lycium ruthenicum Murray (LR; black wolfberry) belongs to the Solanaceae family, and is a perennial thorny shrub with many branches (Yun *et al*., 2022). It has characteristics of drought resistance, cold resistance, wind and sand resistance, salt and alkali tolerance, but poor soil tolerance. It is a tree species with ecological and economic advantages that integrates soil and water conservation, wind and sand fixation, salt-alkali land improvement, and sand industry development (Yun *et al*., 2022). LR usually grows in dry and semi-dry areas above an altitude of 2,000 m, and is widely cultivated in Inner Mongolia, Gansu, Qinghai, Ningxia, Shanxi, Xinjiang, and other regions in China. They are also distributed in countries such as Mongolia, Afghanistan, Kazakhstan, and Tajikistan, but the quantity and coverage are comparatively small (Sharma *et al*., 2022) (Figure 1).

Compared to the berries of *L. barbarum* and *L. chinense*, LR has a purplish-black colour due to their

*Corresponding author.
Email: wenchaocai@foxmail.com ; 19999111099@163.com

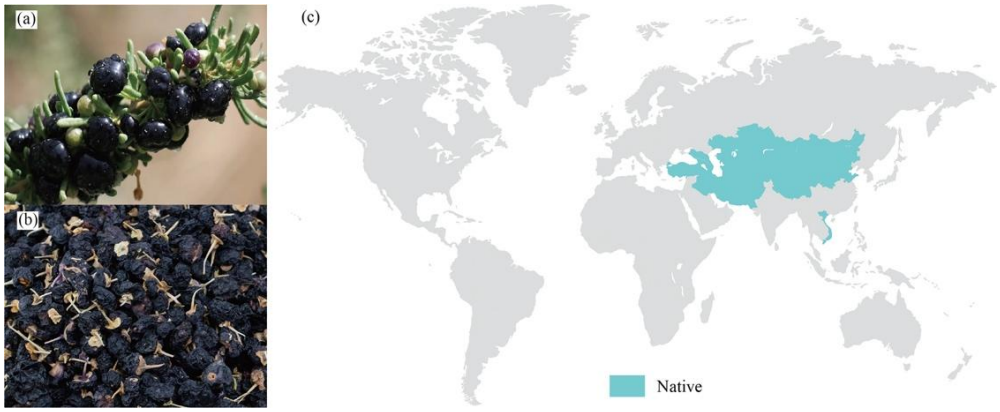

Figure 1. Fresh *Lycium ruthenicum* Murray fruit **(a)**, dried *L. ruthenicum* fruit **(b)**, and world distribution of *L. ruthenicum* fruit.

abundant anthocyanins (Qiang *et al*., 2023). Anthocyanins, which are also important functional components of LR, are known to prevent cardiovascular and cerebrovascular diseases, improve vision, fight cancer, reduce serum lipid levels, delay aging, and combat the effects of radiation (Qiang *et al*., 2023). Besides, LR contains a variety of essential amino acids and biologically active substances, such as polysaccharides and flavonoids (Yun *et al*., 2022). Among these, polysaccharides and flavonoids are the main active components of LR (Du *et al*., 2024). Studies have shown that LR contains a large number of polysaccharides with a variety of biological activities, such as antioxidant activity, antitumor activity, neuroprotective activity, hypoglycaemia, and anti-fatigue (Liu *et al*., 2024). Secondly, LR contains a variety of medicinal bioactive flavonoids with anti-radiation ability, the ability to remove free radicals, anti-inflammatory, and promote immunity effects (Du *et al*., 2024).

In recent years, with people's increasing focus on health and nutrition, the market demand for LR has continued to grow, making it one of the important representatives of high-end food products, with dual medicinal and dietary purposes in China. LR cultivation has grown rapidly, especially in Xinjiang. This has led to the growth of the LR industry. Meanwhile, the amount of research on the composition of LR has increased, and the development of products based on LR fruits is becoming more popular (Liu *et al*., 2020). Currently, processed LR products — including fruit juice, fruit wine, yogurt, bread, and fruit tea — can be divided into two major types: fermented and non-fermented. Fermentation of LR juice products is usually carried out with two types of strains, probiotics and yeasts. There have been a large number of studies exploring the fermentation of LR juice or LR wine by lactic acid bacteria and yeasts, either single or mixed, but there have been few reports on the light fermentation of LR juice by yeasts. Therefore, we chose to mildly ferment LR juice by yeast to explore the changes in its functional constituents and aroma qualities.

Yeast is a unicellular, parthenogenetic, and facultative anaerobic fungus. It can convert sugar into ethanol and CO_2, providing a unique flavour for LR juice (Cai *et al*., 2021a; 2021b; 2022a; 2022b). Secondly, the active ingredients it produces during the fermentation process can decompose some of the macromolecules in the LR juice, and improve the digestibility of the fermented LR beverage (Geng *et al*., 2021). In addition, a variety of nutrients needed by the human body contained in the cells of the yeast can increase the food value of the LR juice, and improve the flavour, aroma, and texture of the product, and enhance its nutritional properties (Qiang *et al*., 2023). Therefore, the use of yeast fermentation of LR juice not only changes the composition of the aroma of the LR juice, thus giving it a unique flavour, but also at the same time, retains the original nutrients in the LR juice so that the LR beverage has slightly fruity with wine and sweet and sour taste.

The fermentation process involved in the preparation of fermented LR beverages was optimised in our laboratory at an early stage. Using a solid/liquid ratio of 1:3, and a fermentation temperature of 20°C, a high-quality fermented LR beverage with an alcohol content of 0.5% could be prepared using the yeast BV818. On this basis, headspace solid-phase microextraction combined with gas chromatography-mass spectrometry (HS-SPME-GC-MS) was performed to compare the

volatile compounds present in raw LR juice and fermented LR beverages. At the same time, the changes in the contents of anthocyanins, total polysaccharides, and total flavonoids before and after fermentation were also analysed. The goal of the present work was to provide technical support for improving the quality of fermented LR beverages. Further, the present work also aimed to guide further research on the synthesis of volatile compounds in mildly fermented LR beverages.

Materials and methods

Materials

Fresh, disease-free LR was purchased at a local market in Shihezi, Xinjiang, China.

Raw LR juice

Ripe and normal-flavour LR fruits were selected. The residual berries were removed, and the flesh was washed with water. LR flesh was blended into pulp after the addition of distilled water 1:3 (v/w) using a high-speed blender (ZG-TJ503, Joyoung Co., Ltd., Shandong Province, P.R. China); 0.08% ascorbic acid (Shanghai Macklin Biochemical Co., Ltd., Shanghai, P.R. China) was added for colour protection. The pulp was subjected to pectinase treatment (Lallemand Group Co., Ltd., France; 0.3 g/L, activated: 10,000 U/g) at 45°C for 2 h. Then, the pulp was filtered and centrifuged at 1,000 *g* for 10 min to obtain the bright raw LR juice, which was used for analysis.

Fermented LR beverage

Citric acid and sucrose (Shanghai Macklin Biochemical Co., Ltd., Shanghai, P.R. China) were added to the pulp to achieve pH 4.0 and 12°Brix. This was followed by the addition of sulphur dioxide (30 mg/L; Shanghai Macklin Biochemical Co., Ltd., Shanghai, P.R. China) for killing bacteria and improving stability. The fermentation broth was mixed with 0.03% (w/w) commercial active dry wine yeast (BV818, Angel Yeast Co., Ltd., China), and the fermentation temperature was 20 ± 1°C. The fermentation mixture was stirred and examined at 12 h intervals. At the end of the fermentation period, the residual amount of sugar was 11°Brix, and the alcohol content was 0.5%. At this point, the fermentation broth was filtered and centrifuged at 1,000 *g* for 10 min to obtain the bright fermented LR beverage, which was used for analysis.

