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# Selection of *Saccharomyces cerevisiae* Isolates from Helan Mountain in China for Wine Production

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**Abstract:** *S. cerevisiae* strains were isolated and identified from vineyards and the spontaneous fermentation must at the eastern foot of Helan Mountain in China, and their oenological properties and fermentation abilities were analyzed. From the total of 199 *S. cerevisiae* strains isolated and identified, 14 isolates (F4-13, F5-7, F5-9, F5-12, F5-18, F5-19, F5-21, F6-8, F6-23, F9-23, SXY-4, HT-10, ZXY-17, MXY-19) exhibited excellent tolerance to sugar, $SO_2$, and ethanol. Among the isolates, the strain F4-13 exhibited the better oenological properties, with low $H_2S$ production (+), suitable flocculation ability (58.74%), and reducing-L-malic acid ability (49.07%), and generated high contents of polyphenol, anthocyanin, tannin, terpenes, and higher alcohols, which contributed to the improvement of the red fruity and floral traits of the wines. The obtained results provide a strategy for the selection of indigenous *S. cerevisiae* for wine fermentation to produce high-quality wine with regional characteristics.

**Keywords:** indigenous *Saccharomyces cerevisiae*; isolation and selection; oenological properties; fermentation abilities



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## 1. Introduction

Wine is the product of complex interaction between grape berries, microbiota on grape peels, and their surroundings, originating from the vineyard and present throughout the fermentation, aging, and packaging processes [1,2]. Yeast plays a vital role during wine fermentation. It converts sugar into alcohol and carbon dioxide and secretes various secondary metabolites (glycerin, higher alcohols, fatty acids, esters, etc.) to modify the organoleptic properties of wine [3,4]. Currently, pure fermentation of the imported commercial *S. cerevisiae* strain has been a common biotechnological strategy for industrial wine production in China due to the excellent fermentative rate, controllable fermentative process, and stable quality compared to spontaneous fermentation [5]. However, the widespread use of imported commercial *S. cerevisiae* as a starter has reduced the biodiversity of indigenous yeasts and hindered the development of indigenous yeast with excellent fermentative properties, resulting in wines with reduced flavor complexity and regional and varietal characteristics [5,6].

Researchers and enologists have attempted methods of producing wines with complex flavor and outstanding organoleptic properties that cater to consumer preferences [7,8]. The co-fermentation of *S. cerevisiae* with non-*Saccharomyces* is an emerging strategy to improve the formation of glycerol, varietal and fermentative aroma compounds, and stable pigments to modify the organoleptic properties of specific wines [9,10]. The addition of exogenous compounds (e.g., enzymes, nitrogen sources, unsaturated fatty acids, amino acids, or polyphenols) during wine fermentation is considered an effective method for the organoleptic properties of wine in enhancing the volatile aroma compounds and modifying physicochemical compositions [11,12]. In addition, the inoculation of the selected indigenous *S. cerevisiae* strain into must is also an economical, practical, and rapid strategy

to strengthen the regional and varietal features of wines, as they are better acclimated to the micro-area conditions of the wine grape production regions [13].

Sun, Hu, Zhang, Zhu and Tao [11] and Wang, et al. [14] indicated that the indigenous *S. cerevisiae* strains with native "*terroir*" traits showed fermentation variations and released distinguishable metabolites, which resulted in various flavor profiles of wines to retain its flavor complexity and its regional and varietal features. The eastern foot of the Helan Mountain (38°47′ N, 106°27′ E), located in Ningxia of China, is a desirable region for wine-grape cultivation (with a significant diurnal temperature difference and sufficient sunlight) and is one of the primary sources of wine grapes in China [15]. As it is a traditional wine-grape cultivation region, hundreds of microbiomes inhabit the region, including indigenous yeasts, which may be better suited to produce wines with regional features due to their long-term adaptive evolution [16,17]. The selected indigenous *S. cerevisiae* strains with excellent oenological property, fermentation ability, and volatile aroma compound production capacity can be used to ferment wines with flavor complexity and regional and varietal characteristics [18,19].

The study aims to isolate and select indigenous *S. cerevisiae* strains from the eastern foot of Helan Mountain that possess excellent oenological properties, fermentation ability, and volatile compound production capacity for local wine production to restore regional features. Firstly, indigenous *S. cerevisiae* strains were isolated from grape peels, leaves, and soils of nine vinicultural regions as well as spontaneous fermentation of Cabernet Sauvignon must from the eastern foot of Helan Mountain, and then identified by morphology on Wallerstein nutrient medium and 26S rDNA D1/D2 domain sequencing. Next, their oenological properties, including stress tolerance, flocculation ability, $H_2S$ production, reducing-L-malic acid ability, and fermentability, were evaluated at laboratory scale. The selected indigenous *S. cerevisiae* strains from the eastern foot of Helan Mountain will provide potential starters for wine production with regional characteristics and a reference for selecting other yeast strains with specific regional features as potential starters.

## 2. Materials and Methods

### *2.1. Sampling and Yeast Isolation*

Grape berries, leaves, and soils of nine vineyards from the eastern foot of Helan Mountain in Ningxia of China (Figure 1A), and spontaneous fermentation wine of Cabernet Sauvignon were sampled as yeast-isolated sources. Specifically, soils were collected from five positions 5~10 cm beneath vineyard soil surfaces, and fresh leaves, dried leaves, ripe grapes, and overripe grapes were sampled from vineyards with sterile bags and stored at 4 °C. Additionally, spontaneous fermentation with 1 ton of Cabernet Sauvignon must (247.22 g/L sugar, 4.00 g/L total acids, pH 5.10) harvested from the Helan Mountain vineyard in 2016 was performed at 28 °C, and 1 mL of wine was taken daily during wine fermentation.

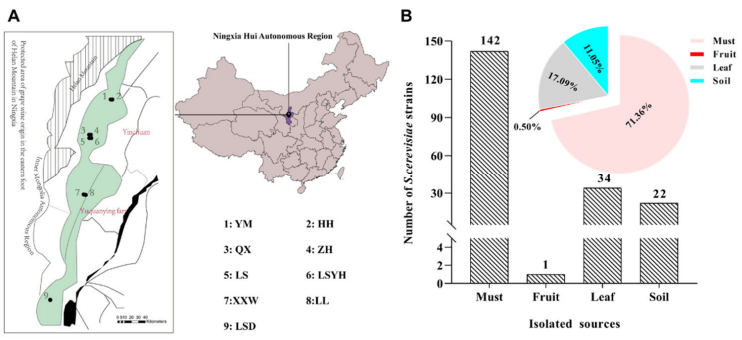


**Figure 1.** Sampling sites (**A**) and the number and rate of *S. cerevisiae* strains isolated from different sources (**B**) at the eastern foot of Helan mountain (YM: Yuma vineyard; HH: Huahao vineyard, QX: Qinxue vineyard, ZH: Zhihuiyuanshi vineyard, LS: Liushi vineyard, LSYH: Lanshanyunhao vineyard, XXW: Xixiawang vineyard, LL: Lilan vineyard, LSD: Luoshan vineyard).

The aseptic flask containing 10 g sample and 90 mL sterile normal saline was shaken at 160 rpm for 30 min at room temperature (approx. 23 °C) to elute microorganisms. Subsequently, 0.1 mL serially diluted samples were spread onto YPD agar plates supplemented with 100 mg/L chloramphenicol to inhibit bacterial growth. After inoculation at 28 °C for 2 d, 20~30 colonies from each plate were randomly chosen for purification. All colonies were preserved on YPD slants at 4 °C for the following experiments.

### *2.2. Grapes, Yeasts and Media*

Cabernet Sauvignon containing 239.92 g/L sugar, 6.75 g/L total acids (expressed as tartaric acid) and pH 3.96, normally harvested from the Helan Mountain region vineyard in 2017, located in Ningxia, China. The indigenous *S. cerevisiae* strains were isolated from grape peels, leaves, soils, and spontaneous fermentation must of Cabernet Sauvignon in 2016 (Figure 1B). *S. cerevisiae* F33 (FSC) from Laffort Ltd. (Actiflore Cervisiae, Laffort Co., Bordeaux, France) was used as a reference strain.

Yeast extract peptone dextrose medium (YPD, 10.00 g/L yeast extract, 20.00 g/L peptone, and 20.00 g/L dextrose) was employed to enrich culture yeast strains.

(1) Wallerstein laboratory nutrient agar medium (WL, 50.00 g/L dextrose, 0.125 g/L $MgSO_4$, 5.00 g/L tryptone, 0.022 g/L bromocresol green, 4.00 g/L yeast extract, 0.0025 g/L $FeCl_3$, 0.55 g/L $KH_2PO_4$, 0.0025 g/L $MnSO_4$, 0.425 g/L KCl, 15.00 g/L agar, and 0.125 g/L $CaCl_2$, pH 5.50) was used to differentiate yeast species according to the colony morphology and the yeast color.
(2) Lysine agar medium (LYS, 10.00 g/L dextrose, 5.00 g/L lysine, 0.10 g/L $KH_2PO_4$, 0.10 g/L $MgSO_4$, 0.10 g/L complex vitamin, and 15.00 g/L agar, pH 4.80) was used to differentiate *S. cerevisiae* and non-*Saccharomyces* strains.
(3) Synthetic grape juice (SGJ, 100.00 g/L dextrose, 100.00 g/L fructose, 1.50 g/L yeast extract, 0.30 g/L citric acid, 5.00 g/L tartaric acid, 5.00 g/L malic acid, 2.00 g/L ammonium sulfate, 5.00 g/L potassium dihydrogen phosphate, 0.50 g/L magnesium sulfate, 0.20 g/L sodium chloride, and 0.05 g/L manganese sulfate, pH 3.20) was used as an alternative to the must for its precise physicochemical composition.
(4) Bismuth sulfite agar medium (BSA, polypeptone 10.00 g/L, beef extract 5.00 g/L, dextrose 5.00 g/L, bismuth sulfite 8.00 g/L, $FeSO_4$ 0.30 g/L, $Na_2HPO_4$ 4.00 g/L, brilliant green 0.025 g/L, and agar 15.00 g/L, pH 7.70 $\pm$ 0.20) was used to test the hydrogen sulfide ($H_2S$) production of *S. cerevisiae* strains.
(5) Yeast exact, L-malic acid peptone medium (YMP, 40.00 g/L L-malic acid, 10.00 g/L yeast extract, 20.00 g/L peptone, and 50 µL of 0.003 g/L bromocresol green solution) was used to evaluate the reducing-L-malic acid ability of *S. cerevisiae* strains.

### *2.3. Morphological and Molecular Identification of S. cerevisiae*

The isolated strains were dotted on WL medium in duplicate and incubated at 28 °C for 5 d, and the colony morphologies were observed. When the front side of the colony was cream with little green, raised and opaque, and the back side was white or with little green, it was initially considered as *S. cerevisiae*. Then, the molecular identification of the isolated strains was performed by the analysis of 26S rDNA D1/D2 domain sequencing. Specifically, genomic DNA in yeast was extracted according to Sun, et al. [20]. The 26S rDNA D1/D2 domain was amplified with primers NL1 (5′-GCA TAT CAA TAA GCG GAG GAA AAG-3′) and NL4 (5′-GGT CCG TGT TTC AAG ACG G-3′) [21]. PCR amplification was performed in a 50 µL volume containing 1 µL DNA template (250 ng/µL), 4.0 µL dNTP (2.5 mmol/L each), 5.0 µL 10×PCR buffer solution ($Mg^{2+}$ plus), 25 µL premix *Taq* polymerase (TAKARA, Beijing, China), and 3 µL each primer (10 µM). The PCR reaction was performed according to the following conditions: denaturation at 95 °C for 5 min, 35 cycles of denaturation at 94 °C for 1 min, annealing at 52 °C for 1 min and extension at 72 °C for 2 min, and a final extension at 72 °C for 10 min. PCR products were analyzed by electrophoresis on a 1% agarose gel, then purified and sequenced by Sangon Biotech (Shanghai, China) Co., Ltd. The 26S rDNA D1/D2 sequence amplified from each yeast strain was compared to

the sequence stored in Genbank® (https://blast.ncbi.nlm.nih.gov/Blast.cgi, accessed on 10 March 2017) by using BLAST software [22]. Generally, the difference in the 26S rDNA D1/D2 domain sequence of the same species is within 1%.

### *2.4. Oenological Properties of S. cerevisiae*

#### 2.4.1. Tolerance to Sugar, $SO_2$, and Ethanol Analysis

The tolerances of the isolated yeast strains and FSC to sugar, $SO_2$, and ethanol were analyzed in a 96-well plate. Specifically, each well contained 200 µL SGJ with different concentrations of sugar (250 g/L), $SO_2$ (100 mg/L), ethanol (12% *v/v*), and $10^6$ CFU/mL yeast cells (prepared by culturing in YPD at 28 °C and 120 rpm for 24 h). After inoculation, plates were incubated at 28 °C for 48 h and microshocked until turbid, and then the absorbance was measured at 600 nm using a Multifunctional microplate reader (Synergy HTX, BioTek, Emeryville, CA, USA). Relative growth rate (RG, %) was used to evaluate the tolerance abilities of isolated yeast strains to sugar and the absorbance of FSC at 600 nm (*AbsS*) at the same sugar concentration as a reference, whereas inhibition rate (IR, %) was used to evaluate the tolerance abilities of isolated yeast strains to $SO_2$ and ethanol. RG (%) and IR (%) were calculated with the following formula:

$$\text{RG } (\%) = \frac{AbsE}{AbsS} \times 100\%;\ \text{IR } (\%) = (1 - \frac{AbsE}{AbsE0}) \times 100\%$$

where *AbsE* is the absorbance of each sample at 600 nm in SGJ with different concentrations of sugar, $SO_2$, or ethanol; *AbsS* is the absorbance of FSC at 600 nm at different concentrations of sugar, and *AbsE*0 is the absorbance of each sample at 600 nm in the SGJ without $SO_2$ and ethanol.

