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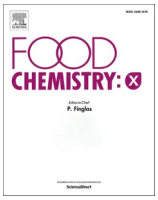

Effects of cofermentation of *Saccharomyces cerevisiae* and different lactic acid bacteria on the organic acid content, soluble sugar content, biogenic amines, phenol content, antioxidant activity and aroma of prune wine

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ABSTRACT

To determine the effect of cofermentation of *Saccharomyces cerevisiae* and different LABs on prune wine quality, this study compared phenolic compounds, organic acids, soluble sugars, biogenic amines and volatile flavor compounds among different treatments. The results showed that inoculation of LAB increased DPPH and total flavonoid content. Malic acid content was reduced in HS, HB and HF. Histamine content in S, F and B was lower than the limits in French and Australian wines. 15 phenolic compounds were identified. Yangmeilin and chlorogenic acid were detected only in HS, HF and HB. 51 volatile flavor compounds were identified, esters being the most diverse and abundant. 14 volatile flavor compounds with OAV > 1 contributed highly to the aroma of prune wine. 9 chemical markers including resveratrol, rutin, and catechin were screened to explain intergroup differences by OPLS-DA. This study provides new insights into the processing and quality analysis of prunes.

1. Introduction

'France' prune (*Prunus domestica* L.) is a plant of the *Prunus* genus in the *Rosaceae* family. This fruit has anti-aging and immune-enhancing properties because it contains a high content of bioactive ingredients, such as vitamins, anthocyanins, and minerals (Celik et al., 2017). Prune juice is used as a regular household juice to relieve constipation in the United States, Canada, and some European countries. In California, prunes are used as a baking ingredient. Prune is native to Europe and was introduced to China for cultivation. Xinjiang is the largest prune cultivation region in China due to its suitable water and soil conditions. At present, the main research for prune fruit has been focused on cultivation technology, postharvest storage quality, and bioactive components in fruit. Moreover, a single product structure and shorter harvesting period constrain the processing and utilization of prunes.

In general, processing fruit into wine is an effective strategy to increase its added value. The fermentation process occurs spontaneously due to the metabolic activities of microorganism naturally presented in the raw material or the environment (Guan et al., 2023). The fermentation of fruit wines retains the flavor and nutritional quality of the fruit, providing more unique qualities for it. With the development of the fruit wine industry, fruit wines fermented with mixed strains are superior to those made with a single strain of *Saccharomyces cerevisiae* (*S. cerevisiae*) (Lleixà et al., 2016). The mixed fermentation of *S. cerevisiae* with *Non-Saccharomyces* in brewing fruit wine is a common strategy for improving its quality (Padilla, Gil, & Manzanares, 2018). It was reported that mixed fermentation with *S. cerevisiae* and *Pichia pastoris* could improve the antioxidant capacity of Cabernet Sauvignon wines, making the flavor more pleasant and more palatable to consumers (Liu et al., 2023). In a previous report from Spain, mixed fermentation with

Abbreviations: MLF, malolactic fermentation; LAB, lactic acid bacteria; ABTS, 2,2′-Azinobis-(3-ethylbenzthiazoline-6-sulphonate); DPPH, 1,1-Diphenyl-2-picrylhydrazyl radical 2,2-Diphenyl-1-(2,4,6-trinitrophenyl) hydrazyl; CK, samples inoculated only with *S. cerevisiae*; S, samples inoculated with both *S. cerevisiae* and *Streptococcus thermophilus*; B, samples inoculated with both *S. cerevisiae* and *Lactobacillus delbrueckii*; F, samples inoculated with both *S. cerevisiae* and *Lactobacillus paracasei*; HS, samples inoculated with *Streptococcus thermophilus* at the end of alcoholic fermentation; HB, samples inoculated with *Lactobacillus delbrueckii* at the end of alcoholic fermentation; HF, samples inoculated with *Lactobacillus paracasei* at the end of alcoholic fermentation; OAV, odour activity value; OPLS-DA, orthogonal partial least squares-discriminant analysis; PCA, principal component analysis.

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Schizosaccharomyces pombe and *S. cerevisiae* was shown to both improve wine flavor and reduce the high levels of biogenic amines produced by malolactic fermentation (MLF) (Mylona et al., 2016).

In recent years, the cofermentation of *S. cerevisiae* and lactic acid bacteria (LAB) has received much attention. An increasing number of studies have shown that LAB, as a mature fermentation aid, plays a vital role in improving the taste and flavor of wines and enhancing the stability of the microbial community (Duan et al., 2023). It was reported that the cofermentation of Chinese rice wine using *S. cerevisiae* and LAB could serve to regulate the carbamate content of the wine (Zhou, Shu, Zhang, & Chen, 2021). LAB are gaining attention in the segment of fruit-based wines, such as cherry wine, *prunus mahaleb* wine, and blueberry wine, due to their enzymatic activities, such as glycosidase, esterase, dehydrogenase, reductase, and decarboxylase.

To date, there are fewer studies on making prune wine, and even fewer reports on mixed fermentation of prune wine with *S. cerevisiae* and LAB. Moreover, it is unknown as to which type of LAB to choose. Therefore, to investigate the effect of mixed fermentation with *S. cerevisiae* and different LABs on the quality of prune wine, this study determined the differences in basic physicochemical indicators, organic acids, soluble sugars, phenolic compounds, antioxidant capacity, biogenic amines, and volatile flavor compounds among different LAB treatments. Overall, this study provides a theoretical basis for brewing prune wine and mixed fermentation with *S. cerevisiae* and LAB.

2. Materials and methods

2.1. Materials and reagents

The prunes used in this study were provided by Xinjiang Guolibao Food Co., Ltd. (Xinjiang Uygur Autonomous Region, China). NaCl, $K_2S_2O_8$, 2,2′-Azinobis-(3-ethylbenzthiazoline-6-sulphonate) (ABTS) and 1,1-Diphenyl-2-picrylhydrazyl radical 2,2-Diphenyl-1-(2,4,6-trinitrophenyl) hydrazyl (DPPH) were purchased from Macklin (Shanghai, China). LC-MS grade water, methanol, and acetonitrile were purchased from Fisher Scientific (Loughborough, UK) and Macklin (Shanghai, China). Organic acid standards (99.0%), sugar standards (99.0%), biogenic amines standards (99.0%), and phenolic standards (99.0%) were purchased from Solarbio (Beijing, China). All reagents and standards were prepared with HPLC-grade water or methanol before use.

2.2. Strains and fermentation

S. cerevisiae CEC010 was purchased from Angel Yeast Co., Ltd. (Hubei, China). *Streptococcus thermophilus* (CICC6220) and *Lactobacillus delbrueckii* subsp. *Bulgaricus* (CICC6098) was purchased from the China Center of Industrial Culture Collection (Beijing, China). *Lactobacillus paracasei* SMN-LBK (CCTCCM2017429) was obtained from Wuhan University (Hubei, China). Three LAB strains were transferred to MRS solid medium and grown in an incubator (THZ—C, Taicang Experimental Equipment Factory, Taicang, China) at 37 °C for 48 h. Then, single colonies were transferred to MRS liquid medium and grown at 37 °C for 24 h. The cultures were re-inoculated (2% *v*/v) into MRS liquid medium. The cells were finally harvested by centrifugation (10,000 rpm, 5 min, 4 °C), washed three times using 0.85% sterile saline, and resuspended in the sterile saline. The freeze-dried *S. cerevisiae* was propagated in yeast malt agar medium (containing 2% glucose, 0.25% yeast extract, 0.25% bacteriological peptone, and 0.25% malt extract) at 25 °C for 48 h and then stored at −80 °C until used.