HS-SPME-GC-MS analysis

Aroma analysis was performed using a slightly modified version of the method used by Peng *et al*. (2024) and Cai *et al*. (2020a; 2020b). For each sample that was to be analysed, 5 mL was placed in a 20 mL headspace bottle, and 3.0 g NaCl was added. The sealed bottle was placed on a magnetic stirrer for 10 min, and the temperature was controlled at 40°C to allow the volatile compounds to evaporate and move to the upper part of the bottle. An SPME with 1 cm fibres coated with 50/30 μm divinylbenzene/carboxen on polydimethylsiloxane (CAR/DVB/PDMS) (57329-U, Supelco, Bellefonte, PA, USA) was passed through the needle, and exposed to the headspace at the top of the bottle. The volatile compounds were adsorbed for 30 min at 50°C. Then, they were immediately injected into the gas chromatography injection port, and adsorbed for 7 min at 250°C, while the instrument was turned on to desorb the volatile compounds.

Target volatile compounds present in the samples were initially analysed using HP5972 GC-MS (Agilent Technologies Inc., PA, USA), as previously described (Zhao *et al*., 2023), but with slight modifications. The GC was used along with a DB-5MS column (30 m × 0.25 mm × 0.25 um; Agilent Technologies Inc., PA, USA), and high purity helium was used as carrier gas. The following conditions were used for GC: a constant flow rate of 1.4 mL/min; injection temperature of 250°C; and the splitless injection mode. The heating procedure was as follows: the initial temperature was 60°C, which was maintained for 2 min; the temperature then increased to 160°C at a rate of 5°C/min, and maintained for 1 min; finally, the temperature was increased to 250°C at a rate of 10°C/min, and maintained for 4 min. The signals were collected in the full scanning mode, and mass spectra were obtained at 70 eV in the electron ionisation (EI+) mode (interface temperature 280°C; ion source temperature 230°C; quad temperature 150°C; scan mass range 45.00 - 450.00 m/z; and scanning frequency 4.58/s).

After GC separation, the unique chromatographic peak of each component was analysed, and a chromatographic map of all its components was obtained. The identity and relative content of each aroma compound present in the samples were determined using computer retrieval and analysis. The chromatographic analysis data for the volatile compounds were processed using

ChemStation software (G1701DA D.02.00.275; Agilent Technologies, Santa Clara, CA, USA).

Relative odour activity value (ROAV) calculation

The ROAV method was used to evaluate the contribution of volatile compounds to the yeast-fermented LR beverage. The ROAV of the volatile compounds with the most obvious influence on the overall flavour of the samples was set to 100. In general, the larger the ROAV value, the greater the contribution of the component to the overall flavour of the sample. ROAV ≥ 1 indicates that the compound is the key flavour compound of the analysed sample, and the components with 0.01 ≤ ROAV < 1 are important modifiers of the overall flavour of the sample (Cai *et al*., 2022c). The ROAV was calculated using Eq. 1:

$$ROAV_i = C_i/T_i \quad \text{(Eq. 1)}$$

where, $ROAV_i$ = relative odour activity value of a volatile compound; C_i = relative compound content (%), and Ti = threshold value of a volatile compound (μg/kg).

Functional analysis

The total flavonoid content was measured using the method described by Cai *et al*. (2020a) and He *et al*. (2025a), with slight modification First, 0.5 mL of the sample solution was added to 0.15 mL of a 5% sodium nitrite solution in a 5 mL volumetric flask, and mixed well at 25°C for 6 min. Following the addition of 0.3 mL 10% aluminium nitrate solution, the mixture was again incubated for 6 min. Then, 2 mL of 4% sodium hydroxide solution (Shanghai Macklin Biochemical Co., Ltd., Shanghai, P.R. China) was added to the volumetric flask. Distilled water was added to reach a volume of 5 mL, and the solution was mixed for 3 min at room temperature. The absorbance of the sample was measured at 510 nm by ultraviolet spectrophotometer. The total flavonoid content was expressed as mg/mL FW.

The total anthocyanin content was determined using the pH differential method, modified from Zhang *et al*. (2020). First, 2.5 mL of the sample was taken, and the volume was increased to 10 mL with potassium chloride-hydrochloric acid buffer (pH = 1) (Shanghai Macklin Biochemical Co., Ltd., Shanghai, P.R. China) or sodium acetate-hydrochloric acid buffer (pH = 4.5) (Shanghai Macklin Biochemical Co., Ltd., Shanghai, P.R. China). After incubation in the dark for 90 min, the absorbance was determined at 525 and 700 nm, respectively. The content of anthocyanins was calculated using Eq. (2):

$$TAC = \frac{(A_{520}-A_{700})_{pH1.0}-(A_{520}-A_{700})_{pH4.5}}{\varepsilon \times I} \times Mw \times DF \times 10^3 \quad \text{(Eq. 2)}$$

where, TAC = total anthocyanin content; Mw = molecular weight of petunidin-3-glucoside (655.2 g/mol): ε = extinction coefficient (20,500 L/mol cm); I = length of the distance travelled by the light passing through the colloid path (1 cm); and DF = dilution factor (DF = 10).

The content of total polysaccharides was determined using a modified version of the protocol used by Chu *et al*. (2024). First, 1 mL of the sample was added to 1.0 mL phenol solution (Shanghai Macklin Biochemical Co., Ltd., Shanghai, P.R. China), followed by the quick addition of 5.0 mL sulphuric acid (added vertically to the liquid without touching the test tube wall, so as to ensure complete mixing with the reaction solution) (Shanghai Macklin Biochemical Co., Ltd., Shanghai, P.R. China). The sample was kept still for 10 min at room temperature. Then, the test tube was placed in a 30°C water bath for 20 min, following which the absorbance of the sample was measured at 490 nm. The glucose content was calculated using a glucose standard curve, and the result was expressed as mg/mL FW.

Statistical analysis

Single-factor analysis of variance (ANOVA) was used to analyse the differences between mean values, and $p < 0.05$ was considered significant. Origin2018 (Origin Lab, Northampton, MA, USA) software was used for principal component analysis (PCA), clustering heat maps, and correlation analysis. All experiments were repeated three times.

Results and discussion

Changes in volatile compounds after fermentation

Figures 2 and 3 show the GC-MS total ion flow diagram of the raw LR juice and fermented LR beverage. The MS data obtained using GC-MS were interpreted through comparison with the National Institute of Standards and Technology (NIST) 2017 and Wiley 8.0 database. The identified volatile compounds and their relative contents are shown in

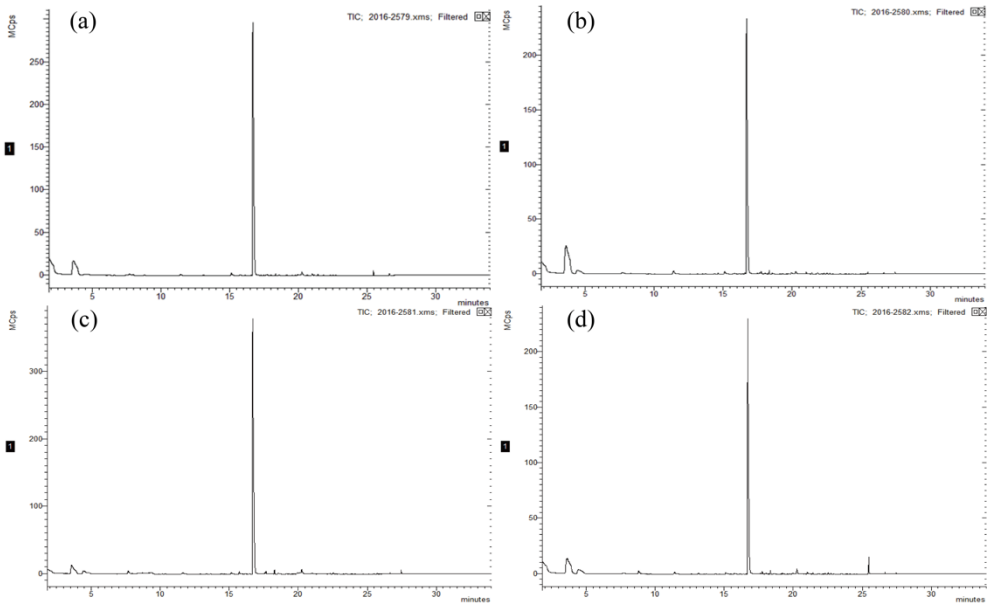

Figure 2. GC-MS total ion current diagram of raw LR juice.

