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Biodiversity and Oenological Property Analysis of Non-*Saccharomyces* Yeasts Isolated from Korla Fragrant Pears (*Pyrus sinkiangensis* Yu)

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Abstract: Oenological yeasts play a critical role in the winemaking process. In this study, the biodiversity of the non-*Saccharomyces* yeast was analyzed and monitored using high-throughput sequencing and culture-dependent approaches. Oenological and fermentation characteristics of these native yeasts were further investigated. A total of 241 fungus species and 5 species of culturable non-*Saccharomyces* yeasts were detected using high-throughput sequencing and culture-dependent approaches, respectively. Five strains of aroma-producing yeasts (K4, K14, K19, K21, and K26) were isolated, and their growth characteristics, carbon source utilization, hydrogen sulfide production performance, and β-glucosidase activity were different. The oenological condition tolerances of most strains were lower than that of commercial *S. cerevisiae* X16. The co-inoculum of these strains and *S. cerevisiae* X16 regulated the volatile aroma characteristics of the fermented Korla fragrant pear (KFP) fruit wine, enriching and complicating the aroma flavor. Thus, the combined inoculation of these indigenous wine yeasts and *S. cerevisiae* has some application potential in the production of KFP wine.

Keywords: Korla fragrant pears; wine yeasts; biodiversity; oenological property; fruit wine

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1. Introduction

Yeasts are single-celled microorganisms used in the production of alcohol through fermentation; they can be classified into *Saccharomyces cerevisiae* (*S. cerevisiae*) and non-*Saccharomyces* yeast according to their fermentation characteristics [1]. *S. cerevisiae* exhibits strong fermentation performance due to its high tolerance to alcohol; thus, it is used to perform alcoholic fermentation. Non-*Saccharomyces* yeast is a general term for all yeasts other than *S. cerevisiae*, such as *Wickerhamomyces anomalus*, *Hanseniaspora uvarum*, and *Pichia kluyveri* [2]. Non-*Saccharomyces* yeast produces volatile and non-volatile constituents, which enhance the flavor and sensory characteristics of wine.

Recently, non-*Saccharomyces* yeasts have attracted considerable attention due to their unique physiological and metabolic features. Some non-*Saccharomyces* yeasts release various hydrolytic enzymes, such as glycoside hydrolases, proteases, and lipases, which effectively regulate the aromatic profile of fermented wines [3]. In addition, some non-*Saccharomyces* yeasts can regulate the types and amounts of acids, alcohol, glycerol, and other physicochemical parameters of fermented wines, which, in turn, affect the richness and complexity of wines [4]. Therefore, several strains of non-*Saccharomyces* yeasts are used in wine production in co-inoculation or sequential inoculation to regulate the quality and characteristics of wines [5].

The Korla fragrant pears (KFPs, *Pyrus sinkiangensis* Yu), a Xinjiang pear species of the genus *Pyrus* in the Rosaceae family, are widely consumed by the public due to their attractive appearance; juicy, crisp, sweet flesh; unique aroma; and rich nutrition, such

as vitamin C, organic acids, and various minerals. The KFPs are national geographical indication products and have entered the first list of geographical indication protection in Central Europe [6]. KFPs are widely accepted by other countries; thus, they are exported to foreign markets as an important agricultural product of Xinjiang.

Currently, research on the KFPs is mainly focused on the agronomic traits [7], preservation, storage [8], and gene function [9]; however, few studies focused on the identification and characterization of functional yeast resources from the KFPs. Fu et al. [10] isolated two excellent indigenous yeasts: XL1 and XL2, from the alcoholic fermentation broth of the KFPs; these two strains can withstand the treatment of 50% wt glucose, 20% *v/v* alcohol, and 250 mg/L sulfur dioxide. The glucose and alcohol tolerances of XL2 were higher than those of XL1; however, the sulfur dioxide tolerance of XL2 was lower than that of XL1. Fu et al. did not identify the species of XL1 and XL2; however, we presumed they were strains of *S. cerevisiae* according to the tolerance characteristics. Thus, additional analysis of the oenological and fermentative properties of the indigenous yeast from the KFPs should be carried out.

In this study, we detected the species composition of non-*Saccharomyces* yeasts and their dynamic changes using high-throughput sequencing. The culturable yeasts were also isolated using a culture-dependent approach. The oenological properties of these culturable yeasts, including growth characteristics, winemaking condition tolerances, and fermentation performance, were investigated to assess the potential applications in the production of KFP fruit wine.

2. Materials and Methods

2.1. Spontaneous Fermentation and Sample Collection

KFPs were grown and obtained from an orchard in Korla (Xinjiang, China). Fresh, mature, and spoiled KFPs were selected, denucleated, and squeezed to obtain their respective juice. Laboratory-scale spontaneous fermentation was performed with 600 mL of KFPs' juice in 1000 mL sterile flasks at 28 °C. The fermentation experiments were repeated thrice under constant conditions. The fermented samples were prepared in 1, 3, 5, and 15 days and designated as SF1, SF3, SF5, and SF15, respectively. The prepared samples were divided into two parts; one part used was for the separation of indigenous wine yeasts, and the other one was used for DNA separation and high-throughput sequencing.

2.2. Illumina High-Throughput Sequencing

The total DNA of each sample was extracted using the EZNA soil DNA Kit (Omega Bio-tek; Norcross, GA, USA). The Kit was purchased from Majorbio BioPharm Technology Co., Ltd. (Shanghai, China) and used according to the manufacturer's instructions. The concentration and purification of the DNA were examined using a NanoDrop 2000 spectrophotometer (Thermo Scientific, Wilmington, DE, USA). Polymerase chain reaction amplification was conducted to amplify the internal transcribed spacer region using primers ITS3F (5′-GCATCGATGAAGAACGCAGC-3′) and ITS4R (5′-TCCTCCGCTTATTGATATGC-3′). Purified and pooled amplification libraries were paired-end sequenced (2 × 300) on the Illumina MiSeq platform (Illumina, San Diego, CA, USA) according to the standard specification given by Majorbio Bio-Pharm Technology Co., Ltd. (Shanghai, China). Raw sequence reads were demultiplexed, quality-filtered, merged, and clustered into operational taxonomic units (OTUs) with a 97% similarity cutoff, and the species composition and differences were analyzed using the Majorbio Cloud Platform (https://cloud.majorbio.com (accessed on 17 July 2022)).

2.3. Culturable Yeasts Isolation and Identification

Spontaneous fermentation samples of KFPs were serially diluted with sterile water, spread on YEPD (Yeast Extract Peptone Dextrose) solid medium, and then cultured at 28 °C for 48 h. Pure isolates were streaked on Wallerstein laboratory (WL) nutrient agar, photographed, and then classified according to their morphotype.

Genomic DNA of the yeasts was prepared using a column yeast DNA purification kit (B518257, Sangon Biotech, Shanghai, China) and quantified using a spectrophotometer (NanoDrop 2000). 26S rDNA D1/D2 domain was amplified with the universal primers NL1 (5′-GCATATCAATAAGCGGAGGAAAAG-3′) and NL4 (5′-GGTCCGTGTTTCAAGACGG-3′). The amplified PCR product was purified and sequenced by Sangon Biotech Co., Ltd. (Shanghai, China). Then, the yeast species were determined by comparing the sequences of the D1/D2 domain of 26S rDNA through a basic local alignment search tool (https://blast.ncbi.nlm.nih.gov/Blast (accessed on 17 July 2022)).

2.4. Growth Curve Determination

The selected yeast strains were inoculated into YEPD liquid medium and incubated at 28 °C with 180 rpm shaking for 36 h, and the optical density (OD) of the cultures was measured at 600 nm every 4 h. The commercial *S. cerevisiae* X16 strain was obtained from Laffort Company (Bordeaux, France) and used as a control, and each experiment was repeated thrice.

2.5. Oenological Condition Tolerance Analysis

The selected yeasts strains were inoculated into YEPD medium at a concentration of 10^6 CFU/mL containing different (1) mass concentrations of glucose (100, 150, 200, 250, and 300 g/L); (2) volume fractions of ethanol (3%, 6%, 9%, 12%, and 15% *v/v*); (3) mass concentrations of malic acid (1%, 1.5%, 2%, 2.5%, and 3%); and (4) mass concentrations of sulfur dioxide (50, 100, 150, 200, and 300 mg/L). All the treated groups were incubated at 28 °C with 180 rpm agitation for 34 h. The OD values for each group were measured at 600 nm. Each treatment was repeated thrice.

