
EXPERIMENTAL ARTICLES

Dynamic Changes in Yeast Populations and Aroma Composition of ‘Beimei’ (*Vitis vinifera* × *Vitis amurensis*) during Spontaneous Fermentation

Y. F. Li^a and X. Z. Liu^{a, *}

^a College of Food and Pharmaceutical Engineering, Guizhou Institute of Technology, Guiyang, 550003 China

*e-mail: liuxiaozhu_840914@163.com

Received November 30, 2023; revised April 24, 2024; accepted April 29, 2024

Abstract—‘Beimei’ is a hybrid cultivar of *Vitis vinifera* and wild *Vitis amurensis* with fungus- and cold-resistant characteristics that is widely grown in many regions of China. However, studies on the yeast diversity and aroma compound composition of this cultivar remain scarce. Here, indigenous yeast diversity, aroma compound composition, and their dynamic changes during the spontaneous fermentation of ‘Beimei’ were investigated and monitored using high-throughput sequencing and gas chromatography-mass spectrometry. The result indicated that non-*Saccharomyces* yeasts were predominant at the initial stages of ‘Beimei’ spontaneous fermentation and then sharply decreased. *Cladosporium herbarum* and *Hanseniaspora uvarum* were identified as the dominant species of filamentous fungi and yeast, respectively, at the beginning and on the first day of fermentation, whereas unclassified_f_Saccharomycetaceae was dominant throughout fermentation, starting from the third day until the end of fermentation. The main volatile aroma chemicals were esters and alcohols, which accumulated abundantly at the late stage of fermentation. Taken together, this study represents the first step in exploring untapped yeast species and aroma compounds, which is helpful for the screening of native yeasts from ‘Beimei’ and producing distinctive aroma wines.

Keywords: ‘Beimei’, spontaneous fermentation, yeasts diversity, volatile aroma compounds, dynamics

DOI: 10.1134/S002626172360458X

Breeding grape varieties with high resistance to harsh cultivation environments is urgently needed because of deterioration of the global climate (Teixeira et al., 2013). ‘Beimei’ is a vinifera hybrid cultivar with high resistance to cold and fungus diseases using *Vitis vinifera* as the female parent and wild *Vitis amurensis* as the male parent (Wang et al., 2014; Jiao et al., 2022). Recently, most studies involving ‘Beimei’ have focused on cultivation and agronomic traits (Li et al., 2017). For example, Kuang et al. (2022) investigated the berry quality traits and metabolites of ‘Beimei’ during berry development and winemaking. They found that the fermented wines with ‘Beimei’ had a typical pleasant rose aroma.

Aroma is one of the most important factors affecting the final quality of wine and the choices of wine consumers (Parker et al., 2017). Generally, wine aroma originates from grapes (Belda et al., 2017), yeast (Mencarelli and Bellincontro, 2017), and winemaking conditions (Peng et al., 2017). It is well known that grape harbors various native oenological yeasts which are closely associated with wine flavor regional distinctiveness (Luzzini et al., 2021). In a study conducted by Liu et al. (2021), the authors compared the diversity and dynamics of yeasts during spontaneous

fermentations of Pinot Noir and Chardonnay using metagenomics and population genetics approaches. They found differentiation of yeast species between different vineyards and grape varieties. Therefore, it is necessary to identify the dynamic changes in yeast population and aroma composition during spontaneous fermentation to produce unique terroir wines. However, the microbiota and aroma components of ‘Beimei’ during spontaneous fermentation remain unclear.

In this study, we identified dynamic changes in the yeast populations of ‘Beimei’ during spontaneous fermentation using a high-throughput sequencing approach. We also detected dynamic compositional changes in the compounds responsible for its aroma (referred to as aroma compounds) during spontaneous fermentation using gas chromatography-mass-spectrometry (GC-MS). Our results provide a theoretical basis for the exploration of these native yeast resources and the production of unique wine flavors.

MATERIALS AND METHODS

Fruit traits of ‘Beimei.’ Berries of ‘Beimei’ were collected from different vineyards in Guiyang region,



Fig. 1. Grape berries morphological characteristics of the variety ‘Beimei.’

Guizhou Province, China. The berry weight of ‘Beimei’ was determined by calculating the average weight of 50 berries. The pH and content of soluble solids (°Brix) of ‘Beimei’ must were determined using a digital pH meter (Sartorius AG, Germany) and a hand refractometer (ATAGO, Japan), respectively.

Spontaneous fermentation and sample collection. Healthy and undamaged grape berries were selected and pressed to obtain grape must. Spontaneous laboratory-scale fermentation was performed by transferring 600 mL of grape must and the pressed skins to sterile 1000-mL flasks, which were statically incubated at 28°C in triplicate. During spontaneous fermentation, five sample stages were prepared at 0, 1, 3, 5, 7, and 15 days (designated as M0, M1, M3, M5, M7, and M15). Before collecting 4-mL aliquots, the samples were thoroughly mixed. Each sample was divided into two parts: one part was used for microbial separation and identification and the other was used for aroma compounds extraction and detection.

Yeast identification. High-throughput sequencing approaches were used for yeast identification (David et al., 2014). Total DNA was extracted from each sample using an E.Z.N.A.[®] Soil DNA Kit (Omega Bio-tek, Norcross, GA, United States). The DNA concentration and purification degree were determined using a NanoDrop 2000 spectrophotometer (Thermo Scientific, United States). Polymerase chain reaction (PCR) amplification of the V5 to V7 region of 18S rDNA was conducted using the primers SSU0817F and 1196R (Table S1) and then purified using a DNA Gel Extraction Kit (Axygen Biosciences, United States). Purified and pooled amplification libraries were paired-end sequenced according to standard protocols published 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 cut-off. Alpha diversity, yeast species composition, and yeast species differences between samples were calculated and analyzed using the Majorbio cloud platform. A phylogenetic tree was constructed by the neighbor-joining method using Molecular Evolutionary Genetics Analysis (MEGA X, United States) software.