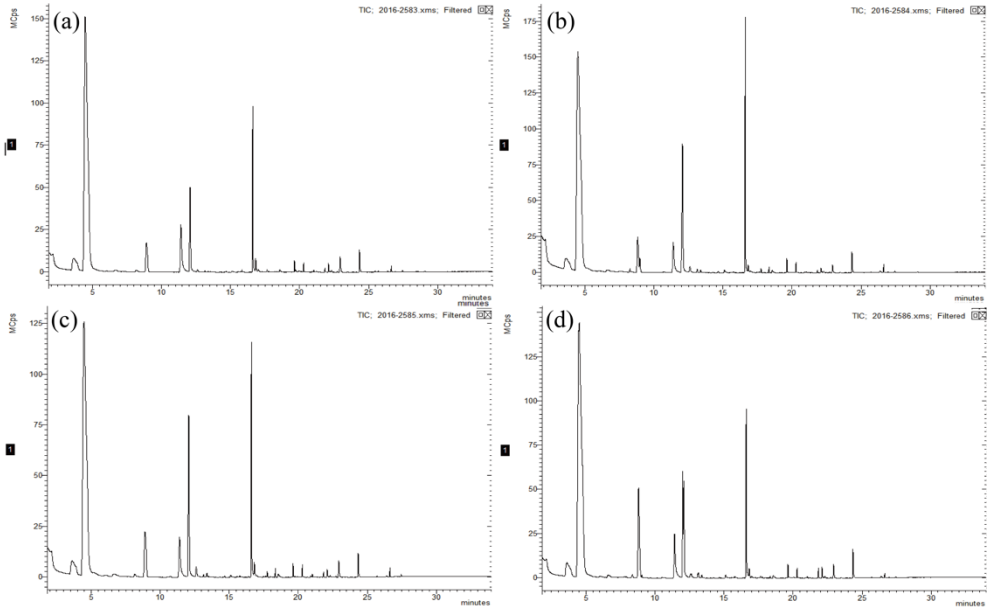

Figure 3. GC-MS total ion current diagram of LR fermented beverage.

Table 1. Based on the structural characteristics of the compounds and comparison with the standard compounds from the NIST database, a total of 24 volatile compounds were identified in the raw LR juice, and 39 volatile compounds were identified in the fermented LR beverage. Identified compounds were classified according to their functional groups as esters (Coded A1 to A17), alcohols (Coded B1 to B8), acids (Coded C1 to C6), aldehydes (Coded D1 to D4), ketones (Coded E1 to E6), and others (F1 to F5). The relative percentage content of each compound was calculated using the area normalisation method.

The changes in volatile compounds before and after fermentation are presented in Figure 4. During the fermentation process, in addition to ethanol, a variety of other by-products were also produced. Among them, the volatile compounds mainly included higher alcohols, esters, and fatty acids. There were 24 volatile compounds in LR juice before fermentation, and 39 after mild fermentation. The fermented LR beverage had 15 more volatile compounds than the raw LR juice (Figure 4c). However, three volatile compounds in raw LR juices were not detected after mild fermentation, namely leaf alcohol, benzyl alcohol, and undecane ester compounds, which are formed by esterification of alcohols and fatty acids, and are one of the main volatile compounds in yeast fermentation of LR juice.

Table 1. Relative contents of volatile compounds before and after mild fermentation of *Lycium ruthenicum* Murr. by *Saccharomyces cerevisiae* BV818.

Peak code[1]	RT (min)[2]	Compound name by class	Relative amount (%)		DSA[4]
			BF	AF	
Ester					
A1	3.584	Ethyl acetate	17.518 ± 0.209	3.706 ± 0.279	*[5]
A2	4.495	Ethyl lactate	2.476 ± 2.038	46.401 ± 2.959	*
A3	8.813	Isoamyl acetate	0.303 ± 0.605	4.572 ± 2.291	*
A4	10.736	Methyl hexanoate	ND[3]	0.023 ± 0.029	NS[6]
A5	12.070	Ethyl hexyl	ND	9.357 ± 2.213	***
A6	13.085	1-Hexyl acetate	0.132 ± 0.127	0.187 ± 0.136	NS
A7	14.285	Propyl hexanoate	ND	0.006 ± 0.009	NS
A8	15.454	1-Heptyl acetate	ND	0.004 ± 0.003	*
A9	16.608	Ethyl caprylate	ND	7.644 ± 1.844	***
A10	17.278	2-Ethylhexyl acetate	0.034 ± 0.068	0.011 ± 0.008	NS
A11	18.216	Ethyl nonanoate	ND	0.017 ± 0.002	***
A12	19.625	Ethyl caprate	ND	0.422 ± 0.057	***
A13	19.890	3-Methylbutylester	ND	0.004 ± 0.004	NS
A14	20.912	1,3-Propanediol diacetate	ND	0.041 ± 0.017	**
A15	21.425	Methyl salicylate	0.178 ± 0.038	0.015 ± 0.008	***
A16	21.842	Phenethyl acetate	0.015 ± 0.030	0.038 ± 0.075	NS
A17	29.086	Diisobutyl phthalate	0.013 ± 0.015	0.014 ± 0.003	NS
Alcohol					
B1	8.338	2-Methyl-1-propanol	0.027 ± 0.035	0.140 ± 0.106	NS
B2	11.418	3-Methyl-1-butanol	0.706 ± 0.325	4.460 ± 0.840	***
B3	15.119	1-Hexanol	0.671 ± 0.133	0.177 ± 0.072	***
B4	15.729	Leaf alcohol	0.136 ± 0.117	ND	NS
B5	18.571	1-Octanol	0.212 ± 0.064	0.193 ± 0.048	NS
B6	21.186	Decyl alcohol	0.064 ± 0.012	0.004 ± 0.009	***
B7	22.530	Benzyl alcohol	0.195 ± 0.044	ND	***
B8	22.935	Phenethyl alcohol	0.036 ± 0.007	0.745 ± 0.212	***
Acid					***
C1	16.682	Acetic acid	70.758 ± 8.126	0.415 ± 0.141	***
C2	19.480	Butyric acid	ND	0.018 ± 0.004	***
C3	22.096	Hexanoic acid	ND	0.326 ± 0.072	***
C4	24.339	Octanoic acid	ND	0.894 ± 0.060	***
C5	26.379	Decanoic acid	ND	0.079 ± 0.021	***
C6	26.949	9-Decylenic acid	ND	0.007 ± 0.006	*
Aldehyde					
D1	7.691	Hexanal	0.509 ± 0.436	0.002 ± 0.004	NS
D2	15.829	Nonaldehyde	0.138 ± 0.232	0.050 ± 0.029	NS
D3	17.582	Decanal	0.083 ± 0.165	0.056 ± 0.040	NS
D4	19.704	Hyacinthin	ND	0.037 ± 0.069	NS
Ketone					**
E1	14.678	Methaptenone	ND	0.055 ± 0.020	**

E2	22.232	Geranyl acetone	0.024 ± 0.029	0.039 ± 0.006	NS
Other					
F1	7.942	Undecane	0.074 ± 0.104	ND	NS
F2	12.607	Styrene	ND	0.175 ± 0.256	NS
F3	15.556	1,3-Propanediol monoethyl ether	ND	0.035 ± 0.008	***
F4	18.345	Therasurane	0.295 ± 0.362	0.045 ± 0.090	NS
F5	26.647	2,4-Di-tert-butylphenol	0.169 ± 0.042	0.193 ± 0.067	NS

[1]Code of every compound; [2]RT: retention time; [3]ND: not detected; [4]DSA: difference significance analysis; [5](*): $p < 0.05$, (**): $p < 0.01$, (***): $p < 0.001$; and [6]NS: non-significant difference.

