RSC Advances

PAPER

View Article Online
View Journal | View Issue

Cite this: *RSC Adv.*, 2026, **16**, 6931

Characterization of flavor metabolites of cocultured fermented high acidity fruit wines, and the correlation between organic acids and esters

Jian Liu,[bd] Can Lyu,[b] Yongli Yang,[c] Shijun Lu,[b] Yuru Wen,[ab] Mengxue Sun,[ab] Zhaohuan Du,[b] Wei Lin,[e] Chensheng Xu,[e] Zhao Chen,[f] Lanmei Zhao *[a] and Ping Dong*[g]

High acidity is a major challenge in producing high-quality fruit wines, particularly those derived from *Prunus mume* (greengage). This study investigated the physicochemical properties, organic acids, and flavor profiles of high-acidity greengage wines fermented by four *Saccharomyces* yeasts, followed by coculture fermentation with *Torulaspora delbrueckii* to evaluate flavor modification. Among the single cultures, ScBV818 exhibited the strongest fermentation capacity, achieving an alcohol content of 13.27%, total acidity of 54.55 g L^{-1}, and significantly enhanced levels of esters, aldehydes, and ketones. Its OAVs of ethyl hexanoate, ethyl octanoate, ethyl benzoate, methyl salicylate, and isoamyl acetate were the highest, contributing to rich fruity and floral aromas. Coculture fermentation further improved flavor complexity, with simultaneous inoculation of ScBV818 and *T. delbrueckii* increasing acetate and ethyl ester contents by 1124.09% and 29.06%, respectively, while enhancing OAVs of ethyl hexanoate, ethyl octanoate, eugenol, linalool, and α-terpineol. Mantel and RDA analyses revealed that high levels of organic acids, such as citric acid and L-malic acid, negatively correlated with acetate and ethyl ester synthesis, while positively influencing ketones, eugenol, and terpenes. These findings highlight the potential of tailored fermentation strategies, such as sequential or simultaneous inoculation, to optimize flavor profiles and sensory quality in high-acidity fruit wines.

Received 13th June 2025
Accepted 27th January 2026

DOI: 10.1039/d5ra04203h

rsc.li/rsc-advances

1. Introduction

The choice of fermentation substrate plays a pivotal role in determining yeast metabolism and the resulting wine characteristics. *Prunus mume* (commonly known as greengage) is a unique fruit that has been cultivated in East Asia for over two millennia, with its use in fermentation dating back approximately 1800 years.[1,2] Greengage is particularly notable for its distinct fruity aroma and high organic acid content, making it a promising candidate for high-acidity fruit wine production.[3] Its health benefits, including antioxidant and antitumor properties, further enhance its appeal as a fermentation substrate.[4] However, the high acidity and antibacterial constituents of greengage often lead to fermentation challenges, such as stuck fermentation and low alcohol yields.[5] Addressing these issues requires innovative fermentation strategies to optimize the balance between acidity and flavor.

Despite the growing interest in greengage wines, research on its fermentation technology remains limited. Traditional methods, such as sugar addition and salt maceration, have been shown to promote the development of indigenous microbiota and enhance aroma complexity through the activity of non-*Saccharomyces* yeasts (such as *Issatchenkia*, *Sordariales*, and *Gliocephalotrichum*), which play a key role in the release of volatile aroma compounds such as ethylphenols, ethyl esters, and monoterpenes.[6] However, these traditional approaches often lack consistency and control, leading to variability in the fermentation process and final product quality. Recent advancements in fermentation technology have highlighted the potential of coculturing *Saccharomyces bayanus* Y4 with *Torulaspora delbrueckii* Y7 to enhance the production of key flavor metabolites, including ethyl esters, acetate esters, and phenylethyl alcohol, thereby improving the flavor and aroma profiles of greengage wines.[1,7] Additionally, innovative fermentation techniques like split-batch fermentation have demonstrated effectiveness in mitigating substrate inhibition, reducing acidity, and enhancing the extraction of bioactive compounds,

[a]*Shandong University of Science and Technology, Qingdao 266590, China. E-mail: lmzhao@sdust.edu.cn*
[b]*Institute of Tobacco Research, Chinese Academy of Agricultural Sciences, Qingdao 266001, China*
[c]*China 19th Metallurgical Group Corporation Limited, Chengdu 610031, China*
[d]*Beijing Life Science Academy, Beijing 102209, China*
[e]*Fujian Tobacco Company Nanping Company, Nanping 353000, China*
[f]*China Tobacco Shandong Industrial Company Limited, Jinan 250104, China*
[g]*China Tobacco Hubei Industrial Company Limited, Wuhan 430040, China. E-mail: dongping@hbtobacco.cn*

such as esters, in greengage wines.[1] Despite these promising developments, the application of coculture fermentation to greengage wine production remains underexplored, leaving significant potential for optimizing fermentation strategies to improve both flavor and quality.

The coculture of *Saccharomyces* and non-*Saccharomyces* yeasts is increasingly attracting attention due to its complex flavor, and it also contributes to address flavor deficiencies or defects associated with traditional fermentation technologies.[8] Non-*Saccharomyces* yeasts, such as *Pichia*, *Wickerhamomyces*, and *Hanseniaspora*, are particularly important for enhancing varietal and fermentative aromas by releasing aroma precursors through enzymatic activities, such as glycosidase and carbon-sulfur lyase, and by synthesizing flavor compounds.[9] Additionally, coculture fermentation has been shown to increase the production of terpenes, acetate esters, and ethyl esters, while reducing higher acetic acid and decanoic acid, thereby contributing to a more pronounced rose or plant aroma in various fruit wines, such as grape, pear, greengage, and kiwi wines.[8,10–12] However, despite a few preliminary studies on coculture fermentation in greengage wine production, the existing studies remains insufficiently explored, particularly in terms of dissecting flavor metabolite characteristics of different yeast coculture systems under high-acidity conditions or optimizing yeast interactions to enhance flavor profiles under acid stress.

Herein, this study aims to address the challenges associated with high-acidity fruit wines production by investigating the effects of different coculture fermentation systems on the physicochemical properties, organic acids, and flavor profiles of greengage wines. The suitable strains were further cocultured with non-*Saccharomyces* yeast to evaluate the potential of modifying the flavor profiles, and the correlation between organic acids and esters was systematically analyzed to better understand the flavor modulation under acid stress. By leveraging different coculture fermentation, this study provides a novel strategy for improving the flavor complexity and quality of greengage wines while addressing the broader research gaps in the fermentation of high-acidity fruit wines.

2. Materials and methods

2.1. Materials and yeasts

The matured greengages with strong aromas were acquired from Dali, China. The strong aromas refer to the sensory evaluation of the aroma intensity of greengages. Specifically, the aroma of unripe greengages is characterized by a prominent green and grassy note, while ripe greengages exhibit a rich floral and fruity aroma. Methyl octanoate, citric acid, L-malic acid, succinic acid, lactic acid, and acetic acid of chromatographically pure were purchased from Sigma-Aldrich Co (St. Louis, MO, USA). Glucose, sodium hydroxide, copper sulfate, potassium sodium tartrate, sodium chloride, sulfuric acid, hydrochloric acid, sodium hydrogen sulfite, and potassium hydrogen phthalate of analytical pure were purchased from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). Four *Saccharomyces* yeasts and one strain of *Torulaspora delbrueckii* were used in this study. The *S. cerevisiae* strains Lalvin ICV D254 (ScD254) and K1 MARQUÉE (ScK1) were purchased from Lallemand S.A.S (Fredericia, Denmark), the Angel BV818 (ScBV818) and SY (ScSY) were purchased from Angel Yeast Co (Yichang, China), and the *T. delbrueckii* strain Zymaflore Alpha was purchased from Laffort SA (Bordeaux, France).

2.2. The fermentation assays

2.2.1. The inoculation strategies. Two consecutive experiments were conducted in this study. First, the fermentation characteristics of four *Saccharomyces* yeasts were compared. Second, the strain specificity and inoculation sequence were further compared, which included: (1) simultaneous inoculation: *Saccharomyces* yeasts ScK1, ScBV818, and ScSY were simultaneously inoculated with *T. delbrueckii*, respectively. (2) Sequential inoculation: *T. delbrueckii* was inoculated first, followed by the same three *Saccharomyces* yeasts were inoculated after 2 days, respectively. Yeast suspensions were amplified with fruit maceration juice, and the initial yeast concentration was adjusted to 5×10^6 CFU mL^{-1} (in the volume of maceration juice) by the hemocytometer, and the ratio of *Saccharomyces* yeasts to *T. delbrueckii* was 1 : 1.