2.6. Determination of the Hydrogen Sulfide (H_2S) Production Performance and β-D-Glucosidase Activity of the Selected Yeasts

The H_2S production performance of the selected yeast strains was examined using Linderholm's method [11]. The strength of H_2S production was determined through the comparison of the color shades on the highly selective bismuth sulfite glucose glycine yeast (BiGGY) agar medium.

The β-D-glucosidase activity of the selected yeast strains was determined using the para-nitrophenyl-β-glucopyranoside (p-NPG) method [12]. The strains were inoculated in YEPD liquid medium, incubated at 28 °C with 180 rpm agitation for 72 h, and centrifuged at 3000× *g* for 10 min. Then, the supernatant was used to determine enzymatic activity. The enzymatic activity unit (U) was defined as the amount of enzyme required to produce 1 μmol para-nitrophenol through the hydrolysis of p-NPG for 1 min at pH 5.0 and 50 °C.

2.7. Laboratory-Scale Fermentation of KFP Fruit Wine

Fresh, ripe, and non-rotten KFPs were selected, pitted, and squeezed to obtain their respective juice. Then, potassium metabisulfite (100 mg/L) and pectinases (20 mg/L) were added to the juice and incubated at room temperature for 12 h. Sugar was added to the mixture to adjust the sugar content to 24 °Brix. The mixture was divided into six groups and transferred into 2 L sterile Erlenmeyer flasks. In the first group, *S. cerevisiae* X16 alone was inoculated at a final concentration of 10^7 CFU/mL as a control. For the second, third, fourth, fifth, and sixth groups, we opted for a mixed fermentation and inoculated the fruit juice with *S. cerevisiae* X16 at a final concentration of 10^7 CFU/mL. The indigenous wine yeasts (K4, K14, K19, K21, and K26) were isolated from KFPs at the final concentration of 10^8 CFU/mL. The fermentation was performed at 22 °C.

2.8. Aromatic Composition Analysis of KFP Fruit Wine

After the fermentation, the supernatant of the KFP fruit wine prepared through centrifugation at 3000× *g* for 10 min was used to analyze the aromatic components. Solid phase microextraction (TQ8040, Agilent, CA, USA) was used to extract the aromatic components

at 40 °C for 30 min. In addition, the aromatic components of KPF wine were determined using a gas chromatography-mass spectrometry system with cyclohexanone as the internal standard [13]. The threshold values of each volatile aromatic component were obtained, and the odor activity value (OAV) of each was calculated.

2.9. Statistical Analysis

The results were expressed as the mean ± standard deviation. Univariate analysis of variance (ANOVA) of the data and the significance of the difference test was performed using SPSS 21.0 software. $p < 0.05$ was considered statistically significant. Principal component analysis (PCA) was performed with SIMCA software.

3. Results and Analysis

3.1. Non-Saccharomyces Biodiversity Determined Using a High-Throughput Sequencing Approach

A total of 290 842 185 sequencing bases, 1 092 195 effective sequences with an average length of 266 nt, and 104 OTUs were obtained from the spontaneous fermentation samples of KFP at the 97% similarity level. Then, coverage was used to assess the cover degree of OTUs with low abundance of the sample, and data demonstrated that the coverage of all the samples was 1.00, indicating the full OTUs coverage of low abundance in this study; this sequencing could represent the real microbial population.

A total of 241 fungus species were obtained from spontaneous fermentation of KFP, and 77, 66, 52, and 46 species were detected in the samples of SF1, SF3, SF5, and SF15, respectively (Figure 1A).

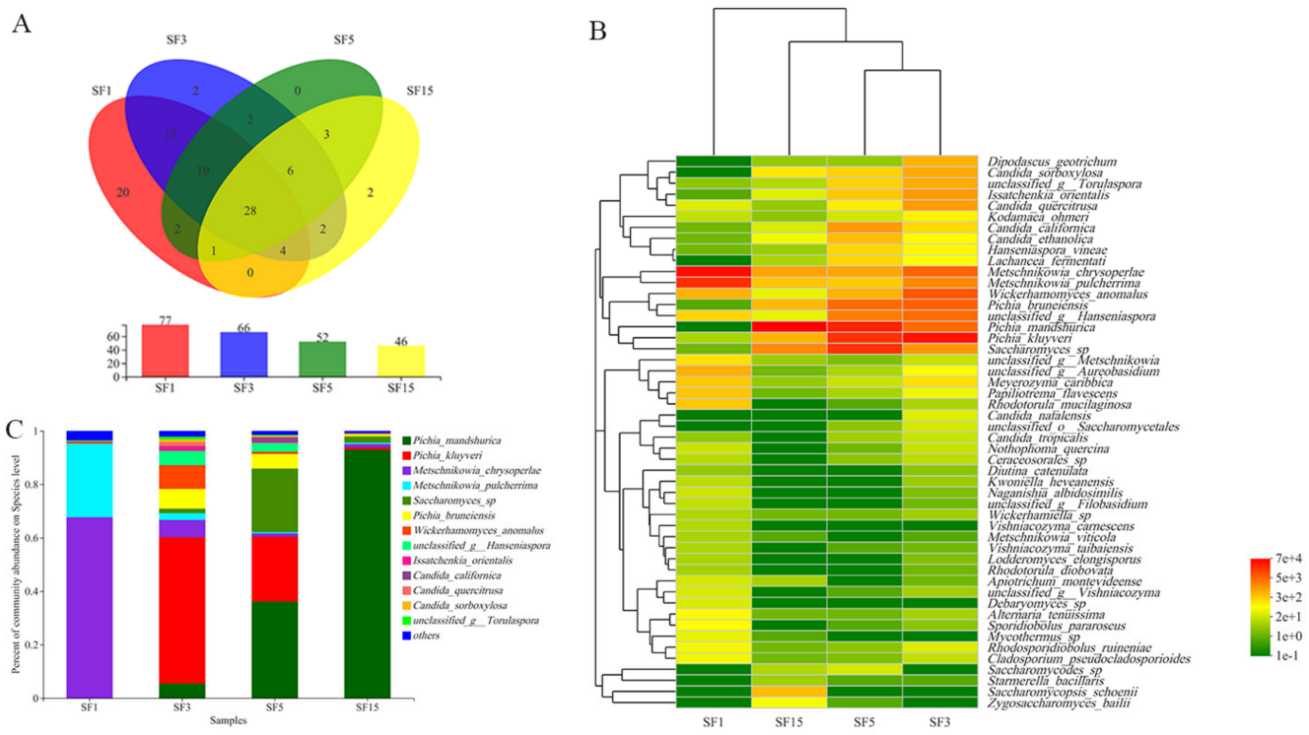

Figure 1. Biodiversity and dynamic changes of non-*Saccharomyces* yeasts of KFP. (**A**) Yeast community distribution from different spontaneous fermentation stages by Venn diagram. (**B**) Community heatmap analysis on species level. (**C**) Relative abundance of yeast community and their dynamic changes for different spontaneous fermentation stages.

Figure 1B shows the clustered heatmap results of the top 50 fungal species. The high species abundance in the sample was indicated in red color, while the low species abundance was indicated in green color. Samples SF5 and SF15 were clustered in a branch, indicating a similar species composition in the two samples; however, samples SF1 and

SF3 were clustered in the separated branches. The major detected fungi were yeasts, such as *Candida sorboxylosa*, *Issatchenkia orientalis*, and *Hanseniaspora vineae*. In addition, some pathogens, including *Nothophoma quercina*, *Diutina catenulate*, *Alternaria tenuissima*, and *Cladosporium pseudocladosporioides*, were detected.

The variety of fungal species on the first day of the spontaneous fermentation (SF1) was a single fungal species because *Metschnikowia chrysoperlae* and *Metschnikowia pulcherrima* were the dominant yeasts, contributing to 67.67% and 27.56% of the total species (Figure 1C). The variety of microorganisms rapidly increased as fermentation continued (SF3 and SF5); however, the species variety decreased at the end of the fermentation (SF15), and the dominant species was *Pichia mandshurica* (92.99%). The changes in the variety of species during the fermentation were confirmed using the results of the diversity index (Table S1). For example, the value of the Shannon index was the highest in SF3; however, the index decreased in SF5 and SF1 and became the lowest in SF15.