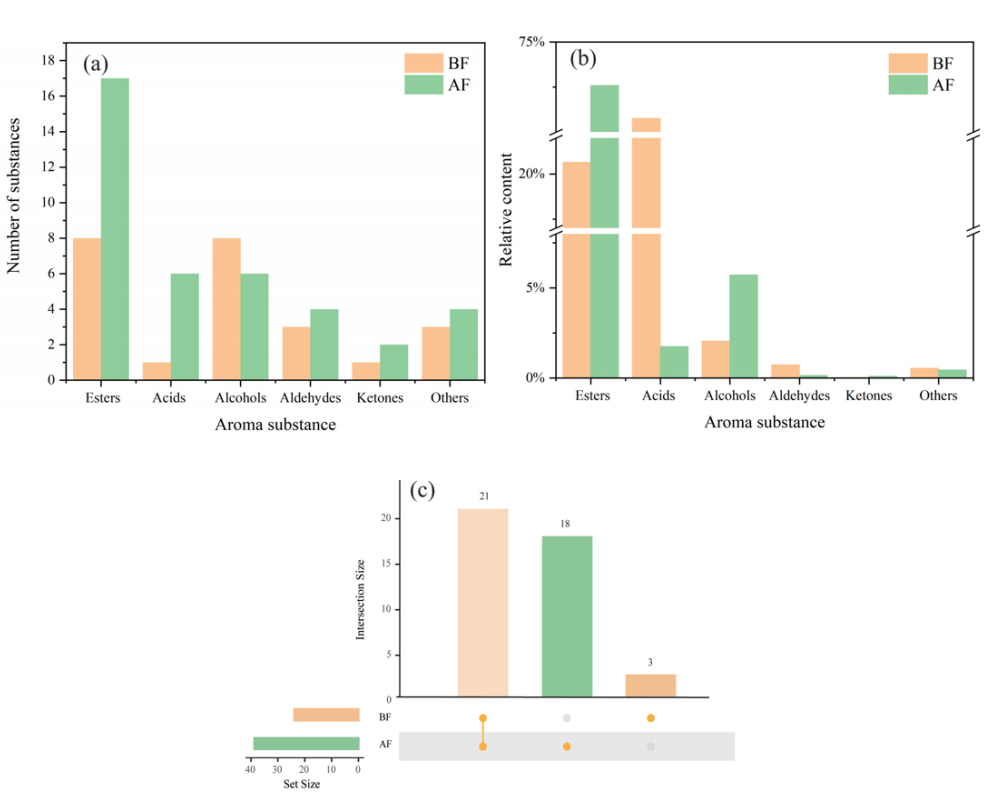

Figure 4. Changes in total number **(a)** and relative contents **(b)** of aroma substances before and after fermentation of LR. Venn diagram showing the quantity of aroma substances before and after fermentation **(c)**. BF represents juice before fermentation, which is LR juice. AF represents juice after fermentation, which is LR beverage.

Esters make a positive contribution to its fruity and floral aroma (Cai *et al*., 2018; 2024). The results showed that both the type and content of esters varied greatly after fermentation. There were eight kinds of esters present in the raw LR juice, and their relative content was 20.65%; of all esters, ethyl acetate had the highest content (Table 1). Ethyl acetate has a strong ether-like smell, and clear and slightly fruity wine aroma. It diffuses easily, and its aroma is not long-lasting. There were 17 types of esters detected in the fermented LR beverage, showing a relative content of 72.59%. Ethyl lactate — a characteristic volatile compound — was the most abundant ester (Table 1). Ethyl lactate has an elegant and soft aroma, and violet and rose fragrance, which confers a unique scent. The esters whose content increased significantly after fermentation were isoamyl acetate (4.57%), ethyl caproate (9.36%), and ethyl caprylate (7.64%) (Table 1). Isoamyl acetate is produced during fermentation, and can make fruit wine sweet and banana-like (Liu *et al*., 2020). Ethyl caproate has a brandy flavour, while ethyl caprylate has a fruity, green leaf-like, and apple fragrance (Zhao *et al*., 2023). The esters surged after fermentation, providing a strong floral and fruity flavour to the fermented LR beverages.

Alcohols were the second largest group of volatile compounds detected in the fermented LR beverage. Alcohols are mainly generated by yeast through the hydrolysis of sugars and amino acids (Wu *et al*., 2023). Of all alcohols, the content of 3-methyl-1-butanol in the LR juice was the highest before and after fermentation, and increased from 0.71 to 4.46% after mild fermentation (Figure 4b). In addition, the content of 2-methyl-1-propanol and phenethyl alcohol also increased. 1-hexanol, leaf alcohol, 1-octanol, decyl alcohol, and benzyl alcohol showed reduced levels after fermentation. Phenethyl alcohol has a pleasant fragrance of flowers, roses, and honey. 3-methyl-1-butanol is considered to have the aroma of fruit, apple, and brandy, and is associated with malt, wine, and onion flavours (Huang *et al*., 2022a). Therefore, although the alcohols in the fermentation broth did not increase as much as the esters, the alcohols still provided a certain fruity and winey flavour to the fermented LR beverages. During fermentation, yeast transforms valine, leucine, and phenylalanine to 2-methyl-1-propanol, 3-methyl-1-butanol, and phenethyl alcohol, respectively, *via* the Ehrlich pathway (Hong *et al*., 2021). It has been shown that phenethyl alcohol and its derivatives are formed from phenylalanine by genetically regulated decarboxylation and deamination, and that yeast extracts have been shown to be a key element in the production of phenylethanol (Bernardino *et al*., 2024). This explains the increased phenylethanol content in fermented LR beverages.

Acids are important for the balance of aroma substances in yeast mildly fermented beverage (Qin *et al*., 2018). In the raw LR juice, we only identified acetic acid, which accounted for 70.75% of all the substances identified (Table 1). After fermentation, the acetic acid content was reduced to 0.42%, and butyric, hexanoic, octanoic, decanoic, and 9-decylenic acids were identified (Table 1). Carboxylic acids improve the flavour of wine, especially by forming esters with ethanol and higher alcohols in the presence of acetyl-CoA and acetyl-transferase (Aldrete-Tapia *et al*., 2022). The acids give the fermented LR beverage a richer flavour and texture (He *et al*., 2025b).

A small number of ketones, aldehydes, hydrocarbons, phenols, and other volatile compounds were detected before and after fermentation in the LR juice. To a certain extent, the production of aldehydes and ketones has a positive effect on the aroma of the fermented LR beverage. Microbial metabolism during fermentation also leads to the production of a small amount of ketones, which have little impact on flavour, but could adversely affect flavour when present in higher quantities (Cai *et al*., 2018). Aldehydes present in the raw material disappear or decrease after mild fermentation, mainly because aldehydes are unstable and are easily reduced to alcohols by yeast dehydrogenase and reductase. Hence, wines tend to have low levels of aldehydes after fermentation (Wu *et al*., 2023).

In general, after yeast fermentation, a large number of new esters and alcohols with apple, rose, brandy flavour, and other aromas have been added to the LR juice, rendering the fermented LR juice more fragrant and softer; at the same time, the decrease in acetic acid and the increase in other acids also made the fermentation juice to have richer and harmonious aroma.

