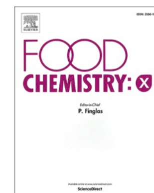
Food Chemistry: X 19 (2023) 100862

Contents lists available at ScienceDirect

Food Chemistry: X

journal homepage: www.sciencedirect.com/journal/food-chemistry-x



Fungal dynamic during apricot wine spontaneous fermentation and aromatic characteristics of *Pichia kudriavzevii* for potential as starter

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ARTICLE INFO

Keywords:
Apricot wine
Spontaneous fermentation
Fungal diversity
Aroma control
Pichia kudriavzevii

ABSTRACT

Microbial activity during spontaneous fermentation in alcoholic beverages have driven in developing the chemical and aromatic characteristic of products but not clear in apricot wines. We have characterised the composition of fungal communities and volatile metabolites in apricot wine spontaneous fermentation among two Shaanxi regions. Results showed that *Aureobasidium*, *Alternaria*, *Pichia* and *Saccharomyces*, were the dominant fungi in apricot wine fermentation. A total of 80 volatiles including esters, alcohols, acids and terpenes were detected from two apricot wines. Their correlations suggested that apricot wine aroma was mainly affected by *Pichia kudriavzevii*, rather than *Saccharomyces cerevisiae* we commonly considered. Furthermore, reinforced inoculation of *P. kudriavzevii* LQD20 has exhibited the commendable potential in enhancing sensory qualities. The results of this study provide fundamental information of the indigenous microbiota in microbial dynamic during apricot wine fermentation, which would be helpful in exploiting the strains with potential for industrial use as starter cultures.

Introduction

Apricot (*Prunus armeniaca* L.) is one of the economically important agricultural commodities for which its relevant products or by-products play the key role in providing a highly valuable nutrient (Zhang, Feng, Li, Qu, Zeng & Xi, 2019), and developing consumer acceptance and marketability (Deng et al., 2021). China, being one of the leading apricot-producing countries globally, harbours extremely abundant apricot germplasm resources of which was widely cultivated in Xinjiang, Shaanxi region, Shandong, etc. (Xi, Feng, Liu, Zhang & Zhao, 2019). Its high values were frequently attributed to the sarcocarp with rich in carotenoids (Su et al., 2020), functional extraction of apricot kernel (Pu et al., 2023), and other business. While the fresh apricot kernel with bitter taste slightly caused an unpleasant mouthfeel in related products and a concern about the potential toxicity of the kernel, current technologies and simple process could completely eliminate these fears (Deng et al., 2021).

Despite the admirable taste of fresh apricots, apricots are not suitable for long-term storage and long-distance transportation after harvest, which mainly attribute to a short harvest time within the hot season and the perishable apricot fruit, restricting the development of regional apricot industry. Apricot wine is considered an importantly enjoyed product as the rising demand for fermented beverage. These fruits with a higher sugar concentration have been fermented to alcoholic beverage, providing the admirable taste and sensory enjoyment (Kesa et al., 2021). This fermentation is also a strong solution for the extension of shelf life and the reduction of refrigerated needs. Generally, the use of commercial *Saccharomyces cerevisiae* resulted in the apricot wine with high levels of ethanol (above 11.0 % v/v) and organic acids (about 9.0 g/L) (Choi, Lee, Choi & Park, 2020; Pu et al., 2023), while apricot juice fermented by *Lactobacillus plantarum* was significantly improved in the nutritional quality and flavour characteristics without ethanol (Sun et al., 2022). However, these commercial starters have also conferred the common and atypical character of products (Lappa, Kachrimanidou, Pateraki, Koulougliotis, Eriotou & Kopsahelis, 2020), which is infaust to the production of regionally multivariate products. One of our aims is to

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https://doi.org/10.1016/j.fochx.2023.100862
Received 24 June 2023; Received in revised form 24 August 2023; Accepted 31 August 2023
Available online 3 September 2023
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screen for specialized yeasts used in apricot winemaking, in order to address the challenges of industrial production caused by a shortage of specialized starters.

Attributing to the cheapness and ready implement of spontaneous methods, researchers seek to enhance the fermented foods of flavour complexity and specificity via spontaneous fermentation, implemented across the whole fermented beverage production chain (Luzzini, Slaghenaufi & Ugliano, 2021; Philipp, Bagheri, Horáček, Eder, Bauer & Setati, 2021), but not yet in the production of apricot wines. In addition, microbial activity was substantially correlated with the spontaneous fermentation process, of which indigenous microbiota (mainly yeast populations) is likely the primary driver of these fermented beverages production (Varela, 2016). The indigenous microbiota colonized in the raw materials could effectively drive the production of fermented beverages in shaping the regional characteristics of products with spontaneous fermentation (Gurakan, Aktuna & Seyedmonir, 2022). Nevertheless, this fermentation method has relied heavily on the raw materials with differently initial microbiota, presenting challenges in ensuring consistency of the final product quality across different batches. Selecting key fermentative species have been regarded as the crux of the transformation from spontaneous fermentation to commercially controlled fermentation, aiming to guarantee the fermentation finish without the interference of polluted species (Chen et al., 2022). Taken together, investigation of microbial diversity with geographical feature in spontaneous fermentation are of high importance for revealing how distinct chemical compositions are formed, and for the selection of expectative starters that can substitute spontaneous fermentation, especially for apricot wines (which has not been proven to date).

Shaanxi, which is located at 105°29′-111°15′ E and 31°42′-39°35′ N, is one of the main apricots planted-region of China with ideal climate conditions except of Xinjiang region. Fruit farmers often struggle to improve their economic efficiency due to the short shelf life of fresh apricots, which results in significant losses if unsold in a timely manner. Moreover, in Shaanxi region, limited information is available on the biodiversity during spontaneous fermentation of apricot wine, and how different fungal populations quantificationally contributes to apricot wines quality. To address these questions, we sampled microbial communities associated with different spontaneous fermentation stages from Jingyang region and Liquan region in Shaanxi. Based on traditional culture-dependent method, yeast colonies isolated during fermentation were taxonomically identified using molecular identification approaches, and further differentiated using interdelta fingerprinting for sub-populations of *S. cerevisiae*. Besides, headspace solid-phase microextraction gas-chromatographic mass-spectrometric (HS-SPME–GC–MS) was analysed to the volatile metabolites of two resultant apricot wine. To elucidate the associations between fungal populations and volatile composition of apricot wine, we used partial least squares regression (PLSR) and structural equation model (SEM) to carry out. We found the key role of *Pichia kudriavzevii* during apricot wine spontaneous fermentation, alongside its potential as starter in apricot wine, and demonstrated that many aromatic volatiles are possibly derived from metabolic activities of *Pichia kudriavzevii*.

Materials and methods

Spontaneous fermentation and sampling collection

Hongmei apricot from each three orchard of two major production regions (JY, Jingyang; LQ, Liquan) in Shaanxi Province of China were chosen to conduct this study (Fig. 1A). The mean distance between two orchards ranged above 55 km. All apricot fruits were collected from the plants at the harvest-stage (June 2021). After collection, all samples were maintained at 4 ℃ and transported to the laboratory within 12 h. The concentration of reducing sugar, total acid (TA) and pH in apricot fruit were measured according to Chen et al. (2022), using neutralization titration method and pH meter (Sartorius PB-10, Shanghai, China), respectively (Table S1).