2.2.2. The elaboration of greengage wines. The matured greengages (1.5 kg) were washed without crushing, coring, and juicing, the whole fruits were mixed with sugar layer by layer in a 2.5 L fermenter at a ratio of 3.75 : 1 (w/w). Solid maceration was carried out at 25 °C for 2 days. After the sugar was solubilized, 150 mg L^{-1} of $(NH_4)_2HPO_4$ was supplemented with maceration juice, and pretreated cultures of yeast were inoculated by single culture and coculture, respectively, and the experiments were performed at 25 °C. When alcohol reached about 10%vol, the fresh wines were separated from the fruit, 120 mg L^{-1} of $NaHSO_3$ was immediately supplemented to fresh wines to stop fermentation, and the fresh wines were preserved in a tank. The physicochemical properties were monitored during fermentation. The inoculation ratio of 1 : 1 was chosen based on previous studies that demonstrated its effectiveness in enhancing the production of key aroma compounds during cocultured fermentation.[1,7,11,13] This ratio ensures a balanced interaction between the two yeast species, allowing both to contribute to the production of aroma compounds. The decision to conduct sequential inoculations a two-day interval was informed by studies indicating that a two-day delay in inoculation can enhance yeast interactions and improve metabolic activity.[1,7,11,14] These findings guided our experimental design to optimize the production of aroma compounds while maintaining a balance between yeast interactions.

2.3. Physicochemical property analysis

Physicochemical properties such as total sugar, total acidity, and alcohol were determined by National Standards.[1] In summary, the total sugar was determined by means of Fehling's solution titration, and the result was expressed as g L^{-1} glucose. The total acidity was determined by titration with a sodium hydroxide solution, and the result was expressed as g L^{-1} tartaric acid. Alcohol was measured by distillation and alcohol

meter, and the result was expressed as a percentage by volume (% vol). Chroma and hue were measured through the UV-vis spectrophotometer (Specoro 200, Analytik Jena AG, Jena, Germany), which chroma = $A_{420} + A_{520} + A_{620}$, and hue = A_{520}/A_{420}.

2.4. Organic acid analysis

The organic acid content was quantified using Cocchi's method.[15] In brief, the samples were subjected to centrifugation at 4 °C and 9338 × *g* for 10 min, after which the resulting solution was filtered using the SPE (Chromclean Silica, Swell Scientific Instruments Co., Ltd, Chengdu, China) column to selectively adsorb impurities, and the SPE column based on the unbonded activated silica gel acted as a positive phase adsorbent. Subsequently treated by the 0.22 μm filter. The treated sample was subjected to high-performance liquid chromatography (Quattro/Acquity, Waters, Milford, Massachusetts, USA) equipped with an organic acid column (Alltech OA-1000, 300 mm × 6.5 mm, Alltech Associates Inc., Columbia, MD, USA) for chromatographic separation. Chromatographic parameters were as follows: the mobile phase consisted of 9 mmol L^{-1} H_2SO_4 with a flow rate of 0.6 mL min^{-1}, the column temperature was at 75 °C, and the UV detector was used at 215 nm. Organic acids were analyzed qualitatively and quantitatively based on retention time and calibration curves of standards.

2.5. Volatile constituent analysis

The volatile constituents were subjected to analysis using the HS-SPME-GC-MS method.[16] In summary, 0.5 mL of sample was placed in a 15 mL vial, followed by the addition of 2 mL of deionized water, 1.5 g of sodium chloride, and 10 μL of internal standard (51 μg mL^{-1} methyl octanoate). The contents of the vial were then mixed thoroughly, after which the vial was sealed with a screw cap containing a silicon septum. The vial was then placed in a 60 °C water bath with a magnetic stirrer for pre-equilibration for a period of 15 min. Following this, an extraction fiber (50/30 μm DVB/CAR/PDMS, Supelco, Bellefonte, PA, USA) was inserted into the vial through the septum and extracted above the sample for a period of 40 min. Subsequent to the extraction, the fiber was inserted into the GC inlet for analysis for a period of 3 min.

DVB/CAR/PDMS fibers (Supelco Inc., Bellefonte, PA, USA) were employed to absorb the volatiles, and GC-MS (7890B-5977A, Agilent Technologies, Santa Clara, CA, USA) equipped with an HP-Innowax (30 m × 0.25 mm × 0.25 μm, Agilent Technologies) were carried out to analyze the volatiles. Helium was utilized as the carrier gas, with a flow rate of 1.0 mL min^{-1} and a splitless mode. The temperature of the inlet and transfer line was set at 250 °C. The sequence of temperatures applied during the heating program was outlined below: an initial temperature of 40 °C was held for 5 min, then increased to 100 °C at a rate of 4 °C min^{-1}, subsequently raised to 220 °C at a rate of 6 °C min^{-1}, and maintained for a further 5 min. The ion source employed was electron impact ionization (EI), with an electron energy of 70 eV, an ion source temperature of 230 °C, and a scanning range of 33–325 amu. The mass spectra of compounds were compared with the NIST (2005) standards library for qualification. The Kováts Retention Index (RI) obtained from C_8–C_{20} *n*-alkanes served to corroborate the results reported in the work. The ratio of the internal standard area to that of the total ion chromatogram was calculated and used to semi-quantify the volatile constituents. The contents of aroma constituents were expressed as μg L^{-1} of methyl octanoate.

2.6. Statistical analysis

The measurements were repeated three times and experimental results were reflected by means ± standard deviation. The odor activity value (OAV) was the ratio of the volatiles concentration to the volatiles odor threshold. One-way analysis of variance and Duncan's multiple comparison tests were used to perform the statistical analysis, and $P < 0.05$ indicated the significant difference based on the IBM SPSS Statistics 25 (IBM Corp., Armonk, NY, USA). Principal component analysis (PCA) was performed to correlate the volatile constituents and fermentation patterns by Simca 14.1 (Mks Umetrics Ab Corp., Malmö, Sweden). The differential volatiles were analyzed by linear discriminant analysis effect scales (LEfSe) and carried out by the Galaxy website (http://huttenhower.sph.harvard.edu/galaxy/). The netheatmap and redundancy analysis (RDA) were performed using the genescloud tools (https://www.genescloud.cn/).

3. Results and discussion

3.1. Effects of *Saccharomyces* yeasts on physicochemical, organic acids, and flavor profiles of greengage wines

There were significant differences in the physicochemical properties of greengage wines fermented by different *Saccharomyces* yeasts (Table 1). Total sugar was lower and the alcohol was higher in ScBV818 and ScSY, while the opposite tendency was observed in ScD254 and ScK1. The alcohol reached 13.27% and the total sugar was the lowest in ScBV818. The strong fermentation capacity of ScBV818, reflected in the highest alcohol content and the lowest residual sugar, suggested its potential for efficient sugar utilization under high-acidity stress, consistent with the performance of acid-tolerant strains in other studies.[17] The total acidity of greengage wine fermented by ScD254 was significantly higher than the other greengage wines. The previous study found that certain *Saccharomyces* yeasts exhibited strain-specific acid production, significantly impacting greengage wine acidity.[5] The chroma of ScD254 was 33.33% higher than that of ScSY, which could negatively affect color preservation of greengage wines. The significant differences in the physicochemical properties of greengage wines fermented with different *Saccharomyces* yeasts highlighted the strain-dependent responses to high-acidity greengage wines.[7]

There were significant differences in the content of different organic acids, with the levels of citric acid and L-malic acid being significantly higher than those of other organic acids (Table 1). The content of organic acids was influenced by the strain specificity of yeast. Among them, the total organic acid content was the highest in ScD254. The organic acid content was the lowest in ScK1, while the contents of the other two

Table 1 The physicochemical properties and organic acids of greengage wines fermented by different *Saccharomyces* yeasts (g L^{-1})[a,b]

