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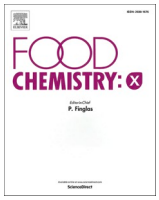

Selection of indigenous *Saccharomyces cerevisiae* strains with good oenological and aroma characteristics for winemaking in Ningxia China

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ABSTRACT

Ningxia is one of the well-known wine producing regions in China. However, the oenological and aroma characteristics of indigenous yeasts remains hidden. The fermentative and oenological properties including stress resistance, hydrogen sulfide, foam production levels; killer phenotype, and flocculation of 89 Ningxia indigenous *Saccharomyces cerevisiae* isolates and ten commercial yeasts were evaluated. The fermentative and oenological properties of the tested strains varied significantly. They could resist 500 g/L glucose, 300 mg/L SO_2, 14% (*v*/v) ethanol and pH 2.8, and produce more esters. They also produce low levels of ethanol and could conduct fermentations vigorously and at a high rate. Cabernet Sauvignon wines made with NXU 21–24 showed the high intensity of tropical fruit, dry fruit, temperate fruit, and spicy flavor. The floral flavor in NXU 21–102 fermented wine is very intense. The indigenous *S. cerevisiae* strains of NXU 21–102 and NXU 21–24 exhibited potential use as starter cultures in wine production.

1. Introduction

Indigenous yeast strains are generally known to be better adapted to their environmental conditions and more easily maintain the typical sensory properties and characteristic profiles of regional wines. In the last 10 years, a lot of effort and attention has been invested in the selection of indigenous yeast species and strains for wine production to defend the specific wine style of a particular wine region (Molinet & Cubillos, 2020; Pretorius, 2000). For example, several important enological properties including stress tolerance, hydrogen sulfide and foam production levels, killer phenotype, and flocculation and fermentation rates of yeast strains isolated from northwest China were evaluated by Feng et al. (2019). In a previous study (Vázquez et al., 2023), autochthonous yeasts from Verdejo grape juice fermentation in Appellation of Origin Rueda were selected, identified, and characterized to exploit the characteristics of the 'terroir'. The findings of these studies have prompted winemakers and wine researchers to use the selected indigenous wine yeast as starter cultures due to their positive effects on wine characteristics (Alves et al., 2015; López-Enríquez, Vila-Crespo, Rodríguez-Nogales, Fernández-Fernandez, & Ruipérez, 2023; Zhang et al., 2022).

Moreover, global warming has led to an increase in the sugar content of grapes, which has resulted in sluggish or stuck fermentations and/or a gradual increase in ethanol content in wines over the past few years (Mozell & Thach, 2014). Moreover, the high ethanol content will affect the flavor characteristics of wine and human health (Hrelia et al., 2023). Therefore, how to effectively reduce the ethanol content in wine has attracted increasing attention. *Saccharomyces cerevisiae* plays a predominant role in winemaking and has an impact on the fermentation process, wine quality, and wine characteristics. To solve the excessive alcohol in wine, it is of great significance to select low-yield ethanol *S. cerevisiae*, and deeply study the regulation mechanism of ethanol metabolism in *S. cerevisiae*. Hence, the selection of indigenous *S. cerevisiae* strains with the potential to produce wines from grapes with high initial sugar content has recently attracted more attention (Noti, Vaudano, Pessione, & Garcia-Moruno, 2015). An important step in the selection of indigenous *S. cerevisiae* strains with excellent enological properties has been done (Feng et al., 2019), and four indigenous *S. cerevisiae* strains were identified as the potential for use as starter cultures in the production of wines from grapes with high initial sugar content in several important wine-producing regions in northwest China. There are limited studies on the use of indigenous *S. cerevisiae* strains in China facing climate change, where the wine industry is growing dramatically.

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Ningxia is one of the oldest and well-known wine producing regions in China. The winegrape growing area in Ningxia, China, has reached 583,000 mu with an annual production of 138 million bottles according to the statistics of People's Daily (2023). The typical geographical and climatic characteristics have facilitated to the formation of rich and diverse yeast communities (Li et al., 2023; Sun et al., 2017). In recent years, several case studies have explored the diversity and oenological properties of indigenous *S. cerevisiae* in Ningxia (Feng et al., 2019; Li, Zhang, Liu, Qin, & Liu, 2019; Liu et al., 2016; Zhang et al., 2022). Although the enological characteristics of a few *S. cerevisiae* strains were investigated, the potential wealth of wine yeast biodiversity was still hidden. Therefore, more efforts and attention must be put into the selection of yeast species and strains, and their role on wine sensory profiles as well as expressing the 'terroir' of a wine producing region.

As a first step to select indigenous *S. cerevisiae* for winemaking in northwest China, Feng et al. (2019) used a schematic exclusion test to evaluate the 52 indigenous *S. cerevisiae* strains isolated from northwest China. Four strains (XJ3, SAX2, SAX5, and SAX6) were considered as the potential starters to produce wines from grapes with high initial sugar content in the Xinjiang and Ningxia regions. However, none of them was Ningxia indigenous *S. cerevisiae*. The present work aimed to evaluate the enological properties of 89 indigenous *S. cerevisiae* strains isolated from Ningxia, China, and to establish a strain collection for use as starter cultures during wine production with regional characteristics. Firstly, this study evaluated the phylogenetic diversity, fermentative, and oenological properties of these indigenous *S. cerevisiae* and commercial strains. Based on the results of stress resistance (ethanol, SO_2, sugar, and acid), killer phenotype, hydrogen sulfide, foam production levels, and flocculation, strains with good fermentative and oenological characteristics were selected for further studies. Finally, the chemical composition and sensory analysis of wines fermented by the selected strains were evaluated.

2. Materials and methods

2.1. Yeast strains

The 89 indigenous *S. cerevisiae* isolates used in this study were isolated from spontaneously fermented must of the main grape varieties (Merlot and Cabernet Sauvignon) grown in Ningxia. The spontaneous fermentations of grape must were conducted at 25 to 28 °C for 8 to 12 days until dry. Fermentations were sampled at the early, middle, and end stages of fermentation. Serial 10-fold dilutions (from 10^{-1} to 10^{-6}) were inoculated onto Wallerstein Laboratory Nutrient medium (Hopebiol, China) and incubated for five days at 28 °C. These yeasts were classified and differentiated by colony morphology and color. The *S. cerevisiae* isolates were confirmed by classical biochemical test (culture on 1ysine medium) and PCR-RFLP of the 5.8S-ITS rDNA using restriction enzymes *Hae* III and *Hinf* I (TaKaRa Biotechnology (Dalian) Co. Ltd.) as described by Sun and Liu (2014). This set of *S. cerevisiae* isolates belongs to the laboratory of Wine Microbial Resources and Breeding at Ningxia University. UCDVEN 522 (Montrachet) was from the Wine Yeast and Bacteria Collection of the Department of Viticulture and Enology at the University of California, Davis. This strain was previously identified as killer-sensitive strain. Ten commercial *S. cerevisiae* were used as control strains. They were FX10 (LAFFORT); BDX, BXQA23, BM4*4, L2323 (Lallemand); DS, TXL, XR, FR, LABGL (LAMOTHE). This set of commercial yeasts is commonly used in Ningxia, China. All of the yeast isolates were maintained at −80 °C after the addition of glycerol at 20% (*v*/v) final concentration. They were subcultured at 1-month intervals on YPD agar (glucose, 20 g/L, peptone, 20 g/L, yeast extract, 10 g/L, agar 20 g/L) and maintained at 4 °C.