Handpicked apricots were immediately denucleated and crushed prior to being loaded in clean and decontaminated 10 L tanks. A diagram of the production and sampling points with sample names are presented in Fig. 1B. Briefly, two amounts of initial sugars were reached by sugar regulation to 135 g/L. Potassium metabisulfite (PMS, Solarbio, Beijing, China) was added to the apricots at smash to yield approximately 40 mg/L total sulfur dioxide (SO_2) to prevent the intervention of useless bacteria on fermentation process. The zymohydrolysis was generated by

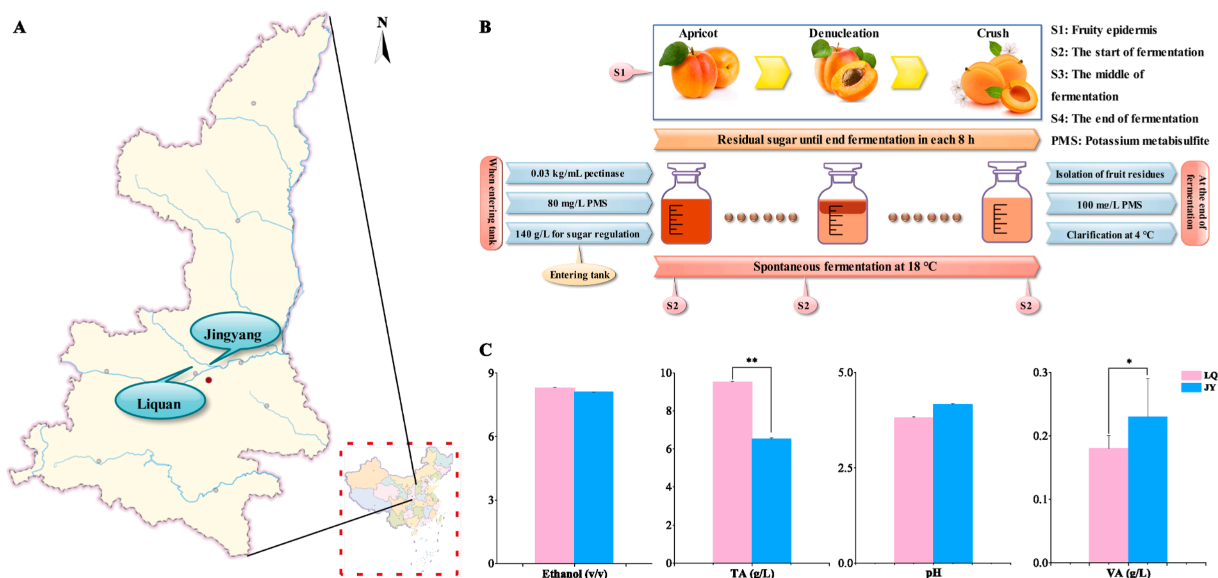

Fig. 1. Sample information. (A) Sampling map of Hongmei apricot in Jingyang and Liquan region, Shaanxi of China. (B) The production diagram of apricot wine used in this study with four sampling points indicated during fermentation process. Sample 1, apricot epidermis. Sample 2, at the start of fermentation (about 24 h after bottling). Sample 3, at the middle of fermentation (around 1/3 of sugar fermented). Sample 4, at the end of fermentation (residual sugar below 4 g/L). (C) Parameters of the resulting apricot wine, involving ethanol (v/v %), titratable acid (TA, g/L), pH, and volatile acid (VA, g/L). The relative difference between two apricot wines was measured, and only TA concentrations showed significant differences between two apricot wines ($p < 0.01$) (JY, Jingyang region; LQ, Liquan region).

0.03 kg/mL pectinase (Yuanye Bio-Technology Co Ltd, Shanghai, China) at 20 ℃ for 1 h. Spontaneous fermentations were carried out at 18 ℃ in triplicate without addition of any commercial strain following similar fermentation protocols. The fermentation process was monitored by measuring daily sugar concentration of each bottle for each 8 h until the end of fermentation. Samplings were performed for traditional microbial cultivation and DNA extraction at four stages (designated as JYn-[1, 2, 3] and LQn-[1, 2, 3], n = 1, 2…4): apricot epidermis (Sample 1), at the start of fermentation (Sample 2, about 24 h after bottling), at the middle of fermentation (Sample 3, around 1/3 of sugar fermented), and at the end of fermentation (Sample 4, residual sugar below 4 g/L).

DNA extraction, PCR amplification and sequencing

Total DNA was extracted from the fermented samples using HiPure Soil DNA Kits (D3142, Magen Biotechnology Co., Ltd., Guangzhou, China) following the manufacturer's instructions. To analyse the fungal communities, partial fungal internal transcribed spacer (ITS) region was amplified using the universal primer pairs ITS2. PCR was carried out in a final volume of 50 μL, containing 5 μL 10 × KOD buffer, 3 μL $MgSO_4$ (25 mM), 5 μL dNTPs (2 mM), 1.5 μL forward primer (10 μM) and 1 μL reverse primer (10 μM), 1 μL KOD polymerase, and 1 μL of the template DNA. Thermal cycles were as follows: 94 ℃ for 2 min; followed by 30 cycles at 98 °C for 10 s, 62 °C for 30 s, 68 °C for 30 s; and a final extension at 68 °C for 5 min. The PCR products were sent to Gene Denovo Biotechnology Co., Ltd. (Guangzhou, China), then sequenced on an Novaseq 6000 with PE250 Model (USA). Raw sequences were processed using QIIME software (Version 1.9.2) to obtain high-quality clean reads. Operational taxonomic units (OTUs) were further clustered using Usearch software based on Uparse algorithm with a threshold of 97% pairwise identity (Ji, Yu, Wu & Xu, 2022). The taxonomy annotation was assigned to OTUs in RDP software using the UNITE fungal ITS database (Version 7.2).

Microbiological enumeration, isolation and identification of yeast

Each four apricots was obtained by fruity epidermis samples and mixed with 400 mL sterile water in a agitation rate of 300 rpm at controlled low temperature for 30 min. All samples from epidermis and fermentation process were spread out the serial dilution (from 10^{-2} to 10^{-6}), then cultured on fresh Wallerstein Laboratory Nutrient (WLN) agar medium (ThermoFisher Scientific, USA) for microbiological enumeration (Chen et al., 2022). Each single colony morphology (238 isolates in total) was recorded via streaking on WLN agar medium for differentiating yeast species.

Yeast colonies with representative morphotypes were used to extract DNA following previous descriptions without any modification (Liu, Legras, Zhang, Chen & Howell, 2021). All selected colonies were identified by sequencing of the 26S rRNA D1/D2 region, which was amplified using the universal primer pair, NL1 (5′-GCATATCAATAAGCGGAGGAAAAG-3′) and NL4 (5′-GGTCCGTGT TTCAAGACGG-3′). A 20 μL reaction volume of PCR amplification was carried out in the mixture containing 10 μL Phanta Max Buffer, 0.8 μL of each primer, 0.4 μL HighFidelity DNA Polymerase, 0.4 μL dNTP, 6.8 μL ddH_2O and 0.8 μL DNA template (100 ng/μL). The conditions of PCR procedures were set up the following: 95 ℃ for 3 min, then followed by 35 cycles at 95 ℃ for 10 s, 52 ℃ for 1 min, 72 ℃ for 90 s, ultimately held 72 ℃ for 7 min after terminating the cycle. The PCR products were transported to Tsingke Biotechnology Co., Ltd., Beijing, China. The results were compared against NCBI database (National Centre for Biotechnology Information, http://www.ncbi.nlm.nih.gov) using BLAST at an identity threshold of at least 98%, and tracked to the closest known relatives of these isolates. A set of 64 *S. cerevisiae* strains, listed in Table S2, was characterized for interdelta figerprinting using delta12 (5′-TCAACAATGGAATCCCAAC-3′) and delta21 (5′-CATCTTAA-CACCGTATATGA-3′) primers (Chen et al., 2022). The PCR was run in a final volume of 20 μL containing 10 μL 2 × Phanta Max Master Mix, 1.0 μL of each primer, 7.0 μL ddH_2O and 1.0 μL DNA template (100 ng/μL). The conditions of PCR procedures were used the previous program with some modification: 95 ℃ for 3 min, then followed by 30 cycles at 95 ℃ for 30 s, 45 ℃ for 30 s, 72 ℃ for 90 s, and a final extension at 72 ℃ for 5 min. 2.0 μL of PCR products with 1 μL 10 × DNA Loading Buffer were separated via electrophoresis on 2.0 % agarose gels (100 V for 2.5 h) and photographed under UV light, comparing to the genetic profiles of the tested *S. cerevisiae*.