Volatile aromatic composition analysis. The volatile aroma components of ‘Beimei’ were extracted at 40°C for 30 min using headspace solid-phase microextraction. The aromatic chemical contents were determined using a gas chromatography-mass spectrometer (TQ8040, Agilent, United States) with cyclohexanone as the internal standard (Li et al., 2023). The odor activity value (OAV) of each aroma component was calculated by determining the threshold values for each volatile aroma component (Li et al., 2022).

Statistical analysis. The results of fruit traits and contents of volatile aroma components of ‘Beimei’ were expressed as the mean $\pm$ standard deviation. Univariate analysis of variance (ANOVA) of the data and significance of the difference test were performed using the SPSS software (version 21.0). Statistical significance was set at $P < 0.05$.

RESULTS

Fruit Traits of ‘Beimei’

The berry color of ‘Beimei’ was dark blue (Fig. 1) and the average berry weight was 1.61 g. The grape must color was red, emitting a rose-like fragrance. The pH of the must was 3.21 and the soluble solids content was 18.5 °Brix.

Yeast diversity composition of ‘Beimei’ during spontaneous fermentation. We used high-throughput sequencing for the identification of yeast diversity composition of ‘Beimei’ during spontaneous fermentation. The Shannon, Simpson, Sobs, Ace, and Chao indices were used to assess the species diversity of samples in high-throughput sequencing (Liu et al., 2020). As may be seen from Table 1, the Shannon indices of samples M0 and M1 were larger, whereas the Simpson index was smaller, indicating richer yeast species diversity at the beginning of fermentation (M0) and the first day of spontaneous fermentation (M1). In contrast, in the middle (M7) and late (M15) stages of spontaneous fermentation, the Shannon and Simpson indices decreased, indicating the presence of fewer species in these samples. In addition, the Sobs, Ace, and Chao indices of M0 were the highest, followed by M1, M3, M5, M7, and M15, consistent with the data on the Shannon and Simpson indices. Taken together, these results indicated that the yeast species diversity and richness gradually decreased with the continuous spontaneous fermentation of ‘Beimei.’

In total, 149 genera and 154 yeast species and filamentous fungi were found from spontaneous fermen-

Table 1. Diversity index of yeast in samples

Sample	Shannon index	Simpson index	Sobs index	Ace index	Chao index
M0	2.27	0.20	43.00	43.76	43.33
M1	1.21	0.55	38.67	43.07	44.67
M3	0.72	0.50	16.00	37.86	29.75
M5	0.38	0.80	11.67	28.42	17.00
M7	0.03	1.00	9.67	23.85	13.83
M5	0.08	0.98	5.67	3.00	5.67

tation of ‘Beimei’ samples. The top 50 species of yeasts and filamentous fungi were clustered in Fig. 2 in a cladogram. Samples M0 and M1, having similar species composition, were clustered in the same branch. The middle and late fermentation stages (M3, M5, M7, and M15) clustered in another branch. High abundance of yeast species detected in spontaneous fermentation of ‘Beimei’ samples included *Hanseniaspora uvarum*, *Candida boidinii*, *Pichia terricola*, *Candida agrestis*, *Golubevia pallescens*, *Bullera unica*, *Sporidiobolus pararoseus*, *Vishniacozyma peneaus*, *Kodamaea ohmeri*, *Starmerella bacillaris*, *Schizosaccharomyces*, *Clavispora*, and *Saccharomycetaceae*.

Dynamic changes in yeast community composition of ‘Beimei’ during spontaneous fermentation. As shown in Fig. 3, the highest diversity of microbial species was observed at the beginning of fermentation (M0) and on the first day of fermentation (M1). However, as fermentation progressed, the diversity of microorganisms decreased significantly (M3 and M5). During the middle stage of fermentation, most microorganisms disappeared. *H. uvarum* was primarily present during the initial stage of spontaneous fermentation and reached its maximum concentration on the first day (M1). Subsequently, it gradually decreased and remained at a very low level until the end of fermentation. At the beginning of fermentation, the abundance of the unclassified *Saccharomycetaceae* was very low. It increased significantly from the third day of fermentation (M3) and remained at a consistently high level throughout the fermentation process (M5, M7, and M15). We further analyzed the species of unclassified *Saccharomycetaceae* by constructing a phylogenetic tree and found that they were highly similar to *Saccharomyces cerevisiae* (Fig. 4).

Aroma components composition of ‘Beimei’ during spontaneous fermentation. As demonstrated in Table 2 and Fig. 5a, a total of 75 volatile compounds were identified in six spontaneous fermentation stages of ‘Beimei’ grapes. These compounds were categorized as esters (20), alcohols (22), aldehydes (7), and others. Among these compounds, there were 23 other com-

pounds, which were most prevalent. Twenty ester compounds were found in the spontaneously fermented samples. Five, 6, 15, 14, 15, and 10 ester compounds were observed in M0, M1, M3, M5, M7, and M15, respectively (Fig. 5b). The content of ester compounds was 45.99 ± 5.52 mg/L in the unfermented grape sample (M0), and 142.07 ± 6.07 , 5013.09 ± 579.27 , 5457.64 ± 127.48 , 6474.24 ± 172.70 , and 7525.78 ± 237.65 mg/L in the fermented samples M1, M3, M5, M7, and M15, respectively. In addition, 22 alcohol compounds were detected in the spontaneously fermented samples and 12, 16, 15, 13, 12, and 11 alcohol compounds were detected in the M0, M1, M3, M5, M7, and M15 samples, respectively (Table 2, Fig. 4b).