2.2. Evaluation of enological characteristics

All enological characteristics were carried out in triplicate.

Screening of low ethanol yield yeast strains. The selection of low ethanol yield strains was conducted according to 2,3,5-Triphenyl Tetrazolium Chloride (TTC) agar described previously (Tanaka, Kiyoshi, Kadokura, Suzuki, & Nakayama, 2021). Yeast suspensions were inoculated into TTC substratum medium (YPD agar) at 28 °C for 2 to 4 days. Then, the TTC upper medium (glucose 0.5 g/L, agar 15.0 G/L, TTC 0.5 g) was poured and incubated at 28 °C for 6 h. Darkness or lightness in colonies color were considered as high or low alcohol production capacity, respectively.

H_2S production. Bismuth Glucose Glycine Yeast (BIGGY) agar was used to evaluate the H_2S production of the strains. Overnight culture was prepared and spotted on BIGGY agar. Plates were incubated at 28 °C for five days. The H_2S production of the yeast strains was evaluated based on their colony color. The color of colonies was identified according to the following six scales: 1 (white), 2 (cream), 3 (light brown), 4 (brown), 5 (dark brown), and 6 (black) described previously (Li et al., 2019). Brown and black colonies were considered as H_2S producers.

Ester production. *Saccharomyces cerevisiae* strains were inoculated on an ester-producing medium and kept at 28 °C for 72 h according to a previous study (Pu, Hu, Jia, & Yan, 2013). According to their colony color on the ester-producing medium, ester producing capability was identified as follows: dark yellow (low ester), cream yellow (medium ester), and light yellow (high ester).

Foam production. *Saccharomyces cerevisiae* strains were inoculated in test tubes containing 10 mL YPD liquid medium and cultured at 28 °C for 24 h. Foam height was measured every 4 h. Yeast strains were classified into three scales according to the maximum foam height: F0 (< 2 mm), F1 (2 mm to 4 mm), and F2 (> 4 mm). (Feng et al., 2019).

Flocculation evaluation. The flocculation property was determined by spectrophotometry following Bony, Barre, and Blondin (1998) with some modifications. Yeasts were inoculated into YPD medium and incubated at 30 °C until the cultures reached the logarithmic phase of growth. Yeast cells were collected and deflocculated by two washes in Na-citrate (50 mmol/L, pH 3.0) and EDTA buffer (5 mmol/L). Then yeast cells were suspended in Na-citrate, calcium chloride and EDTA buffer. Cell suspension was collected in a 100 mL test tube for 30 min, and was pipetted 350 μL from the bottom of the concave liquid surface, followed by the optical density measurement at 600 nm. The flocculation degree was determined as the ratio between the OD600 of the culture suspension before and after shaking the bottle (ODA/ODB × 100). The flocculation levels were identified as follows: no flocculence (ratio > 90%), low flocculence (ratio between 70% and 90%), medium flocculence (ratio between 30% and 70%), high flocculence (ratio < 30%).

Killer activity. Firstly, the sensitive strain *S. cerevisiae* UCD522 was plated onto YPD-MB agar at 10^7 cells/mL. The cell cultures of tested strains, which were activated for 24 h in an incubator at 28 °C, were then spotted on a plate and incubated at 28 °C for 48 h. The killer activity of the tested strains was judged according to their inhibition zone (Petering, Symons, Langridge, & Henschke, 1991).

2.3. Stress tolerance

Ethanol, SO_2, sugar, and acid tolerance tests were performed in this study. The capabilities of the strains to grow at different concentrations of ethanol (10, 12, 14, and 16% [*v*/v]), SO_2 (60, 120, 180, 240, and 300 mg/L), glucose (100, 150, 200, 250 and 300 g/L), and pH 2.8, 3.2, 3.6, 4.0, respectively, was observed. Seed inoculum was inoculated into YPD and incubated at 28 °C for 48 h. The optical density was measured at 600 nm by Multiskan FC (Thermo Scientific) (Origone et al., 2017). All tolerance tests were carried out in triplicate.

2.4. Performance in micro-vinification with selected strains

Fermentations were performed in 500 mL Erlenmeyer flasks filled with 350 mL modified synthetic grape juice Triple M medium

(Spiropoulos, Tanaka, Flerianos, & Bisson, 2000), which contained 10 mg/L ergosterol, 1 mL/L Tween 80, 110 g/L D-fructose and D-glucose, 6 g/L L(+) tartaric acid, 3 g/L malic acid, 0.5 g/L citric acid，1.7 g/L YNB (without amino acids and ammonium sulfate), 1.0 g/L Vitamin-free Casamino acids, 6.0 mg/L myo-inositol, 0.2 g/L anhydrous $CaCl_2$, 0.107 g/L L-arginine*HCl, 1.0 g/L L-proline, 0.1 g/L L-tryptophan and 0.015 g/L Ammonium phosphate, at pH 3.25 The Cabernet Sauvignon grapes were harvested on 23rd Sep 2023 in Yinchuan, Ningxia, China. Mature and healthy grapes were destemmed, crushed with 40 mg/L potassium metabisulfite and 30 mg/L pectinase after crushing, and then kept at 5 °C for 3 days in a 10,000 L stainless fermenter. After the cold maceration, 7.5 L of grape juice was taken and transferred to the laboratory in a sterile and cooler environment. Prior to inoculation, the grape juice was treated for 10 min at 110 °C. Then, fermentations were performed in 1.0 L Erlenmeyer flasks filled with 750 mL Cabernet Sauvignon grape juice (240.47 ± 1.10 g/L sugar, 3.83 ± 0.06 g/L total acidity expressed as tartaric acid, 103.78 ± 1.15 mg N/L yeast assimilable nitrogen, and pH 4.01 ± 0.00) in triplicate.

Triple M medium or Cabernet Sauvignon grape juice was inoculated with 24–48 h pre-cultures to an initial inoculation level of 10^6 cells/mL. All fermentations were carried out at 25 °C and monitored by measuring the weight loss of CO_2 every 24 h. Fermentation was carried out in triplicate, and was considered to be finished at constant weight for two consecutive days. At the end of each fermentation, total acidity, residual sugar, volatile acidity, alcohol content, and pH were determined according to the National Standard of the People's Republic of China (GB/T, 15038–2006).