Apricot wine fermented by P. kudriavzevii

Each 8-kg Hongmei apricots with same batches was carried out to the inoculated fermentation containing two groups of inoculated treatments, according to previously identical management. In the monocultured fermentation, commercial CECE (purchased from Angel Yeast Co., Ltd., Yichang, China) and *P. kudriavzevii* cells (LQD20 and LQD5) were added at the concentration of 2 × 10^8 CFU/mL; among co-fermentation experiments, two *P. kudriavzevii* strains were respectively mixed with CECA at the content of 1 × 10^8 CFU/mL. Fermentation kinetics were monitored by daily determining the content of residual sugar, until the end of fermentation (residual sugar < 4.0 g/L) or the concentrations with no change for three consecutive days. All apricot wine samples, including two samples with spontaneous fermentation, were analysed the further chemical parameters.

Profiling of chemical composition and sensory in apricot wine

Apart from residual sugar (RS), pH and TA, ethanol of fermented apricot wine were previously described by Chen et al. (2022), and volatile acid (VA) was evaluated following the Chinese national standard GB/T 15038–2006.

Headspace solid-phase microextraction-gas chromatography–mass spectrometry (HS-SPME-GC–MS) was carried out in triplicate to identify and quantify the volatile composition of two apricot wines. In brief, upon centrifugation, each wine sample (10 mL) was transferred into a 20 mL crimp-cap vials containing 1.5 g sodium chloride and 5 μL internal standard (2-Methyl-3-heptanone, 7.752 g/L), and then equilibrated at 40 ℃ for 20 min. A CAR/DVB/PAMS (50/30 μm, Sigma, Shanghai, China) SPME fiber was extracted in headspace at 40 ℃ for 10 min with agitation. After desorbing in GC injector at 220 ℃ for 5 min, volatile compounds were separated on a DB-5 GC column (50 m × 0.25 mm × 0.25 μm, Agilent, USA) with helium carrier gas at a flow rate of 1.5 mL/min. An original temperature of 40 ℃ was held for 3 min, then risen to 240 ℃ at the rate of 3 ℃/min and held for 10 min. The temperature of interface was set at 250 ℃, and ion source temperature was 230 ℃. Analyses were conducted with GC–MS QP2020 (Shimadzu, Kyoto, Japan), scanned in a mass acquisition range of 35–750 *m/z* for quantitative analysis of detections. To enhance the reliability of the qualitative, volatile compounds were identified in apricot wines according to the comparation of retention indexes (RI) calculated using *n*-alkanes (C7–C40) and mass spectra matching with NIST17.0 library (the threshold = 85%). Several pure standards were established the calibration curves for quantification. the concentrations of compounds were calculated with previous calibration curves, and the proportion of the peak areas of the internal standard and target compounds (Li, Wan, Yin, Ma & Tao, 2022). The aroma contributions of volatile compounds were evaluated to apricot wines via the odour activity values (OAV), of which were calculated according to the previously described formula (Chen et al., 2022).

The electronic nose (E-nose, PEN3.0, Airsense, Germany) used in the study was coupled with a metal oxide semiconductor (MOS) sensor array of 10 different sensor (W1C, W5S, W3C, W6S, W5C, W1S, W1W, W2S, W2W, W3S, the detailed information was shown in Table S3). For further analysis, each 10 mL wine sample was placed in an airtight headspace bottle at 45 ℃ for 10 min. The E-nose detection process

included cleaning and measurement phases. Clean air was pumped into the sample gas path for 100 s during the cleaning phase, followed by calibration of sensor signals for 10 s. The sensorial compounds were then collected into the sensor chamber at a flow rate of 600 mL/min for 60 s. The full E-nose array response was obtained from the signals of ten distinct sensors.

Data analysis

The data regarding microbiological enumeration was analysed as means expressed in CFU/mL. Analysis of physicochemical parameters, fungal composition, and volatile profiles were performed using SPSS 26.0 (SPSS Inc. Chicago, IBM, USA) and Origin 2021 (OriginLab, USA), including one-way analysis of variance (ANOVA) for statistically significant differences of raw data, and permutational multivariate analysis of variance (PERMANOVA) using distance matrices with 999 permutations. Alpha diversities (characterized by Simpson and Shannon indexes) of fungal communities were calculated with the "vegan" package (Price, Dehal & Arkin, 2009). Using principal coordinate analysis (PCoA) is to evaluate the distribution patterns of fungal communities under different taxonomic levels based on beta-diversity calculated by the bray–curtis distance. Partial least squares regression (PLSR) was made with dominant fungi at the start of fermentation (relative abundance > 0.1%) and volatile compounds identified by ANOVA in the resulting wine, which was carried out in SIMCA 14.1 software. The direct and indirect relationships between fungal populations and yeast populations, *S. cerevisiae*, and apricot wine aroma, were evaluated using the structural equation model (SEM). Corresponding to previous method (Huang et al., 2023), the known causal relations among them was validated by an established priori model. The strength and sign of the relationship between two variables was characterised by a path coefficient. The suitability of the fitted model was assessed by the goodness of fit index (GFI > 0.90), the root MSE of approximation (RMSEA < 0.05), and the χ^2-test ($p > 0.05$). All these analyses were generated in AMOS v25.0 software (AMOS IBM, NY, USA).

Results and discussion

Fermentation kinetics, chemical profiles, and diversity during fermentation

The tested apricots represented in different oenological phenotypes in two regions. All apricot samples were fermented to dryness (residual sugar < 4 g/L), and had significantly impacted on fermentation duration among two regions (Fig. S1). JY samples did not ferment 128 h until the ending throughout the experimental period while the LQ samples for 216 h. Enumeration of yeast was performed using the traditional culture-dependent technique during fermentation. Initially, yeast population was approximately 1.00×10^3 CFU/mL, and 8.20×10^3 CFU/mL on Liquan apricot and Jingyang apricot, respectively (Table S4). Yeast viability gradually increased as fermentation processed, with the maximum population being observed until the ending fermentation stage (LQ4: 2.02×10^8CFU/mL; JY4: 2.90×10^8CFU/mL). The resultant apricot wines were further analysed for the observed characters involving ethanol, titratable acidity (TA), pH, and volatile acidity (VA). All parameters in two apricot wines were within the acceptable ranges with the regional variances, referring the general standard of fruit wine (GB/T 15038–2006) ($p < 0.05$, Fig. 1C). Independent of the brewing practices, the ethanol and TA concentrations in LQ apricot wines were higher compared to JY samples, while the pH and VA were insignificantly distinct from two regions. Interestingly, the TA content had a slight and significant decrease from apricots to apricot wines in JY samples after the ending fermentation (Fig. 1C, Table S1). Generally, microbial reason of this reducing-acid character in TA or pH is attributed to the metabolism of some non-*Saccharomyces* species in fermented samples, e.g. *Hanseniaspora occidentalis* (van Wyk et al., 2022), in which was used to correct the high-acidity fermented food and improve the mouthfeel via inoculating special yeast strains.