Fresh prune fruits were picked, chilled to 12 °C within 4 h, and returned to the laboratory within 24 h for subsequent processing. The fruits were washed with distilled water, removed from the pits, and juiced by a juicer (JYL-C01S, Joyoung Co. Ltd., Jinan, China). The prune juice was filtered and pasteurized in a sterile container at 80 °C for 5 min to ensure microbiological safety. The yeast cultures were inoculated at 1% (*v*/v) with an initial yeast cell count of 10^6 CFU/mL, and the three LAB strains were initially inoculated at approximately 10^7 CFU/mL. Samples inoculated with both yeast and *Streptococcus thermophilus*, *Lactobacillus delbrueckii*, and *Lactobacillus paracasei* were named S, B, and F, respectively. At the end of alcoholic fermentation, samples inoculated with *Streptococcus thermophilus*, *Lactobacillus delbrueckii*was, and *Lactobacillus paracasei* were named HS, HB, and HF, respectively. Meanwhile, a set of trials was set up inoculating only 1% (*v*/v) of the yeast culture as CK. The sugar content of <4 g/L and the L-malic acid content of <0.4 g/L were considered as the end of alcoholic fermentation and MLF, respectively. Three parallel trials for each treatment were set up.

2.3. Determination of basic physical and chemical parameters

The pH of the samples was measured directly by a calibrated pH meter in Shanghai Lei Magnet Biotechnology Co., Ltd. (Shanghai, China). The alcohol content was determined by an alcohol meter manufactured by Huao Instrument Factory (Hebei, China). The soluble solids content was measured by hand-held refractometer and total acid was determined using 0.1 M NaOH. Reducing sugars was determined using the methods of previous generations (Cirlini, Ricci, Galaverna, & Lazzi, 2020). The determination of color attributes was determined based on a slight modification of this method (Kumar, Tian, & Harrison, 2022). Total phenol contents were determined by the Folin-Ciocalteu method and the results were expressed as gallic acid equivalents. Determination of total flavonoids in wine with reference to Sun et al. (2022). All determinations were repeated three times.

2.4. Determination of organic acids

The test conditions for organic acids followed the previous method (Tkacz, Chmielewska, Turkiewicz, Nowicka, & Wojdyło, 2020), the use of high-performance liquid chromatography and a photodiode array detector (HPLC-PDA, Acquity HPLC system, Agilent Technologies, Santa Clara, CA). Chromatographic separation was performed by an Agilent C18 column (250 mm × 4.6 mm × 5 μm; Agilent Technologies, Santa Clara, CA). Briefly, prune wine samples were filtered through 0.45 μm membranes in triplicate. The detection wavelength was 210 nm, the run time was 10 min, the flow rate was 1 mL /min, the column temperature was 30 °C, and the injection volume was 10 μL. Mobile phases A and B were methanol and 0.025 M KH_2PO_4 solution (acidified to pH 2.6 with H_3PO_4), respectively, with isocratic elution. Organic acids were identified and quantified by external standard methods. All determinations were repeated three times.

2.5. Determination of soluble sugars

The concentrations of soluble sugars were analyzed by high-pressure liquid chromatography (HPLC-ELSD, Merck-Hitachi L-7455, Merck KGaA, Darmstadt, Germany) equipped with an evaporative light scattering detector (ELSD, PL-ELS 1000, Polymers Labs Inc., Amherst, MA, US). The assay method used was that of a previous study (Wojdyło, Nowicka, & Bąbelewski, 2018). Briefly, 1.5 mL prune wine was filtered through a 0.22 μm membrane and prepared for detection. The run time was 15 min, the flow rate was 1 mL/min, the column temperature was 40 °C, and the injection volume was 20 μL. The mobile phases A and B were acetonitrile and ultrapure water (75:25), respectively, with isocratic elution. Soluble sugars were identified and quantified by an external standard method. All determinations were repeated three times.

2.6. Determination of phenolic compounds

Ultra-high performance liquid chromatography detection was performed using an ultra-high performance liquid chromatography equipped with a photodiode array detector (UPLC-PDA, Acquity UPLC Systems; Waters, Massachusetts, USA) coupled with a UPLC BEH C18

column (2.1 × 100 mm, 1.7 μm) (Waters, Milford, Massachusetts, USA). Prune wine samples were pretreated by Ju et al. (2023). The mobile phase A was methanol and mobile phase B was 1% aqueous acetic acid. Detection procedures were as follows: 0–10 min, 90% B; 10–20 min, 90–87% B; 20–27 min, 87–82% B; 27–35 min, 82–78% B; 35–47 min, 78–75% B; 47–50 min, 75–68% B; 50–58 min, 68–65% B; 58–62 min, 65–75% B; 62–70 min, 75–75% B; and 62–70 min, 75–75% B. The assay procedure was as follows. The flow rate was 1 mL/min, injection volume was 10 μL, column temperature was 35 °C, and full wavelength scanning was performed.

2.7. Determination of antioxidant capacity

DPPH free radical scavenging capacity was determined as follows, diluted 2 mL of the sample was vortex-mixed with 0.1 mM DPPH ethanol solution (2 mL) and the absorbance was measured by reacting it away from light. DPPH free radical scavenging capacity was expressed as gallic acid equivalents, and the absorbance of the sample was measured at 515 nm. All determinations were repeated three times.

The free radical scavenging ability of ABTS in the samples was determined using the previous method (Chen, Xie, He, Sun, & Bai, 2023). 3.0 mL of ABTS solution was vortexed with 30 μL of the samples and left to stand for 30 min, protected from light, and the absorbance was measured. The absorbance of ABTS solution was detected at 734 nm and diluted with ethanol to 0.7 ± 0.02 before use. All determinations were repeated three times.

2.8. Determination of biogenic amines

Biogenic amines were determined by Waters Alliance HPLC (Waters, Milford, MA, USA) according to the method of Wang, Ye, Zhu, Wu, and Duan (2014). Samples were pretreated as follows: 430 μL of borate buffer solution pH 9.0, 300 μL of methanol, 10 μL of internal standard (2-aminoadipic acid at a concentration of 1 g/L), and 12 μL of diethyl ethoxymethylmalonate (DEEMM) derivatization reagent were mixed with 400 μL of the reagent and ultrasonicated for 30 min. The sample was heated at 75 °C for 2 h, cooled to 25 °C, and passed through a 0.45 μm filter membrane.

2.9. Extraction and determination of volatile flavor compounds

Volatile flavor compounds in prune wines were extracted using the method of headspace solid-phase microextraction (HS-SPME). Briefly, 5 mL wine sample, 1 g NaCl, and 2 μL cyclohexanone (284 μg/L) were added into a glass headspace vial equipped with solid phase extraction needles containing SPME fiber component Divinylbenzene/Carboxene/Polydimethylsiloxane (DVB/CAR/PDMS) in 50/30 μm DVB/CARBOXEN-PDMS size, equilibrated for 15 min at 45 °C and then extracted for 30 min. Cyclohexanone was used as an internal standard to identify volatile flavor compounds.

