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Optimizing yeast strain selection for mulberry wine fermentation: a performance-based approach

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RESEARCH ARTICLE

Abstract

This study focused on identifying the optimal yeast strain for Dechang mulberry wine fermentation blended through a multi-stage evaluation process using five commercial yeasts. Initially, the Durham tube fermentation method and weight-based analysis were employed for initial screening. Subsequently, a secondary evaluation involving physical, chemical, and sensory assessments was conducted. The final stage assessed yeast tolerance to ethanol and sulfur dioxide (SO_2), and the wine's volatile compounds were analyzed using gas chromatography-mass spectrometry. The L13 yeast strain emerged as superior, demonstrating efficient fermentation initiation, rapid primary fermentation, and production of a desirable wine profile, including optimal sugar, acid, and alcohol levels. Notably, the L13 yeast strain showed resilience, tolerating up to 11% ethanol and 170-mg/L SO_2 within 7 days, and contributed to a rich aromatic profile with 23 volatile compounds, including 13 esters. These attributes render the L13 strain particularly suitable for producing mulberry wine with robust fermentation characteristics and a complex aroma, enhancing both quality and sensory appeal of the final product.

Keywords: mulberry wine; yeast strain; selection; GC-MS

Introduction

Traditionally used as an internal disease medicine, mulberries (belonging to the *Moraceae* family) are widely distributed from temperate and subtropical to tropical regions (Lim and Choi, 2019; Wen *et al.*, 2019). Mulberries are fruits from the mulberry tree, belonging to the Morus genus, and are related to figs and breadfruit. These trees are traditionally grown for their leaves, primarily in Asia and North America, but the fruits they produce are also valued highly. Mulberries are known for being rich in various nutrients and bioactive compounds, making them popular as functional foods. They are a rich source of bioactive compounds widely used as functional foods (Kumar *et al.*, 2020). Mulberry fruits also contain vitamins, minerals, fiber, carbohydrates, lipids, and proteins (Yuan and Zhao, 2017). Additionally, mulberry contains numerous flavonoids (such as isoquercitin, quercetin, and rutin), polyphenols, alkaloids, cyanidin-based anthocyanins (especially cyanidin-3-O-rutinoside [C3R] and cyanidin-3-O-glucoside [C3G]), and amino acids (Jiang and Nie, 2015), which have anti-inflammatory, anti-atherosclerosis, neuroprotective, antidiabetic, antioxidant and neuroprotective properties (Chen *et al.*, 2016; Wen *et al.*, 2019).

Mulberry wine, one of the mulberry products, has the advantages of a fruit wine, and because of the potential health benefits and distinct flavor related to its phytochemical compounds, it is a popular alcoholic drink consumed globally (Tchabo *et al.*, 2017). The mulberry alcoholic fermentation results in a wine with a specific purple-black color and rich in aromatic compounds. In this regard, a particular form of yeast cells is very

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important to control the taste and aroma of wines and production of ethanol. Furthermore, during the fermentation process of mulberry wine, the role of yeast is very crucial in determining the quality of the end product, rendering the selection of a yeast strain significantly important, specifically for mulberry wine fermentation (Okunowo and Osuntoki, 2007; Zhang *et al.*, 2020). However, the current market needs specialized yeast strains that exhibit high efficiency in production of alcohol along with tolerance to alcohol and sulfur dioxide (SO_2).

Five commercial yeast strains (such as Excellence FR yeast, Excellence XR yeast, L13 yeast, Excellence SP yeast, and LA Bayanus yeast) are utilized normally in winemaking and have the potential to specialize in the production of mulberry wine. LA Bayanus yeast, a versatile yeast in making all types of wines, ensures complete alcoholic fermentation while avoiding all deviations. Excellence SP yeast produces fresh and fruity red wines with notes of spices, which are suited for producing mid-range wines. Excellence XR yeast is very suitable for producing top-quality red wines because it releases different polysaccharides and has an excellent fermentation capacity. Excellence FR yeast, with low-volatile acidity production, produces high-quality Nouveau-style wines. L13 yeast improves the quality of red wines and is resistant to difficult conditions (Marullo and Dubourdieu, 2022).

This study aimed to investigate commercial yeast strains specifically for mulberry wine fermentation, following a multistage screening process encompassing preliminary, secondary, and tertiary examinations through analysis of aromatic components of fermented mulberry wine. The process culminated in identifying a yeast strain that demonstrated high alcohol yield and resistance to ethanol and SO_2, providing a theoretical foundation for research and production applications in mulberry wine fermentation.