#### 2.4.2. Hydrogen Sulfide Production

$H_2S$ productions of yeast strains were determined with a bismuth sulfite agar medium (BSA, Angel, Yichang, China) [23]. Activated yeast cells were collected by centrifugation (6000 rpm, 5 min) and washed with sterile saline 3 times. Then, 10 µL yeast solution ($10^7$ CFU/mL) diluted with sterile saline was added dropwise on the surface of bismuth sulfite agar medium plate and incubated at 28 °C for 6 d, and the halo color surrounding the yeast colonies was measured. Generally, the strain resulted in higher $H_2S$ production as the color of the halos deepened. The relationships among the symbol, $H_2S$ production, and halo color were indicated as follows: − no (no change); + little (white); ++ low (light brown); +++ medium (green); ++++ high (dark green).

#### 2.4.3. Flocculation Ability

The flocculation abilities of yeast strains were analyzed by the modified Helm method [24]. Specifically, yeast suspension ($10^8$ CFU/mL) was centrifuged at 8000 rpm for 5 min, then the supernatant was discarded and labeled A and B. The sediment in A tube was mixed with an appropriate content of EDTA buffer (0.5 mol/L, pH 7.0) to obtain $10^7$ CFU/mL solution, and then its absorbance at 600 nm (*Abs0*) was measured. The sediment in B tube was washed with 0.51 g/L of calcium sulfate solution, then was mixed with 1 mL buffer (pH 4.5) containing 0.51 g/L of calcium sulfate, 6.80 g/L of sodium acetate, and 4.05 g/L of glacial acetic acid, and stood at room temperature for 15 min. Next, 100 µL of the supernatant was added into 900 µL water and vortexed, and the absorbance at 600 nm (*Abs15*) was measured. Flocculation abilities of yeast strains were evaluated by the following formula.

$$\text{Flocculation ability } (\%) = (1 - \frac{Abs15}{Abs0}) \times 100\%$$

#### 2.4.4. Reducing-L-Malic Acid Ability

The reducing-L-malic acid abilities of yeast strains were assessed using YMP medium with bromocresol green. YMP medium with $10^7$ CFU/mL yeast cells was cultured at 28 °C for 6 d, and the color of the broth was observed. The color of the broth gradually varied from brown to light yellow or dark green as the amount of residual L-malic acid decreased. Residual L-malic acid in broth was detected by high-performance liquid chromatography (HPLC, Shimadzu Corporation, Kyoto, Japan) with Kromasil $C_{18}$ column (250 mm × 4.6 mm, 5 μm), the UV detector (SPD-40/SPD-40V) with 210 nm detection wavelength. A quantity of 10 μL of fermentation supernatant was separated with mobile phase composed of 1% acetonitrile and 0.02 mol/L potassium dihydrogen phosphate solution (pH 2.80) at a ratio of 1:9 and a flow rate of 0.50 mL/min at 30 °C. The abilities of yeast strains to reduce L-malic acid were evaluated by the following formula.

$$\text{Reducing} - \text{L} - \text{malic acid ability } (\%) = (1 - \frac{\text{Residual L} - \text{malic acid content}}{\text{Total L} - \text{malic acid content}}) \times 100\%$$

### *2.5. Laboratory-Scale Production of Wines*

The laboratory-scale vinification was conducted in 1 L sterile flasks containing 800 mL crushed Cabernet Sauvignon (pasteurized at 80 °C for 30 min) with the additional 60 mg/L of $SO_2$, and then macerated at 4 °C for 12 h before inoculation. After the treated must was restored to 25 °C, it was inoculated with the selected *S. cerevisiae* or the commercial *S. cerevisiae* F33 at $10^6$ CFU/mL, and fermented at 25 °C (±1 °C), respectively. The biomass of yeasts was monitored using the diluted coating plate method, and the residual sugar of yeasts was analyzed using the DNS colorimetry method every day during wine fermentation. The final wines were added with 50 mg/L $SO_2$. Wines fermented by 14 *S. cerevisiae* isolated strains and FSC were named as SCw and FSCw, respectively.

#### 2.5.1. Physicochemical Composition Analysis of Wines

Ethanol, total acids (expressed as tartaric acid), and volatile acids (expressed as acetic acid) in wines were determined according to International Organization of Vine and Wine (OIV, Paris, France, 2016). Polyphenols, anthocyanins, and tannins in wines were evaluated by the folin-phenol colorimetry [25], pH differential [26], and colorimetry methods [27], respectively. The pH value of wines was determined by a pH meter (Mettler-Toledo, Shanghai, China).

#### 2.5.2. Volatile Compounds Analysis of Wines

Volatile compounds in wines were detected using gas chromatography-mass spectrometry (GC–MS) coupled with headspace solid-phase microextraction (HS-SPME) adapted from Wang, et al. [28]. Volatile compounds were extracted using a 50/30 μm DVB/CAR/PDMS fiber (Supelco, Bellefonte, PA, USA). A 20 mL gas-tight vial containing 2 g NaCl, 8 mL wine, and 10 μL internal standard (cyclohexanone) was placed in a 40 °C water bath with stirring at 600 rpm for 15 min, extracted for 30 min, and then desorbed in the GC injection port at 250 °C for 5 min using a Shimadzu TQ8040 GC–MS (Shimadzu Corporation, Kyoto, Japan) and a DB-WAX column (30 m × 0.25 mm × 0.25 μm, Agilent J & W, Santa Clara, CA, USA). The GC program was set as follows: the carrier gas was ultrapure helium (99.999%) with a flow rate of 1.00 mL/min; the temperature was increased from 40 °C to 130 °C at 3 °C/min, then ramped to 250 °C at 4 °C/min and maintained for 5 min. The MS was operated in electron impact ionization mode at 70 eV, and ion source temperature was 250 °C. Volatile compounds were identified by comparing their MS fragmentation pattern with those in database NIST 17 Volatile compound concentrations were quantitated by the following formula.

$$\text{Compound concentration (mg/L)} = \frac{\text{GC peak areas of the compond} \times \text{Quantity of internal standard (mg)}}{\text{GC peak area of the internal standard} \times \text{Volume of the sample (L)}}$$

#### 2.5.3. Sensory Evaluation of Wines

Sensory evaluation of the wines was conducted by 17 well-trained panelists (8 males and 9 females) in a tasting room at 20 °C [11]. Wine samples of approximately 20 mL were poured into black INAO glass in triplicate. The wine aromas of wine were described using 4~6 terms, and the intensities of the aromas were scored using a five-point scale (1. very weak; 2. weak; 3. medium; 4. intense; 5. very intense). The radar charts of the aromatic characteristics of wines were plotted.

### *2.6. Statistical Analysis*

All statistical data were analyzed by GraphPad Prism 8 (GraphPad Software Inc., San Diego, CA, USA). Sampling sites were drafted by Adobe Illustrator CS6 (Adobe Software Inc., Adobe, CA, USA). Significant differences ($p < 0.05$) among different *S. cerevisiae* strains were compared by Duncan's multiple tests using SPSS 19.0 software (SPSS Inc., Chicago, IL, USA). Hierarchical Cluster and Heatmap Diagram was used to describe the volatile compound profiles of different wine samples by OmicShare Tools (GENE DENOVO, Guangzhou, China) after Z-score standardization.

## 3. Results and Discussion

### *3.1. Identification of Isolated Yeasts*

A total of 1066 yeast and yeast-like strains (hereby referred to as yeasts) were isolated from grape peels, leaves, soils, and spontaneous Cabernet Sauvignon must at the eastern foot of Helan Mountain in Ningxia, China (Figure 1A). Among these 1066 yeast strains, 199 *S. cerevisiae* strains were identified by the morphology characteristics on WL medium and partial 26S rDNA D1/D2 domain sequence (Tables S1 and S2). The number and isolation ratio of *S. cerevisiae* from different isolated sources were calculated, as shown in Figure 1B. Results indicated that spontaneous fermentation must (FM) was the best source for collecting *S. cerevisiae,* and 142 strains accounting for 71.36% of the total strains were isolated from FM, followed by grape leaves (34 strains, 17.09%), soils (22 strains, 11.05%) and grape peel (1 strain, 0.50%). Previous studies have shown that *S. cerevisiae* was abundant during spontaneous alcoholic fermentation, especially in the mid and late stages [13,29], but not easily found on the berries [30,31].

### *3.2. Oenological Properties of Yeasts*

#### 3.2.1. Tolerance Abilities of Yeasts to Sugar, $SO_2$, and Ethanol

The tolerance abilities analysis of 199 *S. cerevisiae* strains to sugar, $SO_2$, and ethanol showed that 196 strains had decent growth under 250 mg/L sugar (RG > 50%, Figure 2A), and 96 strains had decent growth under 100 mg/L $SO_2$ (IR > 50%, Figure 2B), while 15 strains exhibited decent growth under 12% (*v/v*) ethanol (IR > 50%, Figure 2C). Among them, *S. cerevisiae* F4-13, F5-7, F5-9, F5-12, F5-18, F5-19, F5-21, F6-8, F6-23, F9-23, SXY-4, HT-10, ZXY-17, and MXY-19 showed normal growth under 250 g/L sugar, 100 mg/L $SO_2$, and 12% (*v/v*) ethanol (Figure 2D and Table S3). Among 14 *S. cerevisiae* with good tolerance, 10 strains were isolated from the spontaneous fermentation of Cabernet Sauvignon, suggesting that *S. cerevisiae* from spontaneous fermentation had better stress resistance than those from other isolated sources.

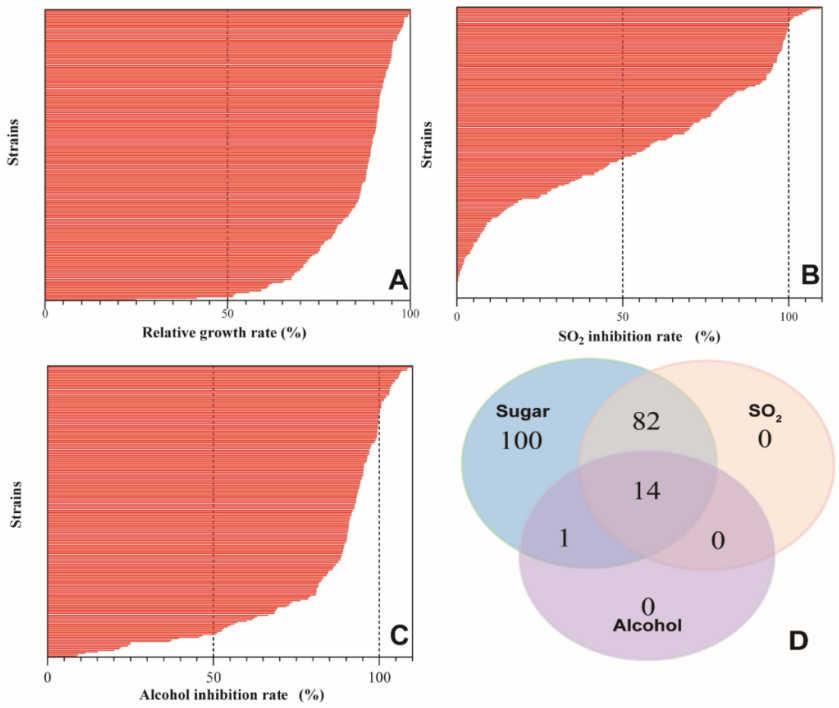


**Figure 2.** The tolerance analysis of the isolated *S. cerevisiae* strains to sugar, $SO_2$, and ethanol ((**A**): sugar tolerance, expressed as relative growth rate in synthetic grape juice containing 250 g/L glucose; (**B**): $SO_2$ tolerance, expressed as inhibition rate in synthetic grape juice containing 100 mg/L total $SO_2$; (**C**): ethanol tolerance, expressed as inhibition rate in synthetic grape juice containing 12% (*v*/*v*) alcohol; (**D**): Venn diagram of tolerance analysis of *S. cerevisiae* strains).

#### 3.2.2. Properties of $H_2S$ Production, Flocculation, and Reducing-L-Malic Acid of Yeasts

Fourteen *S. cerevisiae* strains with good tolerance to sugar, $SO_2$, and ethanol were further selected by analyzing their oenological properties. The results in Figure 3A indicated that 14 strains presented lower $H_2S$ production on BAS plate than FSC, and F4-13, F5-7, HT-10, and ZXY-17 barely produced $H_2S$. High content of $H_2S$ presents "rotten eggs" odor and damages the organoleptic quality of wine, while low $H_2S$ production favors the organoleptic quality of wine [32,33]. Therefore, low $H_2S$ production is one of the evaluating or selecting criteria of that yeast strains to be used as potential starters to ferment musts [34,35].

As shown in Figure 3B, the 14 *S. cerevisiae* strains exhibited 22.17~97.16% flocculation abilities except for F5-9 and ZXY-17, and strains F9-23 (97.16%), F6-23 (92.70%), MXY-19 (89.37%), F5-19 (88.60%), HT-10 (80.58%), F5-18 (76.7%), and F4-13 (58.74%) displayed stronger flocculation abilities than FSC (54.28%). Traditionally, strong flocculation of yeast is in favor of the separation of cell from broth after fermentation [36].

Additionally, the reducing-L-malic acid abilities of 14 selected strains ranged from 11.70% to 72.50%, and HT-10 (72.50%), F6-8 (56.27%), MXY-19 (53.80%), ZXY-17 (53.13%), F4-13 (49.07%), and SXY-4 (44.50%) presented stronger reducing-L-malic acid ability (>44.50%) than FSC (39.60%) (Figure 3D). The results obtained from the color indicator of YMP was highly consistent with that from HPLC analysis (Figure 3C). A reasonable acidity is important to ensure high-quality wine, but high acidity, especially a high level of L-malic acid, will destroy the organoleptic properties of wine [37,38]. Therefore, the reducing-L-malic acid ability is considered a desirable property of yeast strains to achieve a balanced taste in wine [39].