Volatile flavor compounds were determined by gas chromatography–mass spectrometry (GC–MS) (Thermo, CA, USA) equipped with the TR-5MS column (30 m × 0.25 mm, 0.25 μm, J&W Scientific, CA, USA) and quadrupole DSQ II MS. The preheat program is set as follows: hold at 40 °C for 5 min and then warmed to 240 °C at a rate of 5 °C/min and maintained for 5 min. The mass spectrum was performed in electron impact (EI) mode with an ionization energy of 70 eV in full scan mode (45–400 amu). The preliminary identification of the compounds was carried out by comparing the mass spectra with the NIST17 mass spectral database. The internal standard method was used to quantify volatile flavor compounds, and the calculation formula was $C_i = (C_{is} \times A_i)/A_{is}$ (C_i is the mass concentration of any component (μg/kg); C_{is} is the mass concentration of the internal standard (μg/kg); A_i is the chromatographic peak area of any component; A_{is} is the chromatographic peak area of internal standard). All experiments were repeated three times.

Odour activity value (OAV) is used to evaluate the contribution of each volatile flavor compound and calculated using the following equation:

$$\mathrm{OAV} = \frac{C_i}{OT_i} \tag{1}$$

where C_i is the concentration of the various aroma components (μg/g); OT_i is the odour threshold of the aroma components in water.

2.10. Statistical analysis

Each treatment was performed in triplicate and the results were expressed using mean ± standard deviation. One-way analysis of variance (ANOVA) was performed by Statistica 13.1 software (StatSoft, Krakow, Poland). Principal component analysis (PCA) and orthogonal partial least squares-discriminant analysis (OPLS-DA) of the relative abundance of each volatile compound was performed using SIMCA 14. 1 software (Biometric Software Developer Umetrics, Umeå, Sweden). Heat maps were generated via R version 4.2.0 (Vigorous Calisthenics) to analyze the effect of different treatments on the flavor profiles of prune wine.

3. Results and discussion

3.1. Basic physical and chemical indicators

In this study, basic physicochemical indicators in prune wines are shown in Fig. 1. The pH of prune wine in HS, HF, and HB is lower than CK (Fig. 1a). The total acid content of the treated group increased compared with that of the CK (Fig. 1b). The total acid concentration fluctuated from 7.09 to 9.48 mg/mL in each treatment group, which was greater than that in the CK. Moreover, the total acid content of HS, HF, and HB was greater than that of S, F, and B, which may related to MLF, and a previous study reported the similar result (Ricci, Cirlini, Levante, & Dall'Asta, C., Galaverna, G., & Lazzi, C., 2018). Compared to CK, fermentation of prune wine with LAB inoculated at the end of alcoholic fermentation resulted in lower SSC (Fig. 1c). Higher reducing sugars in B (5.00 mg/mL) and HB (5.22 mg/mL) samples compared to CK (4.49 mg/mL) (Fig. 1d).

The alcohol content was significantly higher in F and S than in other samples (Fig. 1e), which may be attributed to the metabolites of LAB during mixed fermentation accelerating the efficiency of sugar conversion to ethanol (Qian et al., 2023). The interaction between *S. cerevisiae* and LAB in fermented products results in producing richer metabolites and improving fermentation efficiency. The color attributes are important indicators for evaluating wine quality. In general, L*, a*, and b* are parameters for evaluating color, indicating brightness, the color degree from green to red, and the color degree from blue to yellow, respectively. As shown in Fig. 1f, g, and h, the L* values fluctuate between 1722.05 and 2870.06, and the L* values of all treatments were lower than that of CK. This is because the mixed fermentation leads to the precipitation of phenols or flavonoids in the wine, which reduces the L* value of the wine (Forino, Picariello, Lopatriello, Moio, & Gambuti, 2020). Moreover, a decrease in L* value was detected in the mixed fermentation samples, especially in sample B. This indicates that mixed fermentation of this strain can have a negative effect on the brightness of prune wine. The b* values also showed significant differences among different treatments, and the b* value was significantly greater in S, F, and H samples than in CK, HS, HF, and HB samples. This could be related to the secretion of mannoproteins by the yeast, which can combine with anthocyanins to have an effect on the color of wines (Li, Zhai, Ma, Duan, & Yi, 2023). The total phenol content of samples B, F and S fluctuated between 144.70 and 152.22 mg/mL, and the total phenol content of samples HB, HF and HS fluctuated between 46.50 and 153.16 mg/mL, whereas the content of group CK was 151.03 mg/mL, which indicated

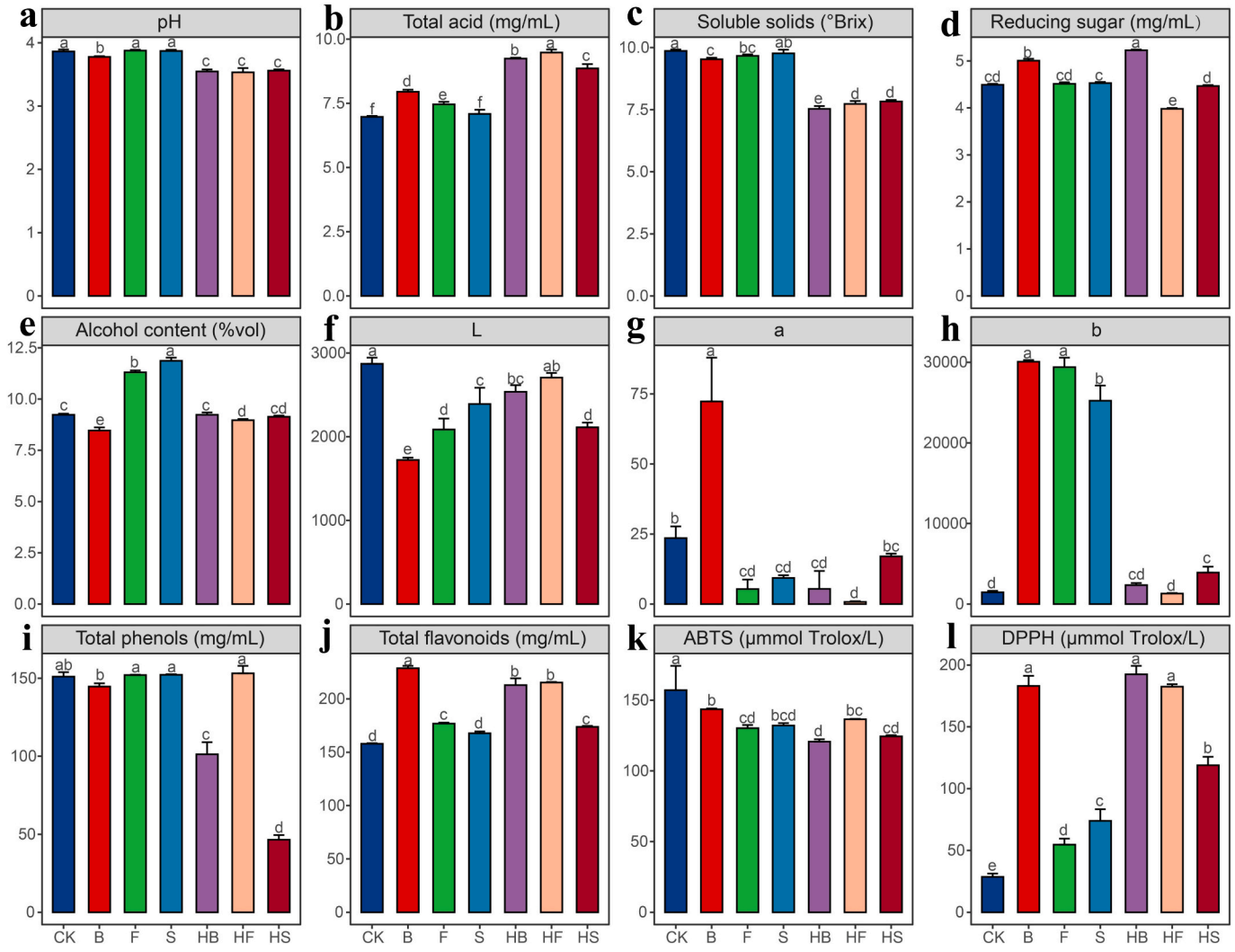

Fig. 1. The results of the basic physicochemical indices for each treatment group and the control group. Different letters at the top of the bars represent statistically significant differences ($P < 0.05$).