Materials and Methods

Materials and equipment

Materials and reagents

Excellence FR yeast, Excellence XR yeast, L13 yeast, Excellence SP yeast, LA Bayanus yeast; Dechang mulberries; granulated sugar; mulberry juice medium: mulberry juice extracted and treated with 0.1 g/100 mL of pectinase at 40°C for enzymatic hydrolysis for 3 h, filtered through a nylon cloth, and centrifuged at 3,000 rpm for 3 min to collect the supernatant; anhydrous ethanol, pectinase, potassium metabisulfite ($K_2S_2O_5$, food grade), potato dextrose broth (PDB), potato dextrose agar (PDA), 0.1% Loeffler's methylene blue stain, and malachite green stain.

Instruments and equipment

BX53F digital biological microscope from Olympus (Japan); LH-B55 digital refractometer from Hangzhou Luheng Biotech Co, Hangzhou China; PH-828 pH meter (Xima Instrument Group Co., South Jordan Utah, USA); desktop gas chromatography (Quad) mass spectrometer (Beijing Dongxi Analytical Instrument Co, Beijing China); CPWAX57CB capillary column (50 m × 0.25 mm × 0.20 μm; Agilent Technologies, Santa Clara, CA, USA); solid phase microextraction (SPME) manual injection handle (SupeLco, Bellefonte, PA, USA); SPME fiber SAAB-57328U DVB/CAR/PDMS (50/30 μm) extraction head (SupeLco).

Experimental method

Preparation of mulberry juice

Fresh, ripe mulberries were squeezed and filtered through four layers of a nylon filter cloth, and the sugar content of the mulberry juice was adjusted to 20°Brix by adding granulated sugar. Citric acid was added to adjust pH to 4.0.

Activation and isolation of yeast cultures

An activated yeast starter culture was obtained by adding 1 g of yeast sample and 99 mL of sterile water into a conical flask and oscillated uniformly in a constant temperature shaker. Yeast culture was produced by inoculating 1 mL of starter culture with potato dextrose broth (PDB). A gradient dilution method was used to dilute step by step the yeast starter culture, obtaining 10^{-3}, 10^{-4}, 10^{-5}, and 10^{-6} dilutions of yeast suspension, which were then evenly spread on PDA plates. Three replicates were performed for each gradient. The plates were incubated invertedly at 28°C for 48 h and then sub-cultured twice through streaking to obtain pure cultures, preserved after microscopic inspection.

Preliminary Screening of Yeast

Durham tube gas production test

A total of 10 mL of 20-Brix mulberry juice medium was inoculated with 1% yeast culture and incubated at 24°C for 48 h. Production of gas in Durham tubes of the five yeast strains was compared using the Durham tube fermentation method to determine the strains with stronger fermentation capabilities.

Fermentation weight loss test

Mulberry juice was inoculated with 1% inoculum of five yeast cultures and fermented at 24°C until cessation of fermentation. Loss of carbon dioxide (CO_2) was measured every 24 h (Peng *et al.*, 2004) until the loss of CO_2 was no higher than 0.2 g. Before measurement, the samples were oscillated for 5 min on a shaker to expel CO_2. Subsequently, the weight loss curves of five strains during the fermentation process were drawn, preliminarily comparing the start and end period of fermentation to determine the fermentation strength of strains.

Secondary screening of yeast

Yeast strains identified in the preliminary screening with strong fermentation capacity and rapid initiation of fermentation were inoculated into mulberry wine at an inoculum rate of 2% and fermented at a controlled temperature of 24°C for 7 days. After primary fermentation, the total sugar, total acid, and alcohol content of the mulberry wine samples were measured following the general analytical methods for wines and fruit wines GB/T 15038-2006 (National Standardization Management Committee, 2007), and the fermented mulberry wine was subjected to sensory evaluation.

Tertiary Selection of Yeast

Alcohol tolerance test for yeast

The Durham tube fermentation method was used, adding anhydrous ethanol to the medium of mulberry juice to achieve ethanol concentrations of 7%, 8%, 9%, 10%, 11%, and 12% (V/V), and then shaking well the mixture. The strains obtained from secondary screening were inoculated into the mulberry medium at an inoculum rate of 1% and incubated at a constant temperature of 24°C for 36 h. The amount of gas produced in Durham tubes was observed, and the length of gas bubbles was measured with a vernier caliper.