To elucidate fungal dynamic during apricot wine fermentation, sufficient samples covering two apricot orchards (Jingyang and Liquan) from the apricot epidermis, the start, middle and end of fermentation, were collected to analyse fungal communities. A total of 1,342,923 effective tags were obtained from all JY samples and 1,188,839 from LQ samples. Microbial diversity indices (Simpson and Shannon), constantly characterised the biodiversity of a region in ecology study, are used to evaluate the alpha diversity within each fermented sample (Fig. 2A). Integrally, the epidermis samples had the highest diversity (Shannon: 4.17, Simpson: 0.86) during Jingyang apricot wine spontaneous fermentation, which indicated that some fungal microorganisms on apricot epidermis samples were struggled to be detected during fermentation. Significant difference in alpha diversity was observed between Jingyang and Liquan region when they were carried out after middle fermentation stage (Shannon: p = 0.006 and 0.001 for Sample 3 and Sample 4, respectively; Simpson: p = 0.005 and 0.003 for Sample 3 and Sample 4, respectively), attributed to the increase of fungal diversity at the end of Liquan apricot wine fermentation (Fig. 2A, Shannon and Simpson indexes: LQ3 < LQ4). Unweighted pair-group method with arithmetic mean (UPGMA) cluster tree based on bray-curtis algorithm was employed to further evaluate the beta diversity of different samples (Fig. S2), indicating that JY1 and LQ1 had close fungal composition, alongside LQ2, JY2, and JY3. These effective tags were assigned to different operational taxonomic units (OTUs) and shown in each sample at a threshold of 97% (Fig. 2B). The distribution of these OTUs in the different samples was evaluated using a Venn diagram, with normalization based on the sample with the lowest abundance of sequences. The microbial community present within two individual regions distinguished different fermentation stages (Fig. 2C, D, E, F). Permutational multivariate analysis of variance (PERMANOVA) confirmed that fungal community structure was indistinctively different between regions based on the Bray–Curtis distance ($R^2 = 0.664$, $p = 0.1$). Fermentation stages strongly and significantly impacted fungal composition Liquan samples ($R^2 = 0.964$, $p = 0.001$) and Jingyang sample ($R^2 = 0.855$, $p = 0.001$). Principal coordinate analysis (PCoA) was conducted to examine the fungal communities across both regions at phylum and genus level during fermentation, with 99.85%, 90.54%, 98.76% and 86.27% of total variance respectively explained by the first two principal coordinate (PC) axes (Fig. 2C, D, E, F). These changes were in accord with the brewing process. These findings imply that the microbial diversity in the dynamic fermentation of apricot wine has a significant impact that is not related to the geographical regions.

Fungal dynamic during spontaneous fermentation

According to culture-independent method

In order to reveal the fungal composition of apricot wine samples, the top relative abundance distribution of fungal composition at phylum and genus levels were shown in Fig. 3. Ascomycota was the most abundant phylum on the apricot comprising 87.70% of all sequences, followed by Basidiomycota (6.57%) (Fig. 3). A further identification at the genus level showed that fungal profiles were dominated by filamentous fungi for apricot epidermis (JY1 and LQ1), mostly of the genera *Aureobasidium*, *Alternaria*, *Mycosphaerella* and *Cladosporium*, of which have awakened and can promote the breakdown of polysaccharides, thereby increasing the concentration of glucose and free amino acids during the start of fermentation (Liang, Li, Cao, Cao, Shen & Wan, 2021; Qian et al., 2023). As the fermentation proceeded, the fermentative yeast populations, especially the grew and dominated *Pichia* genus, thus reshaping the community diversity and composition (Zhao et al., 2022). *Pichia* was detected at the highest abundance (30.29%) in the ending fermentation of Jingyang sample (JY4), while *Issatchenkia* presented the followed abundance (about 20.84%). Likewise, this succession pattern was also observed in spontaneous fermentation of several fruity wine (Gurakan et al., 2022; Skotniczny, Satora, Pańczyszyn & Cioch-

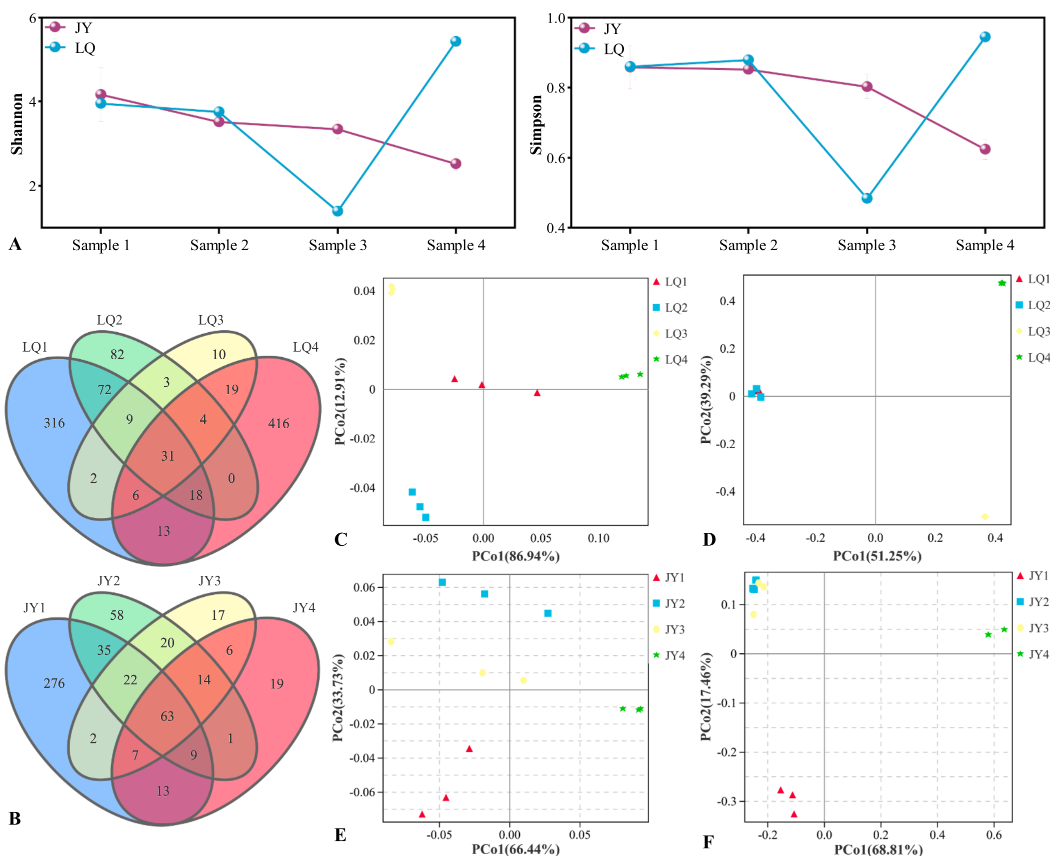

Fig. 2. Fungal diversity of all samples during spontaneous fermentation from two regions. Alpha diversity of 24 fermented samples was characterized by Shannon and Simpson indexes (A). Significant difference in alpha diversity was observed between JY3 and LQ3, JY4 and LQ4, respectively. Venn diagram show the distribution of OTUs among the different samples from Jingyang and Liquan region with a normalization corresponding to the sample with the most abundant sequences (B). Principal coordinate analysis (PCoA) based on Bray-Curtis distances during apricot wine fermentation in LQ (C, D) and JY region (E, F) at phylum (C, E) and genus (D, F) level.