that the MLF had a greater effect on the total phenol content of prune wine ($P < 0.05$), as shown in Fig. 1i. The total flavonoid content varied significantly among the samples and was ranked in order of content as B > HF > HB > F > S > HS > CK (Fig. 1j). In addition, HF had the highest total phenolic content. There was a significant difference ($P < 0.05$) in the total flavonoid content among the different samples, with an average increase of 21.35% in the flavonoid content in each treatment group compared to that in the CK. Inoculation with Lactobacillus mixed fermentation affected the antioxidant capacity of prune wine compared to CK. The results of the ABTS radical scavenging capacity assay showed that inoculation with LAB reduced the ABTS radical scavenging capacity of the wines to varying degrees (Fig. 1k). The average ABTS loss was 14.1%, but B was the least affected. The DPPH activity of each treatment ranged from 54.72 to 192.5 μmol Trolox/L, which was significantly greater than that of CK (28.61 μmol Trolox/L; $P < 0.05$) (Fig. 1l), indicating that mixed fermentation could improve the DPPH radical scavenging capacity of prune wine. Furthermore, the DPPH activity of sample B was much greater than that of F and S under the same treatment and slightly less than that of HB, which implies that inoculation with this strain followed by MLF enhances the DPPH radical scavenging capacity. The differences between different inoculation treatments of the same strain were not significant. During fermentation, microorganisms secrete a variety of polyphenol-related enzymes (tannases, esterases, phenolic acid decarboxylases and glycosidases, etc.) to hydrolyze to smaller phenolic compounds (quercetin, kaempferol, gallic acid, ellagic acid, etc.), which have a higher bioactivity and bioavailability (Leonard, Zhang, Ying, Adhikari, & Fang, 2021). In this study, differences in the ability of different strains to secrete polyphenol-related enzymes may account for this result.

3.2. Organic acids and soluble sugars

The type and amount of organic acids and soluble sugars in a food affect its sensory properties. For example, malic acid in foods has a sharp and sour taste in the mouth, whereas lactic acid is softer. HPLC was employed to characterize the organic acids in the prune wine samples, and a total of 5 were identified: maleic acid, lactic acid, citric acid, quinic acid, and acetic acids. Malic acid content differed significantly ($P < 0.05$) between samples, in the order of content F > B > S > CK > HS > HF > HB (Table 1). The higher malic acid content in F (3.22 g/L), B (2.67 g/L), and S (2.15 g/L) compared to CK (1.22 g/L) implies that inoculation of LAB for prune wine increases the malic acid content, which has an effect on the taste. The malic acid content in HS (0.52 g/L), HF (0.15 g/L), and HB (0.14 g/L) was lower than that in CK (1.22 g/L). The lactic acid content of different samples varied greatly ($P < 0.05$), in the order of HB > HS > HF > CK > F > B > S (Table 1). The higher lactic acid content in HB (2.21 g/L), HS (2.14 g/L), and HF (2.01 g/L) compared to CK (1.16 g/L) implies that inoculation of LAB at the end of

Table 1
Organic acid and soluble sugar content in each treatment group.

	S	B	F	HS	HB	HF	CK
Organic acids (g/L)							
Quinic acid	9.73 ± 0.97a	9.14 ± 0.99a	8.85 ± 0.86a	10.02 ± 1.21a	9.04 ± 0.74a	10.50 ± 0.90a	8.77 ± 0.12a
Malic acid	2.15 ± 0.15c	2.67 ± 0.06b	3.22 ± 0.08a	0.52 ± 0.27e	0.14 ± 0.05f	0.15 ± 0.00f	1.22 ± 0.23d
Lactic acid	1.03 ± 0.06c	1.15 ± 0.05c	1.15 ± 0.16c	2.14 ± 0.02ab	2.21 ± 0.13a	2.01 ± 0.10b	1.16 ± 0.07c
Acetic acid	1.26 ± 0.03a	1.33 ± 0.45a	1.49 ± 0.45a	1.43 ± 0.51a	1.53 ± 0.47a	1.48 ± 0.31a	1.49 ± 0.55a
Citric acid	0.33 ± 0.03a	0.30 ± 0.00a	0.34 ± 0.05a	0.32 ± 0.02a	0.30 ± 0.30a	0.33 ± 0.30a	0.31 ± 0.04a
Soluble sugars (g/L)							
Sucrose	18.92 ± 4.75b	14.20 ± 2.90d	18.52 ± 2.47b	12.02 ± 1.40e	12.43 ± 3.64e	15.44 ± 1.93c	21.29 ± 0.86a
Glucose	28.98 ± 1.45b	26.59 ± 2.47b	26.18 ± 3.06b	27.03 ± 1.48b	38.58 ± 1.80a	38.25 ± 1.06a	38.82 ± 0.59a
Fructose	27.81 ± 1.72a	26.54 ± 0.97a	28.1 ± 1.47a	25.13 ± 4.44a	27.57 ± 0.46a	20.10 ± 3.20b	26.83 ± 2.00a

Note: Data are shown as mean ± standard deviation of three sets of replicated trials, and each set of data was analyzed by one-way ANOVA to mark significant differences ($P < 0.05$).

the alcoholic fermentation increased the lactic acid content in prune wine. Lactic acid content was lower in S (1.06 g/L), F (1.15 g/L), and B (1.15 g/L) than in CK (1.16 g/L). Malic acid has a sharp acidity and lactic acid has a soft acidity, and the MLF in the wines degrades the malic acid to produce lactic acid, which alters the taste of the wines (Zhang, Xing, Chu, Sun, & Wang, 2022). The content of quinic acid and acetic acid in the prune wine samples did not differ significantly.

Sucrose, glucose, and fructose in prune wines were identified by HPLC, and their concentrations are shown in Table 1. The content of sucrose was significantly different among the samples ($P < 0.05$), in order of CK > S > F > HF > B > HB > HS. This implies that inoculation with LAB for fermentation consumes more sucrose. Higher sucrose content in the samples implies lower sucrose utilization. HB and HS had the lowest sucrose content with 12.43 g/L and 12.02 g/L respectively. Fructose content did not show any significant difference between the samples. Changes in the types and contents of organic acids and soluble sugars in prune wine are closely related to the corresponding metabolic pathways. In particular, malic acid is converted to lactic acid by MLF, accompanied by changes in sensory attributes. The consumption of sucrose by LAB was similar in the present study, which may be due to the low utilization of sucrose by the LAB selected in this study. The decrease in soluble sugar content is related to the metabolic activity of the microorganisms and when multiple sugars coexist, fructose is first used as a carbon source, while glucose as a carbon source retards growth (Srinivas, Mital, & Garg, 1990).