In addition, we found that *Metschnikowia chrysoperlae* and *Metschnikowia pulcherrima* were mainly present in the early stage of spontaneous fermentation. The concentration of *Metschnikowia chrysoperlae* and *Metschnikowia pulcherrima* was maximum on the first day (SF1); their concentration decreased and remained constant until the end of fermentation (Figure 1C). In contrast, the abundance of *Pichia mandshurica* was extremely low at the beginning of the fermentation, increased significantly from the third day of the fermentation (SF3), and then remained very at the end of fermentation (Figure 1C).

3.2. Determination of Diversity of Yeasts Using a Culture-Dependent Approach

A total of 32 indigenous yeast isolates were obtained from four stages of the KFP spontaneous fermentation. Five different yeast species were observed using culture-dependent approaches according to the morphological characteristics of their colonies on WL nutrient agar (Figure 2). The colors and morphologies of the colonies found in this study are summarized and listed in Table 1.

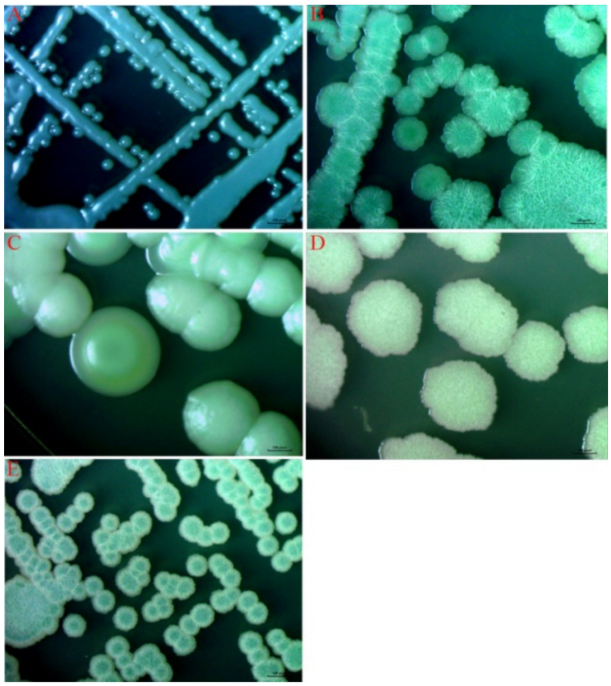

Figure 2. Photographs of yeast colony morphotypes on WL nutrient agar. (**A**) Colony morphotypes of *Metschnikowia sinensis*; (**B**) Colony morphotypes of *Wickerhamomyces anomalus*; (**C**) Colony morphotypes of *Hanseniaspora gulliermondii*; (**D**) Colony morphotypes of *Torulaspora delbrueckii*; (**E**) Colony morphotypes of *Pichia kluyveri*. Scale bar = 100 pixel.

Table 1. Colony morphotypes of indigenous wine yeasts of KFP on WL nutrient agar.

Number	Colony Colour	Colony Topography
A	Steel gray	Knob-like, convex, smooth
B	White on the surface and cyan in the center	Convex, wrinkled surface, irregular edge
C	Slightly yellow to cream	Convex, wrinkled, and opaque, irregularKnob-like, convex, smooth
D	white	Convex, wrinkled surface, irregular edge, opaque surface
E	White on the surface and cyan in the center	Convex, wrinkled surface, irregular edge, opaque surface

Molecular methods were used to further confirm these isolates through the comparison of 26S rDNA D1/D2 domain sequences in GenBank. From the results, five strains of indigenous yeast isolated from KFP were identified as *Metschnikowia sinensis*, *Wickerhamomyces anomalus*, *Hanseniaspora gulliermondii*, *Torulaspora delbrueckii*, and *Pichia kluyveri* (Table S2).

3.3. Growth and Carbon Source Utilization Characteristics of the Selected Yeasts

The intense aroma-producing yeast strains, with greater potential for production of fruit wine production, were screened from 32 indigenous yeast isolates using a sniffing method. Five strains of yeasts, such as K4 (*Metschnikowia sinensis*), K14 (*Wickerhamomyces anomalus*), K19 (*Hanseniaspora gulliermondii*), K21 (*Torulaspora delbrueckii*), and K26 (*Pichia kluyveri*), were obtained due to their strong fruity aroma-producing abilities.

The growth curves of the selected yeasts were tested and shown in Figure 3: the first 4 h represented the demurrage phase, while from 4 to 12 h represented the logarithmic phase. In the logarithmic phase, four indigenous yeast strains (K4, K14, K19, and K21) grew faster than the commercial *S. cerevisiae* (X16); however, they achieved a similar growth rate in their stable phase.

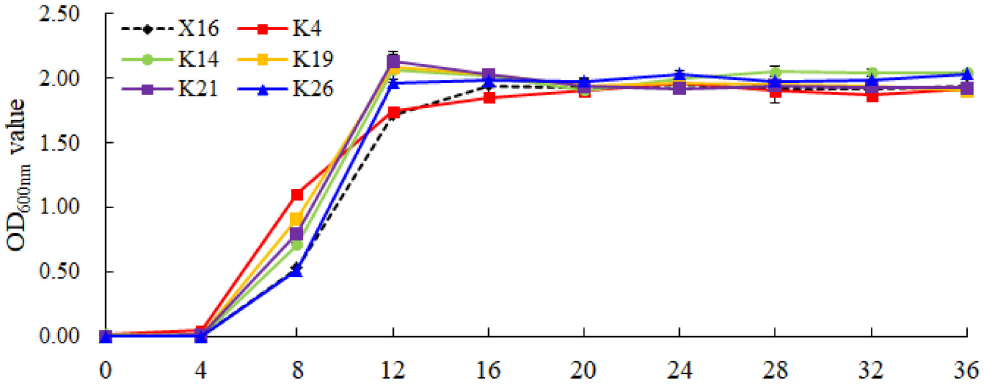

Figure 3. Growth curve of the selected yeasts isolated from KFP.

In addition, we investigated the carbon source utilization characteristics of the selected yeasts. The result showed that K26 could ferment all the tested carbon sources except the arabinose, which was similar to *S. cerevisiae* X16 (Table 2). All the selected yeast strains could not use arabinose as a carbon source to support their growth; however, glucose was the suitable carbon source for all the tested strains.

Table 2. Carbon source utilization characteristics of the selected yeasts isolated from Korla fragrant pear.

Strains	Glucose	Fructose	Sucrose	Maltose	Galactose	Mannose	Arabinose
X16	+	+	+	+	+	+	−
K4	+	+	−	+	+	+	−
K14	+	+	+	+	−	−	−
K19	+	+	+	−	+	+	−
K21	+	−	−	+	+	+	−
K26	+	+	+	+	+	+	−

3.4. Properties of Oenological Condition Tolerance of the Selected Yeasts

To evaluate the oenological condition tolerances of the selected yeasts, we cultured all the tested strains at different concentrations of glucose, ethanol, SO_2, and malic acid treatments. The OD value was measured at the wavelength of 600 nm. The K4 strain grew well in all the glucose concentrations tested (100–300 g/L), which were similar to X16. The $OD_{600\ nm}$ values of K4, K19, and K21 were significantly lower than X16 when they were grown in an additional glucose supply ranging from 100 g/L to 300 g/L (Figure 4A). When analyzing the ethanol tolerances of the five yeast strains, their growth decreased significantly compared to X16 because of the decrease in OD value (from 3% to 12%) after the ethanol treatment (Figure 4B). The growth of selected yeasts decreased significantly compared to X16 at the concentration of SO_2 ranging from 50 mg/L to 300 mg/L (Figure 4C). K14 and K19 were able to withstand 2% of malic acid treatment; however, the malic acid tolerance abilities of K4, K21, and K26 were lower than that of X16 (Figure 4D).