Dynamic changes in the aroma components of ‘Beimei’ during spontaneous fermentation. The number of alcohol and ester compounds first increased and then decreased during the stages of spontaneous fermentation (Fig. 5b). In contrast, aldehydes and other compounds consistently decreased throughout the entire period of spontaneous fermentation (Fig. 5b). Regarding the changes in compound content, it was clear that esters, alcohols, and other compounds increased significantly primarily from the third day (M3) onwards. These higher levels remained until the end of spontaneous fermentation, compared with the initial stage of fermentation. In contrast, the contents of aldehyde compounds declined as fermentation continued (Fig. 4b).

The contents of methyl acetate, ethyl acetate, ethyl hexanoate, ethyl octanoate, ethyl decanoate, the floral, fruity, and vinous aroma compounds increased significantly especially from the 3rd day of spontaneous fermentation. In addition, ester compounds showed the highest level from the 3rd day until fermentation ended among the ester compounds (Fig. 5a). Furthermore, we found that the contents of floral and fruity aroma alcohol compounds, such as citronellol, phenethyl alcohol, and linalool, increased significantly and reached the highest level at the end of the fermentation process (Fig. 6a).

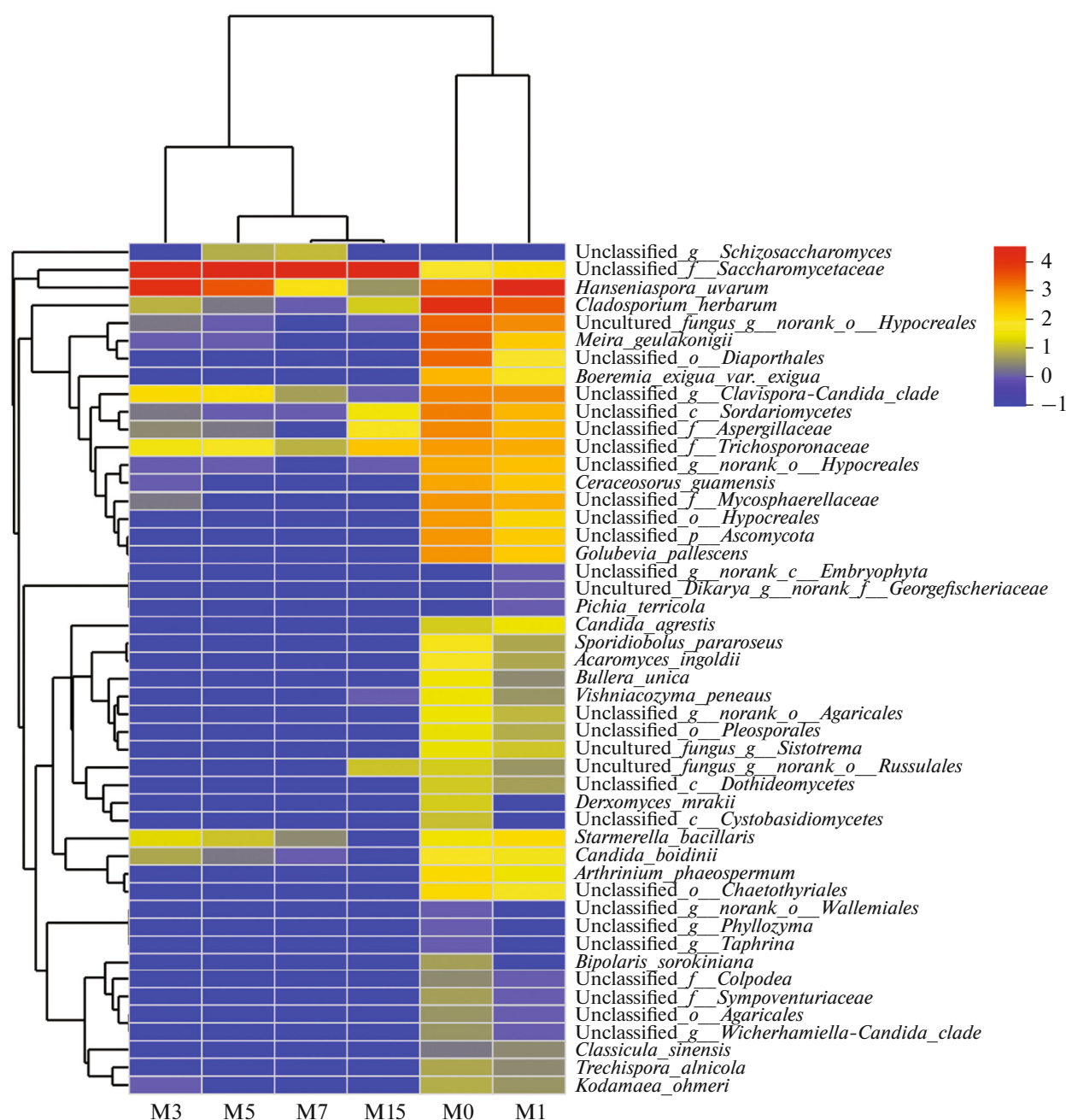


Fig. 2. Heatmap and dendrogram of top 50 species of filamentous fungi and yeasts species in spontaneous fermentation of 'Beimei' specimens.

Contribution of aroma compounds to the flavor of spontaneously fermented 'Beimei' wine. The contributions of the main aroma compounds were analyzed by calculating their OAV values. As shown in Table 3, most aroma chemicals were produced on the third or fifth day of spontaneous fermentation. The OAV of isobutanol gradually increased during fermentation. Its values were <1 at the initial stage of fermentation

(M0 and M1) and then became >1 on the third day of fermentation. 1-Pentanol had an OAV <1 during the entire spontaneous fermentation period. In contrast, three compounds, ethyl acetate, ethyl hexanoate, and hexanol, had OAVs >1 during the entire spontaneous fermentation process, suggesting their important contribution to the flavor of spontaneous fermentation-produced wine.