3.3. Biogenic amine content

Biogenic amines are biologically active, low-molecular-weight nitrogenous organic bases detected mainly in fermented foods such as dairy products, meat products, and alcoholic beverages (Mohedano, López, Spano, & Russo, 2015). In this study, a total of five biogenic amines were measured, and putrescine was not detected in the prune wine samples. The highest biogenic amine content was detected in the HS group, which was significantly different from the biogenic amine content in the other prune wine samples (Fig. 2a). The biogenic amine content was lower in the remaining treatment samples than that in the CK. The MLF samples contained higher levels of biogenic amines compared to the mixed fermentation samples S, B, and F. The MLF samples contained more biogenic amines than the mixed fermentation samples. Inoculation with LAB decarboxylates amino acids to signal the synthesis of biogenic amines (Halász, Baráth, Simon-Sarkadi, & Holzapfel, 1994). In addition, cadaverine content was much higher in the HS group than in the other samples, whereas histamine content decreased in all samples after inoculation, which was particularly noticeable in samples S, B, and F. LAB has tyrosine decarboxylase activity, which may lead to elevated tyramine content during fermentation, but histamine is degraded by LAB to substances such as imidazole acetaldehyde, hydrogen peroxide, and ammonia. The ability of LAB to synthesize biogenic amines in an ethanol environment is affected by pH and strain, especially pH, with the highest production of histamine and tyramine occurring in beer at pH 4 and 5, respectively (Pei et al., 2023). The histamine content in S, F and B fluctuated between 1.27 and 2.94 mg/L, which is lower than the limits for histamine in wines from France (8 mg/mL), Australia (10 mg/mL), and Switzerland (10 mg/mL).

3.4. Phenolic compounds

Polyphenols (including flavonoids) are the most abundant phytochemicals in plant kingdom that serve as a supply source of health-beneficial properties such as antimicrobial and antioxidant activities in the human diet (Brandão et al., 2023). HPLC was employed to characterize the types and contents of phenolic compounds in prune wines. A total of 15 phenolic compounds were identified in CK (14), HS (14), HF (7), HB (13), B (13), F (13) and S (13) and their concentrations are shown in Table 2. The results showed that inoculated LAB mixed fermentation had an effect on the phenolic content of prune wine. The total contents of phenolic compounds in S, B, F, HS, HB, HF, and CK were 8895.19 μg/L, 7319.25 μg/L, 2669.94 μg/L, 8151.14 μg/L, 6390.37 μg/L, 7310.5 μg/L, and 9940.48 μg/L, respectively. Chlorogenic acid and Yangmeiin were identified only in HS, HB, HF and CK. The samples from groups S and HS inoculated with *Streptococcus thermophilus* showed the least decrease in phenolic compounds compared to group CK, indicating better fermentation potential of *Streptococcus thermophilus*.

Inoculation of LAB for prune wine at the end of alcoholic fermentation increased the contents of chlorogenic acid, benzoic acid, and catechins compared to CK. Isorhamnetin was detected only in S, B, and F at 1131.05 μg/L, 2973.75 μg/L, and 38.40 μg/L, respectively (Table 2 and Fig. 2b), which implies that inoculation of LAB for fermentation of prune wine could enhance the phenolic compounds species. Chlorogenic and salicylic acids were not detected in the B, S, and F samples, but were detected in the HF, HS, and HB samples, indicating that fermentation of prune wine by inoculation with LAB at the end of the alcoholic fermentation enhances the synthesis of salicylic and chlorogenic acids. Polyphenols and flavonoids such as ferulic acid, catechins, gingerols and cinnamic acid have antioxidant properties (Kumari, Gaur, & Tiwari, 2023), and an increase in catechin content was detected. In particular, the contents of catechins reached 725.48–748.35 μg/L in the HS, HF, and HB, which were greater than that in CK, S, F, and B (296.97–627.48 μg/L). Iso-rhamnetin was not detected in the CK, HS, HF, and HB. The rutin and salicylic acid contents decreased in all treatment samples. *Trans*-Ferulic acid was detected in all samples, but there was no obvious pattern of change in the content. In this study, inoculation of LAB at the end of alcoholic fermentation increased the content of phenolic compounds in prune wine more than inoculation of LAB at the beginning of fermentation. This phenomenon is consistent with the results of existing studies (Escott et al., 2018). The benefits derived from this treatment are superior to those derived from the mixed inoculation of LAB with *S. cerevisiae* for alcoholic fermentation. During fermentation, microorganisms secrete a variety of enzymes (tannases, esterases, phenolic acid decarboxylases, and glycosidases, among others) that hydrolyze phenolic compounds (quercetin, kaempferol, gallic acid, ellagic acid,

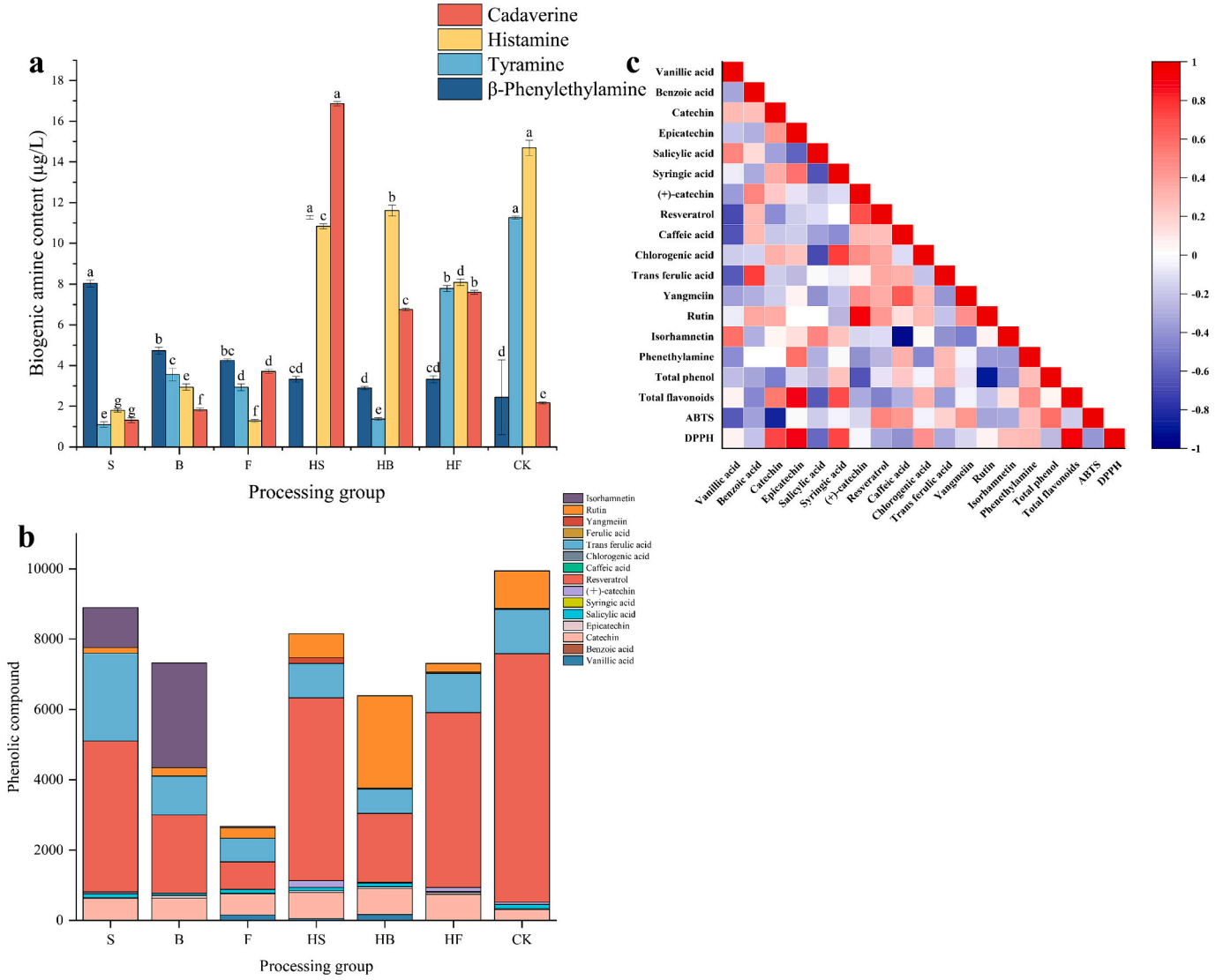

Fig. 2. Histogram of biogenic amines (a), histogram of stacked content of phenolic compounds (b) and heat map of correlation between phenolic compounds and antioxidant capacity (c). Different letters indicate significant differences ($P < 0.05$) between different prune wine samples.