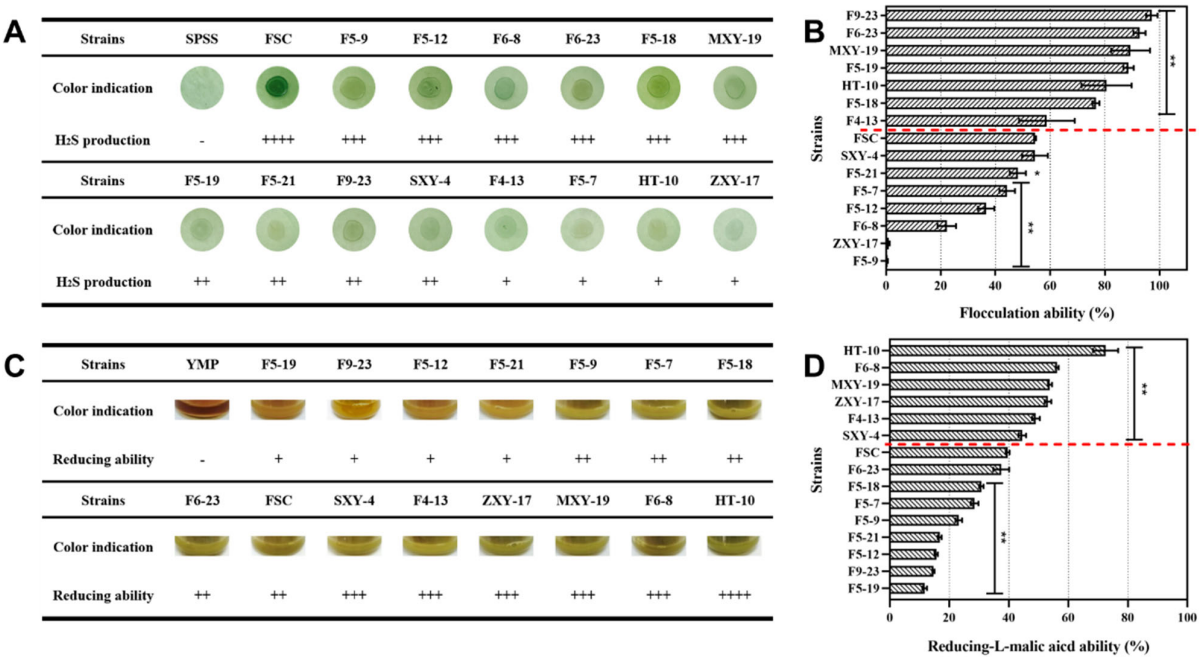


**Figure 3.** Oenological properties analysis of selected *S. cerevisiae* strains ((**A**): $H_2S$ production: stroke-physiological saline solution (SPSS), was used as negative control; (**B**): flocculation ability; (**C**,**D**): Reducing-L-malic acid ability), YMP: yeast extract L-malic acid peptone medium with addition of bromocresol green; all isolates incubated 6 d at 28 °C on YMP medium. - no; + very low/weak; ++ low/weak; +++ medium; ++++ high/strong. * $p < 0.05$, ** $p < 0.01$.

### *3.3. Performance of the Selected Yeasts during Alcoholic Fermentation*

#### 3.3.1. Growth Kinetics and Sugar Consumptions

As shown in Figure 4, the 14 *S. cerevisiae* strains almost reached their maximum biomass (>$10^8$ CFU/mL) between the 1st and 4th days of wine fermentation, and the biomass of F5-7 (6.70 × $10^8$ CFU/mL), F5-9 (5.25 × $10^8$ CFU/mL), and F5-18 (5.05 × $10^8$ CFU/mL) exceeded those of FSC (3.60 × $10^8$ CFU/mL) after one day of wine fermentation (Figure 4C,D,F). The fermentation periods of 14 *S. cerevisiae* isolated strains were 5–8 d, except for F4-13 (8 d), and those of 13 strains were shorter than that of FSC (8 d). Among them, the fermentation period of F5-7, F5-12, F5-18, and MXY-19 was 5 d (Figure 4C,E,F,O), that of F5-9, F5-19, F5-21, F6-23, and ZXY-17 was 6 d (Figure 4D,G,H,J,N), and that of F6-8, F9-23, SXY-4, and HT-10 was 7 d (Figure 4I,K,L,M). These results indicated that 14 *S. cerevisiae* strains displayed excellent growth and fermentation abilities.

#### 3.3.2. Physicochemical Compositions of Wines

Table 1 shows that compared to FSCw, SCw had lower residual sugar content (2.13~3.57 g/L) except for HT-10w and F6-23w, lower ethanol content (11.22~12.50% *v/v*) except for F5-21w and F6-23w, lower pH value (3.82~4.06), higher total acids content (5.39~6.66 g/L) except for ZXY-17w, higher polyphenol content (2.20~2.65 g/L) except for HT-10w, higher anthocyanin content (110.21~249.09 mg/L), and higher tannin content (4.55~5.65 g/L). In addition, the volatile acids content (0.08~0.19 g/L) of all wines was well below 1.2 g/L. The results showed that these physicochemical compositions of the wines met the requirements of National Standard of the People's Republic of China (GB/T 15038-2006, 2006). Interestingly, Qi, et al. [40] found that the wine of the Zhihuiyuanshi vineyard exhibited higher tannins and total phenols than the other three vineyards. Similarly, ZXY-17 obtained from the Zhihuiyuanshi vineyard in the study also showed relatively higher tannins (4.98 ± 0.06 g/L) and total phenols content (2.42 ± 0.03 g/L). In addition, the physicochemical indicators of the wines (pH: 3.05~3.40, alcohol: 11.31~12.65% (*v/v*), total phenolic content: 1.89~2.84 g/L) in our study were consistent with the same

wine-producing region of China (pH: 3.59~3.77, alcohol: 12.10~13.40% (*v/v*), total phenolic content: 1.87~2.04 g/L).

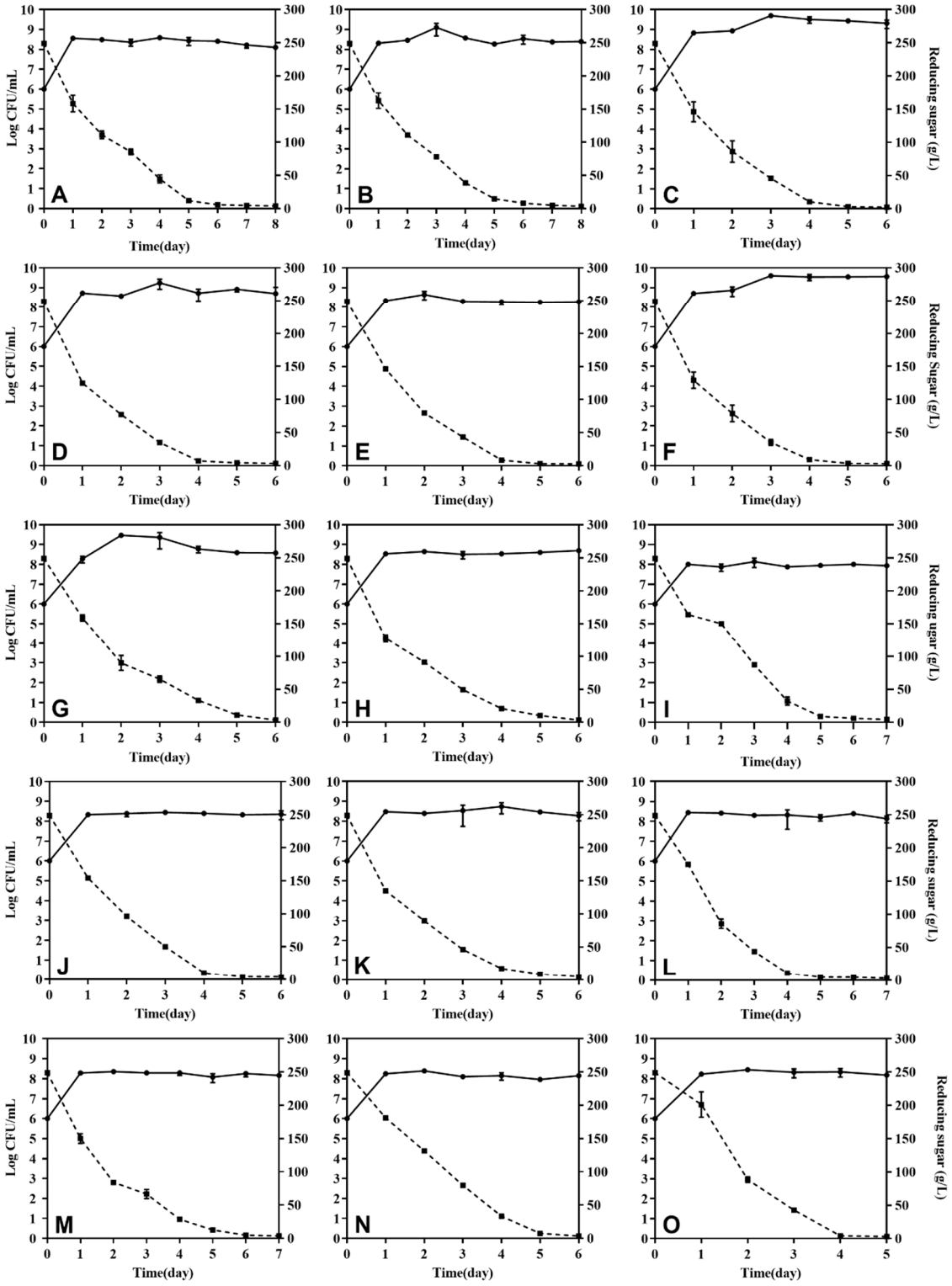


**Figure 4.** The growth curves and sugar consumptions of the selected *S. cerevisiae* strains during pure fermentations (the solid line represents the growth curve; the dashed line represents the sugar consumption curve; (**A**): FSC, (**B**): F4-13, (**C**): F5-7, (**D**): F5-9, (**E**): F5-12, (**F**): F5-18, (**G**): F5-19, (**H**): F5-21, (**I**): F6-8, (**J**): F6-23, (**K**): F9-23, (**L**): SXY-4, (**M**): HT-10, (**N**): ZXY-17, (**O**): MXY-19).

**Table 1.** Physicochemical compositions of wines fermented by the selected *S. cerevisiae* strains.

| Wines | Residual Sugar | Ethanol | Total Acids [A] | Volatile Acids [B] | Polyphenols | Anthocyanin | Tannin | pH |
|---|---|---|---|---|---|---|---|---|
| | g/L | % (*v*/*v*) | g/L | g/L | g/L | mg/L | g/L | |
| FSCw | 3.74 ± 0.05 gh | 12.65 ± 0.34 hi | 4.97 ± 0.23 b | 0.09 ± 0.00 cd | 2.00 ± 0.01 b | 97.13 ± 1.28 a | 3.76 ± 0.30 a | 3.23 ± 0.02 d |
| F4-13w | 3.31 ± 0.09 cd | 11.58 ± 0.34 bc | 5.93 ± 0.23 e | 0.09 ± 0.00 bc | 2.65 ± 0.08 i | 151.96 ± 1.45 d | 4.55 ± 0.08 b | 3.32 ± 0.02 f |
| F5-7w | 2.13 ± 0.03 a | 12.20 ± 0.08 ef | 6.12 ± 0.05 e | 0.09 ± 0.00 bc | 2.50 ± 0.03 fgh | 208.18 ± 1.28 g | 4.73 ± 0.16 bc | 3.32 ± 0.02 f |
| F5-9w | 3.25 ± 0.20 cd | 11.81 ± 0.32 cd | 5.93 ± 0.23 e | 0.07 ± 0.00 a | 2.29 ± 0.03 cd | 110.21 ± 3.64 b | 5.65 ± 0.18 d | 3.38 ± 0.02 gh |
| F5-12w | 2.92 ± 0.04 b | 12.24 ± 0.32 ef | 6.09 ± 0.00 e | 0.11 ± 0.00 e | 2.52 ± 0.13 fgh | 202.61 ± 4.75 g | 4.72 ± 0.09 bc | 3.27 ± 0.04 e |
| F5-18w | 2.89 ± 0.43 b | 12.09 ± 0.15 de | 5.39 ± 0.09 c | 0.08 ± 0.00 b | 2.56 ± 0.12 hi | 156.97 ± 2.21 de | 5.35 ± 0.52 d | 3.35 ± 0.01 fg |
| F5-19w | 3.43 ± 0.07 cd | 11.39 ± 0.08 ab | 6.41 ± 0.00 f | 0.13 ± 0.00 g | 2.33 ± 0.03 de | 154.46 ± 0.83 de | 4.66 ± 0.21 bc | 3.12 ± 0.02 b |
| F5-21w | 3.53 ± 0.04 de | 12.92 ± 0.19 i | 5.23 ± 0.14 c | 0.09 ± 0.00 cd | 2.20 ± 0.03 c | 158.08 ± 9.89 de | 3.90 ± 0.25 a | 3.34 ± 0.01 f |
| F6-8w | 3.40 ± 0.18 cd | 12.20 ± 0.11 gh | 5.39 ± 0.09 c | 0.09 ± 0.00 bc | 2.44 ± 0.02 fg | 224.32 ± 2.55 h | 3.90 ± 0.05 a | 3.06 ± 0.01 a |
| F6-23w | 3.87 ± 0.13 h | 12.65 ± 0.34 hi | 6.12 ± 0.05 e | 0.10 ± 0.00 d | 2.55 ± 0.04 ghi | 249.09 ± 1.74 i | 4.53 ± 0.26 b | 3.17 ± 0.02 c |
| F9-23w | 3.27 ± 0.13 cd | 11.31 ± 0.27 ab | 6.66 ± 0.09 g | 0.09 ± 0.00 bc | 2.84 ± 0.07 j | 242.69 ± 13.26 i | 4.50 ± 0.06 b | 3.24 ± 0.04 de |
| SXY-4w | 2.92 ± 0.04 b | 12.09 ± 0.23 de | 5.67 ± 0.32 d | 0.15 ± 0.01 h | 2.29 ± 0.04 cd | 162.54 ± 2.68 e | 4.98 ± 0.04 c | 3.40 ± 0.02 i |
| HT-10w | 3.61 ± 0.11 fgh | 11.22 ± 0.32 a | 6.34 ± 0.09 f | 0.12 ± 0.00 f | 1.89 ± 0.00 a | 119.40 ± 2.50 c | 3.88 ± 0.18 a | 3.05 ± 0.01 a |
| ZXY-17w | 3.57 ± 0.23 ef | 12.12 ± 0.23 de | 4.75 ± 0.09 a | 0.19 ± 0.00 i | 2.42 ± 0.03 ef | 183.41 ± 3.16 f | 4.98 ± 0.06 c | 3.33 ± 0.02 f |
| MXY-19w | 3.13 ± 0.04 bc | 12.50 ± 0.06 efg | 6.34 ± 0.36 f | 0.12 ± 0.00 fg | 2.20 ± 0.04 c | 150.85 ± 0.48 d | 4.37 ± 0.14 b | 3.38 ± 0.02 gh |

Notes: Values with different lowercase letters in the same row are significantly different according to the Duncan test ($p < 0.05$). A: as acetic acid; B: as tartaric acid.