Indices	ScD254	ScK1	ScBV818	ScSY
Total sugar	94.15 ± 0.38[a]	82.96 ± 0.78[b]	35.25 ± 5.42[d]	52.00 ± 4.48[c]
Alcohol (% vol)	9.10 ± 0.10[c]	12.07 ± 0.15[b]	13.27 ± 0.15[a]	11.53 ± 0.57[b]
Total acidity	64.78 ± 1.69[a]	48.22 ± 1.46[d]	54.55 ± 0.84[b]	51.14 ± 1.46[c]
Chroma (abs)	1.00 ± 0.09[a]	0.81 ± 0.10[ab]	0.87 ± 0.06 [ab]	0.75 ± 0.12[b]
Hue (abs)	0.53 ± 0.00[b]	0.56 ± 0.00[a]	0.53 ± 0.00[b]	0.53 ± 0.00[b]
Citric acid	34.34 ± 1.43[a]	28.08 ± 0.24[c]	30.99 ± 0.23[b]	29.33 ± 0.17[c]
L-Malic acid	25.88 ± 0.53[a]	18.19 ± 0.88[d]	21.70 ± 0.51[b]	19.34 ± 0.16[c]
Succinic acid	4.13 ± 0.11[a]	3.71 ± 0.13[b]	3.49 ± 0.14[c]	3.34 ± 0.02[c]
Acetic acid	2.33 ± 0.23[a]	1.65 ± 0.03[b]	2.33 ± 0.07[a]	1.80 ± 0.03[b]
Total organic acids	66.68 ± 1.57[a]	51.63 ± 1.21[d]	58.51 ± 0.79[b]	53.83 ± 0.37[c]

[a] Data are the means ± standard deviation ($n = 3$). [b] Values in the same row with different letters indicate significant difference ($P < 0.05$) according to Duncan's tests.

greengage wines were between ScK1 and ScD254. The difference in total acidity was consistent with the organic acid content (Table 1). The total acidity of greengage wines fermented by the four *Saccharomyces* yeasts ranged from 48.22 to 64.78 g L^{-1} (Table 1), which is significantly higher than the total acidity of other fruit wines fermented by the same strains (4.29–6.90 g L^{-1}, Table 2). Despite this high acidity, the variations in alcohol among the samples were relatively minor (Table 1 and 2). This indicated that the selected *Saccharomyces* yeasts, including ScBV818, demonstrated high acidity tolerance and maintained efficient alcohol conversion even in the high-acidity environment of greengage wines.

Fifty-two volatile constituents were identified in different greengage wines, which included the esters (27), alcohols (8), acids (7), aldehydes (3), ketones (3), phenols (3), and terpenes (1), total volatiles content was 5.86–7.70 mg L^{-1} (Table S1). Among them, esters, aldehydes, and ketones reached the highest in the greengage wine of ScBV818, and their contents reached 2948.48 ± 282.47, 641.15 ± 30.09, and 90.71 ± 7.12 μg L^{-1}, respectively. There was no significant difference in the total contents of alcohols and acids. The varieties of volatiles were also influenced by the strain specificity of the yeast. 40, 45, 47, and 42 volatiles were detected in ScD254, ScK1, ScBV818, and ScSY, respectively.

The esters were influenced by the strain specificity of the yeast, and the total ester content was between 1.78 mg L^{-1} and 2.95 mg L^{-1}. The ester content reached the highest in ScBV818, due to enhancement of acetate and ethyl esters, and these two constituents reached 1133.20 ± 131.74 and 1688.83 ± 136.99 μg L^{-1}, respectively, both of which were higher than those in other greengage wines (Table S1). These significant increases aligned with findings in previous studies, where enhanced acetate and ethyl ester production was shown to contribute to higher overall ester content and improved aroma profiles during fermentation.[17,21] This evidence underscored the direct impact of acetate and ethyl ester enhancement on the total ester content in ScBV818-fermented greengage wine. Specifically, the contents of isoamyl acetate, ethyl butyrate, ethyl 2-methylbutanoate, ethyl isovalerate, ethyl hexanoate, ethyl 3-hexenoate, ethyl octanoate, ethyl *trans*-4-octenoate, and ethyl cinnamate were significantly increased in ScBV818, and ethyl succinate, ethyl hydrogen glutarate, and ethyl 9-decenoate were increased in ScK1, while ethyl 2-hydroxyisovalerate and ethyl 3-hydroxytridecanoate were higher in ScSY. However, ethyl hydrogen glutarate, ethyl butyl succinate, and ethyl hexanoate were decreased in ScD254. Yeast strains affect the enzymatic and chemical esterification of alcohols and acids during fermentation, leading to differences in esters between wines.[22] The ester compositions in greengage wines are significantly influenced by specific metabolic pathways of yeast strains, particularly those involved in acetate and ethyl ester synthesis.[7,11,23] Strains like ScBV818 exhibited enhanced ester production, potentially linked to optimized acetyl-CoA flux and enzymatic activity during fermentation.[21] Additionally, fermentation conditions, including acidity and sugar utilization rates, further modulate ester profiles, as observed in high-acidity fruit wines.[17] These

Table 2 The physicochemical properties of different fruit wines fermented by *Saccharomyces* yeasts[a]

Fruit wine substrate	Yeast type	Total sugar (g L^{-1})	Alcohol (%vol)	Total acidity (g L^{-1})	Reference
Cabernet sauvignon grape	ScD254	3.90 ± 0.30	14.30 ± 0.10	6.90 ± 0.30	18
Pineapple	ScD254	0.91 ± 0.08	11.0 ± 0.04	5.66 ± 0.11	19
	ScBV818	0.42 ± 0.06	10.9 ± 0.05	5.77 ± 0.12	19
Jujube	ScBV818	—	13.13 ± 0.02	4.51 ± 0.01	20
	ScSY	—	13.15 ± 0.02	4.29 ± 0.02	20

[a] The cited reference only provides data on the total sugar content of jujube before the fermentation and does not include residual sugar levels after fermentation.

findings highlighted the critical role of yeast strain selection and fermentation environment in shaping the aromatic complexity of greengage wines.

The alcohol constituents of ScD254, including isobutyl alcohol, butyl alcohol, isoamyl alcohol, hexyl alcohol, benzyl alcohol, and β-Dihydroionol, were significantly different from the others, while no significant difference was observed among the other strains. The contents of isobutyl alcohol, isoamyl alcohol, hexyl alcohol, and β-dihydroionol in ScK1, ScBV818, and ScSY were significantly higher than those in ScD254, whereas the content of benzyl alcohol in ScD254 was higher than in the other three strains, reaching 290.45 μg L^{-1}. Previous studies have identified benzyl alcohol and isoamyl alcohol as key contributors to fruity and floral aromas.[17,23] In addition, there were differences in the acid constituents due to the strain specificity of the yeast. Among them, octanoic acid and decanoic acid of ScSY were higher than those of ScD254 and ScK1, and octanoic acid of ScBV818 was higher than that of ScD254. Volatile acetic acid was higher in ScD254 and ScBV818, and 2-methylbutyric acid was higher in ScD254 and ScSY. The higher octanoic acid levels in ScSY may negatively impact sensory characteristics due to their association with harsh and rancid notes.[24] These results underscored the importance of strain selection in balancing aroma profiles and mitigating undesirable sensory effects. Except for the esters, alcohols, and acids, there were differences in other volatiles of different strain fermented greengage wines. The contents of aldehydes and ketones of ScBV818 and ScSY were higher than those of ScD254 and ScK1, which was due to the increase of benzaldehyde, 2,4-dimethylbenzaldehyde, *trans*-β-ionone, and dihydro-β-ionone, these constituents were the highest in ScBV818 and reached 272.73 μg L^{-1}, 368.43 μg L^{-1}, 62.18 μg L^{-1}, and 28.53 μg L^{-1}, respectively. In addition, 3-allylguaiacol content was higher in ScK1, and α-terpineol was detected only in ScD254 and ScSY. The elevated benzaldehyde and *trans*-β-ionone levels in ScBV818 suggest enhanced aromatic complexity, as these compounds are linked to almond and floral aromas.[25] Similarly, the unique detection of α-terpineol in ScD254 and ScSY highlights their potential for terpene-related aroma enhancement.[23] These findings provide insights into optimizing yeast strain combinations to tailor volatile profiles in greengage wine fermentation.