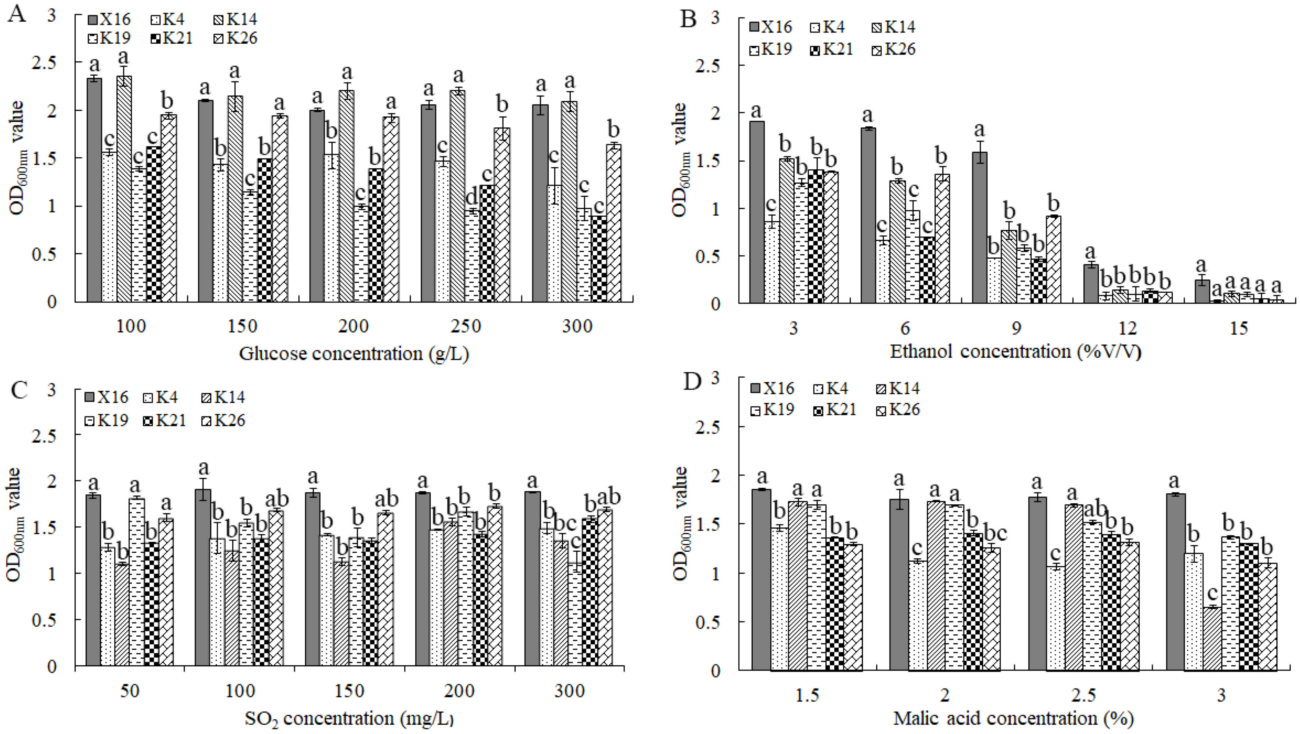

Figure 4. Properties of oenological condition tolerances of the selected yeasts isolated from KFP. (**A**) Glucose tolerance, (**B**) ethanol tolerance, (**C**) SO_2 tolerance, and (**D**) malic acid tolerance. Different lowercase letters above the standard deviation bar indicate a significant difference ($p < 0.05$).

3.5. H_2S and β-Glucosidase Production Capacities of the Selected Yeasts

H_2S production capacity of the selected wine yeasts was compared by examining the colony color on BiGGY agar. The color of X16 was brown, K4 was deep brown, K14, K19,

and K26 were light brown, and K21 was light brown; these results indicated that K14, K19, and K26 were the strains with the weakest capacity to produce H_2S. In contrast, K4 and K21 exhibited strong H_2S production capacities (Figure 5A).

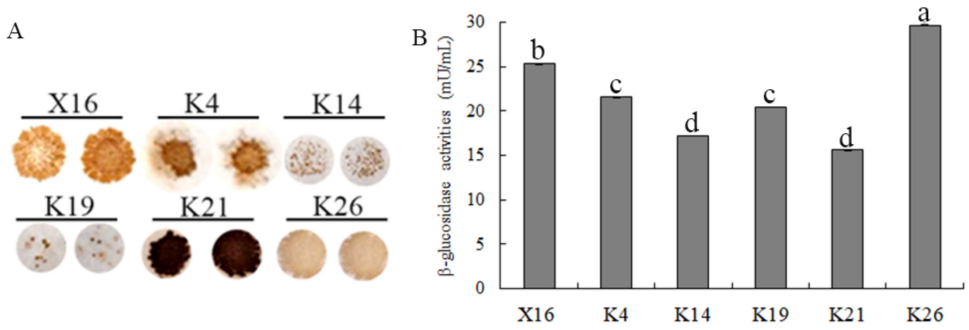

Figure 5. H_2S and β-glucosidase production capacity of the selected yeasts isolated from KFP. (**A**) H_2S production capacity and (**B**) β-glucosidase production capacity. Different lowercase letters above the standard deviation bar indicate a significant difference ($p < 0.05$).

As shown in Figure 5B, compared with X16, K26 exhibited the highest β-glucosidase production ability; however, β-glucosidase production abilities of other strains (K4, K14, K19, and K21) were significantly lower than that of X16.

3.6. Winemaking Properties of the Selected Yeasts

3.6.1. Basic Physicochemical Parameters of KFP Wine

Laboratory-scale fermentation of KFP wines was performed to further evaluate the fermentative properties of the indigenous yeast strains by co-inoculating with *S. cerevisiae* due to their low tolerance to ethanol.

Basic physicochemical parameters of KFP wine are shown in Table 3. The alcohol content of KFP wine fermented with the indigenous yeasts was significantly lower than that of the commercial strain of *S. cerevisiae* (X16) except for the strain of K19, with the concentration of the residual sugar ranging from 8.42 g/L (X16) to 6.88 g/L (K4). The contents of residual sugar produced with strains of X16 and K19 were significantly higher than that of other strains. There were no significant differences among these six types of KFP wine at the pH ranging from 3.89 to 3.81. The total acidity in the wines fermented with X16, K14, and K19 was significantly higher than that of K4, K21, and K26. The contents of volatile acidity in K26 fermented KFP wine were the highest, while the contents of volatile acidity in K19-fermented wine were the lowest.

Table 3. Basic physicochemical parameters of KFP wine.

Strains	Alcohol Content (% *v*/*v*)	Residual Sugar (g/L)	pH	Total Acidity (g/L)	Volatile Acidity (g/L)
X16	13.66 ± 0.19 a	8.42 ± 0.30 a	3.84 ± 0.02 a	4.42 ± 0.10 a	0.34 ± 0.02 b
K4	12.63 ± 0.18 c	6.88 ± 0.30 c	3.89 ± 0.02 a	3.60 ± 0.10 b	0.78 ± 0.10 a
K14	13.09 ± 0.15 b	7.40 ± 0.40 bc	3.81 ± 0.04 a	4.34 ± 0.10 a	0.64 ± 0.03 a
K19	13.95 ± 0.15 a	8.02 ± 0.20 ab	3.84 ± 0.02 a	4.34 ± 0.10 a	0.28 ± 0.03 b
K21	12.75 ± 0.12 bc	7.58 ± 0.30 bc	3.84 ± 0.05 a	3.96 ± 0.02 b	0.77 ± 0.02 a
K26	12.91 ± 0.21 bc	7.19 ± 0.60 bc	3.89 ± 0.01 a	3.84 ± 0.10 b	0.79 ± 0.10 a

Note: Values in the same column with different lowercase letters are significantly different ($p < 0.05$).

3.6.2. Electronic Sensory Characteristics of KFP Wine

Electronic sensory characteristics of different KFP wines were investigated using an electronic tongue system (SA402B, Insent, Japan). No significant differences in electronic sensory characteristics, including sourness, bitterness, astringency, aftertaste-A, aftertaste-B,

umami, richness, and saltiness, were observed among these types of KFP wine fermented with different strains of yeasts (Figure 6).