Sulfur dioxide tolerance test for yeast

The Durham tube fermentation method was used, adding SO_2 to the mulberry juice medium to achieve SO_2 concentrations of 50, 90, 130, and 170 mg/L, and then shaking well the mixture. The strains obtained from secondary screening were inoculated into the mulberry medium at an inoculum rate of 1% and incubated at a constant temperature of 24°C for 48 h. The amount of gas produced in Durham tubes was observed, and the length of gas bubbles was measured with a vernier caliper.

Analysis of volatile flavor compounds in mulberry wine

The mulberry wine fermented by the strains with a good comprehensive evaluation from secondary screening was subjected to headspace solid-phase microextraction (HS-SPME) as a sample pre-treatment method, followed by gas chromatography-mass spectrometry (GC-MS) to analyze the volatile flavor of compounds in mulberry wine (Cao *et al.*, 2017).

Pre-treatment conditions for mulberry wine

In a 20-mL headspace vial, add 10-mL mulberry wine sample, preheat at 45°C for 10 min; insert an aged extraction head (aged at 250°C for 30 min), and perform headspace extraction for 60 min with constant temperature magnetic stirring on a heating plate. After headspace extraction, the aged extraction head is inserted into the GC injection port and desorbed for 10 min, followed by GC-MS analysis.

Analysis conditions and GC conditions

CPWAX57CB quartz capillary column, 50 m × 0.25 m × 0.20 μm; carrier gas is high purity helium, with a constant flow rate of 1 mL/min; column temperature program: start at 40°C, hold for 8 min, then increase to 220°C at 10°C/min, and hold for 10 min; manual injection, injection port temperature 230°C; detection voltage 350 V. MS conditions: e*lectron ionization* (EI) ion source, ion source temperature 200°C, emission current 200 μA, electron energy 70 eV, scan range 20–550 u.

Results and Discussion

Isolation and purification of yeast strains

After activation, the yeast was diluted to 10^{-6}, spread-plated, and purified over two generations. Morphology and microscopic examination results of yeast are shown in Figure 1, while the survival and sporulation rates are presented in Table 1.

Microscopic examination of five yeast strains showed that all strains were vigorous, with the number of living cells significantly exceeding the number of dead cells, and all had a strong ability to produce ascospores.

Preliminary screening of strains

Yeast gas production in Durham tubes

After incubation at a constant temperature and left undisturbed for 48 ho, no significant difference was observed in the production of gas in Durham tubes after 24 and 48 h, the comparison was made using production of gas after 36 h. The results are shown in Table 2.

As observed in Table 2, compared to the FR and LAB strains, the L13, XR, and SP strains produced gas in both experimental and control tubes, and the volume of gas produced was more balanced.

Weight loss results from mulberry wine fermentation

The weight loss method was used for measuring the loss of CO_2 every 24 h during fermentation. After 15 days