The rationale for focusing on OAV > 1 in the paragraph was to highlight compounds with a more significant sensory impact, while the inclusion of OAV > 0.1 in the PCA analysis was intended to provide a broader overview of the volatile profile and capture compounds that may contribute to the overall aroma characteristics, even if their individual impact is less pronounced. The OAVs of six volatiles were above 1.0 which contributed greatly to the overall flavor profile of the greengage wines, including isoamyl acetate, ethyl hexanoate, ethyl octanoate, ethyl benzoate, methyl salicylate, and decanal (Table S1). The OAVs of five volatiles except decanal were higher in ScK1 and ScBV818, and the OAVs of ethyl hexanoate (33.77), ethyl octanoate (73.00), ethyl benzoate (9.68), methyl salicylate (1.22), isoamyl acetate (1.18) were the highest in ScBV818, which contributed to a strong fruity, floral, sweet and banana aroma. The decanal OAV was the highest in ScK1, which promoted the orange peel aroma. The volatiles of four greengage wines were used to perform the PCA analysis, and the variation of 49.60% and 30.40% was explained by PC1 and PC2, respectively (Fig. 1). The separation along PC1 was primarily driven by ethyl hexanoate (loading: 0.3097, R^2VX [2] (cum) = 0.9994), ethyl acetate (loading: 0.3037, R^2VX [2] = 0.9697), ethyl isovalerate (loading: 0.3022, R^2VX [2] = 0.9979), and methyl salicylate (loading: 0.2896, R^2VX [2] = 0.9979). Similarly, the separation along PC2 was dominated by ethyl 9-decenoate (loading: −0.3085, R^2VX [2] = 0.6460), decanal (loading: −0.0665, R^2VX [2] = 0.3671), and α-Terpineol (loading: 0.2238, R^2VX [2] = 0.7963). These volatile compounds played a significant role in distinguishing the yeast strains. ScSY and ScK1 were grouped, while ScBV818 and ScD254 were divided into another two groups. Among them, ScBV818 was associated with ethyl acetate, isoamyl acetate, ethyl butyrate, ethyl isovalerate, ethyl hexanoate, ethyl octanoate, ethyl benzoate, ethyl cinnamate, methyl salicylate, decanoic acid, and benzaldehyde, which indicated that the rich fruity aromas included apple, banana, pineapple, and sweet, honey, almond, flowery and fat aromas in wines of ScBV818. Besides, ScD254 was related to α-terpineol, phenethyl acetate, and ethyl phenylacetate, which imparted the citrus and floral aromas. The ScK1 was associated with the ethyl 9-decenoate and phenylethyl alcohol, which imparted the fruity and rose aromas.

In addition, the contents of total acidity, total organic acids, and chroma of ScD254 increased significantly, which was unfavorable for the acidity control and color protection, and the alcoholic conversion rate was low. Besides, the contents of esters, aldehydes, ketones, and total aroma constituents of ScD254 decreased significantly compared with that of ScBV818. Therefore, ScK1, ScBV818 and ScSY were selected for the subsequent coculture fermentation experiments.

3.2. Effects of coculturing *Saccharomyces* yeasts with *T. delbrueckii* on physicochemical, organic acids, and flavor profiles of greengage wines

There were statistically significant differences in certain physicochemical properties of different greengage wines (Table 3). The total sugar in the coculture of ScK1 was higher than that in the cocultures of ScBV818 or ScSY, except for the sequential inoculation of ScSY and *T. delbrueckii*. The total acidity of greengage wines ranged from 45.78 g L^{-1} to 57.47 g L^{-1}, and its content was slightly higher in the cocultures of ScBV818 or ScSY than that in the coculture of ScK1. Total sugar was reduced and the alcohol was increased in the sequential inoculation of ScK1 and *T. delbrueckii*, while the total sugar was increased in the simultaneous inoculation compared to the ScK1 single culture. Total sugar was significantly decreased and total acidity was increased in the simultaneous inoculation of ScBV818 and *T. delbrueckii*, and only the total sugar was significantly increased in the sequential inoculation of ScSY and *T. delbrueckii* compared with the corresponding single culture. In addition, there were significant differences in total sugar among different simultaneous inoculations, the total sugar of the coculture of ScBV818 with *T. delbrueckii* was significantly decreased, and

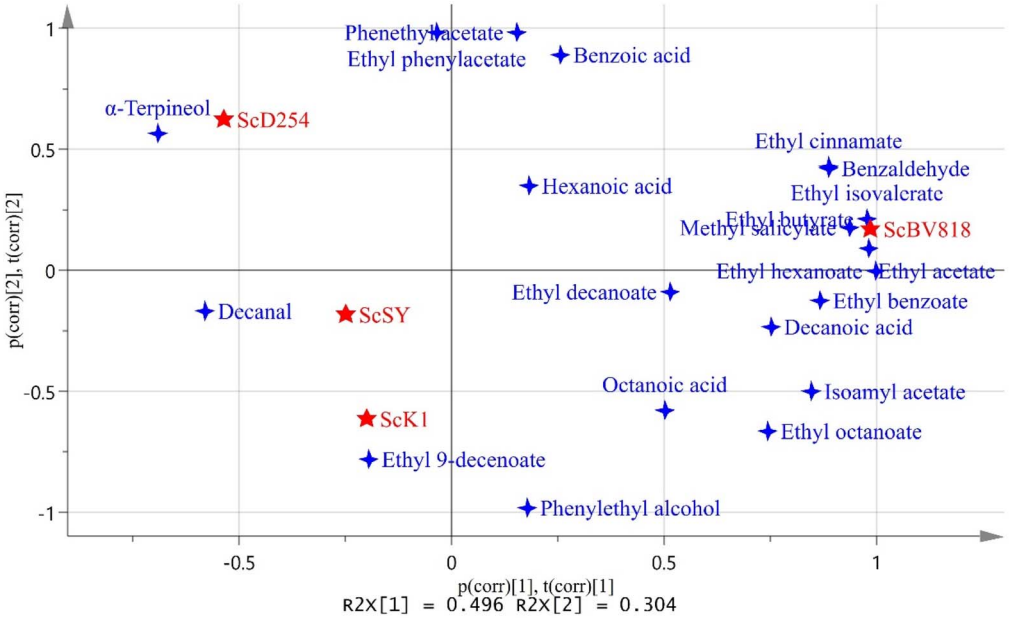

Fig. 1 Principal component analysis (PCA) biplot of volatile constituents of greengage wines fermented by four *Saccharomyces* yeasts (Odor activity value (OAV) > 0.1).

Table 3 The physicochemical properties and organic acids of greengage wines fermented by three *Saccharomyces* yeasts, three simultaneous and sequential coculturing *Saccharomyces* yeasts with *Torulaspora delbrueckii* (g L^{-1})[a,b]

	Single inoculation			Simultaneous inoculation			Sequential inoculation		
Indices (g L^{-1})	ScK1	ScBV818	ScSY	ScK1 + *T. delbrueckii*	ScBV818 + *T. delbrueckii*	ScSY + *T. delbrueckii*	ScK1 + *T. delbrueckii*	ScBV818 + *T. delbrueckii*	ScSY + *T. delbrueckii*
Total sugar	109.89 ± 4.15^{b}	96.74 ± 0.58^{d}	74.37 ± 0.30^{e}	116.25 ± 0.42^{a}	43.40 ± 1.31^{f}	73.39 ± 0.17^{e}	102.66 ± 0.88^{c}	95.42 ± 1.57^{d}	116.54 ± 2.93^{a}
Total acid	49.19 ± 0.84$^{b\ c}$	51.63 ± 5.13^{b}	52.11 ± 0.84^{b}	49.19 ± 0.84$^{b\ c}$	57.47 ± 3.37^{a}	51.14 ± 1.46^{b}	45.78 ± 0.84^{c}	50.17 ± 1.69^{b}	50.17 ± 1.69^{b}
Alcohol (%vol)	10.67 ± 0.06$^{b\ c}$	11.40 ± 0.50$^{a\ b}$	11.40 ± 0.20$^{a\ b}$	10.83 ± 0.12^{b}	12.03 ± 0.55^{a}	9.90 ± 0.10^{c}	12.10 ± 0.62^{a}	11.97 ± 0.71^{a}	11.93 ± 0.64^{a}
Chroma (abs)	0.77 ± 0.15$^{b\ c\ d}$	0.71 ± 0.04$^{c\ d}$	0.87 ± 0.01^{b}	0.84 ± 0.04$^{b\ c}$	0.83 ± 0.03$^{b\ c}$	1.29 ± 0.04^{a}	0.70 ± 0.05^{d}	0.79 ± 0.06^{bcd}	0.81 ± 0.08$^{b\ c\ d}$
Hue (abs)	0.55 ± 0.01^{cd}	0.52 ± 0.00^{g}	0.60 ± 0.00^{b}	0.56 ± 0.00^{c}	0.54 ± 0.00^{e}	0.62 ± 0.00^{a}	0.54 ± 0.00^{e}	0.55 ± 0.00^{d}	0.53 ± 0.00^{f}
Citric acid	34.95 ± 0.16^{c}	31.30 ± 0.08^{g}	30.27 ± 0.05^{h}	36.61 ± 0.08^{b}	35.98 ± 0.05^{b}	36.65 ± 0.07^{a}	34.64 ± 0.06^{d}	33.72 ± 0.04^{e}	33.06 ± 0.06^{f}
L-malic acid	8.95 ± 0.07^{c}	8.55 ± 0.06^{e}	10.39 ± 0.04^{b}	8.81 ± 0.07^{d}	8.81 ± 0.05^{d}	11.30 ± 0.02^{a}	7.95 ± 0.07^{f}	7.99 ± 0.07^{f}	7.79 ± 0.07^{g}
Succinic acid	3.65 ± 0.07^{d}	3.23 ± 0.06^{e}	1.81 ± 0.01^{g}	2.84 ± 0.06^{f}	3.99 ± 0.07^{b}	1.56 ± 0.00^{h}	1.56 ± 0.05^{h}	3.86 ± 0.06^{c}	3.91 ± 0.06$^{b\ c}$

[a] Data are the means ± standard deviation ($n = 3$). [b] Values in the same row with different letters indicate significant difference ($P < 0.05$) according to Duncan's tests.

total acidity and alcohol were increased compared with the others. Total sugar was significantly different between different sequential inoculations, and the total acidity of ScK1 coculturing with *T. delbrueckii* was reduced compared to the other two wines.