### 3.3.3. Volatile Compounds of Wines

As shown in Table 2, the 485.23~1149.74 mg/L volatile compounds were detected from young wines, which included 10 varietal aroma compounds and 47 fermentative aroma compounds. The former contained $C_6$ and terpene compounds, and the later contained higher alcohols, fatty acids, esters, benzene derivatives, and aldoketones.

**Table 2.** Analysis of volatile aroma compounds in wines fermented by the selected *S. cerevisiae* strains (mg/L; means ± SD).

| Aroma Compounds | FSCw | F4-13w | F5-7w | F5-9w | F5-12w | F5-18w | F5-19w | F5-21w | F6-8w | F6-23w | F9-23w | SXY-4w | HT-10w | ZXY-17w | MXY-19w | Threshold * | Description | OVA |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| $C_6$ alcohols | 1.33 ± 0.08 a | 1.04 ± 0.01 abc | 0.68 ± 0.22 c | 0.72 ± 0.01 abc | 0.66 ± 0.06 bc | 0.69 ± 0.40 abc | 0.44 ± 0.23 c | 0.64 ± 0.30 bc | 0.89 ± 0.07 abc | 0.60 ± 0.27 bc | 0.42 ± 0.13 c | 1.10 ± 0.02 ab | 1.09 ± 0.09 ab | 0.88 ± 0.05 abc | 0.91 ± 0.46 abc | | | |
| 1-Hexanol | 0.45 ± 0.03 def | 0.51 ± 0.02 ef | 0.39 ± 0.01 cd | nd | 0.46 ± 0.01 def | 0.27 ± 0.04 ab | 0.19 ± 0.02 a | 0.35 ± 0.04 bcd | 0.47 ± 0.11 def | 0.26 ± 0.02 ab | 0.22 ± 0.02 a | 0.29 ± 0.00 abc | 0.57 ± 0.04 f | 0.27 ± 0.04 ab | 0.41 ± 0.03 de | 8 A | Green, gross | <0.1 |
| cis-2-Hexen-1-ol | 0.88 ± 0.05 a | 0.53 ± 0.03 abc | 0.28 ± 0.21 bc | 0.72 ± 0.01 abc | 0.20 ± 0.07 c | 0.43 ± 0.36 abc | 0.25 ± 0.21 bc | 0.28 ± 0.25 bc | 0.42 ± 0.04 abc | 0.34 ± 0.28 abc | 0.20 ± 0.15 c | 0.81 ± 0.02 ab | 0.52 ± 0.06 abc | 0.61 ± 0.01 abc | 0.50 ± 0.43 abc | 0.4 A | Green, cypress | 0.1–1 |
| Terpenes | 0.56 ± 0.01 a | 0.66 ± 0.04 a | 0.33 ± 0.01 bcd | 0.24 ± 0.12 b | 0.01 ± 0.00 e | 0.64 ± 0.18 a | 0.29 ± 0.11 bcd | 0.42 ± 0.07 d | 0.39 ± 0.00 cd | 0.04 ± 0.00 e | 0.04 ± 0.01 e | 0.28 ± 0.02 bc | 0.27 ± 0.08 bc | 0.03 ± 0.00 e | 0.41 ± 0.01 d | | | |
| Citronellol | 0.09 ± 0.03 abc | 0.14 ± 0.02 b | 0.04 ± 0.00 ac | 0.03 ± 0.01 a | 0.01 ± 0.00 a | 0.04 ± 0.00 ac | 0.03 ± 0.02 a | 0.03 ± 0.01 a | 0.09 ± 0.00 abc | nd | nd | 0.04 ± 0.02 ac | 0.12 ± 0.09 bc | nd | 0.06 ± 0.00 abc | 0.1 A | Lemon green | 0.1–1 |
| Linalool | 0.20 ± 0.00 a | 0.20 ± 0.02 a | 0.13 ± 0.00 c | 0.09 ± 0.07 bcd | nd | 0.12 ± 0.07 bc | 0.12 ± 0.04 bc | 0.04 ± 0.03 d | 0.09 ± 0.01 bcd | 0.04 ± 0.00 d | nd | 0.07 ± 0.02 bcd | 0.06 ± 0.00 bd | nd | 0.08 ± 0.01 bcd | 0.015 A | Floral, fruity, muscat | >1 |
| Nerolidol | 0.03 ± 0.00 a | 0.08 ± 0.00 b | nd | nd | nd | nd | 0.02 ± 0.02 a | nd | 0.03 ± 0.02 a | nd | nd | nd | nd | nd | 0.08 ± 0.02 b | 0.7 A | Rose, apple, citrus | <0.1 |
| cis-Geraniol | 0.10 ± 0.01 a | 0.11 ± 0.01 ab | 0.08 ± 0.00 ab | 0.05 ± 0.02 bc | nd | 0.07 ± 0.02 abc | 0.03 ± 0.01 c | nd | 0.06 ± 0.01 bc | nd | nd | nd | nd | nd | nd | 0.03 A | Tropical fruity, grass | >1 |
| Geranyl acetone | 0.09 ± 0.00 a | 0.03 ± 0.01 a | 0.04 ± 0.00 a | 0.01 ± 0.00 a | nd | 0.34 ± 0.30 b | nd | nd | 0.06 ± 0.01 a | nd | 0.04 ± 0.01 a | 0.03 ± 0.00 a | 0.03 ± 0.00 a | nd | 0.08 ± 0.01 a | 0.06 A | Floral | >1 |
| Citronellol acetate | 0.04 ± 0.00 ab | 0.05 ± 0.01 bc | nd | 0.03 ± 0.02 a | nd | 0.03 ± 0.01 a | 0.02 ± 0.01 a | 0.04 ± 0.00 ab | 0.04 ± 0.00 ab | nd | nd | 0.06 ± 0.00 cd | 0.05 ± 0.01 bc | 0.03 ± 0.00 a | 0.07 ± 0.01 d | 0.25 A | Fresh, fruity, rose | 0.1–1 |
| Nerol acetate | nd | 0.05 ± 0.01 ab | 0.04 ± 0.00 ab | 0.02 ± 0.01 a | nd | 0.04 ± 0.02 ab | 0.06 ± 0.00 bc | 0.31 ± 0.04 d | 0.03 ± 0.01 ab | nd | nd | 0.08 ± 0.01 c | nd | nd | 0.05 ± 0.00 ab | 3 A | Petroleum, floral | <0.1 |
| Fermentative aroma | 1147.85 ± 36.10 d | 994.90 ± 39.98 cd | 664.89 ± 113.19 ab | 550.22 ± 313.48 a | 730.68 ± 88.83 abc | 501.42 ± 201.99 a | 484.50 ± 118.34 a | 955.18 ± 285.05 bcd | 493.74 ± 91.45 a | 659.31 ± 116.11 ab | 503.41 ± 87.35 a | 887.96 ± 62.95 bcd | 690.92 ± 35.70 ab | 716.14 ± 140.83 abc | 1044.55 ± 317.72 d | | | |
| Higher alcohols | 273.39 ± 1.86 abc | 373.92 ± 9.30 d | 286.57 ± 9.48 ac | 157.88 ± 83.94 e | 424.30 ± 15.74 d | 171.99 ± 32.41 e | 288.55 ± 54.81 ac | 424.05 ± 61.17 d | 214.03 ± 29.71 be | 294.55 ± 16.55 a | 219.85 ± 43.71 bce | 263.5 ± 26.53 abc | 370.82 ± 6.01 d | 218.9 ± 37.86 bce | 296.35 ± 13.32 a | | | |
| 1-Butanol | 0.49 ± 0.01 a | 0.24 ± 0.02 b | 0.85 ± 0.03 cd | 0.46 ± 0.26 a | 0.57 ± 0.02 a | 0.92 ± 0.10 cd | 0.70 ± 0.14 ac | 1.50 ± 0.21 e | nd | 1.04 ± 0.12 d | 0.58 ± 0.12 a | 1.49 ± 0.19 e | 2.59 ± 0.13 f | 1.05 ± 0.16 d | 1.51 ± 0.01 e | 150 A | Medicinal, alcohol | <0.1 |
| 1-Decanol | 0.12 ± 0.01 a | 0.11 ± 0.01 ab | 0.09 ± 0.02 bc | 0.05 ± 0.02 ef | 0.05 ± 0.01 efg | 0.07 ± 0.03 cde | nd | 0.05 ± 0.00 efg | 0.09 ± 0.00 b | 0.07 ± 0.01 de | 0.02 ± 0.00 g | 0.05 ± 0.00 ef | 0.04 ± 0.01 fg | nd | 0.08 ± 0.00 bcd | 0.4 A | Orange, flowery | 0.1–1 |
| 1-Nonanol | 0.46 ± 0.04 a | 0.34 ± 0.02 abc | 0.18 ± 0.09 de | 0.26 ± 0.14 be | 0.04 ± 0.00 a | 0.37 ± 0.08 abc | 0.05 ± 0.01 ab | 0.10 ± 0.05 ab | nd | nd | 0.11 ± 0.00 ab | 0.43 ± 0.12 ac | 0.25 ± 0.06 be | 0.14 ± 0.08 def | 0.32 ± 0.10 bc | 0.6 A | Green | 0.1–1 |
| 1-Octanol | 0.48 ± 0.01 abc | 0.61 ± 0.02 b | 0.50 ± 0.10 abc | 0.37 ± 0.20 acde | nd | 0.52 ± 0.21 ab | 0.26 ± 0.05 de | 0.34 ± 0.03 cde | 0.35 ± 0.07 cde | 0.30 ± 0.04 de | 0.21 ± 0.03 d | 0.39 ± 0.08 ace | 0.33 ± 0.01 cde | 0.23 ± 0.04 de | 0.35 ± 0.03 cde | 0.9 A | Intense citrus, rose | 0.1–1 |
| Isobutanol | 7.33 ± 0.06 a | 13.15 ± 0.56 b | 7.02 ± 0.25 ac | 3.42 ± 1.79 d | 11.84 ± 1.56 be | 3.82 ± 0.48 dg | 10.10 ± 2.90 ef | 8.08 ± 1.28 af | 35.30 ± 2.30 h | 10.72 ± 0.80 e | 7.91 ± 1.55 af | 5.91 ± 1.01 acg | 7.25 ± 0.27 a | 4.65 ± 0.80 cdg | 5.92 ± 0.17 acg | 40 A | Alcohol, nail polish | 0.1–1 |
| Isoamylol | 173.94 ± 0.74 ab | 242.62 ± 15.47 cd | 195.45 ± 7.10 abd | 112.15 ± 59.51 e | 308.35 ± 6.20 g | 117.89 ± 22.43 e | 196.23 ± 36.84 ad | 295.50 ± 42.05 fg | 176.94 ± 27.40 ab | 187.96 ± 9.52 ab | 145.6 ± 29.10 be | 187.94 ± 18.17 ab | 257.89 ± 5.10 cf | 158.45 ± 27.94 abe | 205.41 ± 8.41 ad | 30 A | Whisky, nail polish | >1 |
| 2-Methyl-1-butanol | 85.02 ± 0.82 ab | 113.79 ± 5.89 c | 75.57 ± 2.64 ad | 37.52 ± 19.97 e | 100.23 ± 8.49 bc | 43.71 ± 8.32 ef | 78.32 ± 15.19 ad | 111.95 ± 16.87 c | nd | 88.36 ± 5.96 ab | 61.81 ± 12.48 dg | 56.11 ± 5.38 fg | 85.81 ± 1.12 ab | 43.97 ± 8.03 ef | 65.57 ± 2.76 dg | 65 A | Pungent odor | >1 |
| 2-Ethyl-1-hexanol | 0.18 ± 0.03 ab | 0.07 ± 0.02 a | 0.61 ± 0.05 c | 0.37 ± 0.19 bd | nd | 1.15 ± 0.39 e | 0.40 ± 0.07 bcd | 0.06 ± 0.01 a | 0.05 ± 0.01 a | 0.43 ± 0.05 cd | 0.19 ± 0.02 ab | 1.21 ± 0.18 e | 1.13 ± 0.07 e | 0.34 ± 0.03 bd | 0.33 ± 0.01 bd | 8 A | Mushroom; sweet fruity | 0.1–1 |
| 3-Methyl-1-pentanol | 0.33 ± 0.00 ab | 0.37 ± 0.02 ab | 0.52 ± 0.07 bc | 0.28 ± 0.14 ab | 0.52 ± 0.03 bc | 0.32 ± 0.11 ab | 0.24 ± 0.02 a | 2.76 ± 0.37 d | 0.35 ± 0.18 ab | 0.68 ± 0.04 ce | 0.53 ± 0.09 bc | 1.44 ± 0.20 f | 2.65 ± 0.01 d | 0.89 ± 0.12 e | 1.83 ± 0.12 g | 0.5 A | Alcohol, harsh | >1 |
| 4-Methyl-1-pentanol | 0.66 ± 0.28 ab | 0.28 ± 0.03 cde | 0.69 ± 0.16 ab | 0.29 ± 0.24 cde | 0.14 ± 0.01 d | 0.50 ± 0.06 ace | 0.32 ± 0.05 cde | 0.94 ± 0.28 bf | 0.13 ± 0.07 d | nd | 0.22 ± 0.05 de | 0.56 ± 0.00 ac | 0.79 ± 0.24 ab | 0.49 ± 0.21 ace | 1.07 ± 0.03 f | 50 A | Almond, toasted | <0.1 |
| 2,3-Butanediol | 4.38 ± 0.19 a | 2.37 ± 0.29 b | 5.09 ± 0.33 a | 2.70 ± 1.50 b | 2.55 ± 0.47 b | 2.71 ± 0.33 b | 1.93 ± 0.19 bc | 2.76 ± 0.17 b | 0.82 ± 0.31 c | 4.99 ± 0.01 a | 2.68 ± 0.28 b | 7.97 ± 1.48 d | 12.09 ± 0.70 e | 8.69 ± 0.46 d | 13.96 ± 1.74 f | 150 B | Buttery, creamy | <0.1 |
| Fatty acids | 13.05 ± 0.48 ab | 5.98 ± 0.03 c | 9.96 ± 1.18 de | 6.68 ± 3.55 cg | 2.34 ± 0.39 f | 10.55 ± 2.16 ade | 2.27 ± 0.66 f | 4.84 ± 0.47 cf | 11.80 ± 2.93 abe | 8.70 ± 1.22 dg | 5.20 ± 0.25 c | 11.25 ± 0.74 abde | 6.06 ± 0.76 c | 5.46 ± 0.76 c | 13.27 ± 0.94 b | | | |
| Butanoic acid | 0.03 ± 0.01 ab | 0.08 ± 0.01 abcd | 0.32 ± 0.00 e | 0.20 ± 0.09 f | nd | 0.13 ± 0.08 cdf | 0.01 ± 0.00 a | 0.11 ± 0.01 bcd | 0.06 ± 0.03 abcd | 0.05 ± 0.02 abc | nd | 0.02 ± 0.00 a | 0.14 ± 0.11 df | nd | 0.01 ± 0.00 a | 10 A | Cheese | <1 |
| Decanoic acid | 0.90 ± 0.05 a | 0.52 ± 0.01 bcd | 0.34 ± 0.06 bce | 0.33 ± 0.16 bce | 0.06 ± 0.01 e | 0.59 ± 0.20 bcd | 0.14 ± 0.03 e | 0.24 ± 0.01 be | 2.40 ± 0.66 f | 0.79 ± 0.07 ad | 0.34 ± 0.02 bce | 0.65 ± 0.07 acd | 0.26 ± 0.06 be | 0.29 ± 0.05 bce | 0.86 ± 0.05 ad | 15 A | Fatty, unpleasant | <0.1 |
| Hexanoic acid | 4.40 ± 0.12 a | 1.51 ± 0.03 bcd | 3.44 ± 0.41 fg | 2.46 ± 1.34 eh | 1.11 ± 0.19 bd | 3.54 ± 0.57 fg | 0.79 ± 0.16 d | 1.69 ± 0.18 bch | 1.22 ± 0.83 bd | 2.84 ± 0.16 ef | 2.01 ± 0.02 bceh | 3.53 ± 0.33 fg | 2.24 ± 0.24 ceh | 1.70 ± 0.24 bch | 4.19 ± 0.38 ag | 3 B | Cheese, fatty | >1 |
| Octanoic acid | 7.22 ± 0.37 ab | 3.51 ± 0.04 cd | 5.40 ± 0.75 ef | 3.53 ± 1.86 cd | 1.16 ± 0.19 g | 6.07 ± 1.44 af | 1.25 ± 0.49 gh | 2.69 ± 0.29 ch | 8.12 ± 1.41 b | 4.26 ± 0.95 de | 2.65 ± 0.30 ch | 6.78 ± 0.31 abf | 2.73 ± 0.57 ceh | 3.35 ± 0.43 cd | 7.83 ± 0.50 b | 0.5 A | Cheese, rancid, harsh | >1 |
| Isobutyric acid | 0.27 ± 0.02 a | 0.19 ± 0.05 b | 0.22 ± 0.02 bc | 0.09 ± 0.06 de | nd | 0.11 ± 0.03 d | 0.08 ± 0.02 de | 0.11 ± 0.00 d | nd | 0.39 ± 0.00 f | 0.08 ± 0.02 de | 0.10 ± 0.01 de | 0.24 ± 0.03 ac | 0.06 ± 0.00 e | 0.11 ± 0.02 d | 2.3 B | Cheese, butter, rancid | <0.1 |
| Isovaleric acid | 0.23 ± 0.03 a | 0.18 ± 0.02 b | 0.25 ± 0.02 a | 0.07 ± 0.04 cd | nd | 0.12 ± 0.01 c | nd | nd | nd | 0.37 ± 0.01 e | 0.11 ± 0.03 c | 0.17 ± 0.02 b | 0.45 ± 0.02 f | 0.06 ± 0.04 d | 0.27 ± 0.02 a | 0.0334 A | Fatty, sweet | >1 |