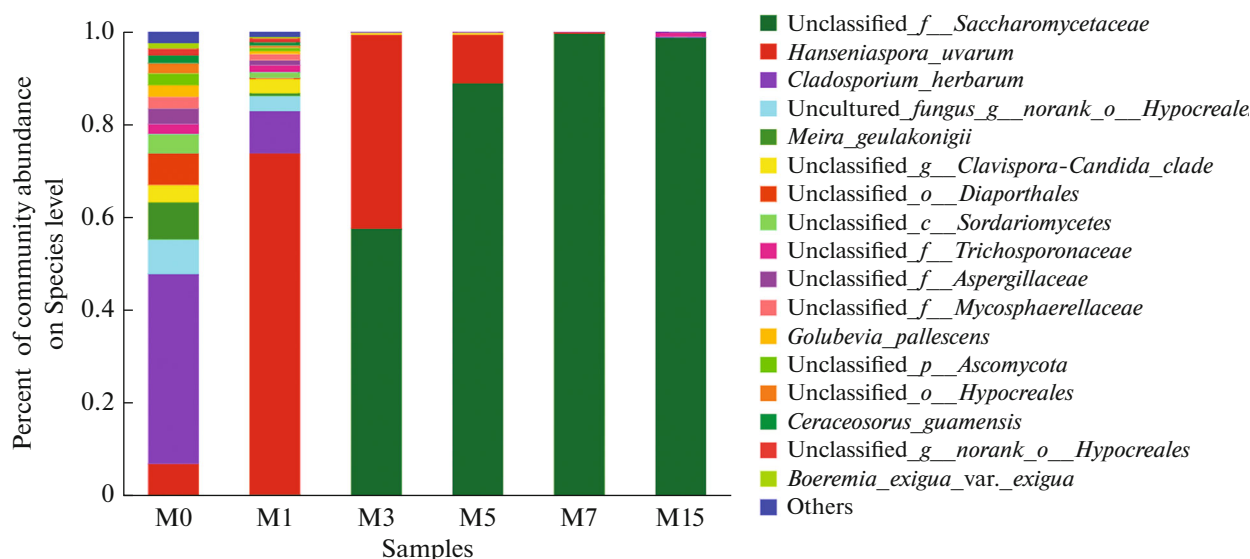


Fig. 3. Histograms showing percentage community abundance at species level during spontaneous fermentation of 'Beimei.'

DISCUSSION

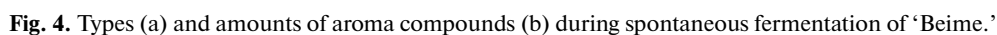
Grape variety is one of the main factors in microbial terroir (del Carmen and Mas, 2016; Zhang et al., 2019), 'Beimei' has been less explored, although it has been widely planted in most wine regions in China; the yeast population composition of this cultivar remains unknown. To our knowledge, this study was the first to use high-throughput sequencing technology to analyze and monitor the diversity of indigenous yeast and the dynamic changes that occur during the spontaneous fermentation of 'Beimei' grape. The analysis indicated that top 50 species of yeasts and filamentous fungi were detected by high-throughput sequencing (Figs. 2 and 3). Moreover, the filamentous fungus *Cladosporium herbarum* was abundantly detected at the beginning of spontaneous fermentation, whereas *H. uvarum* became the dominant species on the first day of fermentation, and unclassified_f_Saccharomycetaceae dominated the fermentation from day 3 to the end of fermentation (Fig. 3). *S. cerevisiae* was the dominant yeast species in the middle and late stages of fermentation owing to its excellent ethanol tolerance. We suspect that the unclassified_f_Saccharomycetaceae species may be *S. cerevisiae*, which will be verified in subsequent experiments using culture-dependent approach.

Usually, most non-*Saccharomyces* yeasts are sensitive to ethanol and dominate the early stages of fermentation. Most die during the middle stage of fermentation, with accumulation of ethanol (Jolly et al., 2014; Mehloakulu et al., 2021). In this study, we detected abundant yeast species in samples M0 and M1, and yeast diversity decreased rapidly in samples M3, M5, M7, and M15 (Fig. 3). We also found a similar phenomenon during the spontaneous fermentation of 'Beihong,' another Eurasian *Vitis* cultivar with

superior disease and cold resistances. In that study, using a culture-dependent approach, we confirmed that the dominant yeast species at the middle and late stages of fermentation was *S. cerevisiae* (Liu et al., 2021).

High-throughput sequencing techniques have been widely applied to investigate microbial diversity in various samples, including many grape cultivars such as *Muscat blanc* (Wang and Liu., 2013), *Vitis davidii* (Wang et al., 2019, and Catalanesca (Di et al., 2007), since they are rapid, simple, and efficient. However, high-throughput sequencing techniques have several disadvantages. For example, many 'uncultured' and 'unclassified' species are observed in the high-throughput sequencing results and this phenomenon was also observed in the present study (Figs. 2 and 3). This may be attributed to the limitations of the reference database. Therefore, culture-dependent technology is required for the full investigation of yeast diversity and dynamics, which will provide further confirmation.