The sequential inoculation of ScK1 and *T. delbrueckii* resulted in reduced total sugar and increased alcohol content, consistent with previous studies highlighting *T. delbrueckii*'s ability to enhance sugar metabolism and ethanol production during fermentation.[23,25] This effect may be attributed to the complementary metabolic activities of *Saccharomyces* and *T. delbrueckii*, where the latter facilitates the breakdown of sugar precursors, accelerating ethanol conversion. In contrast, simultaneous inoculation of ScBV818 and *T. delbrueckii* led to higher total acidity and reduced sugar levels, likely due to enhanced yeast metabolic activity under simultaneous

conditions.[7] These findings underscored the critical role of inoculation strategies in modulating the balance between sugar consumption and ethanol production, offering opportunities to adjust wine sweetness and alcohol content. The observed differences in chroma and hue between simultaneous and sequential inoculations further highlighted the influence of yeast interactions on the sensory and visual characteristics of greengage wines. Previous research showed that *T. delbrueckii* contributed to aromatic complexity by releasing aroma precursors and enhancing ester production.[26] In this study, the sequential inoculation of ScSY and *T. delbrueckii* increased total sugar, potentially contributing to a sweeter sensory profile and improved mouthfeel, consistent with findings from Tian *et al.*[26] Interestingly, simultaneous inoculation of ScBV818 and *T. delbrueckii* resulted in significantly increased alcohol levels, suggesting that *T. delbrueckii*'s metabolic activity under simultaneous conditions may favor ethanol production.[7,25] This contrasted with sequential inoculation strategies, where *T. delbrueckii* appeared to focus more on aroma development.[23] These differences underscored the importance of inoculation timing and yeast compatibility in shaping the physicochemical and sensory profiles of greengage wines. Overall, these results highlighted the potential of coculturing *Saccharomyces yeasts* with *T. delbrueckii* to optimize greengage wine quality. Sequential inoculation strategies offer a promising avenue for enhancing aromatic complexity and sweetness, while simultaneous inoculations may be more suitable for producing wines with higher acidity and alcohol content. These findings contribute to the growing body of knowledge on yeast interactions in fruit wine fermentation and provide practical insights for tailoring fermentation processes to achieve desired wine characteristics.

The acid-base balance of greengage wines was affected by the variety and content of organic acids, and taste and color were influenced (Table 3 and Fig. 2). There were significant differences in organic acid content between different greengage wines. The total organic acids content was between 50.19 g L^{-1} and 63.78 g L^{-1}, and the total proportions of citric acid and L-malic ranged from 94.46% to 95.59%. The organic acids content of greengage wines was influenced by the yeast strain specificity. For example, succinic acid content was decreased and acetic acid was increased in the coculture of ScK1 with *T. delbrueckii*. Citric acid, succinic acid, and total organic acids were significantly increased in the coculture of ScBV818 with *T. delbrueckii*, while citric acid, L-malic acid, and total organic acids were significantly decreased and succinic acid was increased in the coculture of ScSY with *T. delbrueckii*. The variations in organic acid content observed across greengage wines underscored the significant impact of *T. delbrueckii* introduction during greengage fermentation. *T. delbrueckii* is generally considered a low-acid-producing strain even in high-sugar environments, forms an optimal combination with *Saccharomyces* yeasts in co-cultivation, effectively reducing volatile acidity.[23] Additionally, the introduction of non-*Saccharomyces* yeasts could influence metabolic pathways, such as the tricarboxylic acid cycle, as evidenced by the increase in citric acid and succinic acid in the coculture of ScBV818 with *T. delbrueckii*, which contributed to the modulation of organic acid profiles and overall wine balance.[7,23,25]

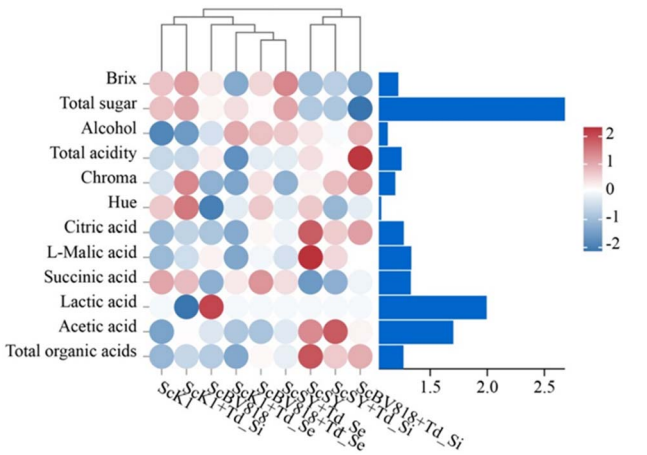

Fig. 2 The heatmap of physicochemical properties and organic acid of greengage wines fermented by three *Saccharomyces* yeasts, three simultaneous and sequential coculturing *Saccharomyces* yeasts with *Torulaspora delbrueckii*, respectively, and the column chart of the fold change of different indices. The data range of −2 to 2 in the heatmap was determined after applying *Z*-score normalization to the physicochemical and organic acid data.

Fifty-three volatile constituents were detected in greengage wines (Table S2), which included esters (24), alcohols (11), acids (8), aldehydes (4), ketones (3), eugenol, and terpenes (2). Esters, aldehydes, ketones, eugenol, and terpenes were significantly affected by coculturing, and changes in contents were influenced by the strain specificity of the yeast. Among them, eugenol, acetate and ethyl esters were significantly enhanced in simultaneous inoculation of ScBV818 with *T. delbrueckii*, their contents increased from 0 μg L^{-1}, 5.10 μg L^{-1}, and 375.68 μg L^{-1} to 32.18 μg L^{-1}, 62.48 μg L^{-1}, and 484.86 μg L^{-1}, respectively. The content of acetate and ethyl esters were increased by 1124.09% and 29.06% compared to the single culture. The reaction between acetyl coenzyme A and higher alcohol has been reported to produce acetate esters during fermentation, which were shown to be a major flavor contributor in greengage wines.[27] The content of ethyl esters was significantly increased, but aldehydes, ketones, and terpenes were decreased in the coculture of ScK1 with *T. delbrueckii*. The proportions of esters and eugenol were increased, and acids, aldehydes, and ketones were decreased by coculturing (Fig. 3), which was consistent with the changes in volatiles. Among them, the proportion of esters was the highest and reached 29.19%, and the proportion of alcohols was the lowest in the simultaneous inoculation of ScBV818 with *T. delbrueckii*. In addition, the varieties of volatiles were also influenced by the coculturing. Thirty-three, 26, and 33 volatiles were detected in the ScK1, ScBV818, and ScSY, respectively, and 31, 41, and 32 volatiles were found in the corresponding simultaneous inoculation, while 27, 32, and 32 volatiles were detected in the sequential inoculation. Eleven and 9 volatiles were not detected, and 9 and 3 volatiles were newly found in the simultaneous and sequential inoculations compared to the ScK1 single culture. While 3 and 2 volatiles

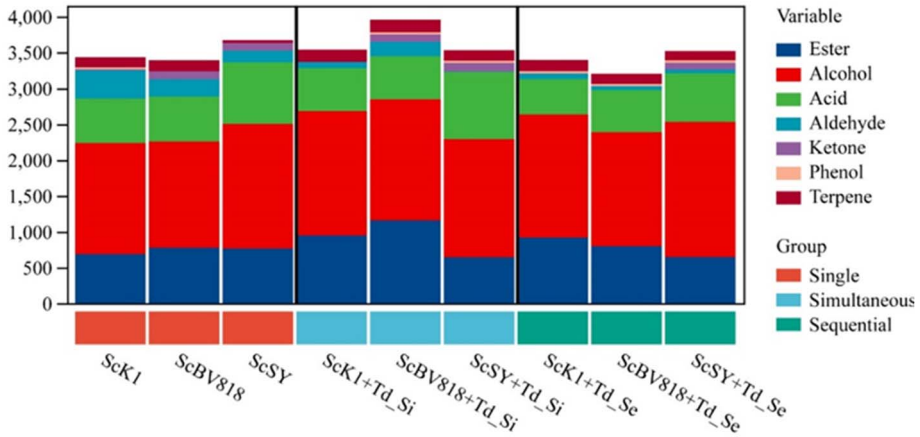

Fig. 3 Volatile constituent profiles of greengage wines fermented by three *Saccharomyces* yeasts, three simultaneous and sequential coculturing *Saccharomyces* yeasts with *Torulaspora delbrueckii,* respectively.

were not detected, 18 and 8 volatiles were newly detected for ScBV818, 8 and 9 volatiles were not detected, and 7 and 8 volatiles were newly detected for ScSY.