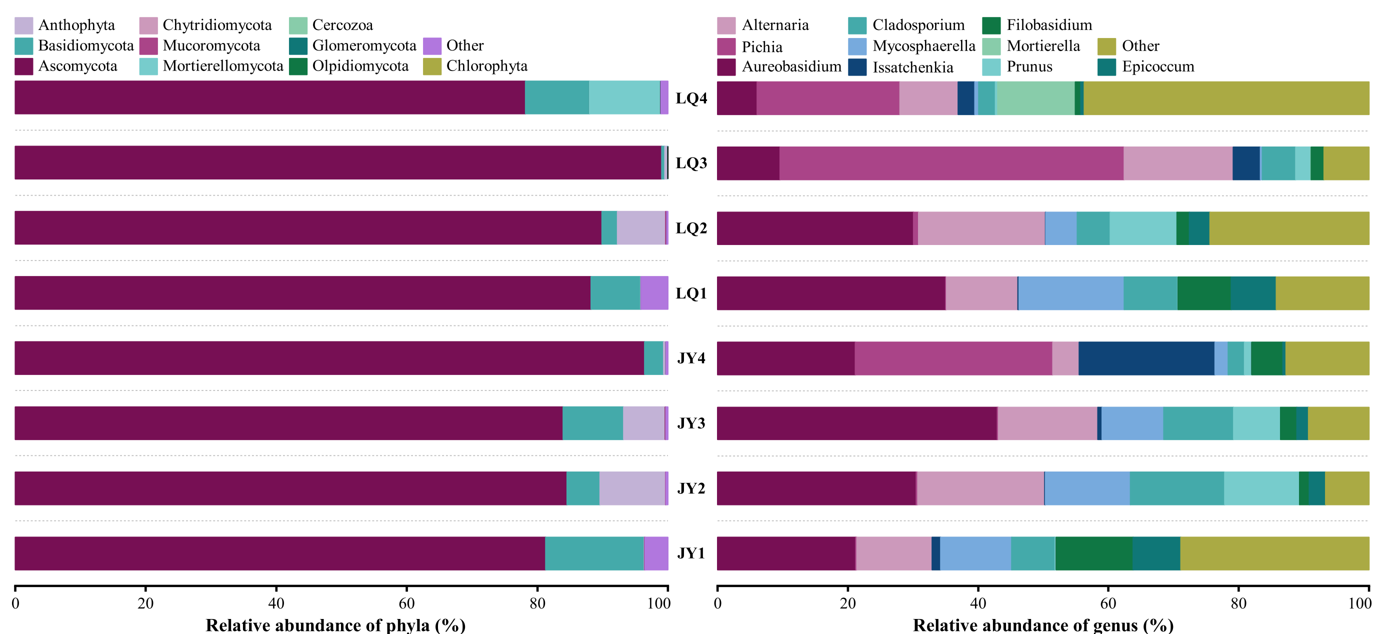

Fig. 3. Microbial community composition at different ferments from Jingyang and Liquan region characterised to the phylum (top) and genus (bottom) level (top 7 phyla and 9 genera).

Skoneczny, 2020), with *Pichia* and *Issatchenkia* showed recoveries in relative abundances between Sample 3 and Sample 4. Correspondingly, regional differences in fungal communities were not significant in all samples (between JY1 and LQ1, PERMANOVA, $R^2 = 0.240$, $p = 0.3$; between JY2 and LQ2, PERMANOVA, $R^2 = 0.700$, $p = 0.1$; between JY3 and LQ3, PERMANOVA, $R^2 = 0.960$, $p = 0.1$; between JY4 and LQ4, PERMANOVA, $R^2 = 0.990$, $p = 0.1$). *S. cerevisiae* populations gradually dominated the alcoholic fermentation of several beverages and impacted the quality of the resultant products, comprising grape (Liu et al., 2023), sugar cane and Chinese rice wine (Qian et al., 2023), etc., which is widely cognizant. However, *S. cerevisiae* was infrequent species during spontaneous fermentation in our ITS sequencing results. These abundantly fermentative yeasts might be the main driver in the formation of apricot flavour, especially certain filamentous fungi that can promote the growth for yeast (Qian et al., 2023; Wu et al., 2022), the genus *Issatchenkia* with the capability of reducing citric acid (He et al., 2022) and *Pichia* with high enzymatic activities (Maicas & Mateo, 2015).

Followed by culture-dependent method

To test the yeast population dynamics during spontaneous fermentations from two regions, 24 duplicate samples containing four fermentation stage among two regions, were collected to analyse the culture-dependent microbiota. Based on colony morphology (Chen et al., 2022), 26S rDNA gene sequencing, and partial interdelta polymorphism fingerprinting (only for *Saccharomyces cerevisiae* isolations), a total of 238 colonies from four fermentation stage were identified to seven species of six genera (Table S2, Table S4), including *Hanseniaspora uvarum* (*H. uvarum*, 63 colonies), *Hanseniaspora opuntiae* (*H. opuntiae*, 3 colonies), *Saccharomyces cerevisiae* (*S. cerevisiae*, 64 colonies), *Pichia kudriavzevii* (*P. kudriavzevii*, 98 colonies), *Kazachstania humilis* (*K. humilis*, 7 colonies), *Candida parapsilosis* (*C. parapsilosis*, 2 colonies), *Aureobasidium pullulans* (*A. pullulans*, 1 colony). These yeasts have been widely detected in several fermented food, especially fermented beverage (Gurakan et al., 2022; Kesa et al., 2021). Notably, *S. cerevisiae* populations, alongside the non-*Saccharomyces* species of *H. uvarum* and *P. kudriavzevii*, dominated the isolates (approximately accounting for 94.02% in JY samples and 94.59% in LQ samples, respectively), whereas other species was sporadically detected. Non-*Saccharomyces* species (*P. kudriavzevii* and *H. uvarum*) dominated the second ferments, 3rd, and 4th ferment point. Although without the detection on apricot epidermis, *S. cerevisiae* had occupied 50% of yeast populations from LQ2 samples. The genera of *H. uvarum* dominated the samples from apricot epidermis (LQ for 60%; JY for total). At the beginning of fermentation, the LQ samples harboured higher species diversity of yeasts than JY samples, with 50% non-*Saccharomyces* species dominating the population in LQ2. As fermentation proceeded, the amount and distribution of yeast species had differed with significant regional differences among different fermentation stages (PERMANNOVA, $R^2_{Region} = 0.433$, $p = 0.001$; $R^2_{Stage} = 0.346$, $p = 0.011$). Furthermore, all 64 *S. cerevisiae* strains were subsequently analysed to the genetic diversity through the interdelta polymorphism fingerprinting. A total number of 9 genotypes were obtained and coded G1 to G9 (Table S5, Fig. S3), total of which were identified at frequencies $< 50\%$ of the isolated population, revealing a higher genetic diversity in LQ region (LQ, 6 genotypes from 28 *S. cerevisiae* strains; JY, 6 genotypes from 36 *S. cerevisiae* strains). These figures present the variance, compared to other study about indigenous *S. cerevisiae* strains from fermented foods, like Feghali et al. (2019) or Yang, Liu, ZhangHe (2020), which might be due to the fewer *S. cerevisiae* isolations. Besides, a clearly regional difference could be observed according to strains clustering. ANOSIM analysis showed the significant genetic differentiation of the regional distribution of *S. cerevisiae* genotypes during different fermentation stages ($R_{region} = 0.998$, $p = 0.031$; $R_{stage} = 0.996$, p = 0.002). Three genotypes (G1, G2, G4) were shared among two regions, while other genetic strains were isolated from within a single region and fermentation stage. Four to eight genotypes were isolated at different fermentation course (Table S5), while each region harboured 6 different genotypes. Within the regional population, four patterns were only shared from the same fermentation process, involving two patterns in LQ2 (G2, G7) and one pattern in LQ3 (G8), alongside two patterns in JY3 (G2, G4). Worthily, G1, the most abundant genotype (followed by G4), was highly dispersed across two regions, frequently isolated from different ferments. The genotype G8 was composed to LQC25 and LQC40, only detected in Liquan region at the middle fermentation stage. These diverse genotypes might originate from the microbial interactions in co-cultured system, which could produce variable amounts of volatile metabolites with positive or negative effects on apricot wine aroma and flavour (Capece, Romaniello, Siesto & Romano, 2012; Sharma, Gupta, Abrol & Joshi, 2012).