2.5. Profiling of volatile compounds

Firstly, the wines fermented from Cabernet Sauvignon grape juice were mixed for the same strain. Then the volatile compound evaluation was conducted in duplicate. The volatile compounds of the wines were extracted by headspace solid-phase microextraction (HS-SPME) and analyzed using gas chromatography–mass spectrometry (GC–MS) following the method described previously (Zhang et al., 2022). In a nutshell, 5.0 mL wine samples were added into a 20 mL glass vial containing 1.5 g NaCl (analytical standard, Guangnuo Chemical Technology, Shanghai, China) and 10 μL internal standards (4-methyl-2-pentanol, ≥ 98.0%, Tokyo Chemical Industry, Tokyo, Japan, 1008.3 mg/L), and equilibrated at 40 °C for 30 min. A 50/30 μm DVB/CAR/PDMS SPME fiber (J&W Scientific, Folsom, CA, USA) was immersed in the headspace for 30 min to extract volatiles, and subsequently desorbed at 250 °C in the GC injector for 8 min. The analyses were performed using Agilent 7890B GC system and Agilent 7000D MS. The temperature procedure is as follows: GC oven was maintained at 50 °C for 1 min, then raised to 220 °C at a rate of 3 °C/min and held for 5 min. Other temperature parameters are 250 °C for the mass spectrometer interface, and 230 °C for the ion source and 150 °C for the quadrupole, respectively. The identification of VOCs was completed by matching the obtained mass spectra to the NIST 17 standard library and comparing the retention indices (RIs), calculated from the C8-C40 alkane (analytical standard, Sigma-Aldrich, Shanghai, China, GC) retention time, with the RIs reported in the NIST Chemistry Web Book (https://webbook.nist.gov/).

2.6. Sensory evaluation

As for sensory analysis, wine sensory analysis was evaluated by a tasting panel of 15 students from the College of Enology and Horticulture at Ningxia University in China. Informed consent was obtained prior to their participation in the study. The tasting room, glasses, steps, and description of aroma characteristics were conducted according to (Zhang, Wang, Zhang, & Sun, 2023). This study does not involve any studies with human or animal subject, and ethical permission was not required for this study.

2.7. SSR analysis for the S. cerevisiae

DNA was extracted from overnight cultures grown in YPD medium using the procedure described by a previous study (Zhou, Sheng, Rao, Wang, & Zhuge, 2004). The genotypes of *S. cerevisiae* isolates were determined using simple sequence repeats (SSR) conducted on the microsatellite sites of SCAAT1, YPL009C, C4, C5, C11, SCAAT3, and SCY0R267c (Jubany et al., 2008; Legras, Ruh, Merdinoglu, & Karst, 2005; Stefanini et al., 2017). The amplifications were performed in a 25 μL reaction volume and touchdown-PCR was performed to amplify the locus according to a previous study (Zhang et al., 2022). After checking on 2% agarose gel of 5 μL sample of the PCR products, the fluorescent fragments were separated using the capillary electrophoresis method of Sangon Biotech Co., Ltd. (Shanghai, China) and measured using an ABI-3730xl Genetic Analyzer (Applied Biosystems, Forster City, CA, USA) with the internal size standard LIZ 500 (GeneScan) to detect PCR products. The lengths of the PCR products and the SSR profiles were analyzed using the GeneMapper 5.0 software (ABI, USA).

Then, the SSR patterns obtained were used for the construction of a presence/absence matrix, taking into account the total number of different bands observed at each locus. Each position was then assigned a “0” or a “1” to indicate the absence or presence of the band, respectively. The 0/1 matrix was then used to generate the dendrograms. Similarities based on the Dice coefficient were calculated, and an unweighted pair group method with arithmetic mean (UPGMA) clustering was obtained using NTSYSpc software (Mercado, Jubany, Gaggero, Masuelli, & Combina, 2010).

2.8. Data analysis

One-way analysis of variance (ANOVA) was performed to identify the differences in wine aroma across treatments with Duncan's test (SPSS 25.0). Principal component analysis (PCA) biplot and heatmap were visualized using Origin 2021 software (OriginLab Corporation, Northampton, MA, USA).

3. Results and discussion

The use of indigenous yeasts for wine production is an advisable choice to defend the ‘terroir’ of a particular wine region. The selection of appropriate indigenous yeasts ensures the maintenance of oenological characteristics while avoiding the risks of stuck or sluggish fermentations. In addition, screening for specific yeast strains with lower alcohol yield or/and higher acidity content is essential in the adaption of winemaking practices to climate change conditions. Therefore, the phylogenetic diversity, fermentative, and oenological properties of 89 indigenous *S. cerevisiae* isolates isolated from Ningxia and ten commercial strains were investigated in this study.

3.1. Selection of S. cerevisiae strains of low alcohol production

To select strains with low alcohol production, we first used TTC agar to quantify their potential production of alcohol. 99 isolates were separated into four levels (I – IV) according to the colony color (Fig. 1A), with 1, 46, 40, and 12 isolates of type I, II, III, and IV, respectively (Table S1). Isolates with light pink color and pink color at the periphery accounted for 87% (86/99) of isolates, whereas isolates with white and pink color accounted for 13% (13/99) of all isolates. Specially, 1 indigenous *S. cerevisiae* isolate of NXU21–102 showed a white colony color on TTC, and 43 indigenous and three commercial yeasts (BM4*4, BDX, FR) showed light pink color, which means they may produce a lower amount of ethanol.

Understanding the genetic diversity of yeast strains could make an important contribution to providing genetic material for further strain development (Li et al., 2023). Moreover, it is very common to use active dry yeast for wine making in China including wine-producing region of