Fifty-three volatiles were significantly different among different inoculation strategies based on the LEfSe analysis (Fig. 4). Among them, the contents of methyl salicylate, ethyl 9-decenoate, ethyl dodecanoate, nonyl alcohol, 9-decenoic acid, tetradecanoic acid, and terpenes were significantly increased in the simultaneous inoculation of ScK1 and *T. delbrueckii*, while the contents of benzyl acetate, ethyl benzoate, and γ-decalactone were enhanced in the sequential inoculation. The contents of esters, phenethyl acetate, ethyl 7-octenoate, ethyl DL-leucate, methyl hexadecanoate, diethyl succinate, 2,3-butanediol, benzaldehyde, and 3,4-dehydro-β-ionone were significantly increased in the simultaneous inoculation of ScBV818 and *T. delbrueckii*.

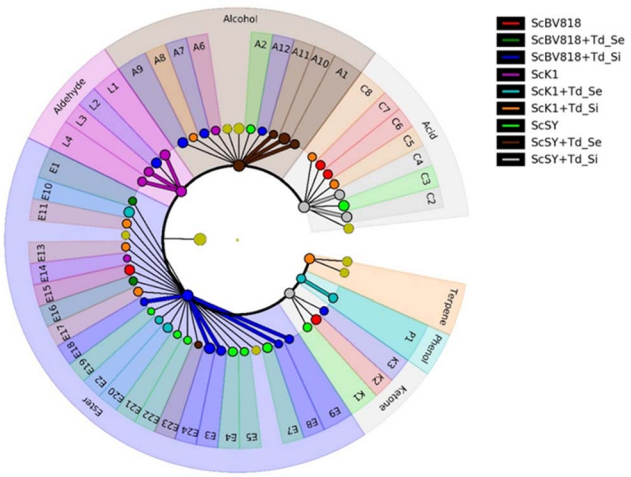

Fig. 4 Linear discriminant analysis effect size (LEfSe) of greengage wine volatiles by three *Saccharomyces* yeasts, three simultaneous and sequential coculturing *Saccharomyces* yeasts with *Torulaspora delbrueckii,* respectively (linear discriminant analysis value (LDA) > 2, $P < 0.05$). The volatiles used for the analysis were listed in Table S2.

Eight volatiles (OAV > 1.0) contributed significantly to the greengage wine flavor profiles, which included ethyl hexanoate, ethyl octanoate, ethyl benzoate, decanal, β-ionone, eugenol, linalool, and α-terpineol (Table S2). OAVs were significantly affected by the coculturing. For example, the OAVs of ethyl hexanoate, ethyl octanoate, ethyl benzoate, linalool, and α-terpineol were enhanced in the simultaneous inoculation of ScK1 and *T. delbrueckii*, while the OAVs of ethyl benzoate, linalool, and α-terpineol were increased in the sequential inoculation and the citrus, sweet and clove aroma was enhanced. The OAVs of ethyl hexanoate, ethyl octanoate, eugenol, linalool, and α-terpineol were increased in the simultaneous inoculation of ScBV818 with *T. delbrueckii*, and the floral, fruity, citrus, and clove aroma were improved. Inoculation strategies significantly influenced the aroma profiles of greengage wine. These findings aligned with previous studies, which showed that simultaneous inoculation enhanced floral and fruity aromas, such as phenethyl acetate, due to synergistic yeast interactions.[13,23,25] Moreover, sequential inoculation further enhanced the quality of greengage wines, imparting rose and plant aroma aromas, supporting its role in diversifying aroma profiles.[12] These results emphasized the potential of tailored inoculation strategies to optimize sensory complexity in greengage wine fermentation.

The variations in volatile profiles between simultaneous and sequential inoculations can be explained by yeast–yeast interactions, such as substrate competition and differences in enzymatic activity timing during fermentation. Sequential inoculation allows *T. delbrueckii* to dominate the initial fermentation stages, during which its β-glucosidase activity breaks down glycosidic precursors, releasing aromatic compounds such as 2-phenylethyl alcohol and terpenols.[23] This process, coupled with the extended metabolic activity of *T. delbrueckii* before introducing *Saccharomyces* yeasts, enhances the production of ethyl esters, such as ethyl octanoate and ethyl hexanoate, which are associated with fatty acid and alcohol metabolism.[28] On the other hand, simultaneous inoculation promotes stronger metabolic interplay between *T. delbrueckii*

and *Saccharomyces* yeasts, resulting in greater acetate ester synthesis, such as benzyl acetate and phenethyl acetate, through acetyl-CoA reactions with higher alcohols.[29] This interaction is influenced by substrate availability, as *Saccharomyces* yeast is more efficient in substrate uptake, limiting *T. delbrueckii* growth in simultaneous fermentations and altering its metabolic outputs.[25] Furthermore, ethanol accumulation during fermentation has been shown to reduce β-glucosidase activity, restricting the release of aroma precursors in simultaneous inoculation settings.[23] These findings suggested that the timing of inoculation directly affected the metabolic pathways of each yeast species, leading to distinct variations in the volatile profiles of greengage wines.

3.3. Correlation between organic acids and esters of single cultured and cocultured greengage wines

The mantel network was performed to evaluate the effect of total acidity and organic acids on the main volatile components (Fig. 5(a)). The heatmap analysis showed that total acidity was closely related to total organic acids and citric acid, while total sugar was negatively related to total acidity, total organic acids, citric acid, L-malic acid, and acetic acid. The mantel analysis showed that the esters content was significantly influenced by total acidity, and the volatile acids were influenced by citric acid, L-malic acid, succinic acid, acetic acid, and total organic acids, while the volatile alcohols and total volatiles were not influenced by the organic acids. As shown in the RDA analysis, citric acid, succinic acid, L-malic acid, and total acidity of single cultured greengage wines were negatively correlated with ethyl decanoate, ethyl 9-decenoate, ethyl hexanoate, ethyl octanoate, and isoamyl acetate, while positively related to terpenes and α-terpineol (Fig. 5(b)). Furthermore, citric acid, L-malic acid, lactic acid, and acetic acid of cocultures were negatively related to phenethyl acetate, ethyl octanoate, ethyl benzoate, ethyl hexanoate, ethyl 9-decenoate, methyl salicylate, ethyl esters and esters, while positively related to acids, hexanoic acid, decanoic

