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Influence of Brewing Methods and *Saccharomyces cerevisiae* on the Aroma and Quality of Shuanghong *Vitis amurensis* Wine

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Abstract: Blending wines is a useful practice in red wine production, however, it is rarely used in *Vitis amurensis* wine. In recent years there has been a tendency to seek new products from the blending of wines to achieve greater complexity. The purpose of this study was to improve the aroma composition and complexity of Shuanghong *V. amurensis* wine, enhance its taste and quality, and attempt to use a new brewing technology. Different brewing methods were used in this study to carry out the fermentation test of 'Shuanghong *Vitis amurensis* wine. Two different kinds of *Saccharomyces cerevisiae* EZ2 and SN3 were selected for the mixed brewing of Shuanghong and Gongniang No. 1 grape juice and separate brewing of Shuanghong grape juice. During the brewing process, total sugar and total acid, reducing sugar, soluble solids, and aroma components of the wine were analyzed, and the sensory evaluation was carried out. It showed that total sugar, reducing sugar, and soluble solids in the fermentation broth decreased during the mixed and separate brewing of different *S. cerevisiae*, whereas total acid content increased initially and then decreased. When Shuanghong grape juice was mixed with Gongniang No. 1 grape juice at a 2:1 ratio and fermented with the EZ2 strain, the acidity of the wine produced was the lowest, at 8.6 g/L, among the four methods. The analysis of aroma components of wine obtained by different brewing methods showed that there were significant differences in aroma components between strains EZ2 and SN3. The mixed brewing made by strain EZ2 contained more ester aroma components and the total content was 19053.76 μg/L. This might indicate that the EZ2 strain is highly capable of synthesizing ester components and it also indicates that the mixed method, combining two different raw materials, can result in wine with richer ester aroma components. According to the comprehensive sensory evaluation results, the wine produced by the mixed fermentation of strain EZ2 had a good taste, rich aroma components, and good acid-reducing effects, which can be further optimized and applied as a new type of *Vitis amurensis* wine fermentation process. The results of this study show that the application of a mixed brewing method comprising Shuanghong and Gongniang No.1 *V. amurensis* at a 2:1 ratio, inoculated with the local *S. cerevisiae* EZ2 strain, can effectively improve the aroma and taste of Shuanghong *V. amurensis* wine, reduce acidity and result in the production of high-quality wine. In this study, a new mixed brewing method suitable for *V. amurensis* wine was developed. The results contribute to improvements in *V. amurensis* wine brewing technology and the quality of wine products, in addition to providing a theoretical basis for the utilization and development of characteristic *V. amurensis* resources in northern China.

Keywords: *Saccharomyces cerevisiae*, *Vitis amurensis* Wine, Brewing Quality, Mixed Brewing, Aroma, Acidity

Introduction

Yeast is an important microbe in the wine brewing process that can convert the sugar in grape juice into ethanol, carbon dioxide, and other flavor substances, such as fuels, aldehydes, acids, and esters (Vernocchi *et al*., 2011). During the fermentation process, the performance of yeast plays a decisive role in the alcohol content and overall quality of wine, of which *Saccharomyces cerevisiae* is particularly important and plays a key role in wine brewing (Morrison-Whittle and Goddard, 2018; Liang *et al*., 2020; Ma *et al*., 2017). Selecting suitable yeast with a good fermentation performance for wine brewing will help to give full play to the excellent quality and characteristics of grape raw materials (Fleet, 2003; Parapouli *et al*., 2010). The fermentation of wine by local yeast is more conducive to the formation of wine product variety, style, and regional characteristics than commercial *S. cerevisiae* and helps somewhat overcome the homogenization of wine (Liu *et al*., 2016; Börlin *et al*., 2016). In this study, two new local *S. cerevisiae* strains, screened from grape-planting areas, were used to produce *V. amurensis* wine, aiming to enhance its aroma and quality and obtain a new method of brewing this wine.