**Table 2.** *Cont.*

| Aroma Compounds | FSCw | F4-13w | F5-7w | F5-9w | F5-12w | F5-18w | F5-19w | F5-21w | F6-8w | F6-23w | F9-23w | SXY-4w | HT-10w | ZXY-17w | MXY-19w | Threshold * | Description | OVA |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Acetates | 240.75 ± 8.61 ab | 202.96 ± 12.81 ab | 92.31 ± 37.42 cd | 84.45 ± 48.89 cd | 92.83 ± 23.27 cd | 45.27 ± 23.16 c | 57.73 ± 17.26 c | 225.88 ± 123.01 ab | 94.18 ± 26.13 cd | 101.88 ± 42.84 cd | 58.18 ± 18.82 c | 169.14 ± 12.17 ad | 81.42 ± 5.60 cd | 197.03 ± 44.92 ab | 279.77 ± 133 b | | | |
| Ethyl acetate | 59.68 ± 2.36 a | 57.27 ± 4.62 a | 39.08 ± 5.48 bc | 29.97 ± 16.75 bcd | 67.16 ± 14.30 ae | 19.14 ± 4.78 d | 21.34 ± 1.95 d | 61.72 ± 14.61 ae | 18.34 ± 1.68 d | 29.55 ± 4.05 bcd | 27.26 ± 1.36 bd | 43.19 ± 2.03 c | 27.51 ± 0.46 bd | 40.26 ± 9.17 bc | 74.62 ± 8.26 e | 7.5 A | Fruity | >1 |
| Butyl acetate | 1.48 ± 0.05 abc | 0.20 ± 0.02 a | 3.35 ± 1.57 cd | 2.64 ± 1.52 bc | nd | 4.97 ± 2.93 d | 2.34 ± 0.82 abc | 0.41 ± 0.20 a | nd | 2.79 ± 1.27 bc | 1.09 ± 0.43 ab | 7.15 ± 0.69 e | 7.64 ± 0.53 e | 2.93 ± 0.69 bcd | 1.63 ± 0.80 abc | 1.6 B | Pleasant, fruity | >1 |
| Hexyl acetate | 1.88 ± 0.00 a | 0.78 ± 0.01 bc | 0.55 ± 0.33 bc | 0.41 ± 0.24 bc | 0.10 ± 0.03 c | 0.33 ± 0.24 bc | 0.32 ± 0.12 bc | 0.97 ± 0.62 bd | 0.54 ± 0.17 bc | 0.63 ± 0.30 bc | 0.20 ± 0.08 c | 1.04 ± 0.05 bd | 0.52 ± 0.07 bc | 1.51 ± 0.33 ad | 1.74 ± 1.16 a | 1.5 A | Pleasant, fruity, pear | 0.1–1 |
| Isobutyl acetate | 1.32 ± 0.03 a | 1.15 ± 0.06 ab | 0.35 ± 0.15 c | 0.32 ± 0.18 c | 0.21 ± 0.07 c | 0.17 ± 0.09 c | 0.27 ± 0.08 c | 0.84 ± 0.46 abc | 0.57 ± 0.07 bc | 0.65 ± 0.27 abc | 0.38 ± 0.13 c | 0.60 ± 0.05 bc | 0.31 ± 0.00 c | 0.78 ± 0.21 abc | 1.34 ± 0.61 a | 1.6 A | Fruity, apple, banana | 0.1–1 |
| Isoamyl acetate | 159.30 ± 5.57 a | 131.16 ± 7.55 acd | 45.17 ± 27.61 be | 47.1 ± 27.79 be | 23.05 ± 8.10 e | 19.08 ± 13.95 e | 30.70 ± 16.75 be | 148.19 ± 97.84 ad | 74.72 ± 24.21 bcde | 61.73 ± 33.50 bce | 26.62 ± 15.40 e | 109.86 ± 8.68 abcd | 41.97 ± 4.21 be | 142.25 ± 32.35 ad | 184.77 ± 112.22 a | 0.03 A | Banana, fruity, sweet | >1 |
| 2-Methybutyl acetate | 17.07 ± 0.61 a | 12.41 ± 0.58 abc | 3.80 ± 2.29 d | 4.02 ± 2.42 d | 2.32 ± 0.77 d | 1.59 ± 1.16 d | 2.76 ± 1.44 d | 13.75 ± 9.27 abc | nd | 6.52 ± 3.44 bd | 2.63 ± 1.41 d | 7.30 ± 0.67 bd | 3.47 ± 0.33 d | 9.29 ± 2.18 bcd | 15.68 ± 9.96 ac | 0.02–0.05 A | Fruity, comfort fat | >1 |
| .1Ethyl esters | 478.95 ± 15.94 a | 270.43 ± 5.26 bcd | 202.36 ± 63.10 bce | 261.46 ± 155.03 bcd | 190.05 ± 43.89 bce | 210.60 ± 128.38 bce | 80.87 ± 32.41 e | 209.05 ± 93.65 bce | 132.04 ± 32.14 be | 175.82 ± 54.74 bce | 129.27 ± 19.66 be | 289.89 ± 16.02 cd | 138.79 ± 11.83 bce | 196.39 ± 42.52 bce | 314.15 ± 167.43 d | | | |
| Ethyl caprate | 101.47 ± 3.59 a | 56.60 ± 0.91 bc | 41.10 ± 3.99 bc | 60.17 ± 36.04 abc | 19.82 ± 1.13 c | 48.72 ± 25.73 bc | 17.85 ± 6.39 c | 37.31 ± 8.33 bc | 31.94 ± 5.51 bc | 49.81 ± 8.93 bc | 34.29 ± 4.67 bc | 69.73 ± 3.05 ab | 27.96 ± 0.31 bc | 36.24 ± 7.60 bc | 64.64 ± 27.20 ab | 0.2 B | Sweet, fruity | >1 |
| Ethyl butyrate | 3.26 ± 0.08 ab | 1.90 ± 0.13 acde | 2.05 ± 1.09 acde | 2.00 ± 1.15 acde | 4.56 ± 1.48 acde | 0.87 ± 0.53 de | 0.50 ± 0.15 d | 2.82 ± 1.73 ac | 3.07 ± 1.04 ab | 1.15 ± 0.50 de | 0.93 ± 0.44 de | 1.01 ± 0.14 de | 0.81 ± 0.05 de | 1.36 ± 0.33 cde | 2.22 ± 1.13 ace | 0.02 A | Strawberry, apple | >1 |
| Ethyl isobutyrate | 0.08 ± 0.00 ab | 0.11 ± 0.02 b | 0.03 ± 0.00 c | 0.02 ± 0.01 ac | nd | nd | 0.02 ± 0.01 c | 0.05 ± 0.03 ac | nd | 0.06 ± 0.05 abc | 0.02 ± 0.01 ac | nd | nd | 0.03 ± 0.02 ac | 0.03 ± 0.01 ac | 0.015 B | Strawberry | >1 |
| Ethyl dodecanoate | 7.36 ± 0.11 a | 4.09 ± 0.13 bcd | 4.29 ± 0.27 bcd | 5.88 ± 3.10 ab | 0.93 ± 0.10 f | 4.32 ± 1.78 bcd | 1.78 ± 0.54 ef | 2.90 ± 0.31 de | 4.07 ± 0.02 bcd | 5.80 ± 0.47 ab | 3.32 ± 0.50 cde | 4.87 ± 0.18 bc | 2.63 ± 0.05 def | 2.42 ± 0.42 def | 5.16 ± 1.49 bc | 0.5 A | Sweet, floral, fruity | >1 |
| Ethyl propionate | 0.89 ± 0.01 a | 0.72 ± 0.08 ab | 0.29 ± 0.11 cd | 0.19 ± 0.10 cd | 0.24 ± 0.08 cd | 0.08 ± 0.04 c | 0.18 ± 0.01 cd | 0.44 ± 0.20 bde | 0.08 ± 0.01 c | 0.60 ± 0.20 abe | 0.41 ± 0.12 de | 0.19 ± 0.05 cd | 0.22 ± 0.01 cd | 0.21 ± 0.04 cd | 0.45 ± 0.13 bde | 1.8 B | Banana; apple | 0.1–1 |
| Ethyl nonanoate | 1.12 ± 0.02 a | 0.54 ± 0.03 bcd | 0.27 ± 0.04 bd | 0.37 ± 0.22 bd | 0.17 ± 0.01 d | 0.31 ± 0.19 bd | 0.14 ± 0.04 d | 0.34 ± 0.12 bd | 0.45 ± 0.07 bd | 0.91 ± 0.22 ac | 0.37 ± 0.04 bd | 0.59 ± 0.00 bc | 0.41 ± 0.04 bd | 0.38 ± 0.07 bd | 0.62 ± 0.32 bc | 1.3 B | Banana, grape | 0.1–1 |
| Ethyl hexanoate | 92.45 ± 2.35 a | 44.8 ± 1.14 bc | 30.01 ± 17.87 bcd | 43.10 ± 25.48 bcd | 44.1 ± 15.53 bc | 22.96 ± 17.09 bcd | 10.19 ± 4.90 d | 44.34 ± 31.14 bc | 32.87 ± 9.57 bcd | 24.16 ± 13.31 bcd | 16.53 ± 9.68 bd | 33.84 ± 2.34 bcd | 21.29 ± 2.52 bd | 37.16 ± 8.10 bcd | 55.35 ± 39.22 c | 0.003 B | Banana, sweet fruity | >1 |
| Ethyl heptanoate | 0.38 ± 0.01 a | 0.12 ± 0.01 bcd | 0.10 ± 0.05 bcd | 0.10 ± 0.06 bcd | 0.06 ± 0.04 d | 0.07 ± 0.05 bd | 0.05 ± 0.01 d | 0.08 ± 0.04 bd | 0.10 ± 0.03 bcd | 0.17 ± 0.05 c | 0.07 ± 0.04 bd | 0.10 ± 0.00 bcd | 0.09 ± 0.01 bd | 0.07 ± 0.02 bd | 0.15 ± 0.09 bc | 0.22 B | Pineapple, fruity | 0.1–1 |
| Ethyl tetradecanoate | 0.13 ± 0.00 ab | 0.09 ± 0.00 cde | 0.08 ± 0.00 ce | 0.09 ± 0.04 cde | 0.02 ± 0.00 d | 0.06 ± 0.02 cde | 0.06 ± 0.01 ce | 0.06 ± 0.00 cde | 0.26 ± 0.01 f | 0.16 ± 0.01 b | 0.13 ± 0.02 ab | 0.09 ± 0.01 cde | 0.06 ± 0.02 cde | 0.05 ± 0.01 de | 0.10 ± 0.00 ac | 2 B | Mild waxy, soapy | 0.1–1 |
| Ethyl hexadecanoate | 0.17 ± 0.01 a | 0.17 ± 0.00 a | 0.11 ± 0.01 ab | 0.10 ± 0.06 ab | nd | 0.09 ± 0.06 ab | 0.07 ± 0.01 b | 0.09 ± 0.03 ab | 0.12 ± 0.01 ab | 0.16 ± 0.01 ab | 0.15 ± 0.00 ab | 0.15 ± 0.01 ab | 0.07 ± 0.02 b | 0.12 ± 0.05 ab | 0.13 ± 0.05 ab | 1.5 B | Fruity, sweet, fatty | 0.1–1 |
| Ethyl octanoate | 268.88 ± 9.71 a | 159.45 ± 4.92 bcd | 122.20 ± 39.52 bcde | 147.82 ± 87.82 bcd | 120.13 ± 27.99 bcde | 132.17 ± 82.41 bcde | 48.48 ± 19.73 e | 120.60 ± 51.72 bcd | 57.00 ± 15.76 e | 88.08 ± 30.22 bce | 72.13 ± 14.77 be | 176.44 ± 10.20 cd | 84.42 ± 8.80 be | 117.72 ± 25.77 bcde | 182.21 ± 96.53 d | 0.002 B | Floral, pineapple, pear | >1 |
| Ethyl 3-hydroxybutanoate | 0.05 ± 0.00 ab | 0.09 ± 0.06 bc | 0.03 ± 0.00 ab | 0.03 ± 0.01 ab | 0.02 ± 0.01 ab | 0.06 ± 0.01 ab | nd | 0.03 ± 0.00 ab | 0.05 ± 0.01 ab | nd | 0.02 ± 0.00 ab | 0.13 ± 0.10 a | 0.03 ± 0.01 c | 0.01 ± 0.01 ab | 0.05 ± 0.04 a | 20 B | Sweet fruity, grape | <0.1 |