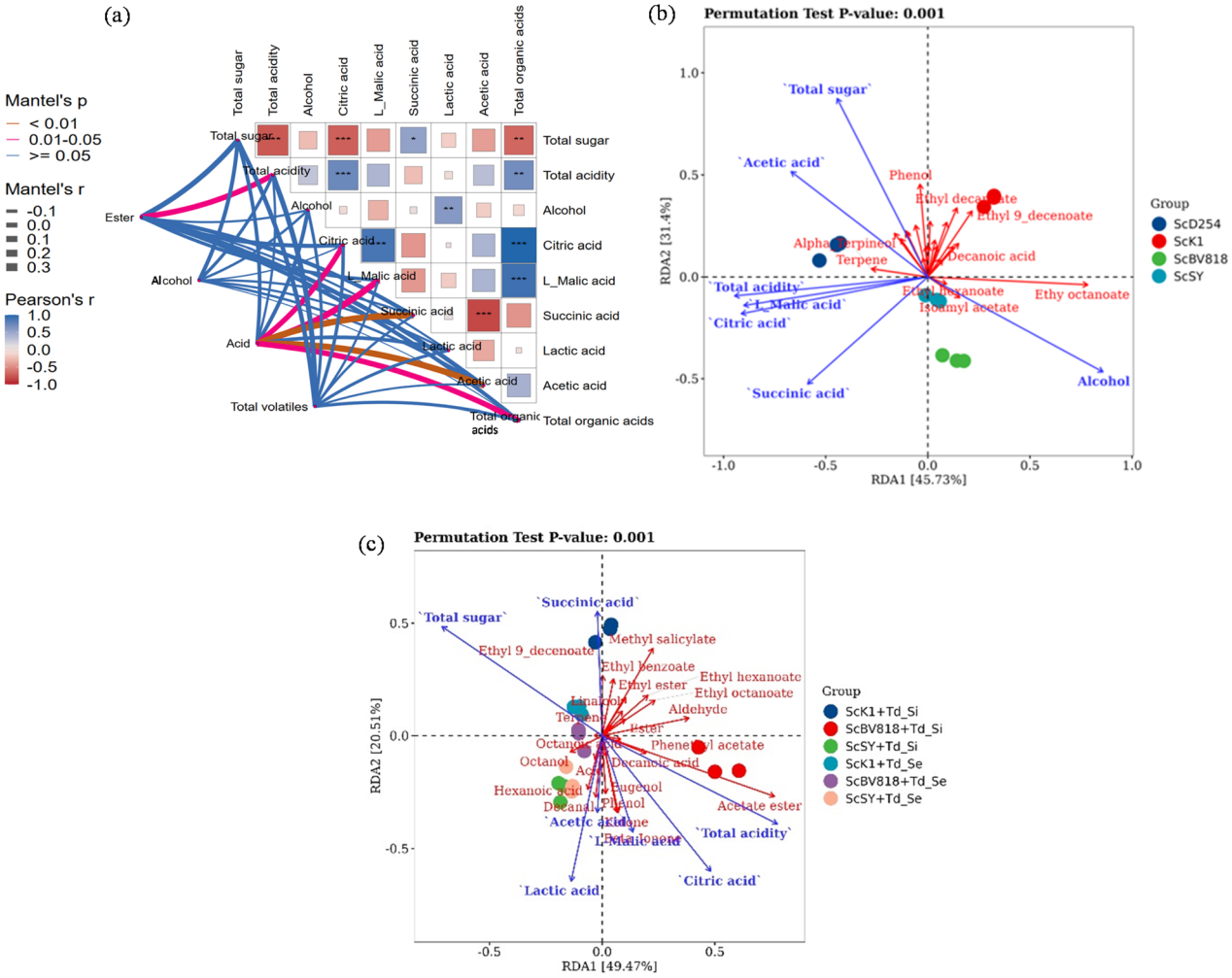

Fig. 5 Correlation between physicochemical properties, organic acids, and volatile constituents (Odor activity value (OAV) > 0.1). (a) Pearson correlation and mantel analysis of greengage wines fermented by four *Saccharomyces* yeasts, three simultaneous and sequential coculturing *Saccharomyces* yeasts with *Torulaspora delbrueckii*, respectively. (b) Redundancy analysis (RDA) of greengage wines fermented by four *S. cerevisiae* strains. (c) RDA of greengage wines fermented by three simultaneous and sequential coculturing *Saccharomyces* yeasts with *T. delbrueckii*, respectively.

acid, ketones, β-ionone, and eugenol (Fig. 5(c)). These results indicated that high levels of organic acids were detrimental to the synthesis of acetate and ethyl esters, both for the single culture and the coculture.

The correlation analysis demonstrated that organic acids, particularly citric acid, L-malic acid, and succinic acid, play a important role in shaping the volatile profiles of greengage wines. High organic acid levels were found to negatively correlate with the synthesis of acetate and ethyl esters (the main esters produced in this study), which are key contributors to fruity and floral aromas, such as ethyl hexanoate and ethyl octanoate.[25,29] Conversely, organic acids positively influenced the formation of ketones, phenols, and terpenes, which impart earthy and plant-like aromas.[7] These findings highlighted the importance of balancing organic acid content to optimize flavor profiles, as excessive acidity can suppress ester synthesis and limit the aromatic complexity of the wine.

Sequential inoculation strategies, where *T. delbrueckii* is introduced before *Saccharomyces* yeasts, have been shown to effectively modulate organic acid levels and enhance ester production. This approach allows *T. delbrueckii* to dominate the initial fermentation stages, leveraging its β-glucosidase activity to release glycosidic aroma precursors, such as 2-phenylethyl alcohol and terpenols, while reducing volatile acidity.[12,17,23] These findings provide practical applications for tailoring fermentation strategies to improve the sensory attributes of greengage wines. By optimizing yeast strain combinations and inoculation timing, producers can modulate the balance between organic acids and volatile compounds, reducing harsh acidity while enhancing aromatic complexity. This approach is particularly valuable for high-acidity fruit wines, offering a pathway to create wines with balanced acidity and rich, desirable flavor profiles.

4. Conclusions

This study demonstrated that the physicochemical properties, organic acids, and flavor profiles of greengage wines were significantly influenced by yeast strain specificity and fermentation strategies. Among the single cultures, ScBV818 exhibited the strongest fermentation capacity, achieving an alcohol content of 13.27% and the highest contents of esters, aldehydes, and ketones. The coculture fermentation further enhanced the flavor complexity, with simultaneous inoculation of ScBV818 and *T. delbrueckii* resulting in a remarkable increase of acetate and ethyl esters by 1124.09% and 29.06%, respectively, compared to the single culture. These esters, particularly ethyl hexanoate and ethyl octanoate, are key contributors to fruity and floral aromas, underscoring the importance of optimizing yeast combinations to enhance flavor metabolites. Mantel and RDA analyses revealed that high levels of organic acids, such as citric acid and L-malic acid, were negatively correlated with the synthesis of acetate and ethyl esters, while positively influencing the formation of ketones, eugenol, and terpenes. This highlighted the need to balance organic acid content during fermentation to achieve desirable flavor profiles, particularly in high-acidity fruit wines.

The findings of this study provide practical guidance for tailoring fermentation strategies to improve the sensory quality of greengage wines and other high-acidity fruit wines. Sequential or simultaneous inoculation of *T. delbrueckii* and *Saccharomyces* yeasts offers a promising approach to modulate organic acid levels and enhance the production of key flavor metabolites. However, limitations remain, such as the lack of investigation into the dynamic metabolic interactions between yeast strains during fermentation. Future research should focus on elucidating these mechanisms and exploring genetic or metabolic engineering of yeast strains to further optimize flavor profiles and fermentation efficiency in high-acidity fruit wines.

Author contributions

Jian Liu: methodology, software, validation, formal analysis, data curation, writing – original draft. Can Lyu: validation, writing – review & editing. Yongli Yang: conceptualization, writing – review & editing. Shijun Lu: resources. Yuru Wen: investigation. Mengxue Sun: investigation. Zhaohuan Du: investigation. Wei Lin: supervision. Chensheng Xu: visualization. Zhao Chen: validation. Lanmei Zhao: conceptualization, writing – review & editing, project administration, funding acquisition. Ping Dong: funding acquisition.

Conflicts of interest

There are no conflicts to declare.

Data availability

Data is provided within the manuscript or supplementary information (SI) files. Supplementary information is available. See DOI: https://doi.org/10.1039/d5ra04203h.

Acknowledgements

This work was supported by Agricultural Science and Technology Innovation Program (ASTIP-TRIC-QH-2022B06), project ZR2023QC207 and ZR2021QE125 supported by Shandong Provincial Natural Science Foundation, project 23-2-1-52-zyyd-jch supported by Qingdao Natural Science Foundation, Science and Technology Project of Beijing Life Science Academy Company Limited (2023000CC0090), Science and Technology Project of Hunan Tobacco Company Chenzhou Company (CZYC2023JS05), Science and Technology Project of China Tobacco Corporation Fujian Company (2023350000200091), Science and Technology Project of Fujian Tobacco Company Nanping Company (2021350700240083), Science and Technology Project of China Tobacco Shandong Industrial Company Limited (202402008).