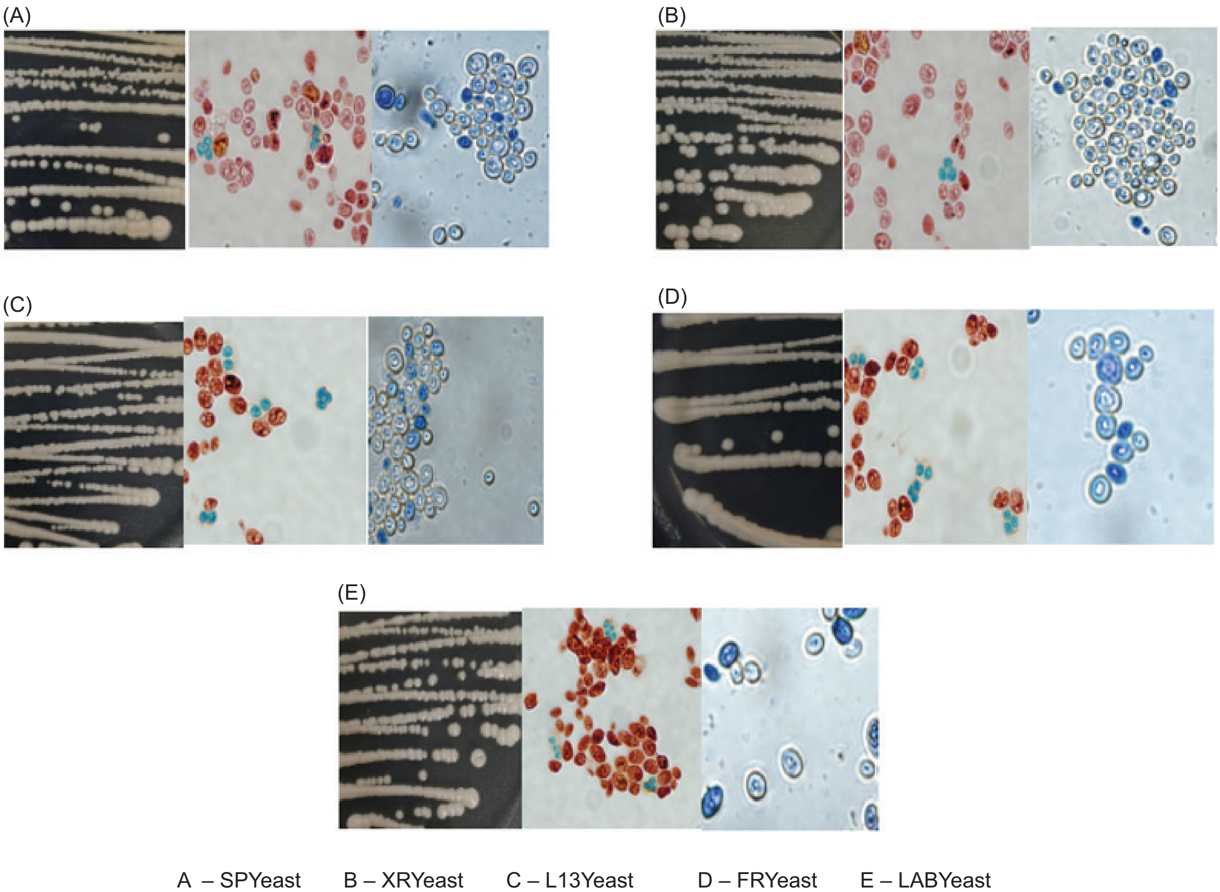

Figure 1. Morphology and microscopic examination results of yeast.

Table 1. Survival and sporulation rates of yeast.

Strains	Survival rate (%)	Sporulation rate (%)
Excellence SP	87.99	12.69
Excellence XR	89.14	10.02
L13	77.58	15.47
Excellence FR	65.85	22.93
LAB	56.10	11.18

of fermentation, the CO_2 weight loss for the mulberry wine fermented by the five yeast strains was less than 0.2 g. The fermentation capacity results for the five yeast strains are shown in Figure 2.

In the weight loss test, the initiation time and fermentation duration were used as the criteria to determine the fermentation capacity of the strains. It was found that the L13, XR, and SP strains had stronger fermentation initiation abilities, compared to the FR and LAB strains.

Combining the Durham tube gas production results and fermentation capacity findings, it was concluded that the L13, XR, and SP yeasts produced a relatively large and balanced amount of gas after 36 h of fermentation, and these three yeast strains also showed stronger fermentation initiation abilities and longer fermentation period. Therefore, the yeast strains selected for secondary screening were L13, XR, and SP.

Secondary screening of strains

The three strains were selected from the preliminary fermentation screening of mulberry wine. After primary fermentation, the total sugar, total acid, and alcohol content of the mulberry wine fermented by three yeast strains was measured, and a sensory evaluation was performed. The results of physicochemical indicators are shown in Table 3, and the sensory evaluation results of mulberry wine are presented in Table 4.

The results established that the mulberry wine fermented by the L13 yeast strain had the lowest remaining total sugar content and the highest alcohol content. According to the indicators where lower residual sugar content corresponds to higher alcohol content, the following order was observed: L13 yeast, XR yeast, and SP yeast. The two strains with higher alcohol content indicators produced mulberry wine with a distinct aroma and a taste that had the typical flavor of mulberry wine. Thus, L13 and XR yeast were the two strains selected for the third round of screening.

Table 2. Gas production results from the Durham tube fermentation.

	FR	L13	LAB	XR	SP
24 h	-	-	-	-	-
36 h	+++/-	++/++	+/-	+++/++	+/+++
48 h	+++/+++	+++/+++	+++/+++	+++/+++	+++/+++

Note: "-": gas production of less than 1/3 of the tube volume; "+": gas production of about 1/2 of the tube volume; "++": gas production of about 2/3 of the tube volume; and "+++": full tube gas production.