Apricot wines volatile and sensory profiles

Using HS-SPME-GC–MS, we analysed the volatile compounds of two apricot wine samples (at the end of fermentation) in triplicate to represent product metabolite profiles. A total of 80 volatile compounds that may contribute to apricot wine flavour were detected in six apricot wine samples, involving 13 higher alcohols, 6 fatty acids, 39 esters, 13 terpenes, and 10 carbonyl compounds (Table S6). The data have been organised into five chemical families: higher alcohols, carbonyl compounds, terpenes, esters (ethyl esters, acetates, and others), and volatile acids (Fig. 4A). The concentrations of chemical families were higher in JY samples than in LQ samples, except for the ester cluster with significant difference (especially ethyl esters and acetate esters), suggesting that LQ samples provided a higher fruit/flower flavour. Volatile acids and terpenes content were significantly distinct from two regions, attributing to acetic acid, hexanoic acid, octanoic acid, dodecanoic acid, alpha-terpineol, geraniol, farnesol. All apricot wines contained 48 geographically differential compounds based on regions (ANOVA, $p < 0.05$), shown in heatmap (Fig. 4B). JY wines were clustered to the most terpenes except of farnesol, and several esters comprised of methyl palmitate, and *n*-butyl butanoate, etc.. LQ samples presented the high correlation with many esters (mainly ethyl esters: ethyl acetate, ethyl hexanoate, ethyl caprylate, etc.; other esters: isoamyl acetate, hexyl acetate, methyl octylate, etc.), and some higher alcohols (A3: 2,3-butanediol, A11: 1-dodecanol, A12: 1-decanol, etc.). Within compound types (Table S6, Fig. 4B), the most prevalent higher alcohol in two apricot wines (61.98% and 49.67% of total higher alcohols in JY sample and LQ sample, respectively) was 3-methyl-1-butanol, followed by phenylethyl alcohol. The production of phenylethyl alcohol, potentially regarded as a signaling molecule (Cordente et al., 2018), is generally attributed to mixed fermentations with several strains but not necessarily their respective monocultures (Comitini et al., 2011; Morata et al., 2019). Currently, the level of phenylethyl alcohol in analysed JY apricot wine was weakly higher than in LQ apricot wine. Albeit present at lower concentration than 3-methyl-1-butanol and phenylethyl alcohol, 1-hexanol was usually considered the major flavour contributors of fermented alcoholic beverage attributed to their low odour threshold (Chen et al., 2022). Separately, five high alcohols had a significant difference between JY and LQ, including 2,3-butanediol ($p = 0.003$), 3-methyl-2-propyl-1-pentanol ($p = 0.002$), 1-dodecanol ($p = 0.002$), 1-decanol ($p = 0.007$), and 1-hexadecanol ($p = 0.01$). The concentrations of total esters (especially ethyl esters of fatty acids) had significantly differed among the apricot wines (ANOVA, $p = 0.003$). The esters, imparting fruity and floral flavour due to their low threshold values (Bowen, Reynolds & Lesschaeve, 2016), was usually formed from the esterification reaction of acids and alcohols. The production of ethyl acetate, ethyl 9-decenoate, and ethyl hexanoate in Liquan apricot wines, were essential for the overall flavour of LQ samples, and they provide a fruity odour and mask rancid odour, while 9 terpenes in a higher proportion in JY samples was identified. Moreover, content of all LQ apricot wines with lower odour threshold compounds (mainly ethyl esters: ethyl hexanoate, ethyl caprylate, ethyl salicylate, ethyl caprate, etc.) was higher than in JY

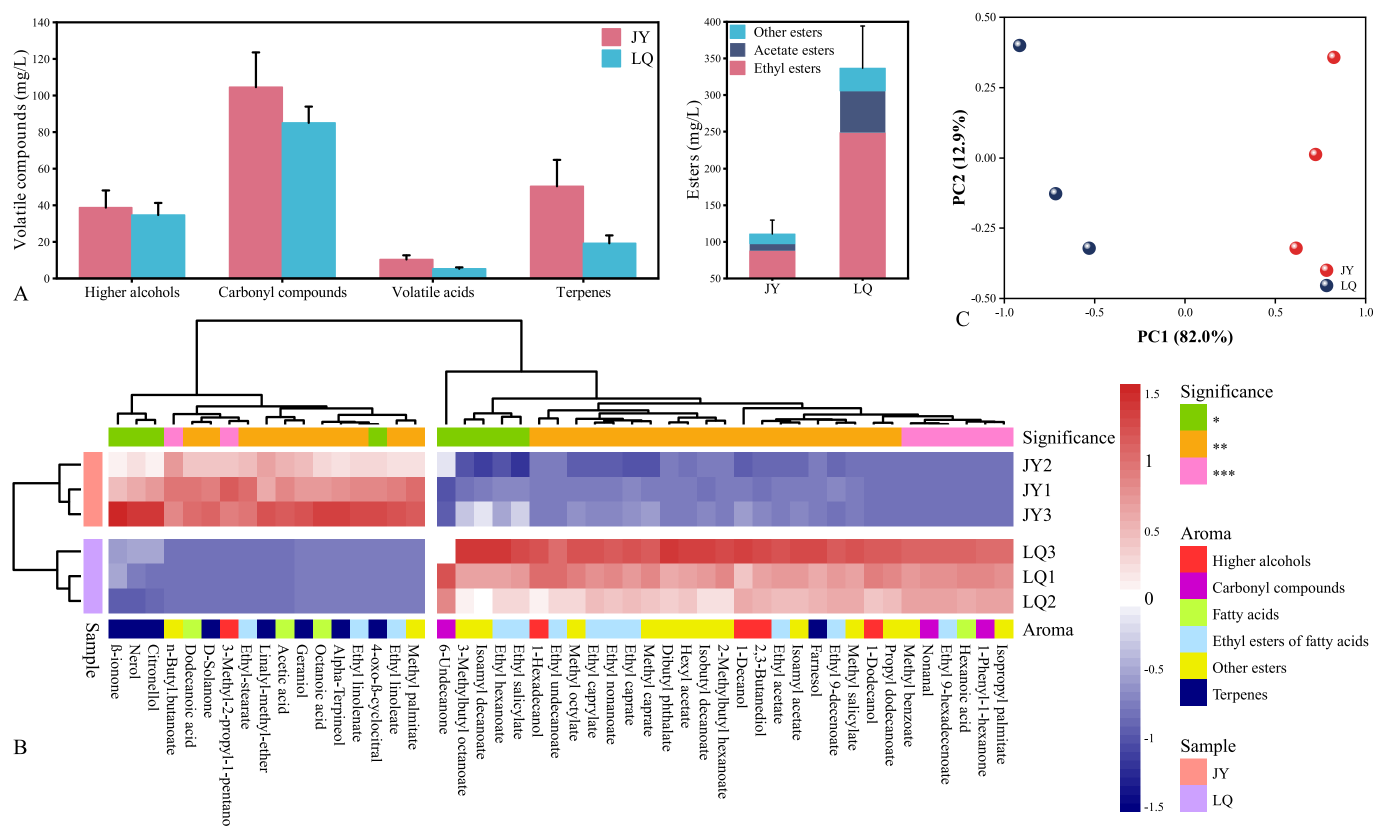

Fig. 4. Volatile profiles of two apricot wines. (A) Sum of higher alcohols, carbonyl compounds, volatile acids, terpenes, and esters (ethyl esters, acetates esters, and other esters) in two apricot wines. The values represent means of triplicates with standard deviation. (B) Heatmap analysis of main volatile compounds with significant difference ($p < 0.05$) in two apricot wines. (C) Principal component analysis of volatile composition in two apricot wines. JY, Jingyang region; LQ, Liquan region.

samples (Chen et al., 2022), suggesting that apricot wines from Liquan region were more acceptable. Volatile profiles of apricot wines are clearly separated with principal component analysis (PCA), where PC1 explained 82.0% of the total variance according to regions (Fig. 4C). Compared with sensory analysis, the E-nose method as a powerful tool could more precisely and objectively differentiate aroma at faster speeds. The response trend of the E-nose to the apricot samples within two apricot samples was consistent, but the obvious differences could be seen between two profiles of the fingerprints within the most of the 10 sensors (Fig. S4, Fig. S5). The five sensors (W1S, W5S, W1W, W2S, and W2W) in the response values of LQ samples were significantly larger than those of JY samples, contributed to the flavour discrimination of those two wines. This indicated that the aroma of regional apricot wines had changed, which agreed with the results obtained through previous volatile analysis.