References

1 J. Liu, M. Liu, P. Ye, C. He, Y. Liu, S. Zhang, J. Huang, J. Zhou, R. Zhou and L. Cai, Characterisation of the metabolite profile and microbial community of repeated batch and coculture-

fermented greengage wine, *Process Biochem.*, 2021, **109**, 117–129.
2 X. Zheng, M. Zhang, Z. Fang and Y. Liu, Effects of low frequency ultrasonic treatment on the maturation of steeped greengage wine, *Food Chem.*, 2014, **162**, 264–269.
3 X. Han, Q. Peng, H. Yang, B. Hu, C. Shen and R. Tian, Influence of different carbohydrate sources on physicochemical properties and metabolites of fermented greengage (*Prunus mume*) wines, *LWT–Food Sci. Technol.*, 2020, **121**, 108929.
4 J. T. Jeong, J. H. Moon, K. H. Park and C. S. Shin, Isolation and characterization of a new compound from *Prunus mume* fruit that inhibits cancer cells, *J. Agric. Food Chem.*, 2006, **54**, 2123–2128.
5 X. Han, Y. Zhao, B. Hu, H. Yang, Q. Peng and R. Tian, Influence of different yeast strains on the quality of fermented greengage (*Prunus mume*) alcoholic beverage and the optimization of fermentation conditions, *LWT–Food Sci. Technol.*, 2020, **126**, 109292.
6 K. Chen, C. Liu, Y. Wang, Z. Wang, F. Li, L. Ma and J. Li, Predominance of indigenous non-*Saccharomyces* yeasts in the traditional fermentation of greengage wine and their significant contribution to the evolution of terpenes and ethyl esters, *Food Res. Int.*, 2021, **143**, 110253.
7 J. Liu, M. Liu, Y. Liu, C. He, J. Huang, S. Zhang, R. Zhou, J. Zhou and L. Cai, Split batch and coculture fermentation to regulate the organic acids and flavor profile of fruit wine-a case study of *Prunus mume* Sieb. et Zucc (greengage) wine, *Food Sci. Technol.*, 2023, **43**, e107622.
8 J. Liu, M. Liu, P. Ye, F. Lin, J. Huang, H. Wang, R. Zhou, S. Zhang, J. Zhou, L. Cai, S. cerevisiae, S. bayanus, S. uvarum and T. delbrueckii, Characterization of major properties and aroma profile of kiwi wine co-cultured by *Saccharomyces* yeast, *Eur. Food Res. Technol.*, 2020, **246**, 807–820.
9 M. C. López, J. J. Mateo and S. Maicas, Screening of β-Glucosidase and β-Xylosidase Activities in Four Non-*Saccharomyces* Yeast Isolates, *J. Food Sci.*, 2015, **80**, 1696–1704.
10 K. Medina, E. Boido, L. Fariña, O. Gioia, M. E. Gomez, M. Barquet, C. Gaggero, E. Dellacassa and F. Carrau, Increased flavour diversity of Chardonnay wines by spontaneous fermentation and co-fermentation with *Hanseniaspora vineae*, *Food Chem.*, 2013, **141**, 2513–2521.
11 J. Liu, M. Liu, P. Ye, C. He, Y. Liu, S. Zhang, J. Huang, J. Zhou, R. Zhou and L. Cai, Ethyl esters enhancement of Jinchuan pear wine studied by coculturing *Saccharomyces bayanus* with *Torulaspora delbrueckii* and their community and interaction characteristics, *Food Biosci.*, 2022, **46**, 101605.
12 Z. Ni, P. Ye, J. Liu, J. Huang and R. Zhou, Research on improving the flavor of greengage wine based on co-cultivation of *Torulaspora delbrueckii* and *Saccharomyces cerevisiae*, *Eur. Food Res. Technol.*, 2021, **247**, 2765–2776.
13 X. C. Wang, A. H. Li, M. Dizy, N. Ullah, W. X. Sun and Y. S. Tao, Evaluation of aroma enhancement for "Ecolly" dry white wines by mixed inoculation of selected *Rhodotorula mucilaginosa* and *Saccharomyces cerevisiae*, *Food Chem.*, 2017, **228**, 550–559.
14 V. Englezos, K. Rantsiou, F. Cravero, F. Torchio, A. Ortiz-Julien, V. Gerbi, L. Rolle and L. Cocolin, *Starmerella bacillaris* and *Saccharomyces cerevisiae* mixed fermentations to reduce ethanol content in wine, *Appl. Microbiol. Biotechnol.*, 2016, **100**, 5515–5526.
15 M. Cocchi, P. Lambertini, D. Manzini, A. Marchetti and A. Ulrici, Determination of carboxylic acids in vinegars and in Aceto Balsamico Tradizionale di Modena by HPLC and GC methods, *J. Agric. Food Chem.*, 2002, **50**, 5255–5261.
16 M. C. Niu, J. Huang, Y. Jin, C. D. Wu and R. Q. Zhou, Volatiles and antioxidant activity of fermented Goji (*Lycium* Chinese) wine: Effect of different oak matrix (barrel, shavings and chips), *Int. J. Food Prop.*, 2017, **20**, 2057–2069.
17 T. Tian, D. Wu, C. T. Ng, H. Yang, J. Liu, J. Sun and J. Lu, Uncovering mechanisms of greengage wine fermentation against acidic stress *via* genomic, transcriptomic, and metabolic analyses of *Saccharomyces cerevisiae*, *Appl. Microbiol. Biotechnol.*, 2020, **104**, 7619–7629.
18 W. P. Duan, B. Q. Zhu, R. R. Song, B. Zhang, Y. B. Lan, X. Zhu, C. Q. Duan and S. Y. Han, Volatile composition and aromatic attributes of wine made with *Vitisvinifera L.*cv Cabernet Sauvignon grapes in the Xinjiang region of China: effect of different commercial yeasts, *Int. J. Food Prop.*, 2018, **21**, 1423–1441.
19 X. Lin, Q. Wang, X. Hu, W. Wu, Y. Zhang, S. Liu and C. Li, Evaluation of different *Saccharomyces cerevisiae* strains on the profile of volatile compounds in pineapple wine, *J. Food Sci. Technol.*, 2018, **55**, 4119–4130.
20 Z. Wang, Z. Yang, M. Chen, G. Chen, H. Lei, H. Xu and X. Yue, Effects of five different commercial strains of *Saccharomyces cerevisiae* on the physicochemical parameters, antioxidant activity, phenolic profiles and flavor components of jujube wine, *LWT–Food Sci. Technol.*, 2024, **198**, 115989.
21 W. Lei, R. Huang, L. Qu, K. Ma and J. Li, Exogenous Mogroside V Drove Microbial Carbohydrate Metabolism and Consequently Enhanced Fruity Aroma in Greengage Wine, *J. Agric. Food Chem.*, 2025, **73**, 11248–11264.
22 V. I. Petropulos, E. Bogeva, T. Stafilov, M. Stefova, B. Siegmund, N. Pabi and E. Lankmayr, Study of the influence of maceration time and oenological practices on the aroma profile of Vranec wines, *Food Chem.*, 2014, **165**, 506–514.
23 B. Padilla, J. V. Gil and P. Manzanares, Past and Future of Non-*Saccharomyces* Yeasts: From Spoilage Microorganisms to Biotechnological Tools for Improving Wine Aroma Complexity, *Front. Microbiol.*, 2016, **7**, 411.
24 M. T. Liberatore, S. Pati, M. A. Del Nobile and E. La Notte, Aroma quality improvement of Chardonnay white wine by fermentation and ageing in barrique on lees, *Food Res. Int.*, 2010, **43**, 996–1002.
25 P. Renault, J. Coulon, G. de Revel, J. C. Barbe and M. Bely, Increase of fruity aroma during mixed *T-delbrueckii/S-cerevisiae* wine fermentation is linked to specific esters enhancement, *Int. J. Food Microbiol.*, 2015, **207**, 40–48.

26 T. Tian, J. Sun, D. Wu, J. Xiao and J. Lu, Objective measures of greengage wine quality: From taste-active compound and aroma-active compound to sensory profiles, *Food Chem.*, 2021, **340**, 128179.
27 H. Li, *Wine Tasting*, Science Press, Beijing, China, 2006.
28 I. Loira, A. Morata, P. Comuzzo, M. J. Callejo, C. González, F. Calderón and J. A. Suárez-Lepe, Use of *Schizosaccharomyces pombe* and *Torulaspora delbrueckii* strains in mixed and sequential fermentations to improve red wine sensory quality, *Food Res. Int.*, 2015, **76**, 325–333.
29 M. Azzolini, E. Tosi, M. Lorenzini, F. Finato and G. Zapparoli, Contribution to the aroma of white wines by controlled *Torulaspora delbrueckii* cultures in association with *Saccharomyces cerevisiae*, *World J. Microbiol. Biotechnol.*, 2015, **31**, 277–293.