Aroma is an important parameter affecting the final quality of wine (Lyu et al., 2021). In this study, we found many esters with fruity and floral aromas, such as ethyl hexanoate and ethyl octanoate largely accumulated at the late stage of the 'Beimei' spontaneous fermentation (Table 2, Fig. 5). Moreover, several rose-scented alcohols were also abundantly detected at the late stage of the 'Beimei' spontaneous fermentation. For example, citronellol (192.68 ± 22.92 mg/L) and phenethyl alcohol (4481.46 ± 336.20 mg/L) were detected in the sample of M15 (Table 2). These data indicated that the fermented wine with 'Beimei' had intense and elegant rose scents, which could attract a large number of consumers.



beginning and on the first day of fermentation, whereas unclassified *Saccharomycetaceae* dominated the whole fermentation from the third day to the end day. The main volatile aroma chemicals were esters and alcohols, which accumulated abundantly at the

Table 2. Volatile compounds (mg/L) during spontaneous fermentation of ‘Beimei’ wine

Num- ber	Volatile compound	Content, mg/L						
		M0	M1	M3	M5	M7	M15	
1	1-Butanol, 3-methyl-, acetate	/	/	2245.90 ± 285.21	/	/	823.64 ± 27.60	
2	Ethyl acetate	33.60 ± 4.73	113.69 ± 5.55	2101.07 ± 245.51	2209.90 ± 47.20	2071.31 ± 48.69	3159.16 ± 87.22	
3	Methyl acetate	0.28 ± 0.01	1.54 ± 0.06	8.35 ± 0.01	10.27 ± 0.62	9.93 ± 0.22	12.36 ± 0.01	
4	Isobutyl acetate	/	/	27.99 ± 2.76	17.50 ± 0.50	12.51 ± 0.18	/	
5	n-Propyl acetate	/	/	5.68 ± 0.67	/	/	/	
6	Ethyl propionate	/	/	21.30 ± 3.41	20.43 ± 0.56	29.94 ± 0.84	116.76 ± 10.63	
7	Ethyl isobutyrate	/	/	1.92 ± 0.1	/	/	48.47 ± 0.14	
8	Ethyl butyrate	0.59 ± 0.08	6.70 ± 0.18	15.85 ± 0.51	29.23 ± 2.36	49.43 ± 0.27	/	
9	Isoamyl acetate	/	/	/	868.71 ± 21.07	855.97 ± 22.87	/	
10	Methyl hexanoate	/	/	/	16.07 ± 0.05	13.29 ± 0.37	/	
11	Isoamyl propionate	10.90 ± 0.65	13.75 ± 0.21	13.02 ± 0.68	4.38 ± 0.02	/	/	
12	Ethyl hexanoate	0.62 ± 0.05	0.86 ± 0.01	136.12 ± 10.65	941.45 ± 31.44	1086.98 ± 38.41	1067.47 ± 42.80	
13	Hexyl acetate	/	5.53 ± 0.06	40.74 ± 5.73	37.19 ± 0.73	47.11 ± 1.20	/	
14	Ethyl octanoate	/	/	253.28 ± 12.65	920.27 ± 4.62	1475.72 ± 10.83	1395.30 ± 37.97	
15	Methyl octanoate	/	/	6.72 ± 0.14	15.93 ± 0.57	18.01 ± 0.13	/	
16	Ethyl decanoate	/	/	87.27 ± 1.10	332.24 ± 13.45	627.47 ± 41.04	846.82 ± 28.83	
17	Phenethyl acetate	/	/	47.88 ± 10.14	34.07 ± 4.29	27.56 ± 0.35	27.71 ± 1.39	
18	Ethyl dodecanoate	/	/	/	/	137.21 ± 6.78	/	
19	Diethyl succinate	/	/	/	/	/	28.09 ± 1.06	
20	6-Octen-1-ol, 3,7-dimethyl-, acetate	/	/	/	/	11.80 ± 0.52	/	
	ΣEsters	45.99 ± 5.52	142.07 ± 6.07	5013.09 ± 579.27	5457.64 ± 127.48	6474.24 ± 172.70	7525.78 ± 237.65	
21	3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)-, (R)-	/	/	14.84 ± 0.69	24.26 ± 1.14	/	/	
22	Methanol	1.03 ± 0.17	1.81 ± 0.26	9.11 ± 1.94	27.40 ± 1.47	41.78 ± 0.40	91.61 ± 5.81	
23	Isobutanol	1.08 ± 0.20	4.96 ± 0.09	197.07 ± 12.11	324.02 ± 3.73	431.41 ± 4.61	940.45 ± 13.22	
24	Citronellol	/	/	46.38 ± 1.28	91.48 ± 1.33	119.15 ± 2.57	192.68 ± 22.92	
25	1-Butanol	0.55 ± 0.04	0.89 ± 0.09	/	/	/	/	

Table 2. (Contd.)