Calculation of ROAVs

In order to investigate the differences in the aroma actives of LR juice before and after fermentation, the ratio of the concentration of volatile compounds to the olfactory threshold (ROAV) was calculated (Table 2), and volatile compounds with ROAV $\geq$ 0.01 were considered to contribute to the overall flavour of the samples (Huang *et al*., 2022b). We found that most of the volatile compounds with ROAV $\geq$ 0.01 belonged to esters and aldehydes in both fermented and unfermented LR juices, with a total of four volatile compounds with ROAV $\geq$ 0.01 in raw LR juices, namely: isoamyl acetate (ROAV = 0.1), nonaldehyde (ROAV = 0.04), decanal (ROAV = 0.02), and 2,4-di-tert-butylphenol (ROAV = 0.34); and a total of six volatile compounds with ROAV $\geq$ 0.01 in the fermented LR juices volatile compounds, namely: isoamyl acetate (ROAV = 1.52), ethyl caprate (ROAV = 0.02), 3-methyl-1-butanol (ROAV = 0.02), nonaldehyde (ROAV = 0.01), decanal (ROAV = 0.01), and 2,4-di-tert-butylphenol (ROAV = 0.39).

Compared with the pre-fermentation period, the ROAV value of isoamyl acetate increased more than 15-fold after fermentation, while the ROAV values of nonanal and decanal both decreased slightly. Isoamyl acetate is a representative volatile compound in LR wine with a strong banana, fruity, and sweet flavour, which is produced from heterohydric alcohols by the reaction of acetyl

Table 2. Odour thresholds and odour activity values of volatile compounds in AF and BF.

Peak code[1]	Compound names by class	Odour threshold (μg/kg)	Odour activity value		Odour description
			BF[2]	AF[3]	
Ester					
A1	Ethyl acetate	7500[m]	< 0.01	< 0.01	Pineapple*
A2	Ethyl lactate	50000[m]	< 0.01	< 0.01	Fruit*
A3	Isoamyl acetate	3[m]	0.10	1.52	Banana*
A4	Methyl hexanoate	75[m]	ND	< 0.01	Fruit, fresh, sweet*
A5	Ethyl hexyl	0. 5[m]	ND	18.71	Apple peel, fruit*
A6	1-Hexyl acetate	40[m]	< 0.01	< 0.01	Fruit, herb*
A7	Propyl hexanoate	/	ND	ND	Fruit*
A8	1-Heptyl acetate	90[m]	ND	< 0.01	Apricot, fresh, fruit, green※
A9	Ethyl caprylate	10000[m]	ND	< 0.01	Fruit, fat*
A10	2-Ethylhexyl acetate	1400[n]	< 0.01	< 0.01	Earthy, herbal, humus, dirty★
A11	Ethyl nonanoate	1200[m]	ND	< 0.01	Fruity, natural, rose, rum※
A12	Ethyl caprate	20[m]	ND	0.02	Grape*
A13	3-Methylbutylester	3[m]	ND	< 0.01	Banana*
A14	1,3-Propanediol diacetate	/	ND	ND	/
A15	Methyl salicylate	60[m]	< 0.01	< 0.01	Peppermint*
A16	Phenethyl acetate	20[m]	< 0.01	< 0.01	Rose, honey, tobacco*
A17	Diisobutyl phthalate	/	ND	ND	/
Alcohol					
B1	2-Methyl-1-propanol	8000[m]	< 0.01	< 0.01	Wine, solvent, bitter*
B2	3-Methyl-1-butanol	250[m]	< 0.01	0.02	Whiskey, malt, burnt*
B3	1-Hexanol	200[m]	< 0.01	< 0.01	Resin, flower, green*
B4	Leaf alcohol	200[m]	< 0.01	ND	Grass*
B5	1-Octanol	900[m]	< 0.01	< 0.01	Chemical, metal, burnt*
B6	Decyl alcohol	230[m]	< 0.01	< 0.01	Fat*
B7	Benzyl alcohol	5500[m]	< 0.01	ND	Sweet, flower*
B8	Phenethyl alcohol	10000[m]	< 0.01	< 0.01	Honey, spice, rose, lilac*
Acid					
C1	Acetic acid	50000[m]	< 0.01	< 0.01	Sour*
C2	Butyric acid	3190[m]	ND	< 0.01	Rancid, cheese, sweat*
C3	Hexanoic acid	80000[m]	ND	< 0.01	Sweat*
C4	Octanoic acid	5000[m]	ND	< 0.01	Sweat, cheese*
C5	Decanoic acid	120000[m]	ND	< 0.01	Rancid, fat*
C6	9-Decylenic acid	4300[n]	ND	< 0.01	Fatty, fruity, soapy※
Aldehyde					
D1	Hexanal	210[m]	< 0.01	< 0.01	Grass, tallow, fat*
D2	Nonaldehyde	3.5[m]	0.04	0.01	Fat, citrus, green*
D3	Decanal	5[m]	0.02	0.01	Soap, orange peel, tallow*
D4	Hyacinthin	90[m]	ND	< 0.01	Hawthorn, honey, sweet*
Ketone					
E1	Methaptenone	/	ND	ND	/

E2	Geranyl acetone	100[m]	< 0.01	< 0.01	Magnolia, green*
Other					
F1	Undecane	10000[n]	< 0.01	ND	Alkane*
F2	Styrene	120[m]	ND	< 0.01	Balsamic, gasoline*
F3	1,3-Propanediol monoethyl ether	50000[n]	ND	< 0.01	Fruit※
F4	Therasurane	/	ND	ND	/
F5	2,4-Di-tert-butylphenol	0.5[n]	0.34	0.39	/

[1]Code of every compound; [2]LR juice before fermentation; [3]LR juice after fermentation; [m]odour thresholds from the "Compendium of Compound Odour Thresholds"; [n]odour thresholds from "Odour Thresholds and Odour Activity Values of Key Aroma-Active Compounds Compilations of Odour Threshold Values in Air, Water and Other Media (Edition 2011)"; *Odour descriptions from http://www.flavornet.org/flavornet.htmlflavornet; ※Odour descriptions from https://foodb.ca/compounds; ★Odour descriptions from http://www.thegoodscentscompany.com.

coenzyme A (Ouyang *et al*., 2017). Nonanal and decanal, which have strong greasy odour and sweet orange notes, are aldehydes that contribute to a greater extent to the aroma of LR wine. After alcoholic fermentation, the content of both LR juice decreased, presumably because the aldehydes reacted with ethanol, and were converted to esters. The decrease in the content of nonanal and decanal in LR wine was accompanied by the production of new volatile compounds, ethyl decanoate and isopentyl alcohol, respectively. We observed that decanoic acid was not present in the pre-fermentation samples, whereas in the post-fermentation samples, not only a decrease in decanal content but also a small amount of new decanoic acid was added, so it was speculated that the ethyl decanoate was generated from decanal through a reduction reaction to produce decanoic acid, which in turn underwent an esterification reaction with ethanol. This also confirms that the decrease in aldehyde content in fermented LR juice is one of the reasons for the increase in ester content. Isoamyl alcohol is formed by the deamination and decarboxylation of isoleucine during the fermentation process, and provides a slightly alcoholic, fruity-sweet flavour to the fermented LR beverage (Ouyang *et al*., 2017). In addition, we found that ethyl hexyl was absent in unfermented LR juice, whereas the ROAV in fermented LR juice was as high as 18.71. Ethyl hexyl has an apple-skin, fruity flavour, thus giving a strong apple-fruit flavour in fermented LR beverage.

Therefore, the aroma of LR juice after mild fermentation by yeast mainly showed strong fruity flavour, and slight wine and greasy flavour, and the main aroma substances were esters and aldehydes.