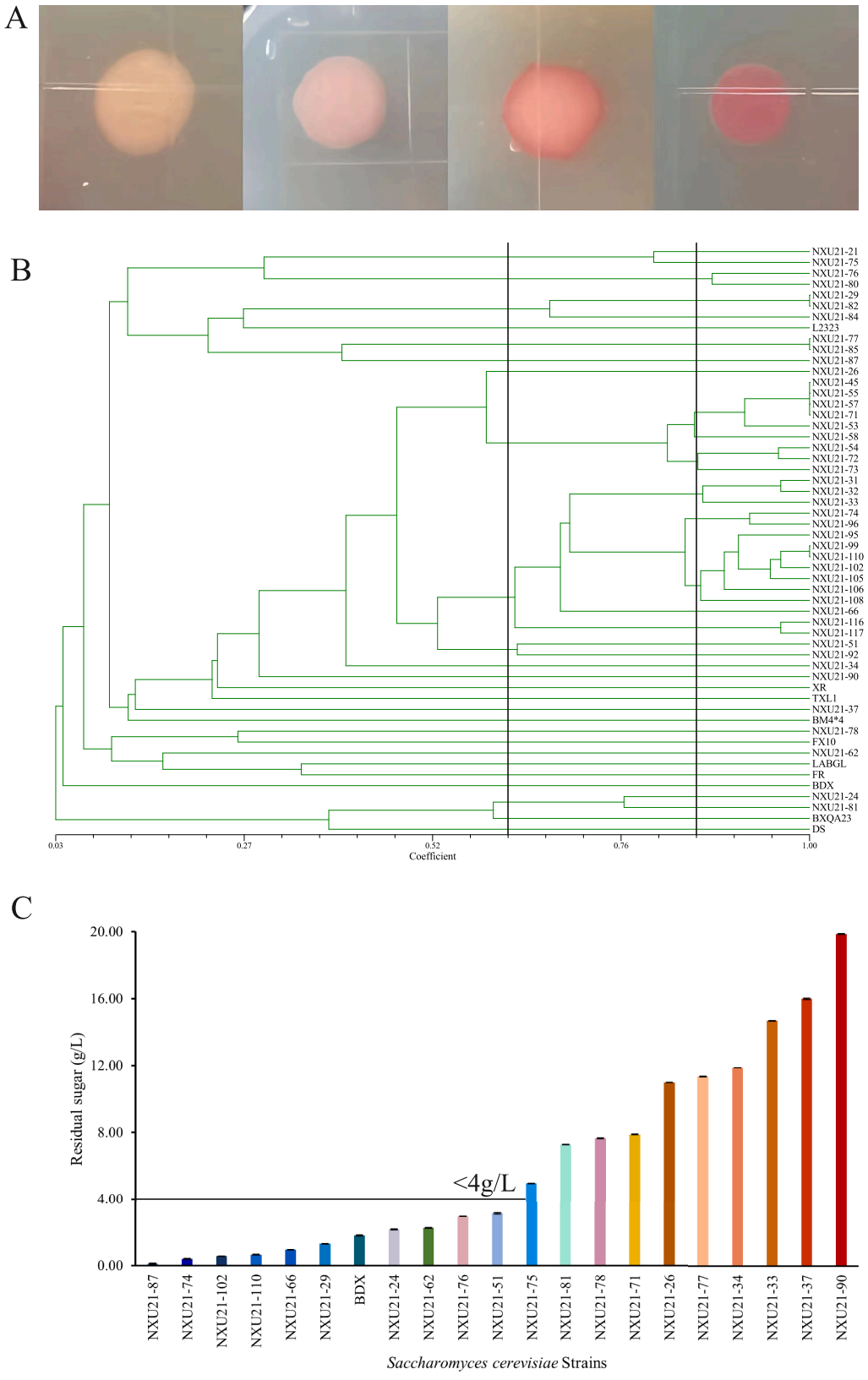

Fig. 1. (A) Colony colors on TTC agar. Ethanol production of *S. cerevisiae* strains were identified as low (white, light pink), medium (light pink, pink at the periphery), and high (pink). The deeper the color, the higher the ethanol yield; (B) Unweighted pair group method with arithmetic mean dendrogram showing genetic relatedness of the 54 isolates. Different groupings of strains were evident with a dice coefficient of 0.616 and 0.856. The bold black line represents the dice coefficient of 0.616 and 0.856, respectively; (C) The content of residual sugar of the tested 21 *S. cerevisiae* strains using triple M. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Ningxia. Therefore, SSR analysis and UPGMA clustering were used to analyze the genetic diversity of 44 indigenous *S. cerevisiae* isolates with low production of ethanol and ten commercial *S. cerevisiae* strains in this study. The database of genotypes is shown in Table S2. Similarities based on the Dice coefficient and UPGMA clustering of SSR profiles are presented in Fig. 1B.

The dendrogram indicated that SSR technique discriminated the 54 isolates into 48 profiles or strains (Fig. 1B). Different groupings of isolates were evident with a Dice coefficient of 0.616 and 0.856, respectively. Four clusters of NXU 21–29 and NXU 21–82, NXU 21–77 and NXU 21–85, NXU 21–45, NXU 21–55, NXU 21–57 and NXU 21–71, and NXU 21–99 and NXU 21–110 showed highly conserved SSR sequence patterns, indicating that they are likely genetically distinct derivatives of the same strain. Moreover, none of the SSR profiles of these indigenous strains were identical to those of the ten commonly used commercial yeasts in Ningxia, China, which could have been attributed to the utilization of these commercial yeasts by the local wineries for wine production in recent years. The cluster of commercial yeast BDX had a low degree of similarity with other Chinese indigenous genotypes and other commercial strains. The different *S. cerevisiae* isolates showed the same genotype, which might originate from the same colony; therefore, only one isolate was selected for the following study. The isolates that showed a clear separation were of special interest in our study. Therefore, 20 indigenous *S. cerevisiae* strains of NXU 21–24, NXU 21–26, NXU 21–29, NXU 21–33, NXU 21–34, NXU 21–37, NXU 21–51, NXU 21–62, NXU 21–66, NXU 21–71, NXU 21–74 – NXU 21–78, NXU 21–81, NXU 21–87, NXU 21–90, NXU 21–102, NXU 21–110, and commercial yeast BDX were selected to conduct fermentation using Triple M.

At the end of fermentation, the basic physicochemistry of wines was measured (Table S3). One objective of this study was to produce dry wine with indigenous *S. cerevisiae*. According to the National Standard of the People's Republic of China (GB/T, 15037–2006), the content of residual sugar in dry wine should not exceed 4.0 g/L. According to their physicochemical indexes of Triple M, 11 indigenous *S. cerevisiae* of NXU 21–24, NXU 21–29, NXU 21–51, NXU 21–62, NXU 21–66, NXU 21–74, NXU 21–75, NXU 21–76, NXU 21–87, NXU 21–102, NXU 21–110 and commercial BDX could complete the fermentation and showed acceptable ranges of residual sugar (Fig. 1C). In addition, the concentration of volatile acid for all selected strains showed acceptable ranges. Therefore, these 12 *S. cerevisiae* strains were used for the evaluation of enological characteristics in the following study. The selection strategy in phenotypic studies was conducted based on genotypes to reduce labor and increase efficiency. A previous study evaluated indigenous *S. cerevisiae* strains for winemaking in Northwest China using a schematic exclusion test (Feng et al., 2019). However, the suitable selection strategy always depends on the characteristics that a wine strain is supposed to hold and the number of strains to be screened (Schuller & Casal, 2005).

3.2. Evaluation of enological characteristics of different S. cerevisiae strains

Numerous oenological characteristics were proposed for the evaluation of indigenous *S. cerevisiae* strains for winemaking in Ningxia, China in our selection strategy. As summarized by a previous study (Schuller & Casal, 2005), technologically relevant data could be obtained by monitoring the fermentation progress, and quantitative characteristics were obtained by analyzing the chemical traits at the end of fermentation. The selection of excellent strains with perfect data on all oenological characteristics is impossible. Sometimes, winemakers and wine researchers tend to get strains with good oenological characteristics according to their needs. In this study, the enological characteristics of 11 indigenous *S. cerevisiae* and BDX (control) are summarized in Table 1. All indigenous strains were killer positive and classified as low foam production (< 2 mm foam production), and resisted 300 mg/L SO_2, which showed acceptable results for all strains. Interestingly, although the fact that Feng et al. (2019) noticed the growth of *S. cerevisiae* strains in the presence of 250 mg/L SO_2, all of our tested strains were capable of growing when cultivated in the presence of 300 mg/L SO_2. The ethanol tolerance and osmotic tolerance were of our priority in selecting these *S. cerevisiae* strains. NXU 21–24, NXU 21–76, NXU 21–102, NXU 21–110, and BDX could resist 14% (*v*/v) ethanol and 500 g/L glucose, which meet the goals of our needs. Similar to a previous study, Suranská, Vránová, and Omelková (2016) noticed the growth of *S. cerevisiae* strains in the presence of 50% glucose and six of our strains were capable of growing when cultivated under the same concentration of glucose. As for the resistance to ethanol, the *S. cerevisiae* strains in our study showed resistance to a lower ethanol content (14%) compared to 16% in previous studies (Feng et al., 2019; Suranská et al., 2016).