| Ethyl 9-decenoate | 2.71 ± 0.08 a | 1.75 ± 0.06 ab | 1.81 ± 0.14 ab | 1.59 ± 0.95 ab | nd | 0.89 ± 0.46 a | 1.54 ± 0.62 ab | nd | 2.03 ± 0.14 ab | 4.76 ± 0.79 c | 0.90 ± 0.14 b | 2.75 ± 0.15 a | 0.81 ± 0.01 b | 0.61 ± 0.11 b | 3.06 ± 1.23 a | 0.1 B | Fruity | >1 |
| Other esters | 2.62 ± 0.08 a | 1.54 ± 0.01 abcd | 1.15 ± 0.14 bcde | 1.44 ± 0.83 bcde | 0.60 ± 0.06 be | 1.18 ± 0.56 bcde | 0.44 ± 0.14 e | 1.26 ± 0.35 bcde | 0.88 ± 0.13 bcde | 1.23 ± 0.27 bcde | 0.71 ± 0.00 bce | 1.76 ± 0.18 acd | 0.84 ± 0.10 bcde | 0.78 ± 0.19 bce | 1.93 ± 0.83 ad | | | |
| Diethyl succinate | 0.40 ± 0.01 a | 0.17 ± 0.02 bc | 0.17 ± 0.01 bcd | 0.14 ± 0.07 bc | 0.11 ± 0.03 bc | 0.19 ± 0.01 bd | 0.07 ± 0.02 bc | 0.05 ± 0.03 d | 0.08 ± 0.01 bc | 0.11 ± 0.02 bc | 0.05 ± 0.00 d | 0.35 ± 0.11 a | 0.13 ± 0.04 bc | 0.08 ± 0.02 bc | 0.30 ± 0.06 ad | 200 B | Light fruity, wine | <0.1 |
| Isopentyl hexanoate | 0.91 ± 0.04 a | 0.54 ± 0.00 abc | 0.36 ± 0.11 bc | 0.46 ± 0.27 abc | 0.27 ± 0.05 bc | 0.31 ± 0.23 bc | 0.13 ± 0.05 c | 0.50 ± 0.23 abc | 0.29 ± 0.06 bc | 0.37 ± 0.16 bc | 0.20 ± 0.04 bc | 0.49 ± 0.02 abc | 0.26 ± 0.04 bc | 0.27 ± 0.10 bc | 0.63 ± 0.35 ab | 0.32 D | Fennel, fruity, spices | >1 |
| 3-Methylbutyl octanoate | 1.31 ± 0.03 a | 0.83 ± 0.04 abc | 0.62 ± 0.03 bc | 0.84 ± 0.00 abc | 0.22 ± 0.02 c | 0.68 ± 0.32 bc | 0.24 ± 0.07 c | 0.71 ± 0.14 bc | 0.51 ± 0.07 bc | 0.75 ± 0.13 abc | 0.46 ± 0.04 bc | 0.93 ± 0.05 abc | 0.45 ± 0.02 bc | 0.43 ± 0.07 bc | 1.00 ± 0.41 abc | 0.125 D | Sweet | >1 |
| Benzene derivatives | 137.28 ± 8.94 a | 138.93 ± 12.40 a | 71.02 ± 1.59 cd | 37.35 ± 20.79 bg | 20.52 ± 5.48 g | 60.61 ± 15.26 cd | 53.86 ± 13.02 bc | 88.82 ± 6.24 ef | 40.72 ± 0.40 b | 74.93 ± 0.36 de | 89.38 ± 4.89 ef | 148.21 ± 6.48 a | 91.14 ± 11.28 ef | 95.62 ± 13.99 f | 134.75 ± 2.12 a | | | |
| Benzyl alcohol | 0.12 ± 0.05 ab | 0.08 ± 0.01 acd | 0.07 ± 0.01 acd | 0.05 ± 0.04 cd | nd | 0.05 ± 0.00 cd | 0.05 ± 0.00 cd | 0.04 ± 0.00 cd | 0.05 ± 0.00 cd | 0.08 ± 0.06 cd | 0.03 ± 0.01 acd | 0.08 ± 0.01 c | 0.07 ± 0.03 abd | 0.07 ± 0.00 cd | 0.12 ± 0.03 cd | 200 A | Almond | <0.1 |
| Benzaldehyde | 0.12 ± 0.01 ab | 0.09 ± 0.00 a | 0.20 ± 0.05 c | nd | nd | 0.17 ± 0.01 bc | 0.10 ± 0.01 a | 0.26 ± 0.03 de | 0.11 ± 0.04 a | 0.21 ± 0.02 c | 0.09 ± 0.01 a | 0.22 ± 0.04 cd | 0.21 ± 0.02 c | 0.19 ± 0.04 c | 0.28 ± 0.03 e | 0.3 B | Sweet, ferity | 0.1–1 |
| Benzene acetaldehyde | 0.05 ± 0.01 a | 0.07 ± 0.03 ab | 0.04 ± 0.03 a | 0.05 ± 0.03 a | nd | 0.16 ± 0.13 c | nd | 0.05 ± 0.00 ab | 0.02 ± 0.00 a | 0.07 ± 0.02 ab | 0.05 ± 0.01 a | 0.06 ± 0.01 ab | 0.07 ± 0.00 ab | 0.06 ± 0.01 ab | 0.13 ± 0.04 bc | 0.004 A | Sugar, floral | >1 |
| Phenylethyl alcohol | 108.40 ± 7.57 ab | 117.85 ± 10.97 b | 55.46 ± 2.00 cd | 31.53 ± 17.85 fg | 19.90 ± 5.40 f | 45.38 ± 10.74 cg | 37.13 ± 8.11 g | 67.78 ± 4.17 de | 32.60 ± 1.14 fg | nd | 77.17 ± 5.16 e | 99.59 ± 3.62 a | 77.03 ± 10.27 e | 67.74 ± 9.12 de | 98.23 ± 0.05 a | 10 A | Rose, pollen, perfume | >1 |
| 2-Phenethyl acetate | 28.60 ± 1.33 a | 20.84 ± 1.40 b | 15.25 ± 0.37 c | 5.72 ± 2.87 d | 0.63 ± 0.08 f | 14.85 ± 4.63 c | 16.58 ± 4.91 be | 20.69 ± 2.03 b | 7.93 ± 0.71 de | 74.58 ± 0.34 i | 12.04 ± 0.27 ce | 48.26 ± 2.82 h | 13.77 ± 0.95 c | 27.58 ± 4.85 a | 35.99 ± 2.20 f | 0.25 A | Floral, pleasant | >1 |
| Aldoketones | 1.81 ± 0.19 aef | 1.14 ± 0.17 bc | 1.52 ± 0.28 ace | 0.96 ± 0.45 bc | 0.04 ± 0.00 d | 1.22 ± 0.06 bc | 0.78 ± 0.04 b | 1.28 ± 0.16 abc | 0.09 ± 0.01 d | 2.20 ± 0.13 f | 0.82 ± 0.02 b | 4.21 ± 0.83 g | 1.85 ± 0.12 ef | 1.96 ± 0.59 ef | 4.33 ± 0.08 g | | | |

**Table 2.** *Cont.*

| Aroma Compounds | FSCw | F4-13w | F5-7w | F5-9w | F5-12w | F5-18w | F5-19w | F5-21w | F6-8w | F6-23w | F9-23w | SXY-4w | HT-10w | ZXY-17w | MXY-19w | Threshold * | Description | OVA |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Decanal | 0.11 ± 0.02 abc | 0.07 ± 0.00 abc | 0.17 ± 0.06 bcd | 0.05 ± 0.04 ab | 0.04 ± 0.00 a | 0.12 ± 0.08 abc | 0.03 ± 0.00 a | 0.07 ± 0.02 abc | 0.09 ± 0.01 abc | 0.07 ± 0.00 abc | 0.03 ± 0.01 a | 0.17 ± 0.03 cd | 0.23 ± 0.02 d | 0.05 ± 0.01 ab | 0.14 ± 0.09 abcd | 0.00125 C | Soap, orange peel | >1 |
| Nonanal | 0.04 ± 0.01 a | 0.05 ± 0.01 ab | 0.08 ± 0.00 bc | 0.10 ± 0.01 c | nd | 0.08 ± 0.06 bc | 0.05 ± 0.01 ab | 0.05 ± 0.01 ab | nd | nd | 0.02 ± 0.00 a | 0.17 ± 0.02 d | 0.16 ± 0.01 d | 0.02 ± 0.01 a | 0.02 ± 0.00 a | 0.015 C | Green, slightly pungent | >1 |
| 2,3-Butanedione | 1.67 ± 0.22 abc | 1.02 ± 0.15 cd | 1.27 ± 0.22 cde | 0.81 ± 0.40 c | nd | 1.03 ± 0.08 cd | 0.70 ± 0.05 c | 1.16 ± 0.13 cde | nd | 2.13 ± 0.12 c | 0.77 ± 0.02 c | 3.88 ± 0.79 f | 1.46 ± 0.09 abd | 1.89 ± 0.58 bc | 4.16 ± 0.00 f | 0.2–0.3 D | Cream, essences | >1 |
| Total aroma | 1149.74 ± 36. 19 d | 996.60 ± 40.03 cd | 665.90 ± 113.42 ab | 551.25 ± 313.65 a | 731.32 ± 88.85 abc | 502.78 ± 202.62 a | 485.21 ± 118.64 a | 956.22 ± 285.42 bcd | 495.02 ± 91.52 a | 659.95 ± 116.38 ab | 503.87 ± 87.49 a | 889.34 ± 62.99 bcd | 692.28 ± 35.87 ab | 717.05 ± 140.88 abc | 1029.04 ± 318.19 d | | | |

Note: Values with different lowercase letters in the same row are significantly different according to the Duncan test ($p < 0.05$). nd indicates not detected, NF indicates not found, *: mg/L. A: [41]; B: [42]; C: [43] D: [44].

## Varietal Aromas Compounds of Wines

The $C_6$ compound contents in SCw (0.42~1.10 mg/L) were slightly lower than those in FSCw (1.33 mg/L) (Table 2 and Figure 5A), which mainly presented an unpleasant green odor in the wines. The terpene contents in the wines were 0.01~0.66 mg/L, and F4-13w (0.66 mg/L) and F5-18w (0.64 mg/L) generated higher terpene contents than FSCw (0.56 mg/L) (Figure 5B). Terpenes with floral and fruity traits had a positive effect on the varietal aroma of the wines [41,45]. Linalool, cis-geraniol, and geranyl acetone were the odor-active compounds (OAV $\geq$ 1) of the wines (Table 2). Liu, et al. [46] reported that the selected indigenous *S. cerevisiae* strains improved terpene synthesis and contributed a pleasant aroma to the wines. These results mean that the selected *S. cerevisiae* strains slightly decreased the green flavor of wine, and *S. cerevisiae* F4-13 and F5-18 had great potential in highlighting the varietal aroma characteristics of wines.