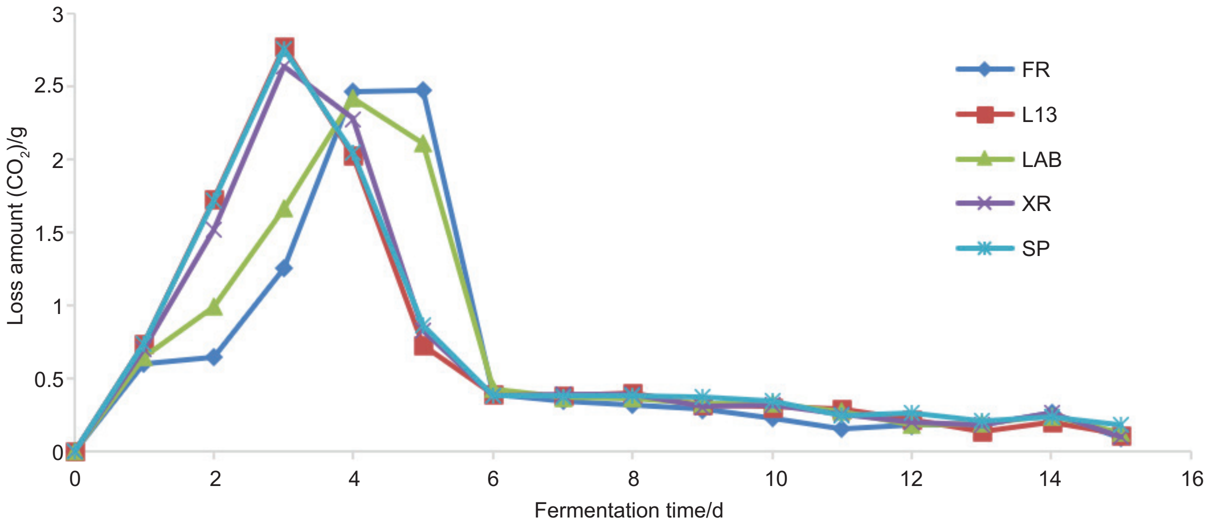

Figure 2. Determination of fermentation capacity of five yeast strains.

Tertiary screening of strains

Yeast alcohol tolerance results

By adding a certain amount of anhydrous ethanol to the mulberry juice medium, strains tolerant to higher alcohol levels could be selected. Tolerance results of the strains are shown in Table 5, where the amount of gas produced in Durham tubes was measured using a vernier caliper.

In the tertiary screening of yeast, both strains could produce a full tube of gas in the mulberry medium with an alcohol content of 7–9%. At an alcohol concentration of 10%, the alcohol tolerance of L13 was slightly weaker. When the alcohol content in the mulberry medium increased to 11%, fewer bubbles were observed in Durham tubes, but when the alcohol content reached 12%, neither of the strain produced bubbles. Therefore, the maximum alcohol concentration tolerated by the two strains was 11%, with the yeast strain XR showing slightly stronger alcohol tolerance.

Yeast sulfur dioxide tolerance results

The national standard for food additives specifies the maximum permitted use of SO_2 additives in fruit wines as 250 mg/L (GB 2760-2014; National Standardization Management Committee (NSMC), 2014), calculated as residual SO_2. The results of SO_2 tolerance of different yeast strains are shown in Table 6, with the amount of gas produced in Durham tubes measured using a vernier caliper.

Table 3. Results of physicochemical indicator tests.

Strain	Total sugar content (g/L)	Total acid content (g/L)	Alcohol content (%)
L13	16.45	13.63	3.5
XR	20.18	12.51	3.3
SP	24.37	12.32	3

In this experiment, potassium metabisulfite was added to adjust SO_2 concentration in the mulberry medium. The results showed that both L13 and XR yeasts, obtained through secondary screening, have good SO_2 tolerance. However, L13 has a slightly stronger tolerance for SO_2, producing more bubbles in Durham tubes at an SO_2 concentration of 170 mg/L.

Analysis of volatile flavor compounds in mulberry wine

Volatile flavor compounds are critical in determining the quality and appeal of fruit wines. These compounds

Table 4. Sensory evaluation results of mulberry wine.

Strain	Color	Transparency	Aroma	Taste	Typicality
L13	Purple-red	Clear	Rich fruit aroma, rich wine aroma	Full-bodied, smooth	Typicality
XR	Purple-red	Transparent	Rich fruit aroma, quite rich wine aroma	Relatively full-bodied, with a slight sourness	Typical
SP	Purple-red	Slightly turbid	Lighter fruit aroma, lighter wine aroma	Thinner body, sour taste	General

Table 5. Alcohol tolerance results of different yeast strains.