Table 2
Phenolic compounds content in each treatment group. The contents were expressed with μg/L.

Phenolic compounds	S	B	F	HS	HB	HF	CK
Vanillic acid	5.24 ± 0.1f	5.05 ± 0.07f	155.54 ± 6.02b	49.24 ± 0.92c	167.22 ± 1.98a	14.31 ± 0.86d	9.74 ± 0.41e
Benzoic acid	2.1 ± 0.20a	0.12 ± 0.00d	0.08 ± 0.00d	1.51 ± 0.03b	0.2 ± 0.01 cd	0.32 ± 0.01c	0.11 ± 0.01d
Catechin	616.09 ± 3.55d	627.48 ± 5.28c	595.24 ± 5.33e	748.35 ± 7.12a	725.71 ± 7.44b	725.48 ± 1.02b	296.97 ± 1.49f
Epicatechin	31.03 ± 2.07d	78.31 ± 2.48a	23.37 ± 0.88e	44.79 ± 0.4c	57.16 ± 1.47b	57.05 ± 4.4b	32.13 ± 1.66d
Salicylic acid	98.38 ± 0.63c	55.99 ± 0.58d	108.67 ± 1.48b	94.65 ± 5.03c	114.35 ± 2.33a	16.85 ± 0.66e	110.58 ± 1.73ab
Syringic acid	3.25 ± 0.08d	4.66 ± 0.19c	0.66 ± 0.02f	0.42 ± 0.02 g	7.88 ± 0.10b	11.07 ± 0.12a	2.59 ± 0.06e
(+)-catechin	55.18 ± 0.22d	3.95 ± 0.13f	0.77 ± 0.05f	190.28 ± 9.53a	17.6 ± 0.49e	105.37 ± 0.3b	69.44 ± 0.64c
Resveratrol	4288.56 ± 39.24d	2222.05 ± 5.03e	779.82 ± 1.63 g	5200.28 ± 32.47b	1953.49 ± 29.3f	4975.79 ± 17.76c	7064.34 ± 15.46a
Caffeic acid	2.2 ± 0.14a	2.35 ± 0.05a	2.19 ± 0.02bc	2.3 ± 0.05ab	0.03 ± 0.00e	2.07 ± 0.08 cd	1.96 ± 0.01d
Chlorogenic acid	nd	nd	nd	0.24 ± 0.05b	0.22 ± 0.02b	0.78 ± 0.02a	0.15 ± 0.02b
Trans ferulic acid	2496.66 ± 6.47a	1103.91 ± 6.89c	666.05 ± 2.57e	975.86 ± 17.76d	696.12 ± 3.44e	1114.41 ± 12.52c	1258.09 ± 54.98b
Ferulic acid	0.01 ± 0.00e	0.14 ± 0.01c	0.13 ± 0.00d	0.18 ± 0.00a	nd	0.16 ± 0.00b	0.16 ± 0.00b
Yangmeiin	nd	nd	nd	157.82 ± 1.54a	22.71 ± 0.58d	36.46 ± 0.52b	25.66 ± 0.57c
Rutin	165.44 ± 0.53f	241.48 ± 2.53e	299.02 ± 3.07d	685.22 ± 5.88c	2627.73 ± 13.66a	250.38 ± 3.21e	1068.65 ± 26.15b
Isorhamnetin	1131.05 ± 17.28b	2973.76 ± 11.07a	38.4 ± 0.56c	nd	nd	nd	nd

Note: Data are shown as mean ± standard deviation of three sets of replicated trials, and each set of data was analyzed by one-way ANOVA to mark significant differences ($P < 0.05$). nd means that the substance was not detected.

and so on) in food products into smaller phenolic compounds, with variations in catabolic capacity between strains.

Fluctuations in phenolic content can affect the quality of wine. The correlation heatmap in Fig. 2c shows that there is a correlation between different phenolic compounds and antioxidant capacity, total phenolic content, and total flavonoid content. There was a positive correlation between rutin and benzoic acid, catechin, resveratrol, Yangmeilin, (+)-catechin, and caffeic acid in this study. The stronger correlation between rutin and (+)-catechin implies that the synthetic pathway of rutin and (+)-catechin may be upstream or downstream of a certain metabolic pathway in this study. The assay found that ABTS free radical scavenging capacity was negatively correlated with the content of catechins and vanillic acid, whereas the opposite conclusion was obtained in the study of Sun et al. (2022). Resveratrol, caffeic acid content and total phenol content were also positively correlated in this study. DPPH free radical scavenging capacity was positively correlated with catechin, epicatechin, salicylic acid and total flavonoid content.

3.5. Volatile flavor compounds

Probiotic fermentation of fruits and vegetables alters their volatile flavor compounds, thereby improving their sensory quality. HS-SPME-GC–MS was employed to detect volatile flavor compounds of prune wine. A total of 51 volatile flavor compounds were identified (Table S1), including alcohols (9), aldehydes (4), acids (9), esters (22), ketones (4), terpenes (1) and others (3). The esters were the most diverse and the highest in content. There were significant differences ($P < 0.05$) in the total volatile flavor compounds among the samples (Table S1). The total volatile flavor compounds in HF, HS and HB were lower than those in CK, B, F and S. In addition, HS was the most affected, with only 68.18% of the total volatile flavor compounds of CK, resulting in a more severe loss of volatile flavor compounds. A larger proportion of esters was identified in all the samples than in the CK group, but different fermentation treatments produced esters in different proportions. In this study, the HB and B samples were inoculated with *Lactobacillus delbrueckii* subsp. *Bulgaricus* were the least affected. Alcohols are the most important volatile flavor compounds in wine and contribute significantly to the overall flavor composition of wine (Zhuan et al., 2022). Mixed fermentation of prune wine with LAB inoculated before and after alcoholic fermentation had an effect on the type and content of volatile flavor compounds in prune wine samples (Table S1). MLF decreases the alcohol content, whereas mixed fermentation increases the alcohol content while producing unique alcohols that improve the flavor of prune wine (Zhang et al., 2022). In the prune wine samples, only group S showed an increase in alcohol content, while all other samples showed a decrease in alcohol content (Table S1). The alcohol with the greatest decrease in content was 2,3-butanediol. This compound has a fruity and creamy aroma, and a decrease in its content leads to a change in flavor in the prune fruit wine samples.