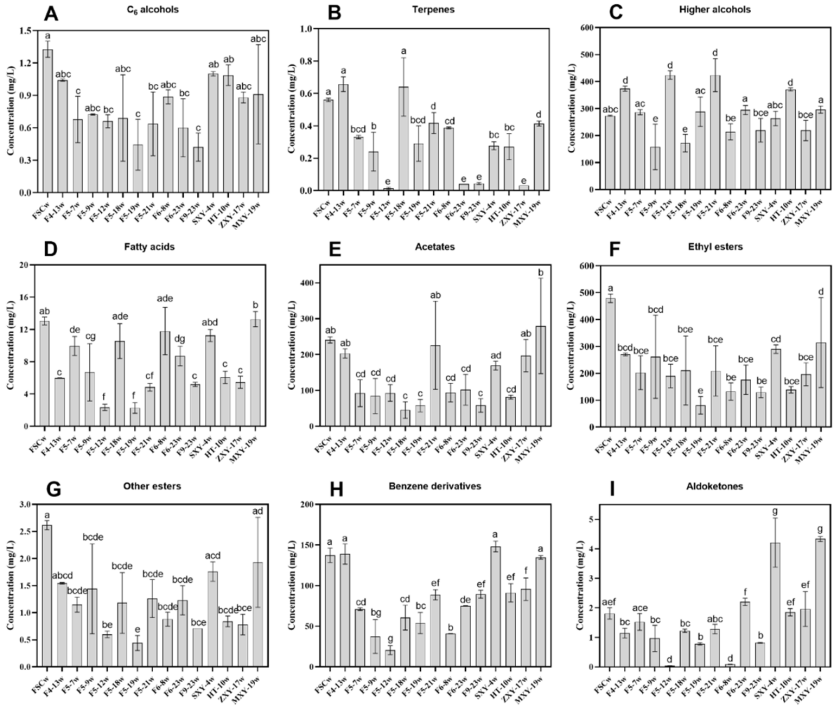


**Figure 5.** Categories and contents of the aroma compounds in wines fermented by the selected *S. cerevisiae* strains ((**A**): $C_6$ compounds; (**B**): terpenes; (**C**): higher alcohols; (**D**): fatty acids; (**E**): acetates; (**F**): ethyl esters; (**G**): other esters; (**H**): benzene derivatives; (**I**): aldoketones). Different lowercase letters indicate significant differences ($p < 0.05$).

## Fermentative Aroma Compounds of Wines

As shown in Table 2, FSCw had the highest content of fermentative aroma compounds (1147.85 mg/L), followed by MXY-19w (1044.55 mg/L), F4-13w (994.90 mg/L) and F5-21w (955.18 mg/L), while the other SCw had 42% to 77% of that in FSCw.

Proper higher alcohol content contributes to mellow, fruity, and floral traits in wines, but excessive higher alcohols (>400 mg/L) causes pungent odors and unpleasant smells [47]. Moreover, higher alcohols are also the precursors of ester biosynthesis, which contributes the fruity trait [48]. The higher alcohol contents in most SCw (157.88~373.92 mg/L) were lower than 400 mg/L except for F5-12w (424.30 mg/L) and F5-21w (424.05 mg/L), and those in F4-13w, F5-7w, F5-19w, F6-23w, HT-10w, and MXY-19w (286.57~373.92 mg/L) were higher than that in FSCw (273.39 mg/L) (Figure 5C). As shown in Table 2, the isoamylol, 2-methyl-1-butanol and 3-methyl-1-pentanol were the odor active higher alcohols (OAV > 1), which contributed to the organoleptic properties of the wines.

Volatile fatty acids mainly present wax, butter, and sweat traits [47]. The volatile fatty acid content in SCw (2.2~11.80 mg/L) except for MXY-19w (13.27 mg/L) was lower than that in FSCw (13.05 mg/L) (Figure 5D), and octanoic acid and hexanoic acid were the odor-active fatty acids (OAV > 1) (Table 2).

Esters mainly contribute fruity and floral traits to wines, and include acetates, ethyl esters, and other esters [9]. Compared with that in FSCw (240.75 mg/L), the acetate content in MXY-19w (279.77 mg/L) was increased, that in F5-21w (225.88 mg/L), F4-13w (202.96 mg/L) and ZXY-17w (197.03 mg/L) showed an insignificant difference, and those in other SCw (45.27~169.14 mg/L) were decreased. Ethyl acetate, isoamyl acetate, and 2-methylbutyl acetate were the main odor-active acetates (OAV > 1) (Figure 5E). The ethyl ester contents in SCw (80.87~314.15 mg/L) were significantly lower than in FSCw (478.95 mg/L) (Figure 5F). Ethyl octanoate, ethyl hexanoate, and ethyl caprate were the main odor active ethyl esters (OAV > 1). The other ester contents in F4-13w (1.54 mg/L), SXY-4w (1.76 mg/L), and MXY-19w (1.93 mg/L) were insignificantly different from those in FSCw (2.62 mg/L) (Figure 5G), but those in other SCw (0.44~1.44 mg/L) were lower than those in FSCw. Isopentyl hexanoate and 3-methylbutyl octanoate were the main odor esters (OAV > 1).

Benzene derivative mainly contribute rose, strawberry, and pleasant traits to wine [49]. Benzene derivative contents in F4-13w (138.93 mg/L), SXY-4w (148.21 mg/L) and MXY-19w (134.75 mg/L) were insignificantly different from that in FSCw (137.28 mg/L), while those in other SCw (20.52~95.62 mg/L) were lower than those in FSCw (Figure 5H). Phenylethyl alcohol, 2-phenethyl acetate, and benzene acetaldehyde were the main odor-active benzene derivatives (OVA > 1).

Aldoketones, including aldehydes and ketones, mainly contribute nut, fat, and butter traits to wine [50]. The aldoketone contents in MXY-19w, SXY-4w, F6-23w, ZXY-17w, and HT-10w (1.85~4.33 mg/L) were higher than those in FSCw (1.81 mg/L), while those in other SCw (0.04~1.52 mg/L) were lower than in FSCw (Figure 5I).

As charted in Figure 6, hierarchical clustering analysis of the volatile compound content in SCw indicated that all the SCw were classified into three categories. MXY-19w, SXY-4w, FSCw, and F4-13w were clustered together with the highest contents of aroma compounds. F6-23w, HT-10w, F5-18w, F5-7w, and F5-9w were clustered together with medium contents of aroma compounds. F6-8w, F5-12w, F5-21w, ZXY-17w, F9-23w, and F5-19w were grouped together with the lowest contents of aroma compounds.

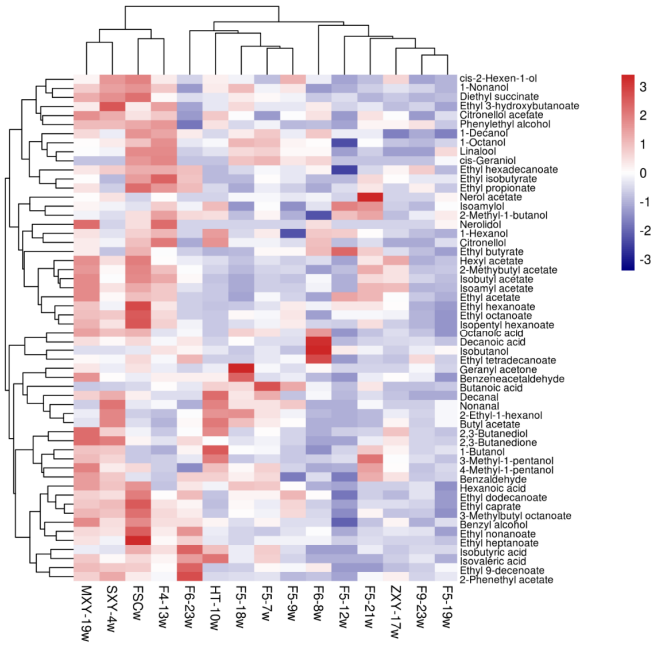


**Figure 6.** Hierarchical clustering heatmap of volatile aroma compounds of wines fermented by the selected *S. cerevisiae* strains (all data were normalized).

3.3.4. Sensory Evaluation of Wines

The sensory evaluation of SCw indicated that red fruity, black fruity, floral, green, spices, and yeasty were the main aroma traits of wines (Figure 7). Compared with those in FSCw, the red fruity trait in F4-13w, F5-21w, F5-18w, F5-12w, HT-10w, MXY-19w, F6-8w, F6-23w, and ZXY-17w was improved, especially in F4-13w, the floral trait was enhanced in F4-13w and SXY-4w, and the green trait with negative odor in SCw was reduced [51]. The spices trait in SCw was almost the same as that in FSCw, apart from F9-23w, F5-12w, F5-19w, and MXY-19w. However, MXY-19w, F9-23w, and SXY-4w presented a mild yeasty trait, which exhibited strong flocculation ability (Figure 3D). Bauer, et al. [52] indicated that strong flocculation ability led to the early deposition of yeast cells during alcoholic fermentation, which resulted in yeasty traits in wines.

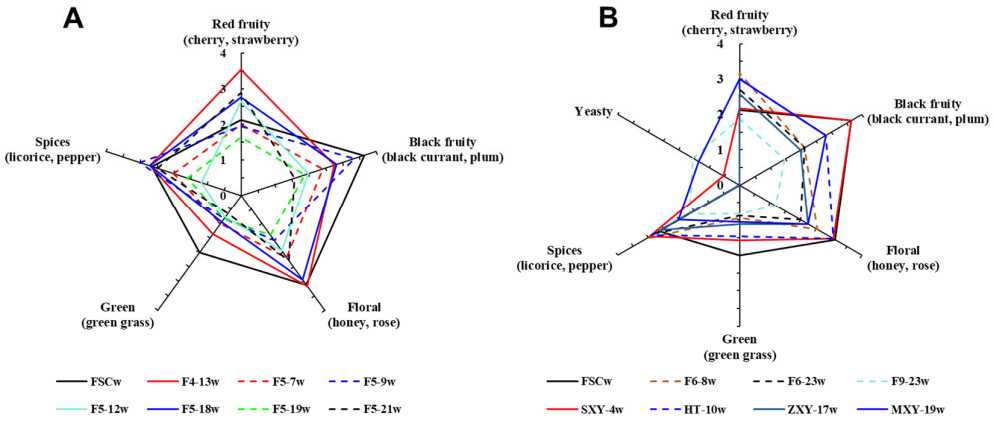


**Figure 7.** Aroma sensory evaluation of wines fermented by the selected *S. cerevisiae* strains (**A**): sensory evaluation of FSCw, F4-13w, F5-7w, F5-9w, F5-12w, F5-18w, F5-19w, and F5-21w; (**B**): sensory evaluation of FSCw, F6-8w, F6-23w, F9-23w, SXY-4w, HT-10w, ZXY-17w, and MXY-19w).

## 4. Conclusions

A total of 199 *S. cerevisiae* strains were isolated and identified from the vineyards and spontaneous fermentation must at eastern foot of Helan Mountain in China, and spontaneous fermentation must was the best source for collecting *S. cerevisiae.* Among the *S. cerevisiae* strains, 14 isolates exhibited excellent tolerance abilities to 250 mg/L sugar, 100 mg/L $SO_2$, and 12% (*v/v*) ethanol. F4-13 had the better oenological properties, with low $H_2S$ production (+), suitable flocculation ability (58.74%), and a certain L-malic-acid-reducing ability (49.07%), and generated high contents of polyphenol, anthocyanin, tannin, terpenes, and higher alcohols in wines, which resulted in the improvement of the red fruity and floral aromas of the wines, suggesting that F4-13 had the potential to be a starter for winemaking. The presented results provide a strategy for the selection of indigenous *S. cerevisiae* for wine fermentation to produce high-quality wine with regional characteristics. However, a large-scale wine fermentation characteristic of F4-13 should be carried out in the future.

**Supplementary Materials:** The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/fermentation9040376/s1, Table S1: Descriptions of the colony morphologies for *S. cerevisiae* strains on WL medium (28 °C, 5 d); Table S2: Sequencing results of the 26S rDNA D1/D2 domain apart of the isolated *S. cerevisiae* strains; Table S3: Tolerance analysis of the selected *S. cerevisiae* strains.

**Author Contributions:** L.H.: conceptualization, data curation, formal analysis, methodology, writing—original draft, writing-review & editing. X.J.: data curation, investigation, methodology. J.L.: data curation, formal analysis. Y.J.: formal analysis, software, writing—original draft. X.W.: conceptualization, writing—review & editing. X.Z.: conceptualization, funding acquisition, project administration, resources, supervision, writing—review & editing. All authors have read and agreed to the published version of the manuscript.