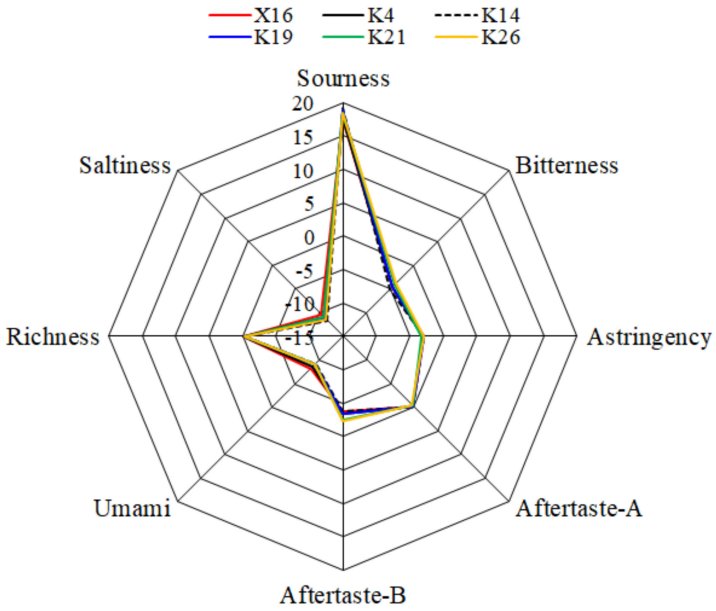

Figure 6. Electronic sensory characteristics of KFP wine fermented with different strains.

3.6.3. Volatile Aroma Characteristics of KFP Wine

The volatile aroma profiles of the KFP wines fermented with different strains of wine yeasts were further measured using the solid-phase microextraction gas chromatography-mass spectrometry (SPME-GC-MS) method (Table 4). A total of 45 volatile compounds, including 16 esters, 14 alcohols, four acids, two aldoketones, and nine other compounds, were detected in KFP wines. The number of volatile compounds in K19-produced wine was the lowest (30), while the number of volatile compounds in K14-fermented wine was the highest (33). Ethyl isovalerate and methyl caprylate were detected only in X16-fermented wine, whereas glycolaldehyde was specific in K4-fermented wine. The numbers of other volatile compounds increased when the indigenous yeasts were used as KFP wine fermentation starters compared with using the sole X16 as a starter.

Esters

Esters are indispensable for various fermented wines, and most of them release floral and fruity aromatic fragrances [14]. A number of esters in KFP wine were identified, and 13 esters were identified in X16-fermented wine, 11 esters were identified in K4 and K21, and 10 esters were identified in K14 and K26 (10). The total concentration of esters ranged from 185.93 mg/L (X16) to 59.61 mg/L (K14) (Table 4). Ethyl ester compounds, such as ethyl acetate, ethyl caprylate, and ethyl caprate, were the main components of esters in all the groups of KFP wines. Ethyl propionate was the main ester compound in the KFP wine with K14 as the starter, and isoamyl acetate was specific to the KFP wine produced with the strain of K21.

Alcohols

Fourteen alcohols were identified in 6 different KFP wines, and a total number of 13, 13, 14, 12, 12, and 13 alcohols were detected in X16-, K4-, K14-, K19-, K21-, and K26-fermented wines, respectively (Table 4). The concentration of alcohols ranged from 277.29 mg/L (K4) to 116.25 mg/L (K21). The content of alcohols in K4-fermented wine was similar to that of X16, which was higher than other types of wine. 1-Pentanol, phenethyl alcohol, isoamylol, and isobutanol were the main alcohols in KFP wines. Most alcohols except for 1-butanol and (Z)-3-Hexen-1-ol could be detected in all kinds of KPF wines. Phenylethyl

alcohol concentration, a rose-scented chemical compound, was high in X16-, K4-, and K19-fermented wines.

Table 4. Volatile compounds (mg/L) in KFP wines fermented with different yeasts.