The fermentation of prune wine by mixed inoculation of LAB produced specific flavor compounds such as ethyl 7-octanoate, isopropyl caproate, n-propyl propionate, and 2-methylhexanoate. Ethyl 7-octanoate was identified only in B, while ethyl caproate was highest in B. The ethyl caproate was detected only in HB, while ethyl caproate was detected only in HB. Isopropyl hexanoate and 2-methylhexanoate were detected only in HB, both volatile flavor compounds had fresh berry flavor and contributed to the improvement of sample flavor. 2-Methyl-2-butanol, a commonly used flavor ingredient, was identified only in S (Peinado, Moreno, Bueno, Moreno, & Mauricio, 2004). During fermentation microorganisms secrete metabolites such as esterase, β-glucosidase, etc., which will increase the expression of aroma substances in the metabolic pathway (Robinson et al., 2014). A reduction in ester content was observed in all fermentation samples, with the least reduction of 1.3% in B. This can also be a side effect to prove that there is a significant difference between the samples. Alcohols are an important class of volatile flavor compounds.

In Fig. 3a, the groups can be classified into two major groups and four subgroups based on the strength of their correlations. HF, HB, and CK samples were better separated, indicating that inoculation of different LAB at the end of alcoholic fermentation affected the flavor of prune wine. Similarly, S, B and F were separated from CK, and the flavor of the sample group was also improved to some extent. The relationship between volatile flavor compounds and samples was further investigated using PCA, and the results are shown in Fig. 3b. A total of 51 compounds were analyzed, with PC1 and PC2 explaining 48.2% and 15.6% of the overall variance, respectively. The first quadrant (HF group) and the

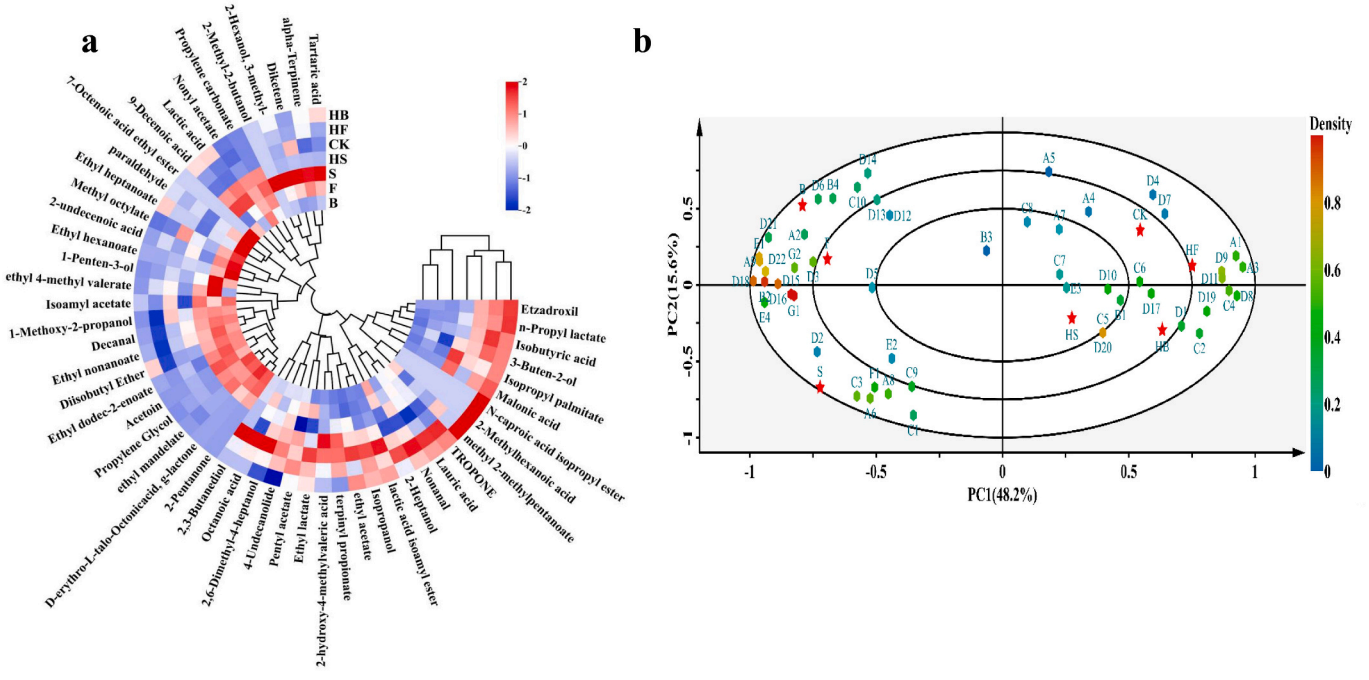

Fig. 3. Clustering heat map (a) and PCA plot (b) of volatile flavor substances. Compound number in figure b are available in Table S1.

second quadrant (B and F samples) had a large number of volatile flavor compounds. Unlike the second quadrant, the substances in the second quadrant were mainly esters and acids, including isopropyl caproate, ethyl mandelate, and 2-undecenoic acid.

The odour activity value (OAV) of volatile flavor compounds is often used to indicate the extent to which the volatile flavor compounds contributes to the overall flavor. A total of 14 volatile flavor compounds with OAV > 1 were identified in CK (10), HB (7), HF (7), HS (6), B (6), F (7) and S (7) (Table S2). This suggests that mixed fermentation affects the OAV of volatile flavor compounds in prune wines, but that this effect is unpredictable. In this study, the lactic acid and lauric acid contents increased to varying degrees after fermentation, but neither exceeded the corresponding thresholds. Aldehydes and terpenoids are predominantly found in fresh fruits. The reduced content of aldehydes in HS and HB may be attributed to the fact that aldehydes are unstable and microbial activity can oxidize them to acids or reduce them to alcohols. Terpenes are usually combined with glycosides to produce non-volatile compounds during wine fermentation. Different fermentation methods result in different OAVs for volatile flavor compounds, based on which the overall organoleptic characteristics vary and can play a significant role in aroma enhancement or inhibition, which is consistent with the findings of the previous study (Chen & Liu, 2016). OAV reflects the 'sensory intensity' of an aroma substance, and volatile flavor compounds with an OAV > 1 contribute to the flavor, the larger the OAV, the greater the contribution of the ingredient to the flavor of the food. The larger the OAV, the greater the contribution to the flavor of the food. Meanwhile, due to the synergistic and antagonistic effects between volatile flavor compounds, the final flavor of the food is full of uncertainty (Culleré, Cacho, & Ferreira, 2007).

3.6. Chemical marker screening

OPLS-DA is often used to analyze differences between samples and screen for chemical markers (El-Shamy & Farag, 2022). As shown in Fig. 4a, the hierarchical cluster analysis showed that the samples of prune wine fermented with different LAB mixtures were classified into four categories: S and HB were each classified as one category (Cluster 1, Cluster 3), and B and F were classified as one category (Cluster 2). HF, HS, and CK were in a single category (Cluster 4). Meanwhile, it was possible that HF and CK could be subdivided into a single category and that HS was in a separate category.