Num- ber	Volatile compound	Content, mg/L					
		M0	M1	M3	M5	M7	M15
26	Isoamylol	15.47 ± 1.26	80.89 ± 1.53	3169.88 ± 17.49	6997.47 ± 87.71	9233.55 ± 111.03	/
27	1-Pentanol	0.82 ± 0.07	2.68 ± 0.21	54.45 ± 2.09	/	/	/
28	1-Octen-3-ol	0.57 ± 0.01	/	9.76 ± 0.93	/	/	/
29	1-Octanol	/	/	212.73 ± 3.69	361.43 ± 5.83	347.76 ± 11.71	495.65 ± 18.49
30	2-Heptanol	0.34 ± 0.06	1.49 ± 0.04	/	/	/	/
31	Prenol	/	1.28 ± 0.02	/	/	/	/
32	2,3-Butanediol	/	/	/	66.62 ± 7.78	62.73 ± 3.43	294.73 ± 2.21
33	1-Hexanol	105.48 ± 6.92	231.40 ± 12.74	322.86 ± 2.79	275.58 ± 3.89	233.43 ± 6.84	525.57 ± 10.62
34	(E)-3-Hexen-1-ol	1.13 ± 0.03	2.01 ± 0.06	/	/	/	/
35	(Z)-3-Hexen-1-ol	1.01 ± 0.04	3.33 ± 0.01	3.60 ± 0.02	/	/	/
36	(E)-2-Hexen-1-ol	4.28 ± 0.61	/	/	/	/	/
37	(Z)-2-Hexen-1-ol	0.29 ± 0.05	1.37 ± 0.02	/	/	/	/
38	Sulcatol	/	1.31 ± 0.04	23.34 ± 0.33	42.24 ± 0.66	46.18 ± 0.53	55.13 ± 1.05
39	Linalool	/	3.24 ± 0.49	29.02 ± 3.44	75.13 ± 3.57	99.31 ± 2.14	197.23 ± 25.95
40	Alpha-terpineol	/	0.80 ± 0.03	13.79 ± 0.54	23.11 ± 1.59	39.03 ± 1.47	78.73 ± 8.68
41	(Z)-3,7-dimethyl-2,6-octadien-1-ol	/	5.07 ± 0.18	40.16 ± 2.35	53.11 ± 3.19	49.04 ± 1.34	45.97 ± 0.41
42	Phenethyl alcohol	/	1.30 ± 0.06	292.95 ± 41.27	1257.12 ± 74.49	2039.71 ± 238.56	4481.46 ± 336.20
	ΣAlcohols	132.05 ± 9.46	343.83 ± 15.87	4439.94 ± 90.96	9618.97 ± 196.38	12743.08 ± 384.63	7399.21 ± 445.56
43	Acetaldehyde	/	/	18.47 ± 1.45	42.87 ± 4.80	29.54 ± 2.93	57.94 ± 2.70
44	Isobutyraldehyde	0.26 ± 0.05	0.98 ± 0.04	/	/	/	/
45	2-Methylbutyraldehyde	1.30 ± 0.33	2.38 ± 0.27	/	/	/	/
46	3-Methylbutyraldehyde	1.36 ± 0.14	0.83 ± 0.03	/	/	/	/
47	Hexanal	44.18 ± 4.37	/	/	/	/	/
48	Acetaldehyde ethyl isoamyl acetal	/	/	/	12.88 ± 0.78	19.42 ± 0.13	/
49	2-Hexenal	95.30 ± 6.84	1.75 ± 0.35	/	/	/	/
	ΣAldehydes	142.40 ± 11.73	5.94 ± 0.69	18.47 ± 1.45	55.75 ± 5.58	48.96 ± 3.06	57.94 ± 2.70

Table 2. (Contd.)

Num- ber	Volatile compound	Content, mg/L					
		M0	M1	M3	M5	M7	M15
50	1-Ethoxy propane	/	/	15.06 ± 0.57	/	/	103.05 ± 3.51
51	Ethoxy ethene	/	/	15.95 ± 0.55	44.44 ± 9.04	78.00 ± 8.36	11.29 ± 0.06
52	Acetoin	0.39 ± 0.05	0.82 ± 0.01	24.66 ± 0.34	15.57 ± 0.09	/	/
53	2-Heptanone	/	/	4.01 ± 0.47	3.93 ± 0.06	/	/
54	2-Pentanone	/	3.54 ± 0.11	/	/	/	/
55	2,3-Pentanedione	/	/	3.73 ± 0.15	9.98 ± 0.22	16.91 ± 1.87	/
56	2,3-Heptanedione	/	/	/	7.23 ± 0.36	/	/
57	6-Methyl-5-hepten-2-one	0.72 ± 0.05	11.08 ± 0.14	100.02 ± 9.27	132.44 ± 1.86	104.32 ± 3.23	93.29 ± 7.04
58	1-Methylethyl-benzene	0.41 ± 0.02	/	/	/	/	/
59	Styrene	0.72 ± 0.08	16.52 ± 0.84	24.15 ± 0.61	26.73 ± 2.15	27.57 ± 3.68	/
60	Alpha-terpinene	0.90 ± 0.06	/	/	/	/	/
61	Gamma-terpinene	1.31 ± 0.23	1.05 ± 0.06	/	/	/	/
62	Para-cymene	5.91 ± 0.47	3.27 ± 0.02	/	/	/	/
63	2,4-Di-tert-butylphenol	0.45 ± 0.07	0.37 ± 0.02	/	/	/	/
64	Hydrazinecarboxamide	/	/	/	/	722.45 ± 54.11	851.64 ± 20.72
65	Carbamic acid, monoammonium salt	/	/	40.56 ± 1.93	/	90.88 ± 2.98	/
66	n-Hexylmethylamine	/	/	353.59 ± 26.25	616.97 ± 15.10	/	/
67	Octane	1.57 ± 0.58	/	/	/	/	/
68	2,4-Dimethyl heptane,	1.39 ± 0.26	/	/	/	/	/
69	2,4,5-Trimethyl-1,3-dioxolane,	1.64 ± 0.10	2.24 ± 0.09	72.72 ± 2.15	157.56 ± 18.56	463.58 ± 5.17	1120.66 ± 74.98
70	Toluene	2.42 ± 0.17	2.05 ± 0.00	4.17 ± 0.31	6.99 ± 0.75	/	/
77	Ethylbenzene	0.97 ± 0.04	1.09 ± 0.02	/	/	/	/
72	o-Xylene	1.11 ± 0.03	0.90 ± 0.04	/	/	/	/
73	p-Xylene	0.35 ± 0.00	/	/	/	/	/
Σ	Others	20.26 ± 2.21	42.93 ± 1.35	658.62 ± 42.60	1014.61 ± 48.19	1503.71 ± 79.40	2179.93 ± 106.31