As regards the resistance to pH, strains of NXU 21–24, NXU 21–75, NXU 21–102, and BDX could grow under pH 2.8 (Table 1). The results presented here indicate that the indigenous *S. cerevisiae* strains NXU 21–102 and NXU 21–24 are inferior to that of the control commercial strain BDX. In summary, they could resist 500 g/L sugar, 300 mg/L SO_2, 14% (v/v) ethanol, and pH 2.8. Considering the results of stress resistance of these strains, NXU 21–102, NXU 21–24, and BDX (control strain) were selected as starter cultures for further grape must fermentation.

3.3. Physicochemical parameters of cabernet sauvignon wines

The fermentations were carried out in 750 mL Cabernet Sauvignon grape juice inoculated with NXU 21–102, NXU 21–24, or the control strain (BDX). All physicochemical parameters of the resulting wines were within acceptable ranges according to the National Standard of the People's Republic of China (GB/T, 15038–2006). All strains completed the fermentations within 12 days (<4.0 g/L of sugar) (Fig. 2A). As shown in Table S4, no significant difference in the volatile acid was

Table 1
The enological characteristics of the 11 indigenous *S. cerevisiae* 1 comercial strains in YPD medium.

Yeast strains	Killer phenotype	Foaming	Flocculation	H_2S producting	Ester producting	Ethanol tolerance (%, *v*/v)	SO_2 tolerance (mg/L)	Osmotic tolerance (g/L)	pH tolerance
BDX	+	low	high	low	high	14	300	500	2.8
NXU 21–24	+	low	high	high	high	14	300	500	2.8
NXU 21–29	+	low	medium	high	high	< 10	300	300	4.0
NXU 21–51	+	low	medium	low	low	< 10	300	< 200	4.0
NXU 21–62	+	low	medium	high	high	10	300	300	4.0
NXU 21–66	+	low	medium	high	high	< 10	300	300	4.0
NXU 21–74	+	low	medium	high	high	< 10	300	< 200	4.0
NXU 21–75	+	low	medium	high	high	10	300	500	2.8
NXU 21–76	+	low	medium	low	low	14	300	500	3.6
NXU 21–87	+	low	medium	high	high	< 10	300	< 200	4.0
NXU 21–102	+	low	high	high	high	14	300	500	2.8
NXU 21–110	+	low	medium	medium	medium	14	300	500	3.6

+: Positive killer activity.

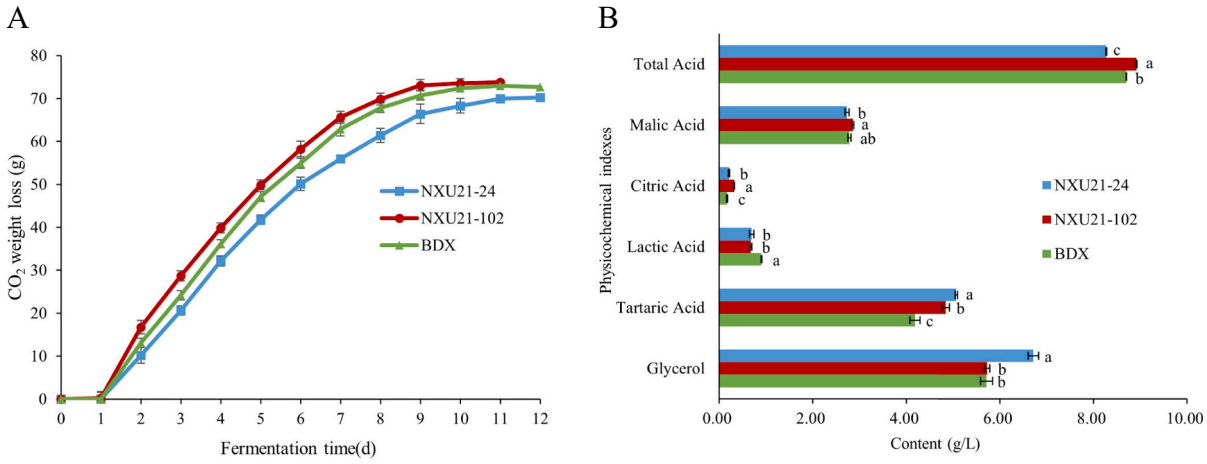

Fig. 2. (A) The CO_2 weight loss of NXU 21–102, NXU 21–24, and BDX (control strain) using 750 mL of Cabernet Sauvignon grape juice; (B) Physicochemical indexes of Cabernet Sauvignon wines. The values with different letters of a, b, ab, and c in figures represent the differ statistically according to Tukey's test ($P < 0.05$).

observed in the wines, while significant differences in the content of residual sugars, total acids, alcohol, citric acid, and tartaric acid were found between the three fermentation treatments. The lowest ethanol content of the fermented wine was 12.93 ± 0.06 conducted by NXU 21–102. The findings differed from the observations of Liu et al. (2016), in which higher ethanol content (13.52 ± 0.11%–14.12 ± 0.09%) was found under an initial sugar content of 244.6 ± 0.7 g/L. This study firstly used TTC agar to quantify the potential production of alcohol for different *S. cerevisiae* strains, and the results of this study showed that it is an efficient step to select strains with low alcohol production. In this study, the highest content of total acid, malic acid and citric acid were also found in NXU 21–102 wines, which have the potential to produce wines with higher acidity (Fig. 2B). Moreover, NXU 21–102 produced medium amount of glycerol in Cabernet Sauvignon wines. In summary, NXU 21–102 produced ethanol 0.5–1.5% lower than other strains, and could conduct fermentations vigorously and at a high rate. Therefore, adaptive laboratory evolution and fermentation performance of NXU 21–102 and NXU 21–24 are now underway to help further select ideal indigenous strains as starters.