The key role of P. kudriavzevii in formation of apricot wine aroma revealed by SEM and PLSR

To elucidate the correlation between fungal communities (the top 20 genera) and volatile metabolites (OAVs > 1.0) in apricot wine, partial least squares regression (PLSR) was used to generate a correlative model. of which metabolites shown in the plots explained 93.90% of the variance in the top two components (Fig. 5A) and were identified by ANOVA (Table S6). Meanwhile, a separation of different fungal species and volatile metabolites was seen along component 1, accounting for 82.0% of the total explained variance and indicating the high covariable relationships between fungal species (especially yeasts) and volatile compounds. In fact, the genera of *Pichia*, *Issatchenkia* and *Kazachstania*, correlated strongly with three ethyl esters (ethyl 9-decenoate, ethyl caprylate, and ethyl caprate) and other esters (isoamyl acetate, ethyl acetate, methyl salicylate, methyl octylate); *Gibberella* and *Mortierella* with octanoic acid and *n*-decanoic acid; and previous genera presented the high connections with linalool and 1-hexanol. VIP (Variable importance for predictive components) value was used to define the key species among the relationships (Huang et al., 2023). In our research, the VIP values of *Buckleyzyma*, *Mycophaerella*, *Epicoccum*, and *Pihcia*, were > 1.0 and regarded as key species during apricot wine fermentation (Fig. 5B). Further, structural equation modelling (SEM) provided a strong tool to disentangle the role of comprehensive microbiota (fungal population, yeast population, and *S. cerevisiae*) on apricot wine metabolites. The SEM explained 37.1% of the variance found in the regional pattern of apricot wine aroma (Fig. 5C). Fungal populations in the apricot juice indirectly drove the aroma profiles by effects on yeast populations (path coefficient = 0.485**), and weakly influenced on *S. cerevisiae* (path coefficient = -0.386**). Yeast populations had the highest direct positive effects on the resulting apricot wine aroma characteristics (path coefficient = 0.350***), while fungal populations had the lowest but significant effects (path coefficient = -0.502**). The prime focus has been the fermentative yeasts (especially *S. cerevisiae*) in past researches about the fermented beverages given its association and significance to fermentation (Li, Jing, Hu, Huang & Xu, 2018; Pu et al., 2023). And *S. cerevisiae* populations were the most important driver of volatile compositions in fermented wine (Liu et al., 2021). Inversely, here we observed that the yeast populations significantly generated a higher association with apricot wine aroma profiles than *S. cerevisiae* species (path coefficient = 0.299**), displayed the most important effects on apricot wine characteristics at this scale (Fig. 5C). Advertently, the fungal species, although the unvouched nonparticipation during apricot wine fermentation, could carry out the synthesis of some

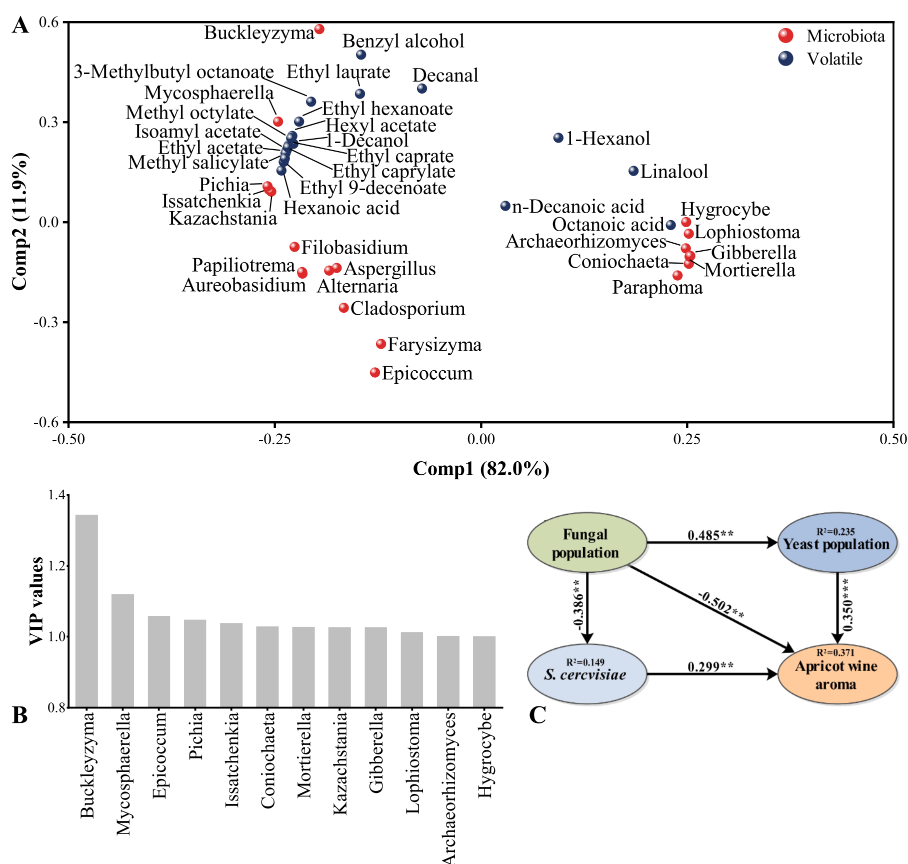

Fig. 5. Analysis of the key microorganism during apricot wine fermentation. (A) Partial least squares regression (PLSR) demonstrates microbial influence on volatile compounds (OAV > 1.0) of apricot wines. (B) VIP values (variable importance for predictive components) of the key microbiota by PLSR modeling. (C) Structural equation model (SEM) fitted to apricot wine aroma composition. Numbers adjacent to arrows are path coefficients and indicative of the effect size of the relationship.

sensory-active compounds associated with fermentative aroma formation (Verginer, Leitner & Berg, 2010), or benefit the crop production via their interactions (Liu, Zhang, Chen & Howell, 2019). The non-*Saccharomyces* of *P. kudriavzevii* populations seems to be the role microbiota for driving the fermentation, but not found in previous studies.