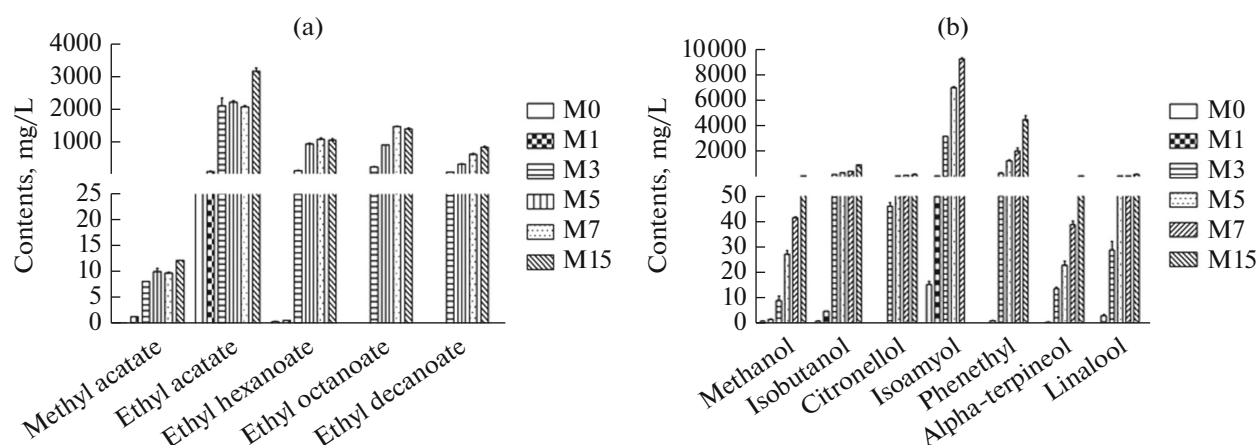


Fig. 6. Dynamic changes of main aromatic esters (a) and alcohols (b).

Table 3. The OAVs for the aroma compounds of spontaneously fermented 'Beimei' wine

Number	Volatile compound	Odor descriptor	Odor threshold, mg/L	OAV					
				M0	M1	M3	M5	M7	M15
A1	Ethyl acetate	Pineapple, fruity, solvent, balsamic	0.75	44.8	151.59	2801.42	2946.53	2761.74	4212.21
A2	Ethyl butyrate	Apple, banana	0.02	29.25	335	792.50	1461.5	2471.50	/
A3	Ethyl hexanoate	Sweet, fruity	0.05	12.40	17.20	2722.4	18829	21739.6	21349.4
A4	Ethyl octanoate	Sweet, fruity	0.58	/	/	436.69	1586.67	2544.34	2405.69
A5	Ethyl decanoate		1.12	/	/	77.92	296.64	560.24	756.09
A6	Isobutyl acetate	Sweet, fruity, apple, banana	1.6	/	/	17.49	10.94	7.82	/
A7	Isoamyl acetate	Banana, fruity, sweet	0.16	/	/	/	5429.43	5349.81	/
A8	Phenethyl acetate	Floral	1.8	/	/	26.6	18.93	15.31	15.39
B1	2,3-Butanediol	Fruity	150	/	/	/	0.44	0.42	1.96
B2	Hexanol	Floral, green, cut grass	8	13.19	28.93	40.36	34.45	29.18	65.70
B3	Isobutanol		40	0.03	0.12	4.93	8.10	10.79	23.51
B4	Isoamylol	Burnt, alcohol	30	1.30	2.70	105.66	233.25	307.79	/
B5	Phenethyl alcohol	Floral, roses	10	/	0.13	29.30	125.71	203.97	448.1.5
B6	Linalool	Floral	0.02	/	16.2	1451	3756.5	4965.5	9861.5
B7	Citronellol	Floral, roses	0.1	/	/	463.8	914.	1191.5	1926.8
B8	1- Pentanol	Alcohol, pungent	80	0.01	0.03	0.58	/	/	/
B9	1-Octanol	Jasmine, lemon	0.12	/	/	1772.75	3011.92	2898	4130.42

late stage of fermentation. This study represented the first step in exploring untapped yeast species and aroma compounds, which was helpful for the screening of native yeasts from 'Beimei' and producing distinctive and aromatic wines.

AUTHOR CONTRIBUTION

Contributions to the conception of the work, analysis and writing of manuscript were done by X.L. Analysis and interpretation of data for the work was done by Y.L. All authors read and approved the final version of the manuscript.

FUNDING

This work was supported by the Guizhou Provincial Science and Technology Foundation [ZK (2023)137].

DATA AVAILABILITY

Data will be provided by the corresponding author upon request.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This work does not contain any studies involving human and animal subjects.

CONFLICT OF INTEREST

The authors of this work declare that they have no conflicts of interest.

OPEN ACCESS

This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons license, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons license and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this license, visit <http://creativecommons.org/licenses/by/4.0/>

REFERENCES

Barata, A., Malfeito-Ferreira, M., and Loureiro, V., The microbial ecology of wine grape berries, *Int. J. Food Microbiol.*, 2012, vol. 3, pp. 243–259.

Belda, I., Ruiz, J., Esteban-Fernández, A., Navascues, E., Marquina, D., Santos, A., and Moreno-Arribas, M.V., Microbial contribution to wine aroma and its intended use for wine quality improvement, *Molecules*, 2017, vol. 2, p. 189.

David, V., Terrat, S., Herzine, K., Claisse, O., Rousseaux, S., Tourdot-Maréchal, R., Masneuf-Pomarede, I., Ranjard, L., and Alexandre, H., High-throughput sequencing of amplification for monitoring yeast biodiversity in must and during alcoholic fermentation, *J. Ind. Microbiol. Biotechnol.*, 2014, vol. 5, pp. 811–821.

del Carmen, P.M. and Mas, A., Analysis of microbial diversity and dynamics during wine fermentation of Grenache grape variety by high-throughput barcoding sequencing, *LWT-Food Sci. Technol.*, 2016, vol. 72, pp. 317–321.