Aroma is one of the most important factors applied to evaluate wine quality, and differentiated volatile profiles are an important indicator in a yeast selection program. Therefore, a qualitative and quantitative analysis of the volatile compounds in the Cabernet Sauvignon wines resulting from NXU 21–102, NXU 21–24, and the control strain (BDX) was performed using HS-SPME-GC–MS. The content, threshold values, and aroma characteristics of the aroma components are listed in Table 2. A total of 33 volatile components were detected in this study, including 18 esters, 10 alcohols, 3 acids, and 2 other compounds. The total odorant concentrations of esters were significantly different among these three strains, while those of alcohols and acids were not. The total acid content of NXU 21–102 (2237.48 ± 474.44 μg/L) was significantly lower than that of NXU 21–24 (2934.83 ± 206.25 μg/L) and BDX (2924.98 ± 347.47 μg/L). The total content of esters in NXU 21–24 (103,677.85 ± 5109.70 μg/L) was significantly higher than that in NXU 21–102 (91,767.12 ± 2010.37 μg/L) and BDX (79,695.33 ± 4346.25 μg/L). Clustering analysis was performed to search for the discriminative aromas that contributed to the distinction among the wines obtained from different strains (Fig. 3). The clustering analysis shows that the wine samples fermented from the same strain cluster together. Compared with BDX, the contents of the volatile compounds in NXU 21–102 and NXU 21–24 are more similar.

Thirteen volatile compounds in both NXU 21–102 and NXU 21–24 were detected differing significantly from the control yeast BDX in terms of content (Table 2). For strain NXU 21–102, three alcohols (3-methyl-1-butanol, 1-butanol, 1-propanol) and eight esters (isoamyl acetate, ethyl lactate, ethyl hexanoate, hexyl acetate, isopentyl hexanoate, ethyl nonanoate, ethyl caprate, and ethyl laurate) were significantly higher than those in the BDX inoculation wines, while 2,3-butanediol and decanoic acid were significantly lower. For strain NXU 21–24, two alcohols (isobutanol and 2,3-butanediol) and three esters (isoamyl acetate, hexyl acetate, and diethyl succinate) were significantly lower than those in the BDX inoculation wines, while eight esters (ethyl hexanoate, ethyl caprylate, isopentyl hexanoate, ethyl nonanoate, ethyl caprate, isoamyl octanoate, ethyl laurate and ethyl hexadecanoate) were significantly higher. Consequently, the content of ethyl hexanoate, isopentyl hexanoate, ethyl nonanoate, ethyl caprate and ethyl laurate in NXU 21–102 and NXU 21–24 were significantly higher than those in the BDX inoculation wines, while 2,3-butanediol was significantly lower. This difference indicates that those compounds could be considered characteristics of indigenous yeast strains.

3.4. Odorous evaluation of cabernet sauvignon wines

The odor activity value (OAV = mass concentration/threshold) of volatile compounds is used to measure their contribution to wine aroma. It has been suggested that aroma compounds with an OAV > 0.1 could be used as potential compounds affecting aroma in wines, which in turn positively affect wine aroma (Ye, Wang, Zhan, Tian, & Liu, 2022). Firstly, principal component analysis (PCA) was conducted using the OAVs >0.1 to detect the main variations and reveal the different aromas of wines fermented by NXU 21–102, NXU 21–24, and BDX (control strain). Fig. 4A shows the scores and loadings of the first two principal components. Supplementary Table S5 and Table S6 showed the component score coefficient matrix and eigenvalues of the principal components, respectively. PCA1 and PCA2 in Fig. 4A explain 47.7% and 30.4% of the total variability of the wines, respectively. PCA showed that none of the wines fermented by the three *S. cerevisiae* strains overlapped, reflecting the significant impact of yeast strains on the aroma of the wine.

Of the OAVs of the 8 volatile compounds clustered near NXU 21–24, four of them, including ethyl caprylate, ethyl caprate, isoamyl octanoate, and ethyl laurate, were larger than 1 (could be recognized by human beings), significantly higher than the wines fermented by BDX. According to the previous study on the odor of volatile compounds shown in Table 2, these volatile compounds could introduce floral, fruity, and cream flavors to wine (Legras et al., 2005; Liu et al., 2015). 8 volatile compounds were clustered near NXU 21–102. The OAVs of isoamyl acetate and ethyl hexanoate were larger than 1, and significantly higher in the wines fermented by NXU 21–102. An odor of anise and several fruity aromas such as banana, green apple, and strawberry

Table 2
Qualitative and quantitative analysis of aroma composition in Cabernet Sauvignon wines from NXU 21–102, NXU 21–24 and BDX.