Effect of fermentation by P. kudriavzevii on apricot wine aroma

Several non-*Saccharomyces* yeasts increased in fermentation, but except for this, one of culture-dependent species belonging to *Pichia* simultaneously played an important role in correlativity. Considering the potential of *Pichia* as the starter, involving ethanol tolerance and aroma improvement (Li et al., 2022), the dominant of *Pichia* in the middle and end of fermentation could carry out the fermentation to dryness. Our aim in this section is to confirm the role of this keystone specie in the aroma formation of apricot wine. In this study, the subsequent research is due to the fact that *P. kudriavzevii* LQD20 and LQD5, demonstrates an accelerated fermentation process and exhibits notable resistance to high levels of ethanol than LQD33 (Fig. 6A). It is remarkable that, in all experimental designs, the groups associated with the LQD5 strain were unable to ferment apricot wine to dryness (Fig. 6B). Previous studies did not identify any significant antagonism or competitive relationship between *P. kudriavzevii* and *S. cerevisiae* yeasts (Chagas Junior, Ferreira, de Aguiar Andrade, do Nascimento, de Siqueira & Lopes, 2021; Liu et al., 2023). However, our research indicated that under the presence of *P. kudriavzevii* LQD5, *S. cerevisiae* populations were unable to dominate and consequently depletes all available sugars (Fig. 6B). Subsequently, we opted to employ the naturally fermented Liquan apricot wine with a higher level of acceptance as a control, in order to appraise the impact of *P. kudriavzevii* LQD20 on the apricot wine flavour. Overall, based on volatile profiles and PCA, the samples were clearly separated and the aroma compounds were mainly formed during fermentation with different inoculated treatments (Fig. 6C). The variation in PC1 (55.8%) and PC2 (30.7%) were together represented 86.5 % of the total variability of total samples. The volatile composition in the apricot wine fermented and co-fermented by LQD20 were closely clustered. Given that *P. kudriavzevii* LQD20 was capable of individually completing the fermentation, it could be feasible to consider direct fermentation of apricot wine using non-*Saccharomyces* species, instead of traditionally relying on *S. cerevisiae*. We further analysed 27 volatile compounds with OAVs > 1.0, and observed that apricot wines with the participation of *P. kudriavzevii* LQD20 contained a higher content of aromatic compounds (Fig. 6D), such as phenylethyl alcohol, ethyl acetate, ethyl laurate, ethyl benzoate, isoamyl acetate, and linalool, alongside a lower concentration of "green" compounds, involving decanal and 1-hexanol (Huang et al., 2023). The contents of several ethyl esters have been considerably decreased. However, this reduction has not implied a weakening of the pleasurable flavour, as an excessive concentration of ethyl compounds can also affect the harmony of the aroma in alcoholic beverages (Li et al., 2022; Liu et al., 2023). As sensory analysis indicated, there is a marked presence of unpleasant odours in spontaneous apricot wines (Fig. 6E). Almost all of these attributes were perceived at the lowest responses in apricot wines fermented by CECA, except of "cheese" and "bake" flavour. Notably, the higher intensities of "floral" and "sweet fruity" aroma, and lower olfaction with "green" and "unpleasant" flavour, were in LQD20 apricot wines. The responses of the favorable attributes (i.e., "honey", "fruity", and "floral") in LQD20 apricot wines were comparable to spontaneous apricot wines, with the lowest responses in "cheese" flavour.

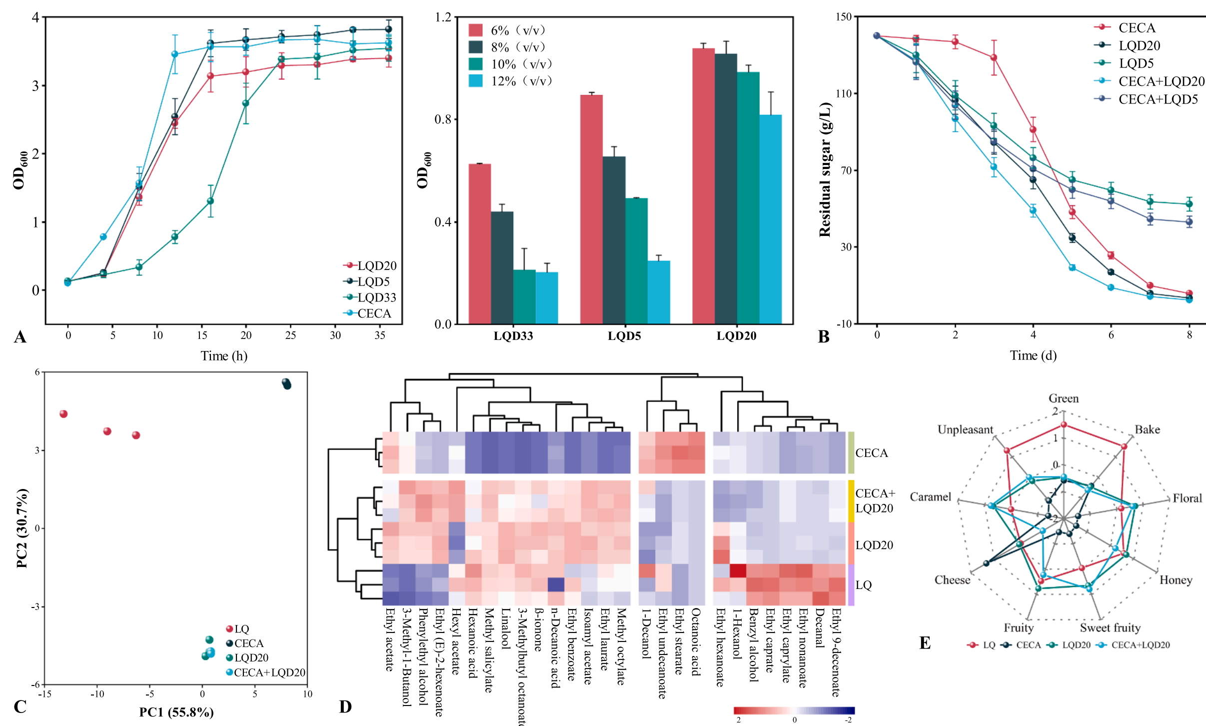

Fig. 6. Effect of *P. kudriavzevii* species on apricot wine fermentation. (A) Selection of *P. kudriavzevii* strains based on growth kinetics and ethanol tolerance in YPD medium. (B) Fermentation kinetics of two *P. kudriavzevii* species with different treatments in apricot wine fermentation. A commercial CECA strain was the control. (C) Principal component analysis of volatile composition in four apricot wines. LQ samples were another control. (D) Heatmap analysis of volatile compounds with OAV > 1.0 among these apricot wines (significant difference, $p < 0.05$). (E) Aroma attributes of apricot wines in different treatments.

Conclusion

Fresh fruits with a short shelf life are of increasing concern in relevant sectors, and the production of an acceptable fermented beverage shows potential to address these issues. Our study describes the production of apricot wine through spontaneous fermentation, and diversity of fungal communities during the fermentation process in carefully selected cultivars (Hongmei apricot) originated from two regions of Shaanxi. We observed the difference of fungal profiles was significant in the fermentative stage and regionality in apricot wine spontaneous fermentation, also indicated that the continually changeable fermentation environment played a significant role in shaping the sub-population diversity of *S. cerevisiae*. Fungal communities were weakly correlated with apricot wine volatile metabolites in the whole, but of which yeast populations (especially *Pichia* species) are the main drivers of apricot wine aroma and sensory characteristics, instead of *S. cerevisiae* populations. This study investigated the aroma regulation of apricot wine through the reinforced inoculation of *P. kudriavzevii*, of which *P. kudriavzevii* LQD20 has exhibited the commendable potential in enhancing olfactory qualities. Additionally, *P. kudriavzevii* LQD5 also showed the strong interaction with *S. cerevisiae*, despite the lack of in-depth research on its underlying mechanism and the impact on aroma. Our studies provided a better understanding of the spontaneous fermentation mechanism of apricot wine, which could be useful for optimizing the production of plant food and beverages, and for the selection of admirable starter of special alcoholic beverage.

Funding

This project was supported by Key Research and Development Project of Ningxia Hui Autonomous Region (2022BBF02015; 2023BCF01025), National Natural Science Foundation of China (32372312), National Natural Science Key Foundation of China (U21A20269), and China Agriculture Research System of MOF and MARA (CARS- 29-jg-03).

CRediT authorship contribution statement

Yu Chen: Formal analysis, Data curation, Investigation, Writing – original draft, Visualization. **Jiali Qi:** Formal analysis, Data curation, Software, Investigation. **Hanyu Yang:** Software, Investigation. **Xingmeng Lei:** Software, Investigation. **Jiao Jiang:** Resources. **Yuyang Song:** Supervision. **Yi Qin:** Writing – review & editing, Supervision, Project administration. **Yan-Lin Liu:** Conceptualization, Writing – review & editing, Supervision, Project administration, Funding acquisition.

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

No data was used for the research described in the article.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2023.100862.

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