On this basis, a significant difference between the samples can be seen in the OPLS-DA score graph (Fig. 4b). The reliability of the OPLS-DA model was demonstrated by the 200-permutation test (Fig. 4c). The results of the 200 permutation test showed that $R^2X = 0.983$, $R^2Y = 0.833$, $Q^2 = 0.997$. Q^2 represents the predictability of the model, while R^2Y and R^2X represent the explanatory rate of the Y and X matrices, respectively. R^2Y (0.833) scores were higher than 0.8, which indicated that the OPLS-DA model has a good ability to explain the differences between groups. Q^2 (0.997) score is higher than 0.5, indicating that the OPLS-DA model has good predictive power to further search for chemical markers between groups. In this study, nine chemical markers including resveratrol, rutin, trans ferulic acid, catechin, isoamyl lactate and ethyl caproate were screened based on the criteria of VIP > 1 and $P < 0.05$ (Fig. 4d). Ethyl caproate is an important ester in wine that is mainly produced by microbial metabolism and is generated in three ways (Fan et al., 2018): 1) esterases secreted by yeast, bacteria, and molds catalyse the esterification reaction between hexanoic acid and ethanol; 2) the catalysis of ethanol and acyl-CoA by hexanoyl transferase

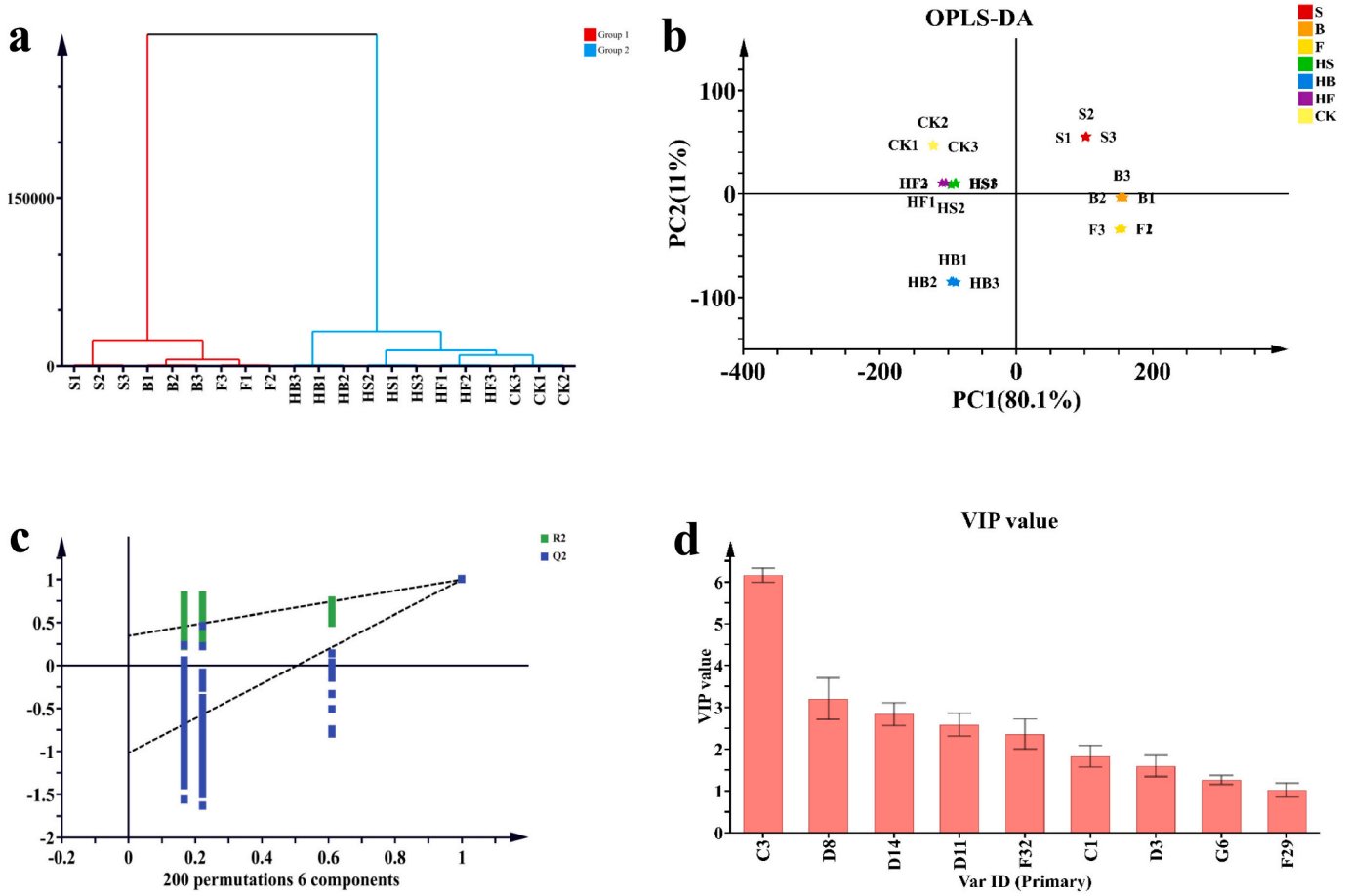

Fig. 4. Plots of multivariate statistical analysis of prune wine. (a) Hierarchical cluster analysis plot; (b) OPLS-DA score plot; (c) OPLS-DA model replacement test plot; (d) VIP plot.

in microorganisms; and 3) the use of ethyl acetate as the acceptor, in which ethanol is added to continuously extend the carbon chain to synthesize first ethyl butyrate and finally ethyl caproate. The production of ethyl caproate in wines is mainly achieved by esterification. The content of ethyl caproate in B was significantly different from that in the other samples ($P < 0.05$). The content of ethyl caproate in the HB, HF, and HS of the MLF group was lower than that in the B, S, and F. This difference is related to MLF and may be related to the inhibition of the esterification of ethyl caproate (Furukawa, Yamada, Mizoguchi, & Hara, 2003).

In wine, rutin and resveratrol are important products of microbial metabolism. These compounds have physiological functions such as anti-inflammatory, antioxidant, and cardiovascular disease prevention (Semwal, Joshi, Semwal, & Semwal, 2021). The highest content of rutin was found in HB, and the difference between the samples was significant ($P < 0.05$). The synthesis pathway of rutin in microorganisms is as follows: from phenylalanine, dihydrosorbitol and dihydromyricetin are synthesized, and finally rutin is synthesized via the dihydromyricetin pathway (Guadagni, Miers, & Venstrom, 1969).

4. Conclusion

In this study, inoculation of LAB into prune wine increased DPPH and total flavonoid content. In addition, inoculation of LAB at the end of alcoholic fermentation improved the brightness of the wine and had an effect on the color. The inoculation of LAB to prune wine at the end of alcoholic fermentation has a certain effect of acid reduction and taste improvement, as the content of malic acid in prune wine decreases and the content of lactic acid increases compared with CK. The histamine content in S, F, and B is lower than the limits for histamine in wines from France, Australia, and Switzerland. 15 phenolic compounds were identified, and mixed fermentation increased the phenolic types. Inoculation of LAB for prune wine at the end of alcoholic fermentation increased the contents of chlorogenic acid, benzoic acid, and catechins compared to CK. A total of 51 volatile flavor compounds were identified, inoculation LAB mixed fermented prune wine produces specific volatile flavor compounds including ethyl 7-octanoate, isopropyl caproate, and methyl 2-caproate. OAV of 14 volatile flavor compounds was >1. There were 9 chemical markers including resveratrol, rutin, trans ferulic acid, catechin, DPPH, isoamyl lactate and ethyl caproate were screened based on OPLS-DA. This research provides novel insight into the processing and utilization of prunes.

CRediT authorship contribution statement

Jianqiao Jiang: Writing – original draft. **Ruonan Yin:** Resources. **Yun Xie:** Resources. **Xiaomei Ma:** Methodology. **Miao Cui:** Software. **Yiwen Chen:** Methodology. **Yongkang Li:** Software. **Yue Hu:** Data curation. **Jianming Niu:** Investigation. **Weidong Cheng:** Writing – review & editing. **Feifei Gao:** Writing – review & editing.

Declaration of competing interest

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

Data availability

The authors are unable or have chosen not to specify which data has been used.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2024.101502.

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