Volatile compounds		Concentration(μg/L, mean ± SD)			Threshold (μg/L)	OAV			Odor description	Reference
		NXU 21–24	NXU 21–102	BDX		NXU 21–24	NXU 21–102	BDX		
Alcohols	Phenethyl alcohol	32,564.94 ± 738.74^{a}	32,417.55 ± 1281.11^{a}	29,252.02 ± 1210.33^{a}	1400	> 1			Floral, rose	(Hu, Jin, Mei, Li, & Tao, 2018)
	Isobutanol	625.65 ± 129.55^{b}	1155.34 ± 9.86^{a}	1382.64 ± 88.05^{a}	40,000	< 0.1			Mild sweet, alcohol	(Noguerol-Pato, Gonzalez-Barreiro, Cancho-Grande, & Simal-Gándara, 2009)
	3-Methyl-1-butanol	26,968.52 ± 1031.36^{b}	31,884.16 ± 884.54^{a}	28,161.16 ± 1454.07^{b}	30,000	> 0.1			Alcohol, bitter, nail polish	(Noguerol-Pato et al., 2009)
	1-Hexanol	2984.77 ± 35.63^{a}	3167.78 ± 262.98^{a}	2716.56 ± 141.24^{a}	8000	> 0.1			Flower, green, cut grass	(Tristezza et al., 2016)
	2,3-Butanediol	941.57 ± 105.34^{b}	878.81 ± 15.30^{b}	1724.19 ± 196.58^{a}	120,000	< 0.1			Rubbery, creamy, fruity	(Tao & Zhang, 2010)
	1-Octanol	7.86 ± 0.57^{a}	10.58 ± 3.01^{a}	7.02 ± 0.60^{a}	900	< 0.1			Intense citrus, rose	(Hu et al., 2018)
	2-Heptanol	4.15 ± 0.62^{a}	5.23 ± 0.83^{a}	5.53 ± 0.81^{a}	200–300	< 0.1			Soil, mushroom	(Hu et al., 2018)
	1-Butanol	1071.14 ± 6.92^{ab}	1339.38 ± 217.15^{a}	859.31 ± 68.58^{b}	150,000	< 0.1			Medicinal, alcohol	(Wang, Tao, Wu, An, & Yue, 2017)
	1-Heptanol	14.67 ± 4.7^{a}	11.90 ± 1.79^{a}	6.81 ± 0.65^{a}	1000	< 0.1			Green, sweet	(Wang et al., 2017)
	1-Propanol	377.28 ± 31.2^{b}	642.76 ± 66.08^{a}	252.95 ± 2.64^{b}	50,000	< 0.1			Fresh alcohol	(Wang et al., 2017)
	Total alcohol content	65,560.53 ± 2084.66^{a}	71,513.49 ± 2742.66^{a}	64,368.18 ± 3163.54^{a}						
Esters	Ethyl acetate	3313.72 ± 580.83^{a}	4296.72 ± 89.98^{a}	3889.70 ± 305.48^{a}	7500	> 0.1			Fruity , sweet	(Wang et al., 2017)
	Ethyl butyrate	307.32 ± 32.30^{a}	273.66 ± 29.24^{a}	339.61 ± 26.93^{a}	20	> 1			Sour fruit, strawberry, fruity	(Wang et al., 2017)
	Isoamyl acetate	6024.66 ± 119.58^{c}	9914.56 ± 228.86^{a}	8562.11 ± 497.24^{b}	30	> 1			Banana	(Hu et al., 2018)
	Ethyl lactate	1754.62 ± 284.11^{ab}	2088.68 ± 44.30^{a}	1492.62 ± 33.18^{b}	154,636	< 0.1			Lactic, raspberry	(Wang et al., 2017)
	Ethyl hexanoate	13,545.68 ± 526.09^{a}	13,755.38 ± 486.94^{a}	11,751.38 ± 651.17^{b}	14	> 1			Green apple, fruity, strawberry, anise	(Wang et al., 2017)
	Hexyl acetate	669.15 ± 23.15^{c}	990.23 ± 10.24^{a}	808.93 ± 40.07^{b}	1500	> 0.1			Fruity,pear	(Wang et al., 2017)
	Ethyl caprylate	47,408.70 ± 1663.29^{a}	37,074.32 ± 669.6^{b}	34,485.88 ± 1626.28^{b}	5	> 1			Pineapple, pear, floral	(Wang et al., 2017)
	Isopentyl hexanoate	873.78 ± 0.92^{a}	757.07 ± 4.53^{b}	663.93 ± 34.69^{c}	–	–			–	–
	Diethyl succinate	549.92 ± 73.17^{b}	740.96 ± 135.05^{ab}	882.54 ± 24.96^{a}	6000	<0.1	>0.1	>0.1	Light fruity, wine	(Wang et al., 2017)
	Ethyl nonanoate	937.16 ± 34.99^{a}	826.79 ± 10.03^{a}	593.60 ± 56.67^{b}	1300	> 0.1			Waxy, fruity	(Hu et al., 2018)
	Ethy heptanoate	0.76 ± 0.12^{a}	0.69 ± 0.08^{a}	0.67 ± 0.22^{a}	220	< 0.1			Pineapple, fruity	(Hu et al., 2018)
	Ethyl caprate	22,315.79 ± 1346.35^{a}	16,271.73 ± 36.03^{b}	12,831.38 ± 825.74^{c}	200	> 1			Fruity, fatty, pleasant	(Hu et al., 2018)
	Isoamyl octanoate	1104.12 ± 66.15^{a}	720.88 ± 12.26^{b}	595.85 ± 41.84^{b}	125	> 1			Fruity,cream, chess	(Wang et al., 2017)
	Phenethyl acetate	578.53 ± 32.85^{a}	678.13 ± 120.33^{a}	456.60 ± 84.41^{a}	250	> 1			Honey,sweet	(Wang et al., 2017)
	Ethyl laurate	4057.81 ± 281.32^{a}	3103.71 ± 102.11^{b}	2117.76 ± 83.62^{c}	1500	> 1			Sweet. floral, fruity, cream	(Wang et al., 2017)
	Ethyl tetradecanoate	19.42 ± 2.53^{a}	20.29 ± 1.05^{a}	17.15 ± 1.44^{a}	2000	< 0.1			Mild waxy, soapy	(Wang et al., 2017)
	Butanedioic acid,1-ethyl ester	194.36 ± 38.37^{a}	234.94 ± 25.93^{a}	194.81 ± 11.06^{a}	–	–			–	–
	Ethyl hexadecanoate	22.36 ± 3.58^{a}	18.38 ± 3.76^{ab}	10.80 ± 1.25^{b}	1500	< 0.1			Fatty, rancid, fruity, sweet	(Wang et al., 2017)
	Total esters content	103,677.85 ± 5109.70^{a}	91,767.12 ± 2010.37^{b}	79,695.33 ± 4346.25^{c}						
Acids	Hexanoic acid	346.12 ± 130.64^{a}	282.24 ± 16.58^{a}	456.60 ± 84.41^{a}	420	> 0.1			Sweet	(Tristezza et al., 2016)
	Octanoic acid	1929.16 ± 59.39^{a}	1434.27 ± 430.49^{a}	1548.48 ± 113.53^{a}	500	> 1			Rancid, harsh, cheese, fatty acid	(Wang et al., 2017)
	Decanoic acid	659.55 ± 16.22^{ab}	520.98 ± 27.38^{b}	919.91 ± 149.53^{a}	1000	> 0.1			Fatty, unpleasant	(Wang et al., 2017)
	Total acid content	2934.83 ± 206.25^{a}	2237.48 ± 474.44^{a}	2924.98 ± 347.47^{a}						

(*continued on next page*)

Table 2 (*continued*)

Volatile compounds		Concentration(μg/L, mean ± SD)			Threshold (μg/L)	OAV			Odor description	Reference
		NXU 21–24	NXU 21–102	BDX		NXU 21–24	NXU 21–102	BDX		
Others	Linalool	83.56 ± 13.54[a]	80.92 ± 9.27[a]	65.44 ± 6.74[a]	25	> 1			Flowery, fruity, muscat	(Peng, Wen, Tao, & Lan, 2013)
	β-Damascenone	87.07 ± 35.77[a]	89.20 ± 37.26[a]	89.22 ± 3.39[a]	0.05	> 1			Flowery, sweet, honey, apple	(Peng et al., 2013)
	Other total content	170.63 ± 49.31[a]	170.12 ± 46.53[a]	154.66 ± 10.14[a]						

Values are presented as mean ± standard deviation ($n = 2$).
OAV, odor activity value (OAV = mass concentration/threshold).
Values displayed in the same row and tagged with different letters are significantly different ($p < 0.05$).
"–"no related reference.