Di, M.E., Ercolini, D., and Coppola, S., Yeast dynamics during spontaneous wine fermentation of the Catalanesca grape, *Int. J. Food Microbiol.*, 2007, vol. 2, pp. 201–210.

Jiao, H., Liu, A., and Xie, C., A review of Beimei and Beihong wine grapes in Ningxia, *Farm Products Processing*, 2022, vol. 12, pp. 104–105.

Jolly, N.P., Varela, C., and Pretorius, I.S., Not your ordinary yeast: non-*Saccharomyces* yeasts in wine production uncovered, *FEMS Yeast Res.*, 2014, vol. 2, pp. 215–237.

Kuang, Y., Ren, C., Wang, Y., Kirabi, G.E., Wang, Y., Wang, L.J., Fan, P.G., and Liang, Z.C., Characterization of the berry quality traits and metabolites of 'Beimei' interspecific hybrid wine grapes during berry development and winemaking, *Horticulturae*, 2022, vol. 6, p. 516.

Li, Y., Huang, Y., Long, H. Yu, Z., Huang, M., and Liu, X., Biodiversity and oenological property analysis of non-*Saccharomyces* yeasts isolated from Korla fragrant pears (*Pyrus sinkiangensis* Yu), *Fermentation*, 2022, vol. 8, p. 388.

Li, Y., Ding, P., Tang, X., Zhu, W., Huang, M., Kang, M., and Liu, X., Screening and oenological property analysis of ethanol-tolerant non-*Saccharomyces* yeasts isolated from *Rosa roxburghii* Tratt., *Front. Microbiol.*, 2023, vol. 14, p. 1202440.

Li, Z., Zhou, T., Yang, C., Li, Y., Sun, Q., Ren, Y., Zhao, B., Zhang, L., and Li, G., Histological studies on the infection process of the grape downy mildew between susceptible cultivar and resistant cultivar, *Acta Horticulturae Sinica*, 2017, vol. 5, pp. 861–870.

Liu, D., Legras, J.L., Zhang, P., Chen, D.L., and Howell, K., Diversity and dynamics of fungi during spontaneous fermentations and association with unique aroma profiles in wine, *Int. J. Food Microbiol.*, 2021, vol. 338, p. 108983.

Liu, X., Li, Y., Yu, Z., Hardiem W., and Huang, M., Biodiversity of non-*Saccharomyces* yeasts during natural fermentation of *Rosa roxburghii*, *Acta Microbiol. Sin.*, 2020, vol. 60, pp. 1696–1708.

Liu, X., Li, Y., Zhang, Y., Zeng, S., and Huang, M., Yeast diversity investigation of 'Beihong' (*V. vinifera* × *V. amurensis*) during spontaneous fermentation from Guiyang region, Guizhou, China, *Food Sci. Technol. Res.*, 2021, vol. 6, pp. 887–896.

Luzzini, G., Slaghenaufi, D., Pasetto, F., and Ugliano, M., Influence of grape composition and origin, yeast strain and spontaneous fermentation on aroma profile of Corvina and Corvinone wines, *LWT-Food Sci. Technol.*, 2021, vol. 143, p. 111120.

Lyu, J., Chen, S., Nie, Y., Xu, Y., and Tang, K., Aroma release during wine consumption: factors and analytical approaches, *Food Chem.*, 2021, vol. 346, p. 128957.

Mehlomakulu, N.N., Hoff, J.W., Erten, H., Beukes, L., and Jolly, N.P., Screening non-*Saccharomyces* yeasts as low ethanol producing starter cultures, *S. Afr. J. Enol. Vitic.*, 2021, vol. 1, pp. 56–66.

Mencarelli, F. and Bellincontro, A., Recent advances in postharvest technology of the wine grape to improve the wine aroma, *J. Sci. Food Agric.*, 2020, vol. 14, pp. 5046–5055.

Parker, M., Capone, D., Francis, I., and Herderich, M.J., Aroma precursors in grapes and wine: flavor release during wine production and consumption, *J. Sci. Food Agric.*, 2017, vol. 610, pp. 2281–2286.

Peng, B., Li, F., Cui, L., and Guo, Y., Effects of fermentation temperature on key aroma compounds and sensory

properties of apple wine, *J. Food Sci.*, 2015, vol. 12, pp. S2937–S2943.

Teixeira, A., Eiras-Dias, J., Castellarin, S.D., and Gerós, H., Berry phenolics of grapevine under challenging environments, *Int. J. Mol. Sci.*, 2013, vol. 9, pp. 18711–18739.

Wang, C. and Liu, Y., Dynamic study of yeast species and *Saccharomyces cerevisiae* strains during the spontaneous fermentations of Muscat blanc in Jingyang, China, *Food Microbiol.*, 2013, vol. 2, pp. 172–177.

Wang, C., Wu, C., and Qiu, S., Yeast diversity investigation of *Vitis davidii* Föex during spontaneous fermentations using

culture-dependent and high-throughput sequencing approaches, *Food Res. Int.*, 2019, vol. 126, p. 108582.

Wang, L., Li, S., and Fan, P., Introduction, breeding and production of wine grapes in China, *Acta Hortic.*, 2014, vol. 1046, pp. 241–244.

Zhang, S., Chen, X., Zhong, Q., Zhuang, X., and Bai, Z., Microbial community analyses associated with nine varieties of wine grape carposphere based on high-throughput sequencing, *Microorganisms*, 2019, vol. 12, p. 668.

Publisher's Note. Pleiades Publishing remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.