Functional component analysis

Yeast has an obvious influence on the type and content of nutrients and the flavour of fermented beverage (Qiang *et al*., 2023). As shown in Figure 5, the polysaccharide and flavonoid contents of raw LR juice changed significantly after mild fermentation by yeast. However, the content of anthocyanin did not change significantly. The content of total polysaccharides decreased from 10.88 g/100 g before to 9.99 g/100 g after fermentation. It is worth noting that the content of total flavonoids in LR juice after fermentation was higher than that before fermentation, increasing from 1.0775 to 1.7025 g/100 g. The total polysaccharides in wolfberry include arabinose, galactose, rhamnose, xylose, mannose, and glucose peptide-containing polysaccharides (Yu *et al*., 2022). Some yeasts can convert xylose and arabinose into alcohol *via* pentose metabolism (Wu *et al*., 2023). Xylose isomerase (XI) can directly convert xylose into xylulose. Xylulose is phosphorylated by xylulokinase (XK) to produce 5-phosphoxylulose, which can enter the pentose phosphate pathway (PPP), and finally enter the glycolysis pathway to produce alcohol *via* the intermediate products 6-glucose phosphate and 3-glyceraldehyde phosphate (Pswarayi and Ganzle, 2022). In addition, arabinose can also be enzymatically converted to xylulose 5-phosphate, which can then enter the PPP (Bedoya *et al*., 2024). This may account for the decrease in the total polysaccharide content after fermentation.

Owing to the high stability of flavonoids under the study conditions when all the flavonoids in the raw materials entered the fermentation broth, the content of flavonoids in the fermented beverage remained basically unchanged. The change in

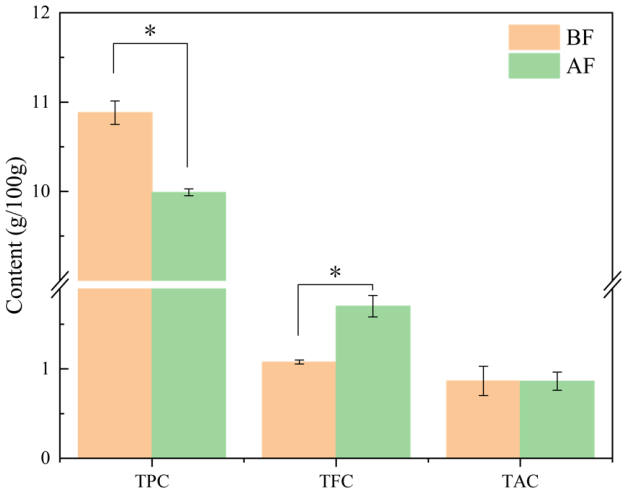

Figure 5. Differences in contents of total polysaccharides, flavonoids, and anthocyanins before and after fermentation. BF represents juice before fermentation, which is LR juice. AF represents juice after fermentation, which is LR beverage.

flavonoid content during fermentation occurred because the amount of flavonoids dissolved into the fermented beverage, increased with the prolongation of the fermentation process and the soaking duration. Meanwhile, the alcohol content of the fermentation broth also increased during the fermentation process, which was conducive for the extraction of flavonoids (Xu *et al*., 2024).

The molecular structure of anthocyanins makes these molecules unstable. Thus, anthocyanins are greatly affected by external stimuli such as temperature, light, and pH, among other factors. During the fermentation process, various factors such as pH, temperature, and biochemical changes can destroy the molecular structure of anthocyanins, leading to a decrease in their content (Fang *et al*., 2025). However, in our study, the anthocyanin content in the fermented LR beverages did not change significantly after mild fermentation at low temperature, consistent with previous findings.

Hierarchical and principal component analysis of volatile compounds

In order to visually analyse the differences in volatile compounds before and after the fermentation of LR juice, heat maps were constructed, and the changes in the aroma of each sample were evaluated. Horizontal clustering was used to reflect the changes in different volatile compounds within each sample, and longitudinal clustering was used to indicate the relationship between the samples for a certain aroma. Red indicated a higher relative value, while blue indicated lower relative values. The heat map and cluster analysis of changes in the relative content of volatile compounds in the eight samples are shown in Figure 6a. The figure shows that there were significant differences in the presence of volatile compounds before and after fermentation. BF1, BF2, BF4, and BF3 were similar, and belonged to the same category, while AF1, AF2, AF3, and AF4 belonged to the same category. In terms of compound clustering, the volatile compounds involved in the analysis could be divided into three categories. The first category was blue before fermentation, including ethyl hexyl, propyl hexanoate, 1-heptyl acetate, ethyl caprylate, ethyl caprate, 3-methylbutylester, 1,3-propanediol diacetate, hexanoic acid, octanoic acid, decanoic acid, 9-decylenic acid, and methaptenone. The second category was blue after fermentation, and included leaf and benzyl alcohol, while the third category showed no obvious change after fermentation, and included hexyl acetate, 1-hexanol, and geranyl acetone.

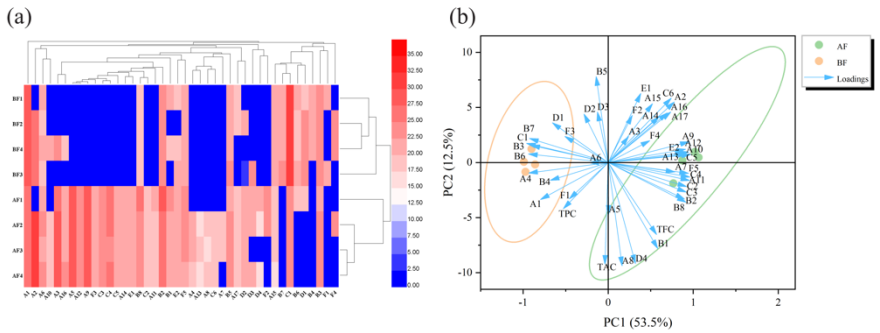

Figure 6. Heat map of aroma substance clustering before and after fermentation **(a)**, principal component analysis (PCA) biplot of aroma compounds and functional components before and after fermentation **(b)**. BF represents juice before fermentation, which is LR juice. AF represents juice after fermentation, which is LR beverage.

To further determine if a relationship existed between aroma, functional composition, and fermentation treatment, PCA was performed (Figure 6b). PCA is an unsupervised statistical technique that provides a good representation of sample similarities, without any classification (He *et al*., 2025c).

From the results obtained, PC1 and PC2 explained 53.5 and 12.5% of the total data variance, respectively, with a cumulative value of 66.0%. As shown in Figure 6b, raw LR juice was located on the negative side of PC1. The number of volatile compounds on this side was small, and mainly included a small number of higher alcohols. The fermented LR beverage was located towards the positive value of PC1, being rich in volatile compounds, and mainly containing a high number of esters and acids. Specifically, the fermented LR beverage samples were in the first and fourth quadrants, close to propyl hexanoate (A7), ethyl caprylate (A9), 2-ethylhexyl acetate (A10), ethyl nonanoate (A11), ethyl caprate (A12), 3-methylbutylester (A13), 2-methyl-1-propanol (B1), 3-methyl-1-butanol (B2), phenethyl alcohol (B8), butyric acid (C2), hexanoic acid (C3), octanoic acid (C4), and decanoic acid (C5). These volatile compounds have pineapple- and apple-like aromas. Anthocyanins were neither close to the raw LR juice nor the fermented LR beverage, indicating that the anthocyanins were not changed following fermentation. Notably, the angle between the arrow clusters of total flavonoids and the arrowheads of 2-methyl-1-propanol (B1), isoamyl alcohol (B2), and phenethyl alcohol (B8) was very small. The results indicated that there was a strong correlation between the content of total flavonoids and 2-methyl-1-propanol (B1), 3-methyl-1-butanol (B2), and phenethyl alcohol (B8). The contents of 2-methyl-1-propanol (B1), 3-methyl-1-butanol (B2), and phenethyl alcohol (B8) significantly increased after fermentation. This result well-validated the analysis presented in previous section "*Calculation of ROAVs*".