Vitis amurensis is an East Asian deciduous vine belonging to the grape family, and is a characteristic Chinese wine grape variety with a good cold and disease resistance (Gu *et al*., 2020; Wu *et al*., 2012). *V. amurensis* has smaller grains, a thicker skin and color, and more unique fruit aromas than European and Asian grapes, such as Cabernet Sauvignon and Chardonnay, and its organic acid, tannin, amino acid, mineral, and resveratrol content is high (Liu and Hua, 2013). The unique ecological conditions in the Yalu River valley in northern China give rise to the excellent quality and unique style of grape and wine in this region (Liu *et al*., 2017). The raw materials of *V. amurensis* have the problem of high acidity during the brewing process, which makes the *V. amurensis* wine taste sourer and sharper. Therefore, the deacidification of *V. amurensis* wine is a key issue in brewing high-quality wine. At present, there are three main methods for deacidification in wine, chemical, physical, and biological (Su *et al*., 2014). Biological deacidification is the use of microorganisms to decompose organic acids in wine during the fermentation process, thereby achieving this primary goal. The use of biological deacidification methods can not only reduce the acidity of wine but also improve the content and proportion of trace components and increase the complexity of the wine flavor (Alice, 2017).

Single-variety brewing is a common way of wine brewing in the world. However, due to the diversity of grape varieties, mixed brewing based on the complementarity of the differences between varieties is also possible (Khalafyan *et al*., 2019; Vilanova and Freire, 2017; Cáceres-Mella *et al*., 2014). The most obvious purpose of blending wines is to balance the taste or obtain a specific taste. Moreover, it improves certain characteristics that are appreciated by consumers, including color, flavor, alcohol content, body, aromatic composition, and overall quality (Monagas *et al*., 2006; Dooley *et al*., 2012; Hopfer *et al*., 2012). The use of the mixed brewing method has also been reported in a study on the deacidification fermentation of *V. amurensis* wine (Yuan *et al*., 2019). Shuanghong and Gongniang No.1 are both excellent varieties of *V. amurensis* in northern China. Mixing Shuanghongshan and Gongniang No.1 *V. amurensis* for brewing can achieve complementarity between the two raw materials, producing a wine with a rich aroma and unique taste.

In this study, ‘Shuanghong’ grapes were used as the raw material. Before fermentation, Gongniang No. 1 grapes were added to form mixed brewing. By adding two *S. cerevisiae* strains separately, namely EZ2 and SN3, four different brewing methods were designed to produce four different types of wine. During the fermentation process, the physical and chemical indexes and volatile aroma components of different wine samples were detected and analyzed and the effects of different brewing methods and yeast strains on the total acid content and quality of ‘Shuanghong' wine were analyzed. The results of this study can help optimize the brewing process of *V. amurensis* and obtain a novel brewing method based on local yeast. Our findings could provide a theoretical basis for improving the quality of *V. amurensis* wine characteristics and increasing the value of raw materials.

Materials and Methods

Materials, Chemicals and Strains

‘Shuanghong’ and ‘Gongniang No.1’ grapes were obtained from Ji’an City, Jilin Province. 3,5-Dinitrosalicylic acid (DNS) reagent, glucose standard, were purchased from Yuanye Bio-Technology Co., Ltd (Shanghai, China); C8~C20 normal alkanes were purchased from Sigma-Aldrich Co., Ltd (USA), 4-octanol was purchased from TCI Co., Ltd (Japan). Phenol and sulfuric acid were chemically pure; potassium pyrosulfite and white granulated sugar were all food grade. The EZ2 and SN3 strains of *S. cerevisiae* were screened beforehand and preserved in our laboratory.

Wine Fermentation Process

Grape raw materials → picking, excepting infarction, crushing → pressing, filtration, juice collection → clarification → juice collection following filtration → inoculation and fermentation → fermentation for approximately 13 d. Once the total sugar content did not change significantly and the alcohol content reached approximately 10°, fermentation was over and SO_2 was added after which the wine was placed in sealed storage.

Brewing Methods

Mixed brewing: 'Shuanghong' and Gongniang No. 1 grape juice were mixed and brewed at a 2:1 ratio, then white granulated sugar was added at a ratio of 8:1. 'Shuanghong' wine fermentation alone: 'Shuanghong' grape juice was mixed with white granulated sugar at a ratio of 8:1. Subsequently, preserved EZ2 and SN3 strains were inoculated in the liquid medium containing 100 mL YPD for activation, then shaken at 180 r/min and 28°C for 8-12 h. When absorbance values of the liquid culture of the different strains at 600 nm were between 0.7-0.8, they were inoculated into the mixed and 'Shuanghong' single brew with 2.5% inoculum respectively. Following fermentation (when there were slight changes in total sugar content and all indicators and alcohol content was 10°), we added 90 mg/L SO_2 to seal and terminate fermentation. Fig. 1 presents the research methodology in a flowchart.