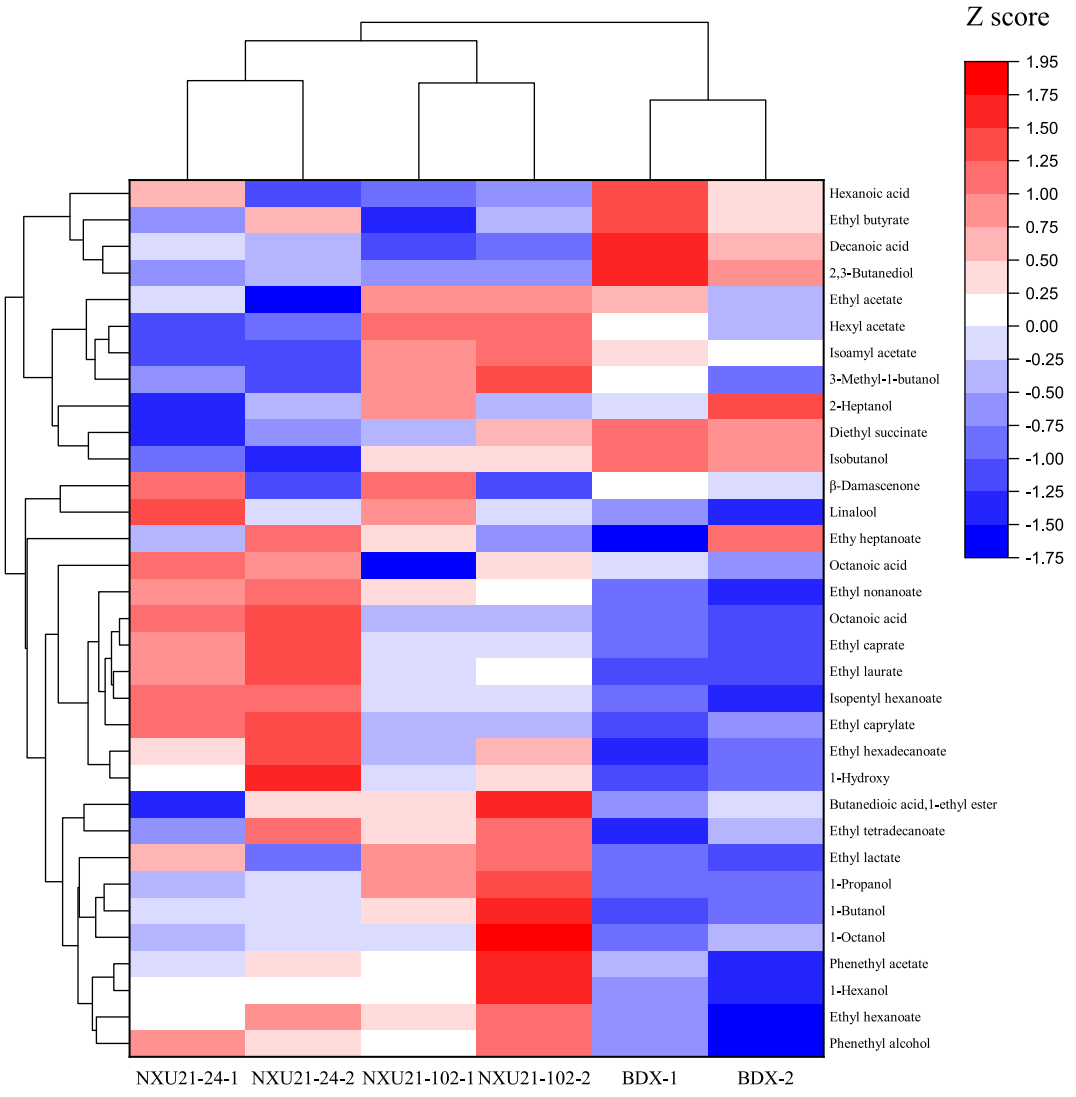

Fig. 3. Clustering analysis of aroma components in Cabernet Sauvignon wine fermented by NXU 21–102, NXU 21–24, and BDX (control strain). The number after each strain represents the two technical replicates.

could be recognized by human beings.

To better assess the organoleptic variability of wines fermented by the different *S. cerevisiae* strains, a sensory evaluation was also performed. The sensory evaluation was conducted with 15 practitioners in blinding taste. The description words were selected from the Davis aroma wheel and the fermentation aroma characteristics were presented in Fig. 4B. Ten families, namely citrus, berry, tropical fruit, temperate fruit, dried fruit, floral, herbal, spice, toasted and undesirable flavors were classified in this study according to the taste of wines fermented by NXU 21–102, NXU 21–24 and BDX (control strain). Although the undesirable flavors were tasted, the intensity was low. Compared with BDX wines, the NX 21–102 wines were characterized by more tropical fruit, floral, and toasted aromas with moderate levels of the other flavors. For NXU 21–24, the intensity of tropical fruit, temperate fruit, dried fruit,

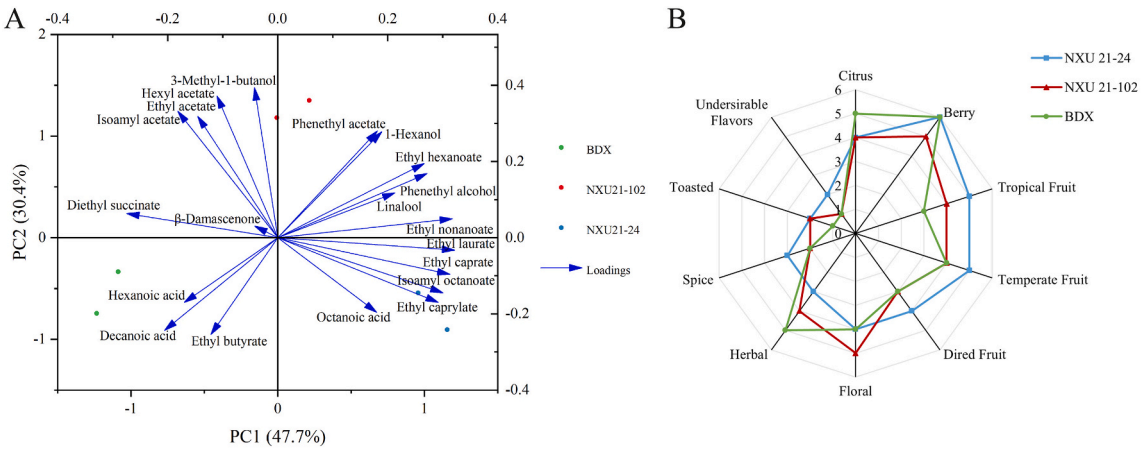

Fig. 4. Odorous evaluation of Cabernet Sauvignon wines. (A) Principal components analysis (PCA) based on the concentration of volatile compounds in wines fermented by NXU 21–102, NXU 21–24, and BDX (control strain). Solid circles represent wines fermented by different strains; (B) Sensory aroma profile of wines fermented by NXU 21–102, NXU 21–24, and BDX (control strain).

spice, and undesirable flavors was higher than the others, while the intensity of herbal flavors was lower.

4. Conclusions

The fermentative and oenological properties of 89 indigenous *S. cerevisiae* isolates isolated from Ningxia and ten commercial strains varied significantly in this study. The SSR typing and UPGMA analysis enabled us to differentiate *S. cerevisiae* at strain level. The selection strategy in this study is suitable for rapid selection of the interested *S. cerevisiae* strains, and could reduce the labor and increase efficiency. The indigenous *S. cerevisiae* strains of NXU 21–102 and NXU 21–24 showed excellent characteristics and could be applied in the wine making process. These two indigenous *S. cerevisiae* strains will be tested as starter cultures in a pilot-scale fermentation in Ningxia, China. Moreover, adaptive laboratory evolution and fermentation performance of NXU 21–102 and NXU 21–24 are now underway to help further select ideal indigenous strains as starters.

CRediT authorship contribution statement

Junyu Liu: Writing – original draft, Formal analysis. **Ruirui Li:** Investigation, Formal analysis, Data curation. **Ying Li:** Formal analysis, Data curation. **Yue Sun:** Writing – review & editing, Supervision, Methodology, Funding acquisition, Conceptualization.

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

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2024.101693.

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