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## References

1. Fleet, G.H. Wine yeasts for the future. *FEMS Yeast Res.* **2008**, *8*, 979–995. [CrossRef] [PubMed]
2. Van Wyk, N.; Grossmann, M.; Wendland, J.; Von Wallbrunn, C.; Pretorius, I.S. The whiff of wine yeast innovation: Strategies for enhancing aroma production by yeast during wine fermentation. *J. Agric. Food Chem.* **2019**, *67*, 13496–13505. [CrossRef]
3. Berbegal, C.; Spano, G.; Tristezza, M.; Grieco, F.; Capozzi, V. Microbial resources and innovation in the wine production sector. *S. Afr. J. Enol. Vitic.* **2017**, *38*, 156–166. [CrossRef]
4. Parapouli, M.; Vasileiadis, A.; Afendra, A.; Hatziloukas, E. *Saccharomyces cerevisiae* and its industrial applications. *AIMS Microbiol.* **2020**, *6*, 1–31. [CrossRef] [PubMed]
5. Di Maio, S.; Polizzotto, G.; Di Gangi, E.; Foresta, G.; Genna, G.; Verzera, A.; Scacco, A.; Amore, G.; Oliva, D. Biodiversity of indigenous *Saccharomyces* populations from old wineries of south-eastern Sicily (Italy): Preservation and economic potential. *PLoS ONE* **2012**, *7*, e30428. [CrossRef]
6. Csoma, H.; Zakany, N.; Capece, A.; Romano, P.; Sipiczki, M. Biological diversity of *Saccharomyces* yeasts of spontaneously fermenting wines in four wine regions: Comparative genotypic and phenotypic analysis. *Int. J. Food Microbiol.* **2010**, *140*, 239–248. [CrossRef]
7. Claus, H.; Mojsov, K. Enzymes for wine fermentation: Current and perspective applications. *Fermentation* **2018**, *4*, 52. [CrossRef]
8. Benito, Á.; Calderón, F.; Benito, S. The influence of non-*Saccharomyces* species on wine fermentation quality parameters. *Fermentation* **2019**, *5*, 54. [CrossRef]
9. Belda, I.; Ruiz, J.; Beisert, B.; Navascués, E.; Marquina, D.; Calderón, F.; Rauhut, D.; Benito, S.; Santos, A. Influence of *Torulaspora delbrueckii* in varietal thiol (3-SH and 4-MSP) release in wine sequential fermentations. *Int. J. Food Microbiol.* **2017**, *257*, 183–191. [CrossRef]
10. Li, N.; Wang, Q.; Xu, Y.; Li, A.; Tao, Y. Increased glycosidase activities improved the production of wine varietal odorants in mixed fermentation of *P. fermentans* and high antagonistic *S. cerevisiae*. *Food Chem.* **2020**, *332*, 127426. [CrossRef]
11. Sun, W.; Hu, K.; Zhang, J.; Zhu, X.; Tao, Y. Aroma modulation of Cabernet Gernischt dry red wine by optimal enzyme treatment strategy in winemaking. *Food Chem.* **2018**, *245*, 1248–1256. [CrossRef] [PubMed]
12. Ling, M.; Qi, M.; Li, S.; Shi, Y.; Pan, Q.; Cheng, C.; Yang, W.; Duan, C. The influence of polyphenol supplementation on ester formation during red wine alcoholic fermentation. *Food Chem.* **2022**, *377*, 131961. [CrossRef] [PubMed]
13. Capece, A.; Pietrafesa, R.; Siesto, G.; Romaniello, R.; Condelli, N.; Romano, P. Selected indigenous *Saccharomyces cerevisiae* strains as profitable strategy to preserve typical traits of Primitivo wine. *Fermentation* **2019**, *5*, 87. [CrossRef]
14. Wang, C.; Liang, S.; Yang, J.; Wu, C.; Qiu, S. The impact of indigenous *Saccharomyces cerevisiae* and *Schizosaccharomyces japonicus* on typicality of crystal grape (Niagara) wine. *Food Res. Int.* **2022**, *159*, 111580. [CrossRef] [PubMed]
15. Song, Z.W.; Sun, M.Y.; Yang, R.J.; Dou, G.Y.; Zhang, Y.; Zhang, L. Evaluation of ecosystem service value of the grape industry at the eastern foot of Helan Mountain, Ningxia, China. *Ying Yong Sheng Tai Xue Bao J. Appl. Ecol.* **2019**, *30*, 979–985.
16. Orlić, S.; Vojvoda, T.; Babić, K.H.; Arroyo-López, F.N.; Jeromel, A.; Kozina, B.; Iacumin, L.; Comi, G. Diversity and oenological characterization of indigenous *Saccharomyces cerevisiae* associated with Žilavka grapes. *World J. Microbiol. Biotechnol.* **2010**, *26*, 1483–1489. [CrossRef]
17. Ilieva, F.; Kostadinovic Velickovska, S.; Dimovska, V.; Mirhosseini, H.; Spasov, H. Isolation of *Saccharomyces cerevisiae* yeast strains from Macedonian "Tikveš" wine-growing region and their impact on the organoleptic characteristics of Vranec and Cabernet Sauvignon wines. *Res. J. Biotechnol.* **2019**, *14*, 100–110.
18. Tofalo, R.; Patrignani, F.; Lanciotti, R.; Perpetuini, G.; Schirone, M.; Di Gianvito, P.; Pizzoni, D.; Arfelli, G.; Suzzi, G. Aroma profile of Montepulciano d'Abruzzo wine fermented by single and co-culture starters of autochthonous *Saccharomyces* and non-*Saccharomyces* yeasts. *Front. Microbiol.* **2016**, *7*, 610. [CrossRef]
19. Agarbati, A.; Canonico, L.; Ciani, M.; Comitini, F. Fitness of selected indigenous *Saccharomyces cerevisiae* strains for white Piceno DOC wines production. *Fermentation* **2018**, *4*, 37. [CrossRef]
20. Sun, Y.; Guo, J.; Liu, F.; Liu, Y. Identification of indigenous yeast flora isolated from the five winegrape varieties harvested in Xiangning, China. *Antonie Van Leeuwenhoek* **2014**, *105*, 533–540. [CrossRef]
21. Brysch-Herzberg, M.; Seidel, M. Yeast diversity on grapes in two German wine growing regions. *Int. J. Food Microbiol.* **2015**, *214*, 137–144. [CrossRef] [PubMed]

22. Raja, H.A.; Miller, A.N.; Pearce, C.J.; Oberlies, N.H. Fungal identification using molecular tools: A primer for the natural products research community. *J. Nat. Prod.* **2017**, *80*, 756–770. [CrossRef]
23. Souza, C.; Chernicharo, C.; Melo, G. Methane and hydrogen sulfide emissions in UASB reactors treating domestic wastewater. *Water Sci. Technol.* **2012**, *65*, 1229–1237. [CrossRef] [PubMed]
24. Vichi, S.; Gallardo-Chacón, J.J.; Pradelles, R.; Chassagne, D.; López-Tamames, E.; Buxaderas, S. Surface properties of *Saccharomyces cerevisiae* lees during sparkling wine ageing and their effect on flocculation. *Int. J. Food Microbiol.* **2010**, *140*, 125–130. [CrossRef]
25. Fliszár-Nyúl, E.; Zaukuu, J.-L.Z.; Szente, L.; Kovacs, Z.; Poór, M. Impacts of β-cyclodextrin bead polymer (BBP) treatment on the quality of red and white wines: Color, polyphenol content, and electronic tongue analysis. *LWT* **2023**, *176*, 114567. [CrossRef]
26. Chen, J.; Du, J.; Li, M.; Li, C. Degradation kinetics and pathways of red raspberry anthocyanins in model and juice systems and their correlation with color and antioxidant changes during storage. *Lwt* **2020**, *128*, 109448. [CrossRef]
27. Liu, G.; Sun, J.; He, X.; Tang, Y.; Li, J.; Ling, D.; Li, C.; Li, L.; Zheng, F.; Sheng, J. Fermentation process optimization and chemical constituent analysis on longan (Dimocarpus longan Lour.) wine. *Food Chem.* **2018**, *256*, 268–279. [CrossRef] [PubMed]
28. Wang, X.; Li, A.; Dizy, M.; Ullah, N.; Sun, W.; Tao, Y. Evaluation of aroma enhancement for "Ecolly" dry white wines by mixed inoculation of selected *Rhodotorula mucilaginosa* and *Saccharomyces cerevisiae*. *Food Chem.* **2017**, *228*, 550–559. [CrossRef]
29. Vaudano, E.; Quinterno, G.; Costantini, A.; Pulcini, L.; Pessione, E.; Garcia-Moruno, E. Yeast distribution in Grignolino grapes growing in a new vineyard in Piedmont and the technological characterization of indigenous *Saccharomyces* spp. strains. *Int. J. Food Microbiol.* **2019**, *289*, 154–161. [CrossRef]
30. Schuller, D.; Valero, E.; Dequin, S.; Casal, M. Survey of molecular methods for the typing of wine yeast strains. *FEMS Microbiol. Lett.* **2004**, *231*, 19–26. [CrossRef]
31. Wei, J.; Niu, C.; Liu, B.; Yuan, Y.; Yue, T. Identification and characterization of epiphytic yeasts on apples in China. *Rsc Adv.* **2017**, *7*, 44766–44772. [CrossRef]
32. Ugliano, M.; Kolouchova, R.; Henschke, P.A. Occurrence of hydrogen sulfide in wine and in fermentation: Influence of yeast strain and supplementation of yeast available nitrogen. *J. Ind. Microbiol. Biotechnol.* **2011**, *38*, 423–429. [CrossRef] [PubMed]
33. Suárez-Lepe, J.; Morata, A. New trends in yeast selection for winemaking. *Trends Food Sci. Technol.* **2012**, *23*, 39–50. [CrossRef]
34. Massera, A.; Assof, M.; Sturm, M.E.; Sari, S.; Jofré, V.; Cordero-Otero, R.; Combina, M. Selection of indigenous *Saccharomyces cerevisiae* strains to ferment red musts at low temperature. *Ann. Microbiol.* **2012**, *62*, 367–380. [CrossRef]
35. Šuranská, H.; Vránová, D.; Omelková, J. Isolation, identification and characterization of regional indigenous *Saccharomyces cerevisiae* strains. *Braz. J. Microbiol.* **2016**, *47*, 181–190. [CrossRef]
36. Verstrepen, K.; Derdelinckx, G.; Verachtert, H.; Delvaux, F. Yeast flocculation: What brewers should know. *Appl. Microbiol. Biotechnol.* **2003**, *61*, 197–205. [CrossRef]
37. Redzepovic, S.; Orlic, S.; Majdak, A.; Kozina, B.; Volschenk, H.; Viljoen-Bloom, M. Differential malic acid degradation by selected strains of *Saccharomyces* during alcoholic fermentation. *Int. J. Food Microbiol.* **2003**, *83*, 49–61. [CrossRef]
38. Su, J.; Wang, T.; Wang, Y.; Li, Y.; Li, H. The use of lactic acid-producing, malic acid-producing, or malic acid-degrading yeast strains for acidity adjustment in the wine industry. *Appl. Microbiol. Biotechnol.* **2014**, *98*, 2395–2413. [CrossRef]
39. Saayman, M.; Viljoen-Bloom, M. The biochemistry of malic acid metabolism by wine yeasts–a review. *S. Afr. J. Enol. Vitic.* **2006**, *27*, 113–122. [CrossRef]
40. Qi, Y.; Wang, R.; Qin, Q.; Sun, Q. Soil affected the variations in grape and wine properties along the eastern foot of Helan Mountain, China. *Acta Agric. Scand. Sect. B Soil Plant Sci.* **2019**, *69*, 494–502. [CrossRef]
41. Hu, K.; Jin, G.-J.; Xu, Y.-H.; Tao, Y.-S. Wine aroma response to different participation of selected *Hanseniaspora uvarum* in mixed fermentation with Saccharomyces cerevisiae. *Food Res. Int.* **2018**, *108*, 119–127. [CrossRef] [PubMed]
42. Kong, C.; Li, A.; Su, J.; Wang, X.; Chen, C.; Tao, Y. Flavor modification of dry red wine from Chinese spine grape by mixed fermentation with Pichia fermentans and S. cerevisiae. *LWT-Food Sci. Technol.* **2019**, *109*, 83–92. [CrossRef]
43. Zhang, W.; Zhuo, X.; Hu, L.; Zhang, X. Effects of Crude β-Glucosidases from *Issatchenkia terricola*, *Pichia kudriavzevii*, *Metschnikowia pulcherrima* on the Flavor Complexity and Characteristics of Wines. *Microorganisms* **2020**, *8*, 953. [CrossRef] [PubMed]
44. Van Gemert, L. Compilations of odour threshold values in air, water and other media. *Odour Threshold.* **2011**, *2*, 1–486.
45. Ruiz, J.; Kiene, F.; Belda, I.; Fracassetti, D.; Marquina, D.; Navascués, E.; Calderón, F.; Benito, A.; Rauhut, D.; Santos, A. Effects on varietal aromas during wine making: A review of the impact of varietal aromas on the flavor of wine. *Appl. Microbiol. Biotechnol.* **2019**, *103*, 7425–7450. [CrossRef]
46. Liu, N.; Qin, Y.; Song, Y.; Ye, D.; Yuan, W.; Pei, Y.; Xue, B.; Liu, Y. Selection of indigenous *Saccharomyces cerevisiae* strains in Shanshan County (Xinjiang, China) for winemaking and their aroma-producing characteristics. *World J. Microbiol. Biotechnol.* **2015**, *31*, 1781–1792. [CrossRef]
47. Jackson, J.; Bakker, R. *Wine: Flavour Chemistry*; John Wiley & Sons: Hoboken, NJ, USA, 2011.
48. Waterhouse, A.L.; Sacks, G.L.; Jeffery, D.W. *Understanding Wine Chemistry*; John Wiley & Sons: Hoboken, NJ, USA, 2016.
49. Hu, K.; Jin, G.-J.; Mei, W.-C.; Li, T.; Tao, Y.-S. Increase of medium-chain fatty acid ethyl ester content in mixed *H. uvarum*/*S. cerevisiae* fermentation leads to wine fruity aroma enhancement. *Food Chem.* **2018**, *239*, 495–501. [CrossRef]
50. Styger, G.; Prior, B.; Bauer, F.F. Wine flavor and aroma. *J. Ind. Microbiol. Biotechnol.* **2011**, *38*, 1145. [CrossRef]

51. Sala, C.; Busto, O.; Guasch, J.; Zamora, F. Factors affecting the presence of 3-alkyl-2-methoxypyrazines in grapes and wines. A review. *J. Agric. Food Chem.* **2004**, *55*, 153–159.
52. Bauer, F.F.; Govender, P.; Bester, M.C. Yeast flocculation and its biotechnological relevance. *Appl. Microbiol. Biotechnol.* **2010**, *88*, 31–39. [CrossRef]