Correlation analysis

In order to further explore the trends in the changes of various substances after fermentation, correlation coefficients were calculated to visualise the relationships between volatile compounds and the functional components before and after fermentation (Figure 7). There was a significant positive correlation between esters and alcohols ($p < 0.05$), with a correlation coefficient of 0.71, while a significant negative correlation was noted between esters and acids ($p < 0.05$), with a correlation coefficient of -0.78. Esters are synthesised in yeast cells *via* alcohol acetyl-transferases (AA Tases) using higher alcohols and acetyl-CoA as substrates (Cai *et al*., 2022d). Yeast contains esterase and produces corresponding esters consisting of small molecule acids and ethanol upon esterase action.

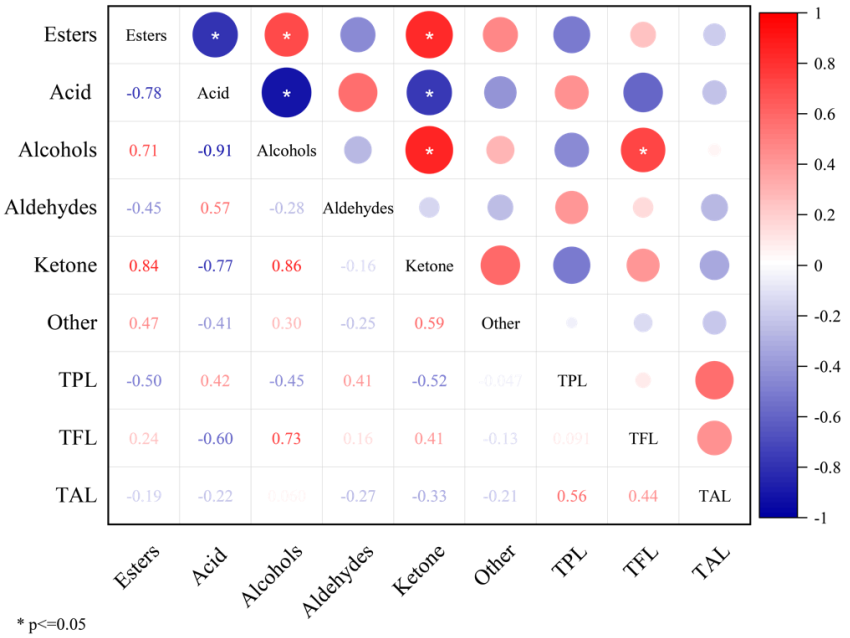

Figure 7. Correlation analysis between volatile compounds and functional components before and after fermentation.

Alcohols and total flavonoids showed a positive correlation, with a correlation coefficient of 0.73 ($p < 0.05$). This was mainly because flavonoids are insoluble in water but easily solubilise in alcohol. As alcohol fermentation progressed, the flavonoids gradually dissolved in the fermentation liquid with the increase in alcohol content (Kelanne *et al*., 2020). Ketones also showed a significant positive association with esters and alcohols, and showed a negative association with acids. However, their relationship has been rarely reported in previous studies.

Conclusion

In the present work, the changes of volatile compounds and functional components of raw LR juice and mildly fermented LR beverage were compared. The present work showed that mildly fermented LR beverage retained some components of the raw LR juice. However, it had higher levels of volatile compounds such as alcohols and esters, which were produced *via* yeast metabolism. After mild fermentation, the number of esters in the juice increased from 8 to 17, and the proportion of esters increased from 20.65 to 72.59%. Compared with the raw LR juice, the number of alcohols in the fermented LR beverage decreased by two. However, it is noteworthy that the content of alcohols increased from 2.05 to 5.72%. The relative content of acids decreased significantly from 70.76 to 1.74%, but the number of acids increased from 1 to 6. A total of seven volatile compounds with ROAV > 0.01 were calculated in fermented and unfermented LR juices, among which isoamyl acetate increased more than 15-fold after fermentation, and ethyl hexyl was detected only in fermented LR juice, but its ROAV value was as high as 18.71. In addition, both volatile compounds had strong fruity sweetness. This explained why the fermented LR beverage had a rich aroma and unique, attractive, and suitable sour taste. In terms of functional components, the total flavonoid content in the fermented LR beverage increased significantly from 1.08 to 1.70 g/100 g ($p < 0.05$) during the fermentation process. On the contrary, the total polysaccharide content decreased significantly after fermentation, from 10.88 to 9.99 g/100 g ($p < 0.05$). However, the content of anthocyanins did not change significantly after fermentation. The present work provides a certain theoretical basis for the development of LR-related drinks, but the formation mechanism of functional components and volatile compounds needs to be further explored by omics technology.

Acknowledgement

The present work was financially supported by the NGHJG Project of Xinjiang Production and Construction Corps (grant no.: 2023AA503), and the Projects of Innovation and Development Pillar Program for Key Industries in Southern Xinjiang of Xinjiang Production and Construction Corps (grant no.: 2022DB007).

References

Aldrete-Tapia, J. A., Escalante-Minakata, P., Miranda-Castilleja, D. E. and Hernández-Iturriaga, M. 2022. Fermentation conditions for yeast selection and effect of yeast-bacterial interaction in developing a starter culture for tequila fermentation. Journal of Food Science 87(11): 5089-5098.

Bedoya, K., Buetas, L., Rozès, N., Mas, A. and Portillo, M. C. 2024. Influence of different stress factors during the elaboration of grape must's *pied de cuve* on the dynamics of yeast populations during alcoholic fermentation. Food Microbiology 123: 104571.

Bernardino, A. R. S., Torres, C. A., Grosso, F., Peixe, L. and Reis, M. A. M. 2024. Optimal conditions for 2-phenylethanol production from L-phenylalanine by *Acinetobacter soli* ANG344B. Journal of Chemical Technology and Biotechnology 99(3): 746-754.

Cai, W. C., Tang, F. X., Guo, Z., Guo, X., Zhang, Q., Zhao, X. X., … and Shan, C. H. 2020a. Effects of pretreatment methods and leaching methods on jujube wine quality detected by electronic senses and HS-SPME-GC-MS. Food Chemistry 330(12): 127330.

Cai, W. C., Tang, F. X., Shan, C. H., Hou, Q. C., Zhang, Z. D., Dong, Y. and Guo, Z. 2020b. Pretreatment methods affecting the color, flavor, bioactive compounds, and antioxidant activity of jujube wine. Food Science and Nutrition 8(9): 4965-4975.

Cai, W. C., Tang, F. X., Shan, C. H., Yang, L. P., Zhan, Q., Zhao, X. X. and Ning, M. 2018.

Changes in volatile compounds of fermented mixed drink using commercial yeast strain. Przemysl Chemiczny 97(8): 1398-1405.

Cai, W. C., Wang, Y. R., Ni, H., Liu, Z. J., Liu, J. M., Zhong, J. A., … and Guo, Z. 2021a. Diversity of microbiota, microbial functions, and flavor in different types of low-temperature Daqu. Food Research International 150: 110734.

Cai, W. C., Xue, Y. A., Tang, F. X., Wang, Y. R., Yang, S. Y., Liu, W. H., … and Shan, C. H. 2022c. The depth-depended fungal diversity and non-depth-depended aroma profiles of pit mud for strong-flavor *baijiu*. Frontiers in Microbiology 12: 789845.

Cai, W. C., Xue, Y. A., Wang, Y. R., Wang, W. P., Shu, N., Zhao, H. J., … and Shan, C. H. 2021b. The fungal communities and flavor profiles in different types of high-temperature Daqu as revealed by high-throughput sequencing and electronic senses. Frontiers in Microbiology 12: 784651.

Cai, W. Q., Li, B., Chen, Y. R., Fu, G. M., Fan, H. W., Deng, M. F., … and Li, M. X. 2022d. Increase the content of ester compounds in blueberry wine fermentation with the ester-producing yeast: *Candida glabrata*, *Pichia anomala*, and *Wickerhamomyces anomalus*. Foods 11(22): 3655.

Cai, W., Fei, L., Zhang, D., Ni, H., Peng, B., Zhao, X., … and Shan, C. 2024. Impact of ultra-high-pressure treatment on microbial community composition and flavor quality of jujube juice: Insights from high-throughput sequencing technology, intelligent bionic sensory system, and metabolomics approach. Food Research International 191: 114688.