Determination of Physical and Chemical Indexes of Wines Following Different Brewing Methods During Fermentation

During the fermentation following the different brewing methods, the total sugar, total acid, reducing sugar, and soluble solid content of the wines were regularly detected. The different wine samples were centrifuged at 6000 r/min and 4℃ for 10 min and the physicochemical indexes of the supernatants were determined. The total sugar of the samples was determined using the phenol-sulfuric acid method (Guo *et al*., 2008). Total acid was measured by titration with 0.1 M NaOH to pH 8.2 (Scacco *et al*., 2012). Reducing sugar was detected using the 3,5-dinitrosalicylic acid method (Sun *et al*., 2016). The soluble solid was determined with a saccharometer (Pal-Bx/Acid 1, Atago, Co., Ltd, Japan).

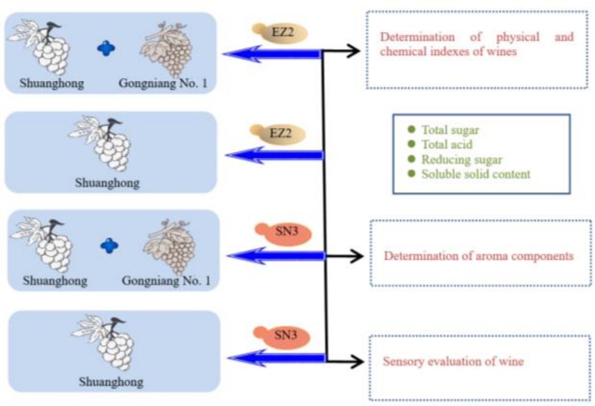


Fig. 1: Flowchart of research methodology

Determination of Aroma Components of Different Wine Samples

The aroma components of the wines were determined by headspace solid-phase microextraction-gas chromatography-mass spectrometry (Lan *et al*., 2016). Before extraction, the extraction fiber (DVB/CAR/PDMS, Supelco Co., Ltd, America) was aged at 260°C for 1h. Then, for testing4 mL of the sample was placed into a 15 mL headspace bottle and 1.2 g of NaCl and 10 µL internal standard (4-octanol) were added. The bottle cap was immediately tightened and sealed with a polytetrafluoroethylene spacer and the bottle was balanced for 15 min at 50℃. Subsequently, we inserted the activated extraction fiber into the headspace of the headspace bottle after the gas-liquid phase aroma substances in the bottle reached equilibrium, and extraction was performed under stirring conditions for 30 min at 50 °C. The extraction fiber was subjected to thermal desorption for 5 min at 260°C and the sample was injected using the non-split method. Gas chromatography-mass spectrometry conditions were as follows (5975C/7890A, Agilent Technologies, Inc, America): The carrier gas was high-purity helium at a flow rate of 1 mL/min. The temperature rise procedure of the column temperature box was as follows: Box was maintained at 50°C for 5 min, then raised to 220°C at 5°C /min for 5 min. The mass spectrometry interface, ion source, and quadrupole temperatures were 240, 230, and 150°C. The ionization mode used was the electronic ionization source. The ion source energy was 70 eV and the mass scanning range was 20~350 (m/z).

Qualitative Method: The aroma to be tested was qualitatively analyzed by mass spectrometry and Retention Index (RI). Quantitative Method: The internal standard method was used for semi-quantitative analysis.

Sensory Evaluation Method of Wine

The sensory evaluation of wine is mainly based on eight aspects closely related to wine quality: Taste (body, balance, bitterness), color, aroma (purity, richness, elegance), and typicality. The ten evaluators used here were intensively trained and had professional knowledge. The sensory analysis of wine samples was performed in a professional room set in order to facilitate the tasters' task of identifying descriptors. The evaluations were made in individual booths under white light (Belda *et al*., 2015). A constant volume of 30 ml of each wine was evaluated in wine-taster glasses at 12℃. The full score of each item is 100 points. Statistical analysis was performed on all results and the average score was determined (Pectka *et al*., 2006).