No.	Compounds	CAS	Groups					
			X16	K4	K14	K19	K21	K26
1	Ethyl acetate	141-78-6	17.82 ± 0.89 b	18.8 ± 1.79 b	16.27 ± 1.87 b	27.47 ± 2.64 a	17.00 ± 4.16 b	27.41 ± 0.18 a
2	Ethyl butyrate	105-54-4	0.96 ± 0.08 a	1.00 ± 0.00 a	0.53 ± 0.05 b	ND	ND	0.52 ± 0.00 b
3	Ethyl octanoate	106-32-1	47.74 ± 1.29 b	62.29 ± 0.88 a	17.90 ± 2.19 c	20.62 ± 0.14 c	16.92 ± 3.28 c	20.37 ± 0.58 c
4	Ethyl caprate	110-38-3	93.12 ± 13.25 a	67.21 ± 0.73 b	15.11 ± 0.35 e	44.02 ± 4.81 c	30.52 ± 1.35 d	32.80 ± 1.95 d
5	Ethyl laurate	106-33-2	1.67 ± 0.00 c	12.63 ± 0.62 a	0.37 ± 0.01 d	2.39 ± 0.19 b	0.20 ± 0.00 e	0.33 ± 0.00 d
6	Ethyl hexanoate	123-66-0	20.91 ± 9.21 a	19.12 ± 0.07 a	5.24 ± 0.13 bc	4.72 ± 0.19 c	3.16 ± 0.23 d	6.04 ± 0.68 b
7	Ethyl isovalerate	108-64-5	0.18 ± 0.00	ND	ND	ND	ND	ND
8	Ethyl pelargonate	123-29-5	1.06 ± 1.49 abc	1.49 ± 0.08 b	2.74 ± 0.54 a	1.13 ± 0.04 c	1.46 ± 0.08 b	1.27 ± 0.00 c
9	Ethyl propionate	105-37-3	ND	ND	0.19 ± 0.00	ND	ND	ND
10	Hexyl acetate	142-92-7	1.25 ± 0.24 a	0.27 ± 0.09 bc	0.11 ± 0.01 c	0.41 ± 0.02 b	0.94 ± 0.28 a	ND
11	Isobutyl acetate	110-19-0	0.07 ± 0.00 b	0.34 ± 0.08 a	ND	ND	0.13 ± 0.05 b	0.02 ± 0.00 b
12	Isoamyl acetate	123-92-2	ND	ND	ND	ND	4.80 ± 0.00	ND
13	Methyl acetate	79-20-9	0.23 ± 0.01 a	0.07 ± 0.00 b	ND	ND	ND	0.03 ± 0.00 b
14	Phenethyl acetate	103-45-7	ND	ND	ND	1.06 ± 0.68 b	29.66 ± 1.46 a	ND
15	Methyl caprylate	111-11-5	0.28 ± 0.10	ND	ND	ND	ND	ND
16	Vinyl formate	692-45-5	0.64 ± 0.03 b	0.69 ± 0.04 b	1.15 ± 0.33 a	0.28 ± 0.12 c	0.26 ± 0.00 c	0.61 ± 0.00 b
	ΣEsters		185.93 ± 26.59 a	183.91 ± 4.38 a	59.61 ± 5.48 c	102.10 ± 8.83 b	105.05 ± 10.89 b	89.40 ± 3.39 b
17	1-Butanol	71-36-3	0.01 ± 0.00 a	0.06 ± 0.06 a	0.06 ± 0.03 a	ND	ND	0.04 ± 0.00 a
18	(R,R)-2,3-Butanediol	24347-58-8	13.89 ± 0.00 a	3.57 ± 0.50 c	3.71 ± 0.72 c	3.39 ± 0.01 c	1.68 ± 0.17	7.33 ± 0.04 b
19	cis-4-Decen-1-ol	57074-37-0	4.86 ± 1.73 ab	5.51 ± 0.46 a	3.79 ± 1.03 b	5.85 ± 0.38 a	1.36 ± 0.36 c	6.01 ± 0.84 a
20	1-Hexanol	111-27-3	6.22 ± 1.19 a	5.37 ± 0.02 a	3.72 ± 0.42 b	0.66 ± 0.12 d	3.54 ± 0.20 b	2.46 ± 0.19 c
21	(Z)-3-Hexen-1-ol	928-96-1	ND	ND	0.23 ± 0.00	ND	ND	ND
22	Isoamylol	123-51-3	18.11 ± 2.34 ab	15.46 ± 2.13 b	2.57 ± 0.46 d	5.64 ± 0.46 c	23.37 ± 3.48 a	5.46 ± 0.42 c
23	Isobutanol	78-83-1	16.08 ± 4.05 a	20.41 ± 1.44 a	6.74 ± 0.70 cd	8.56 ± 1.02 c	6.70 ± 0.07 d	12.26 ± 1.49 b
24	Methyl alcohol	67-56-1	2.07 ± 0.56 a	1.21 ± 0.40 a	1.01 ± 0.56 a	0.04 ± 0.00 c	0.28 ± 0.33 bc	0.38 ± 0.27 b
25	1-Nonanol	143-08-8	2.06 ± 0.31 b	0.43 ± 0.10 d	0.23 ± 0.00 e	3.01 ± 0.17 a	0.91 ± 0.24 c	3.15 ± 0.00 a
26	3,6-Nonadien-1-ol, (E,Z)-	56805-23-3	4.02 ± 0.03 a	2.74 ± 0.00 c	2.31 ± 0.05 d	3.55 ± 0.31 b	3.36 ± 0.00 b	2.38 ± 0.53 cd
27	1-Octanol	111-87-5	5.23 ± 0.86 a	5.31 ± 0.87 a	0.17 ± 0.09 d	4.42 ± 0.74 ab	0.34 ± 0.03 c	3.63 ± 0.45 b
28	1-Pentanol	71-41-0	174.59 ± 9.53 a	193.72 ± 11.75 a	93.97 ± 6.80 b	89.05 ± 1.23 b	63.57 ± 7.07 c	102.83 ± 13.46 b
29	Phenethyl alcohol	60-12-8	18.56 ± 3.05 a	22.14 ± 1.97 a	11.01 ± 0.58 b	24.60 ± 4.05 a	10.97 ± 1.24 b	12.64 ± 1.28 b
30	1-Propanol	71-23-8	1.09 ± 0.52 abc	1.36 ± 0.31 a	0.58 ± 0.01 c	0.79 ± 0.00 b	0.17 ± 0.09 d	0.81 ± 0.23 b
	∑Alcohols		266.79 ± 24.17 a	277.29 ± 20.01 a	130.10 ± 11.45 b	149.56 ± 8.49 b	116.25 ± 13.28 c	159.38 ± 19.20 b
31	Acetic acid	64-19-7	6.91 ± 2.11 c	2.79 ± 0.26 d	6.28 ± 1.54 c	7.48 ± 0.23 c	9.49 ± 0.16 b	14.24 ± 2.46 a
32	Gamma-Linolenic acid	506-26-3	ND	ND	ND	2.32 ± 0.00	ND	ND
33	Hexanoic acid	142-62-1	ND	ND	0.37 ± 0.00	ND	ND	ND
34	Octanoic acid	124-07-2	3.97 ± 0.79 a	0.14 ± 0.02 c	4.14 ± 1.39 a	3.64 ± 0.12 a	0.60 ± 0.11 b	3.39 ± 0.10 a
	∑Acids		10.88 ± 2.90 bc	2.93 ± 0.26 d	10.79 ± 2.93 bc	13.44 ± 0.35 b	10.09 ± 0.27 c	17.63 ± 2.56 a
35	Glycolaldehyde	141-46-8	ND	0.06 ± 0.00	ND	ND	ND	ND
36	4-Hydroxy-2-butanone	590-90-9	ND	ND	ND	0.03 ± 0.00	ND	ND
	∑Aldoketones		ND	0.06 ± 0.00 a	ND	0.03 ± 0.00 a	ND	ND
37	Carbamic acid, monoammonium salt	1111-78-0	ND	3.79 ± 0.89 a	1.18 ± 0.00 d	1.60 ± 0.00 c	1.45 ± 0.12 c	2.34 ± 0.38 b
38	2,4-Di-tert-butylphenol	96-76-4	17.01 ± 0.13 c	9.96 ± 1.42 d	16.87 ± 0.51 c	34.75 ± 4.92 a	35.84 ± 3.88 a	19.45 ± 0.34 b
39	3,3-dimethyl-1,5-dioxaspiro [5.5]undecane	707-29-9	ND	ND	ND	ND	0.30 ± 0.00 b	0.49 ± 0.00 a
40	Ethanol, 2-[2-(ethenyloxy)ethoxy]-	929-37-3	ND	ND	1.99 ± 0.00	ND	ND	ND
41	Ethylbenzene	100-41-4	ND	1.52 ± 0.04 a	0.33 ± 0.00 c	0.27 ± 0.00 d	0.31 ± 0.04 cd	0.97 ± 0.00 b
42	2-methyl-1,5-dioxaspiro [5.5]undecane	6413-26-9	1.71 ± 0.29 f	8.92 ± 0.04 e	23.73 ± 1.18 a	11.87 ± 0.90 d	14.21 ± 0.35 c	19.52 ± 0.47 b
43	2-Phenyl-1-propene	98-83-9	0.64 ± 0.03 a	0.61 ± 0.10 a	0.25 ± 0.00 c	ND	0.36 ± 0.09 c	0.56 ± 0.02 b
44	Propane, 1-ethoxy-	628-32-0	ND	ND	ND	1.00 ± 0.02	ND	ND
45	3-Tridecene	61883-33-8	ND	ND	ND	ND	1.58 ± 0.00	ND
	∑Other compounds		19.36 ±0.45 d	24.80 ± 2.49 c	44.35 ± 1.69 b	49.49 ± 5.84 ab	54.05 ± 4.48 a	43.33 ± 1.21 b

Note: ND represents a compound that is not detected; Values in the same column with different lowercase letters are significantly different ($p < 0.05$).

Acids

Four different acids, namely acetic acid, gamma-linolenic acid, hexanoic acid, and octanoic acid, were detected in KFP wines (Table 4). Acetic acid and octanoic acid were identified in all kinds of wines, while gamma-linolenic acid and hexanoic acid were only found in K19- and K14-fermented wines. The concentration of acids ranged from 17.63 mg/L (K26) to 2.93 mg/L (K4). The concentration of acetic acid in K26-fermented wine was the highest, approximately six times compared to K4-fermented wine.

Aldoketones

Glycolaldehyde and 4-Hydroxy-2-butanone were the main aldoketones detected in KFP wines (Table 4). Glycolaldehyde was mainly detected in the K4-fermented wine, while 4-hydroxy-2-butanone was mainly detected in K19-fermented wine. The concentration of aldoketones in KFP wines was relatively low, ranging from 0.06 mg/L (K4) to 0.03 mg/L (K19). There were no significant differences between K4- and K19-fermented wines.

Other Compounds

A total of nine other compounds were identified in KFP wines (Table 4). Only three compounds were detected in the wine with X16 as fermentation starter, which was lower than other wines. The highest concentration of other compounds was observed in K21-fermented wine, while the lowest was observed in X16-fermented wine.

Aroma Contribution of the Main Compounds in KFP Wine

The OAV assay was performed to investigate the aroma-contributing compounds in KFP wine. The OAVs of 22 main aromatic compounds in KFP wine are displayed in Table S3. The OAV of 13 compounds detected in KFP wine was $\geq$1, while that of the nine compounds detected was <1. Most of the OAV $\geq$ 1 compounds were esters, such as ethyl acetate, ethyl butyrate, and ethyl octanoate. The largest OAV of the compounds in KFP wine was ethyl hexanoate, followed by 1-pentanol, ethyl caprate, and (R,R)-2,3-Butanediol, indicating them as the main contributor to the aroma of KFP wine.

PCA was further applied to analyze the aroma contribution of the main compounds in KFP wine. As shown in Figure 7, most of the volatile compounds were clustered in the positive half axis of p (corr) [1], which were closely related to the aromatics of the X16- and K4-fermented wines; these two types of wines had similar concentrations of esters and alcohols, which were well-differentiated from the other four wines, while K14-, K19-, K21-, and K26-fermented wines were mainly on the negative half axis of p (corr) [1]; however, the K21-fermented wine was separated from these wines using K14, K19, and K26 as fermenters.