Strains	Alcohol content (%)					
	7%	8%	9%	10%	11%	12%
L13	3.000 cm	3.000 cm	3.000 cm	1.910 cm	1.792 cm	0.000 cm
XR	3.000 cm	3.000 cm	3.000 cm	2.988 cm	2.450 cm	0.000 cm

contribute to the aroma and taste of the wine and serve as indicators of the fermentation's effectiveness and the wine's overall character. This study employed the HS-SPME–GC-MS technique to analyze the volatile flavor compounds in mulberry wine produced by selected yeast strains. These strains were chosen for their robust alcohol-producing capabilities and positive sensory evaluations, making them ideal for producing high-quality mulberry wine.

The analysis focused on identifying the diversity and concentration of volatile flavor compounds in the wines fermented by these strains. The results, as detailed in Figure 3, provide a comprehensive comparison of the types and quantities of these compounds. This comparison is essential for understanding how different yeast strains influence the flavor profile of the wine. Further, the study delves into the specific categories of volatile compounds detected, such as esters, alcohols, acids, and phenols, each of which contribute uniquely to the wine's aroma and taste. Esters, for example, are known for imparting fruity and floral notes, while alcohols and acids contribute to the wine's sharpness and freshness. The presence and balance of these compounds are crucial for achieving a harmonious and appealing bouquet. The study also examines how factors, such as duration of fermentation, temperature, and selection of yeast strain impact the formation of these volatile compounds. Such insights are invaluable for winemakers aiming to refine their fermentation processes and enhance the sensory qualities of mulberry wine. In summary, this analysis provides a detailed profile of the volatile flavor compounds in mulberry wine and offers a deeper understanding of the intricate relationship between fermentation of yeast strains and development of wine flavor. This knowledge is pivotal for advancing the quality and distinctiveness of mulberry wine in the competitive fruit wine market.

Table 6. SO_2 tolerance results of different yeast strains.

Strains	SO_2 concentration (mg/L)			
	50 mg/L	90 mg/L	130 mg/L	170 mg/L
L13	2.788 cm	2.296 cm	2.118 cm	1.828 cm
XR	1.232 cm	1.068 cm	0.580 cm	0.000 cm

As observed in Figure 3, 23 volatile flavor compounds were identified in the mulberry wine fermented by the L13 yeast strain, including 13 esters, 3 alcohols, 1 acid, and 6 other types of compounds. In the mulberry wine fermented by the XR yeast strain, 21 volatile flavor compounds were identified, including 12 esters, 2 alcohols, 1 acid, and 6 other types of compounds. The relative contents of volatile flavor compounds in the mulberry wine fermented by the two strains are shown in Table 7.

Table 7 shows that alcohols are the main volatile flavor compounds in the mulberry wine fermented by the L13 yeast strain. At the same time, esters are the main volatile flavor compounds in the mulberry wine fermented by the XR yeast strain.

The alcohol content of L13 and XR yeasts is 50.25% and 32.69%, respectively. Ethanol and phenylethyl alcohol are present in the mulberry wine fermented by both strains; phenylethyl alcohol, which has a floral scent, contributes to the unique flavor of mulberry wine (Li *et al.*, 2010).

In the mulberry wine fermented by L13 and XR yeast strains, the ester content is 42.64% and 43.73%, respectively. Esters are formed through condensation during fermentation, giving mulberry wine its unique aroma (Hao *et al.*, 2012; Yin *et al.*, 2016). The highest ester content in the mulberry wine fermented by the L13 yeast strain is ethyl caprate, followed by ethyl caprylate and ethyl acetate; for the XR yeast, ethyl eicosanoate has the highest content, followed by ethyl caprylate. Ethyl caprate and ethyl acetate have fruity aromas, with ethyl acetate having slight cherry and grape notes and ethyl caprate possessing