Cai, W., Wang, Y., Liu, Z., Liu, J., Zhong, J. A., Hou, Q., … and Guo, Z. 2022a. Depth-depended quality comparison of light-flavor fermented grains from two fermentation rounds. Food Research International 159: 111587.

Cai, W., Wang, Y., Wang, W., Shu, N., Hou, Q., Tang, F., … and Guo, Z. 2022b. Insights into the aroma profile of sauce-flavor *baijiu* by GC-IMS combined with multivariate statistical analysis. Journal of Analytical Methods in Chemistry 2022: 4614330.

Chu, Q. M., Xie, S. N., Wei, H. L., Tian, X. C., Tang, Z. H., Li, D. W. and Liu, Y. 2024. Enzyme-assisted ultrasonic extraction of total flavonoids and extraction polysaccharides in residue from *Abelmoschus manihot* (L). Ultrasonics Sonochemistry 104: 106815.

Du, Y. W., Ma, H. Y., Liu, Y. Y., Gong, R., Lan, Y., Zhao, J. H., … and Sun, G. Y. 2024. Major quality regulation network of flavonoid synthesis governing the bioactivity of black wolfberry. New Phytologist 242(2): 558-575.

Fang, Z. Z., Lin-Wang, K., Lin, Y. J. and Espley, R. V. 2025. Metabolomic and transcriptomic analyses provide insights into temperature and light regulated anthocyanin accumulation in flesh of 'Furongli' plum (*Prunus salicina* Lindl.). Postharvest Biology and Technology 221: 113326.

Geng, J. Y., Zhao, L. and Zhang, H. L. 2021. Formation mechanism of isoprene compounds degraded from carotenoids during fermentation of goji wine. Food Quality and Safety 5: 1-9.

He, C. L., Zhang, D. S., Mao, Y. W., Mkunga, J. J., Fei, L. Y., Chen, P. P., … and Cai, W. C. 2025c. Integrative analysis of metabolite changes and potential health effects in pomegranate juice fermentation. Food Bioscience 64: 105934.

He, C., Mkunga, J. J., Zhang, D., Mao, Y., Fei, L., Chen, P., … and Cai, W. 2025a. Integrative analysis of the fermentation mechanisms, antioxidant properties, and potential health benefits of mulberry wine using multiple bioinformatics approaches. Food Bioscience 66: 106185.

He, C., Peng, B., Zhang, D., Fei, L., Mao, Y., Lu, Z., … and Cai, W. 2025b. Comparative analysis of strong-flavor *baijiu* and brandy: Volatile and nonvolatile metabolic compounds identified using non-targeted metabolomics. European Food Research and Technology 251: 1-15.

Hong, M. N., Li, J., Chen, Y. W., Qi, B. Y., Huang, Y. P., Wu, J., … and Wang, F. 2021. Impact of mixed non-*Saccharomyces* yeast during fermentation on volatile aroma compounds of Vidal blanc icewine. LWT - Food Science and Technology 145(8): 111342.

Huang, J. T., Wang, Y. Q., Ren, Y. C., Wang, X. N., Li, H. C., Liu, Z. D., … and Gao, Z. P. 2022a. Effect of inoculation method on the quality and nutritional characteristics of low-alcohol kiwi wine. LWT - Food Science and Technology 156(7): 113049.

Huang, W. J., Fang, S. M., Wang, J., Zhuo, C., Luo, Y. H., Yu, Y. L., … and Ning, J. M. 2022b. Sensomics analysis of the effect of the withering method on the aroma components of Keemun black tea. Food Chemistry 395: 133549.

Kelanne, N., Yang, B. R., Liljenbäck, L. and Laaksonen, O. 2020. Phenolic compound profiles in alcoholic black currant beverages produced by fermentation with *Saccharomyces* and Non-*Saccharomyces* yeasts. Journal of Agricultural and Food Chemistry 68(37): 10128-10141.

Liu, B., Ma, J. Y., Li, T., Li, P., Yan, D. H., Zhu, J. and Zhang, X. G. 2024. Advances in the preparation, structure and bioactivity of polysaccharides from *Lycium ruthenicum* Murr.: A review. Foods 13: 1995.

Liu, Z. G., Liu, B. L., Wen, H. X., Tao, Y. D. and Shao, Y. 2020. Phytochemical profiles, nutritional constituents and antioxidant activity of black wolfberry (*Lycium ruthenicum* Murr.). Industrial Crops and Products, 154(10): 112692.

Ouyang, X. Y., Yuan, G. S., Ren, J., Wang, L. Y., Wang, M. Z., Li, Y. H., … and Zhu, B. Q. 2017. Aromatic compounds and organoleptic features of fermented wolfberry wine: Effects of maceration time. International Journal of Food Properties 20(10): 2234-2248.

Peng, B., Hao, X. S., Zhang, Q., Cai, W. C., Zhao, X. X., Tang, F. X. and Shan, C. H. 2024. Effects of low-temperature storage on the aroma composition and quality of Golden Empress Hami melons: A transcriptomic and metabolomic analysis. Food Bioscience 57: 103422.

Pswarayi, F. and Ganzle, M. 2022. African cereal fermentations: A review on fermentation processes and microbial composition of non-alcoholic fermented cereal foods and beverages. International Journal of Food Microbiology 378: 109815.

Qiang, X., Xia, T., Geng, B. B., Zhao, M., Li, X., Zheng, Y. and Wang, M. 2023. Bioactive components of *Lycium barbarum* and deep-processing fermentation products. Molecules 28(24): 8044.

Qin, Z. H., Petersen, M. A. and Bredie, W. L. P. 2018. Flavor profiling of apple ciders from the UK and Scandinavian region. Food Research International 105: 713-723.

Sharma, R., Raghuvanshi, R., Kumar, R., Thakur, M. S., Kumar, S., Patel, M. K., … and Saxena, S. 2022. Current findings and future prospective of high-value trans Himalayan medicinal plant *Lycium ruthenicum* Murr: A systematic review. Clinical Phytoscience 8(1): 3.

Wu, Y. Z., Li, Z. G., Zou, S. B., Dong, L., Lin, X. P., Chen, Y. X., … and Liang, H. P. 2023. Chemical composition and flavor characteristics of cider fermented with *Saccharomyces cerevisiae* and Non-*Saccharomyces cerevisiae*. Foods 12(19): 3565.

Xu, M. L., He, Y. F., Xie, L., Qu, L. B., Xu, G. R. and Cui, C. X. 2024. Research progress on active ingredients and product development of *Lycium ruthenicum* Murray. Molecules 29(10): 2269.

Yu, C. X., Hu, X. X., Ahmadi, S., Wu, D. M., Xiao, H., Zhang, H. L., … and Chen, J. L. 2022. Structure and *in vitro* fermentation characteristics of polysaccharides sequentially extracted from goji berry (*Lycium barbarum*) leaves. Journal of Agricultural and Food Chemistry 70(24): 7535-7546.

Yun, D. W., Yan, Y. M. and Liu, J. 2022a. Isolation, structure and biological activity of polysaccharides from the fruits of *Lycium ruthenicum* Murr: A review. Carbohydrate Polymers 291(16): 119618.

Zhang, J. T., Dong, Y. S., Nisar, T., Fang, Z. X., Wang, Z. C. and Guo, Y. R. 2020. Effect of superfine-grinding on the physicochemical and antioxidant properties of *Lycium ruthenicum* Murray powders. Powder Technology 372: 68-75.

Zhao, X. X., Tang, F. X., Cai, W. C., Peng, B., Zhang, P. L. and Shan, C. H. 2023. Effect of fermentation by lactic acid bacteria on the phenolic composition, antioxidant activity, and flavor substances of jujube-wolfberry composite juice. LWT - Food Science and Technology 184: 114884.