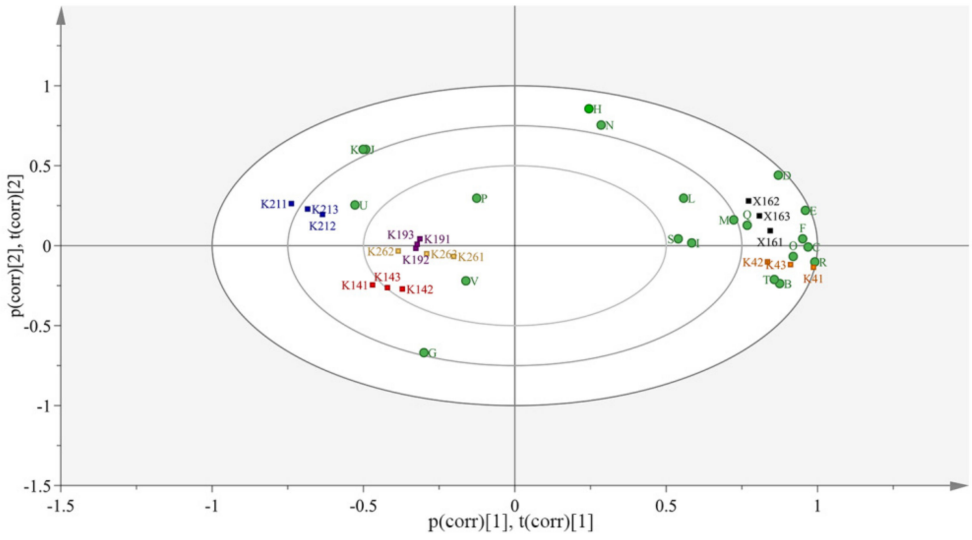

Figure 7. Principal component analysis of aromatic compounds in KFP wine. Abbreviations: A:Ethyl acetate; B:Ethyl butyrate; C:Ethyl octanoate; D:Ethyl caprate; E:Ethyl laurate; F:Ethyl hexanoate; G:Ethyl propionate; H:Hexyl acetate; I:Isobutyl acetate; J:Isoamyl acetate; K:Phenethyl acetate; L: (R,R)-2,3-Butanediol; M:1-Hexanol; N:Isoamylol; O:Isobutanol; P: 1-Nonanol; Q:1-Octanol; R:1-Pentanol; S:Phenethyl alcohol; T:1-Propanol; U:Acetic acid; V: Octanoic acid.

4. Discussion

Non-*Saccharomyces* yeast strains are considered harmful because they are originally isolated from uncontrolled fermentation of spoiled wines or fruits and are associated with the production of off-flavor substances, such as acetic acid, acetaldehyde, and H_2S [15]; however, in-depth research has revealed that non-*Saccharomyces* yeasts can improve the flavor and the overall quality of wine by increasing the amounts of glycerol, total acid, and volatile esters, and reducing the amount of acetic acid [16] Therefore, there has been a great deal of research on non-*Saccharomyces* yeast, and the focus has shifted from grapes and grape wines to other types of fruits and fruit wines, such as mulberry [17], persimmon [18], lychee [19], and cider [20]. The KFP is an important fruit in the Xinjiang region of China; however, research has been limited to analyzing the biodiversity and oenological properties of the native yeasts, particularly the non-*Saccharomyces* yeasts from the KFP fruits. Hence, in the present study, we isolated and identified five strains of non-*Saccharomyces* yeasts, namely *Metschnikowia sinensis*, *Wickerhamomyces anomalus*, *Hanseniaspora gulliermondii*, *Torulaspora delbrueckii*, and *Pichia kluyveri*. The oenological and fermentation characteristics of the five strains were further investigated.

Biodiversity identification of the yeast isolates was performed via two different methods: culture-independent approach (high-throughput sequencing) and culture-dependent approach. The high-throughput sequencing is a powerful, simple, and efficient technique for identifying species of various environmental samples [21–23]. We globally investigated the community composition and the dynamics of the wild wine yeast using a high-throughput sequencing method; however, the insufficient sequencing depth and limited coverage of the comparison database of high-throughput sequencing usually result in 'uncultured' or 'unclassified' species [1]; this phenomenon was also encountered in this research. For example, unclassified-*Torulaspora*, unclassified-*Hanseniaspor*a, and unclassified-*Metschnikowia* were detected (Figure 1B). Therefore, high-throughput sequencing and culture-dependent technologies are indispensable for the investigation of yeast diversity in a comprehensive manner.

During ethanol fermentation, yeast cells are simultaneously and sequentially exposed to a number of stresses: osmotic stresses induced by the high concentrations of sugar substrates at the beginning of the fermentation, acid stress throughout the fermentation process, and ethanol stresses encountered generally from the middle and the end of the fermentation [24]. We investigated the oenological condition tolerances of the native yeasts in KFP wine, and the result showed that the K14 exhibited good tolerance to glucose and malic acid among the glucose concentrations ranging from 100 g/L to 300 g/L and malic acid concentrations ranging from 1.5% to 2.5% compared with the commercial *S. cerevisiae* X16 (Figure 4). The ethanol and sulfur dioxide tolerances of the K14 strain were lower than those of the X16. Compared with the growth of X16, the growth rate of the K4 strain was inhibited under all the treated conditions. All the strains were sensitive to ethanol, and their tolerances were lower than that of X16.

Glycosidases can effectively hydrolyze aromatic compounds containing glycosidic bonds and promote the release of free-state aromatic glycosidic ligands [25]. Our study showed that the β-glucosidase activity of strain K26 was significantly higher than that of the commercial *S. cerevisiae* X16. Conversely, the β-glucosidase activities of other strains were lower than that of X16. Only the β-glucosidase activity was determined in this study. Thus, other glycosidases, such as α-L-arabinofuranosidase [26], α-L-rhamnosidase [27], or β-D-xylosidase [28], need to be investigated.

Several studies have supported the combination of non-*Saccharomyces* starters and *S. cerevisiae* for the production of wine [29,30]. In this research, we co-inoculated non-*Saccharomyces* yeasts with *S. cerevisiae* X16 for laboratory-scale fermentation of KFP wine. The basic physicochemical parameters of Korla fragrant pear wine with different yeast strain combinations were different. For example, the combination of K4 and X16 could reduce the total acidity and contents of alcohol and increase the concentration of volatile acidity

compared with the inoculation of S. cerevisiae alone; however, there were no significant differences in the electronic sensory characteristics among these types of KFP wine.

Ethyl acetate and phenethyl acetate are common ester compounds found in various wines. The moderate concentration of ethyl acetate gives the wine elegant tropical fruit and floral aromas [31]. We found that the concentrations of phenethyl acetate in K19- and K26-fermented wines and the concentrations of ethyl acetate in K19- and K21-fermented wines significantly increased, which indicated that the fragrance characteristics of these wines were more complex and rich than other wines.

5. Conclusions

To the best of our knowledge, this study is the first to systematically analyze the biodiversity and oenological properties of the native non-*Saccharomyces* yeasts of KFP. The high-throughput sequencing method was used to detect varieties of yeast species. Five species of culturable yeasts were isolated by the traditional purification and separation method. The characteristics of growth, carbon source utilization, H_2S production, and β-glucosidase activity of these native yeasts were different. The oenological condition tolerances of most strains were lower than that of commercial *S. cerevisiae* X16. The mixed inoculum of the strains of native yeasts with *S. cerevisiae* X16 can regulate the volatile aroma characteristics of the fermented KFP fruit wine, enriching and complicating the aroma flavor. Thus, the combined inoculation of these indigenous yeasts with *S. cerevisiae* has some application potential in the production of KFP wine.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/fermentation8080388/s1, Table S1: Diversity index of fungus in spontaneous fermentation of KFP; Table S2: Yeast identification by comparing 26S rDNA D1/D2 domain sequences; Table S3: The OAV of the main aromatic compounds in KFP wine.