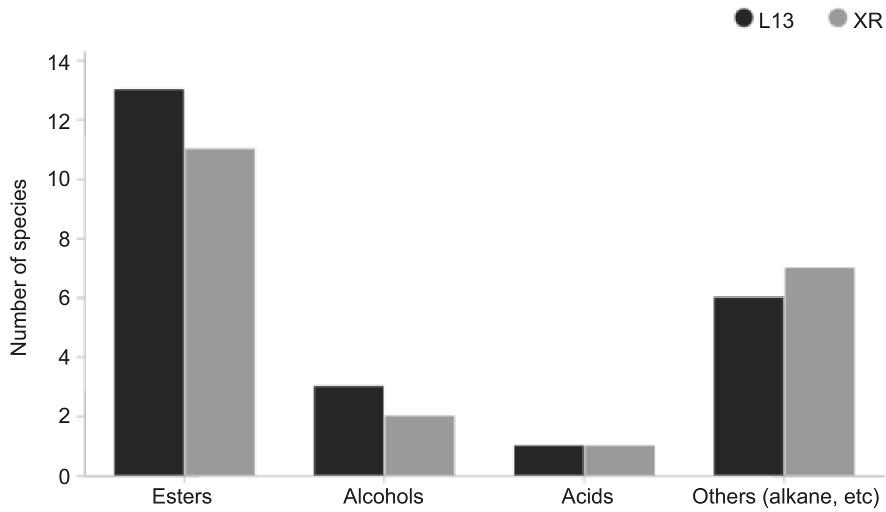

Figure 3. Types of volatile flavor compounds.

a strawberry flavor (Verzera *et al.*, 2008), while ethyl caprylate contributes a creamy and sweet aroma.

The acid content for L13 and XR yeast strains is 4.22% and 5.80%, respectively. Acids in fruit wines often act to harmonize other flavors. Both strains of yeast-fermented mulberry wine have a low content of acetic acid. Acetic acid imparts a pungent taste to fruit wines (Liu *et al.*, 2013). Other compounds account for 3.36% and 9.23% of pungent taste, respectively, mostly hydrocarbons, which also contribute to the flavor of mulberry wine.

Combining the results of tertiary selection, it was concluded that both L13 and XR yeast strains could tolerate an alcohol concentration of 11%, with XR yeast having slightly stronger alcohol tolerance. Both L13 and XR yeast strains have good tolerance to SO_2, with L13 being slightly stronger. Considering the type and quantity of volatile flavor compounds produced by the fermentation of mulberry wine by five strains, it was concluded that the comprehensive fermentation capability of L13 yeast strains was superior to that of XR yeast strain.

The optimization technique played a pivotal role in creating a selenium-enriched mulberry wine, suitable for immediate consumption and capable of contributing to the daily recommended intake of selenium. Additionally, the optimized parameters established through this process act as a foundational guideline for the technological advancement and large-scale production of selenium-enriched mulberry wine (Johnson *et al.*, 2023). According to Wang *et al.* (2013), the response surface methodology (RSM) findings indicated that the ideal conditions for mulberry fermentation include a pH of 3.2, an inoculum size of 0.53%, a fermentation temperature of 31.4°C, and a fermentation duration of 6 days. If fermented under these optimal conditions, the alcohol content of the wine was 12.46%.

Conclusion and Future Trends

The fruit wine industry often needs yeasts specifically tailored for fermenting different types of fruit wines. This gap in the industry is particularly evident in the production of unique fruit wines, such as mulberry wine. This research aimed to identify yeast strains that excelled in fermenting mulberry fruit wine, focusing on achieving optimal fermentation efficiency and flavor profile. After extensive screening of five commercially available yeast strains, the L13 strain was identified as the most suitable strain for fermentation of mulberry fruit wine. This strain distinguished itself by its ability to tolerate high alcohol concentrations of up to 11% and endure SO_2 levels of as high as 170 mg/L. Such resilience is crucial in ensuring consistent and robust fermentation under varied conditions. The superiority of the L13 strain was further evidenced in the quality of the mulberry fruit wine. Compared to wines fermented with other strains, the L13 strain-fermented mulberry wine demonstrated exceptional taste and typicity, capturing the essence of mulberry fruit more accurately and pleasingly. This could be attributed to the L13 strain's unique ability to generate a complex and rich array of aromatic compounds. Notably, it produced the highest number of aromatic compounds among the tested strains, including significant quantities of ethyl caproate and ethyl acetate, which are key contributors to the characteristic aroma profile of high-quality fruit wines. In addition to these sensory advantages, the L13 strain also demonstrated superior fermentation kinetics, characterized by a rapid initiation of fermentation and a steady progression to completion. This efficiency is beneficial for optimizing the production process and contributes to the wine's overall stability and consistency. Considering its robust fermentation capabilities, tolerance to high alcohol and SO_2 concentrations, and exceptional contribution to the sensory profile of

Table 7. Relative contents of volatile flavor compounds in the mulberry wine fermented by different yeast strains.