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References

1. Liu, X.Z.; Li, Y.F.; Yu, Z.H.; Hardie, W.J.; Huang, M.Z. Biodiversity of non-*Saccharomyces* yeasts during natural fermentation of *Rosa roxburghii*. *Acta Microbiol. Sin.* **2020**, *60*, 1696–1708.
2. Morata, A.; Escott, C.; Bañuelos, M.A.; Loira, I.; del Fresno, J.M.; González, C.; Suárez-Lepe, J.A. Contribution of non-*Saccharomyces* yeasts to wine freshness: A review. *Biomolecules* **2020**, *10*, 34. [CrossRef] [PubMed]
3. Borren, E.; Tian, B. The important contribution of non-*Saccharomyces* yeasts to the aroma complexity of wine: A review. *Foods* **2021**, *10*, 13. [CrossRef] [PubMed]
4. Basso, R.F.; Alcarde, A.R.; Portugal, C.B. Could non-*Saccharomyces* yeasts contribute on innovative brewing fermentations? *Food Res. Int.* **2016**, *86*, 112–120. [CrossRef]
5. Liu, X.Z.; Li, Y.F.; Zhou, J.C.; Huang, M.Z. Effects of co-inoculation and sequential inoculation of *Wickerhamomyces anomalus* and *Saccharomyces cerevisiae* on the physicochemical properties and aromatic characteristics of longan (*Dimocarpus longan* Lour.) wine. *Qual. Assur. Saf. Crops* **2021**, *13*, 56–66. [CrossRef]
6. Liu, Y.; Zhang, Q.; Niu, H.; Zhang, H.; Lan, H.P.; Zeng, Y.; Jia, F.G. Prediction method for nutritional quality of Korla pear during storage. *Int. J. Agr. Biol. Eng.* **2021**, *14*, 247–254. [CrossRef]

7. Wang, Y.; Su, S.P.; Chen, G.G.; Mao, H.J.; Jiang, Y. Relationship between cuticular waxes and storage quality parameters of Korla pear under different storage methods. *J. Plant Growth Regul.* **2021**, *40*, 1152–1165. [CrossRef]
8. Wang, Y.; Mao, H.; Lv, Y.; Chen, G.G.; Jiang, Y. Comparative analysis of total wax content, chemical composition and crystal morphology of cuticular wax in Korla pear under different relative humidity of storage. *Food Chem.* **2021**, *339*, 128097. [CrossRef]
9. Mamat, A.; Tusong, K.; Xu, J.; Yan, P.; Mei, C.; Wang, J.X. Integrated transcriptomic and proteomic analysis reveals the complex molecular mechanisms underlying stone cell formation in Korla pear. *Sci. Rep.* **2021**, *11*, 7688. [CrossRef]
10. Fu, L.; Zhang, H.; Ye, H. Studies on the yeast fermentation performance of XL1 and XL2 isolated from Korla pear. *Food Sci.* **2003**, *24*, 61–64.
11. Loira, I.; Morata, A.; Comuzzo, P.; Callejo, M.J.; González, C.; Calderón, F.; Suárez-Lepe, J.A. Use of *Schizosaccharomyces pombe* and *Torulaspora delbrueckii* strains in mixed and sequential fermentations to improve red wine sensory quality. *Food Res. Int.* **2015**, *76*, 325–333. [CrossRef]
12. Liu, X.Z.; Li, Y.F.; Yu, Z.H.; Hardie, W.J.; Huang, M.Z. Screening and characterisation of β-glucosidase production strains from *Rosa roxburghii* Tratt. *Int. J. Food Eng.* **2021**, *17*, 1–9. [CrossRef]
13. Liu, X.Z.; Li, Y.F.; Zhao, H.B.; Yu, Z.H.; Huang, M.Z. Oenological property analysis of selected *Hanseniaspora uvarum* isolated from *Rosa roxburghii* Tratt. *Int. J. Food Eng.* **2020**, *17*, 445–454. [CrossRef]
14. Song, R.R.; Duan, W.P.; Zhu, X.; Liu, Q.; Yang, X.S.; Han, S.Y. Effects of sequential inoculation with *Torulaspora delbrueckii* and *Saccharomyces cerevisiae* on aroma profile of dry red wine. *Food Ferment. Ind.* **2019**, *45*, 1–9.
15. Padilla, B.; Gil, J.V.; Manzanares, P. Past and future of non-*Saccharomyces* yeasts: From spoilage microorganisms to biotechnological tools for improving wine aroma complexity. *Front. Microbiol.* **2016**, *7*, 411. [CrossRef]
16. Tufariello, M.; Fragasso, M.; Pico, J.; Panighel, A.; Castellarin, S.D.; Flamini, R.; Grieco, F. Influence of non-*Saccharomyces* on wine chemistry: A focus on aroma-related compounds. *Molecules* **2021**, *26*, 644. [CrossRef]
17. Hu, J.; Vinothkanna, A.; Wu, M.; Ekumah, J.N.; Akpabli-Tsigbe, N.D.K.; Ma, Y.K. Tracking the dynamic changes of a flavor, phenolic profile, and antioxidant properties of *Lactiplantibacillus plantarum*-and *Saccharomyces cerevisiae*-fermented mulberry wine. *Food Sci. Nutr.* **2021**, *9*, 6294–6306. [CrossRef]
18. Kwon, S.J.; Choi, J.S.; Yeo, S.H.; Par, H.D. Fermentation characteristics of persimmon wine by the mixed culture of *Pichia anomala* JK04 and *Saccharomyces cerevisiae* Fermivin cells. *Korean J. Food Preserv.* **2015**, *22*, 768–777. [CrossRef]
19. Chen, D.; Yap, Z.Y.; Liu, S.Q. Evaluation of the performance of *Torulaspora delbrueckii*, *Williopsis saturnus*, and *Kluyveromyces lactis* in lychee wine fermentation. *Int. J. Food Microbiol.* **2015**, *206*, 45–50. [CrossRef]
20. Xu, Y.; Zhao, G.A.; Wang, L.P. Controlled formation of volatile components in cider making using a combination of *Saccharomyces cerevisiae* and *Hanseniaspora valbyensis* yeast species. *J. Ind. Microbiol. Biot.* **2006**, *33*, 192–196. [CrossRef]
21. Soon, W.W.; Hariharan, M.; Snyder, M.P. Highthroughput sequencing for biology and medicine. *Mol. Syst. Biol.* **2013**, *9*, 640. [CrossRef]
22. Zhao, L.Y.; Li, Y.; Jiang, L.W.; Deng, F.M. Determination of fungal community diversity in fresh and traditional Chinese fermented pepper by pyrosequencing. *FEMS Microbiol. Lett.* **2016**, *363*, fnw273. [CrossRef]
23. Wei, Y.J.; Wu, Y.; Yan, Y.Z.; Zou, W.; Xue, J.; Ma, W.R.; Wang, W.; Tian, G.; Wang, L.Y. Highthroughput sequencing of microbial community diversity in soil, grapes, leaves, grape juice and wine of grapevine from China. *PLoS ONE* **2018**, *13*, e0193097.
24. Auesukaree, C. Molecular mechanisms of the yeast adaptive response and tolerance to stresses encountered during ethanol fermentation. *J. Biosci. Bioeng.* **2017**, *124*, 133–142. [CrossRef]
25. Hu, K.; Qin, Y.; Tao, Y.S.; Zhu, X.L.; Peng, G.T.; Ullah, N. Potential of glycosidase from non-*Saccharomyces* isolates for enhancement of wine aroma. *J. Food Sci.* **2016**, *81*, M935–M943. [CrossRef]
26. Margolles, A.; de los Reyes-Gavilan, C.G. Purification and functional characterization of a novel α-l-arabinofuranosidase from *Bifidobacterium longum* B667. *Appl. Environ. Microb.* **2003**, *69*, 5096–5103. [CrossRef]
27. Gallego, M.V.; Pinaga, F.; Ramón, D.; Vallés, S. Purification and characterization of an α-L-Rhamnosidase from *Aspergillus terreus* of interest in winemaking. *J. Food Sci.* **2001**, *66*, 204–209. [CrossRef]
28. Padilla, B.; Gil, J.V.; Manzanares, P. Challenges of the non-conventional yeast *Wickerhamomyces anomalus* in winemaking. *Fermentation* **2018**, *4*, 68. [CrossRef]
29. Balmaseda, A.; Bordons, A.; Reguant, C.; Bautista-Gallego, J. Non-*Saccharomyces* in wine: Effect upon *Oenococcus oeni* and malolactic fermentation. *Front. Microbiol.* **2018**, *9*, 534. [CrossRef]
30. Varela, C. The impact of non-*Saccharomyces* yeasts in the production of alcoholic beverages. *Appl. Microbiol. Biotechnol.* **2016**, *100*, 9861–9874. [CrossRef]
31. Saerens, S.; Thevelein, J.; Delvaux, F. Ethyl ester production during brewery fermentation, a review. *Cerevisia* **2008**, *33*, 82–90.