No.	Name	Molecular formula	Molecular weight	L13 (%)	XR (%)	Flavor characteristics
Esters						
A1	Ethyl acetate	$C_4H_8O_2$	88	2.43	ND	Strong ether-like smell, clear and slightly fruity wine aroma
A2	Ethyl acetate	$C_8H_{16}O_2$	144	0.88	0.57	Pineapple fruit aroma
A3	Ethyl caprylate	$C_{10}H_{20}O_2$	172	7.27	9.63	Reminiscent of brandy with sweetness
A4	Diethylhexyl sebacate	$C_8H_{18}O_4Si_2$	234	0.44	3.14	
A5	Ethyl nonanoate	$C_{11}H_{22}O_2$	186	0.37	ND	It has a rose fragrance, fruity aroma, and wine scent
A6	Ethyl phenylacetate	$C_{10}H_{12}O_2$	164	0.40	ND	Aroma reminiscent of roses with a honey-like base note
A7	Ethyl decanoate	$C_{12}H_{24}O_2$	200	13.24	6.72	Fruity and winy aroma with nuances of pear and brandy
A8	Ethyl dodecanoate	$C_{14}H_{28}O_2$	228	3.00	2.15	A mild scent with a fatty note, slight leafy and petal-like aromas
A9	Ethyl undecanoate	$C_{13}H_{26}O_2$	214	1.68	ND	Coconut fragrance
A10	(E)-3-(2,6,6-Trimethyl-3-oxo-1-cyclohexenyl)-2-methylacrylate	$C_{13}H_{18}O_3$	222	0.82	ND	
A11	Ethyl eicosanoate	$C_{22}H_{44}O_2$	340	9.19	15.79	
A12	Dibutyl phthalate	$C_{32}H_{54}O_4$	502	1.04	ND	
A13	Not standard nomenclature, likely a specific synthesized ester compound	$C_{19}H_{25}NO_5$	347	1.88	2.28	
A14	Methyl propiolate	$C_4H_4O_2$	84	ND	0.06	
A15	Not standard nomenclature, likely a specific synthesized ester compound	$C_{28}H_{44}O_4$	444	ND	1.83	
A16	Isopropyl palmitate	$C_{19}H_{38}O_2$	298	ND	1.56	Slight fatty odor
Alcohols						
B1	Ethanol	C_2H_6O	46	38.44	16.71	Unique refreshing fragrance of alcohol
B2	Isoamyl alcohol	$C_5H_{12}O$	88	1.98	ND	Fruity and floral aromas with a slightly spicy brandy scent
B3	Phenethyl alcohol	$C_8H_{10}O$	122	9.83	15.98	Has a floral scent reminiscent of gardenia and roses
Acids						
C1	Acetic acid	$C_2H_4O_2$	60	4.22	5.80	It has an irritating odor
Others						
D1	2-Ethyl-3,3-dimethyl-4-methylene-1-trimethylsilyl cyclopentene	$C_{13}H_{24}Si$	208	0.28	1.82	
D2	N,N-diphenylacetoxyundecanediamide	$C_{23}H_{26}N_2O_6$	426	0.41	ND	
D3	N-benzoxycarbonyl-L-tyrosine	$C_{17}H_{17}NO_5$	315	0.30	ND	
D4	10-Methyl-eicosane	$C_{21}H_{44}$	296	1.29	ND	
D5	Bis(trimethylsilyl)ethane	$C_8H_{22}O_2Si_2$	206	0.36	ND	
D6	3-(1a,2,7,7a-Tetrahydro-2-methoxy-1-phenyl-1,2,7-methoxy-1H)-cyclopropane	$C_{24}H_{19}NO$	337	0.72	ND	
D7	1-Iodododecane	$C_{12}H_{25}I$	296	ND	2.22	
D8	5-Propyltridecane	$C_{16}H_{34}$	226	ND	2.20	
D9	Nonadecane	$C_{19}H_{40}$	268	ND	2.03	
D10	2,2,4-Trimethyl-1,2-dihydroquinoline	$C_{12}H_{15}N$	173	ND	0.96	

"ND": not detected.

mulberry fruit wine, the L13 strain emerges as a highly suitable candidate for commercial mulberry wine production. Its utilization could significantly enhance the quality and distinctiveness of mulberry fruit wines, potentially setting new standards in the fruit wine industry.

Data Availability Statement

The data used to support the findings of this study are available from the corresponding author upon request.

Conflict of Interest

The authors declared no conflict of interest. The funders had no role in the study's design, and in the collection, analyses, or interpretation of data, writing of the manuscript, or decision to publish the results.

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