Data Processing and Analysis

SPSS 19 was used for statistical and Principal Component Analyses (PCA) and Origin 9.0 was used for graphic rendering.

Results

Analysis of Changes in Total Sugar Content of Wine During Fermentation Using Different Brewing Methods

For the fermentation of Shuanghong wine and Shuanghong mixed with Gongniang No. 1, *S. cerevisiae* strains EZ2 and SN3 were used alone. The total sugar content of the samples was detected on days 1, 3, 5, 7, 9, 11, and 13 of fermentation. The results showed that total sugar decreased gradually during the fermentation process. The total sugar content of strain EZ2 in both fermentation methods decreased rapidly from the beginning of fermentation to day 3, indicating that the initial fermentation speed of this strain was faster. The total sugar content of strain EZ2 in the mixed fermentation wine decreased significantly on day 11 and the total sugar content of strains EZ2 and strain SN3 samples on day 13 in the mixed fermentation samples were 77.6 g/L and 80.4 g/L, respectively. The total sugar of the Shuanghong wine brewed alone using the EZ2 strain decreased most and the residual sugar content was 41.9 g/L after brewing. The changes in total sugar content of the wine samples fermented using different brewing methods are shown in (Fig. 2).

Analysis of the Changes in Total Acid Content in Wine During Fermentation Using Different Brewing Methods

The EZ2 and SN3 strains of *S. cerevisiae* alone were used to ferment Shuanghong wine and Shuanghong mixed with Gongniang No. 1. The total acid content of the samples was detected on days 1, 3, 5, 7, 9, 11, and 13 of fermentation. The results showed that the total acid content increased first and then decreased during fermentation. The total acid content of mixed wine fermented using the EZ2 strain showed an increasing trend from days 1-5 and then decreased gradually. Its acidity declined from day 7, that of the mixed wine fermented using the SN3 strain and Shuanghong wine alone fermented using EZ2. The acidity of Shuanghong wine alone fermented using SN3 decreased from day 9. On day 13 of fermentation, the total acid content of the mixed wine fermentation samples of strains EZ2 and SN3 were 8.6 and 10.3 g/L, respectively. The changes in the total acid content of the wine samples by different fermentation methods are shown in (Fig. 3). According to the results, the brewing of the wine mix mixed fermented using the EZ2 strain had a good deacidification effect.

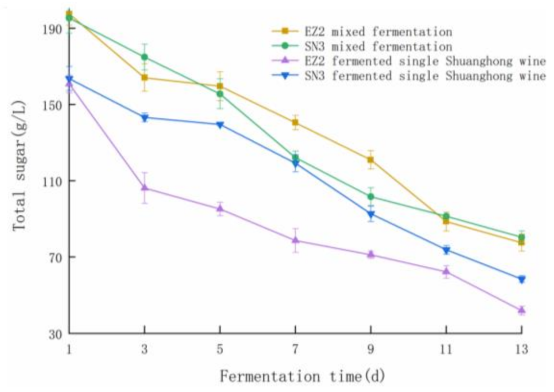


Fig. 2: Changes in total sugar content following different brewing methods

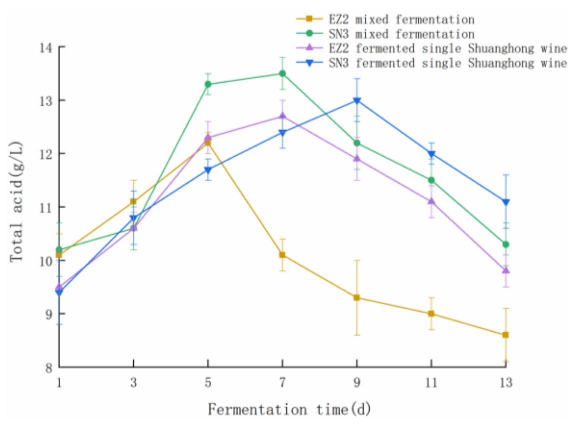


Fig. 3: Changes in total acid content following different brewing methods

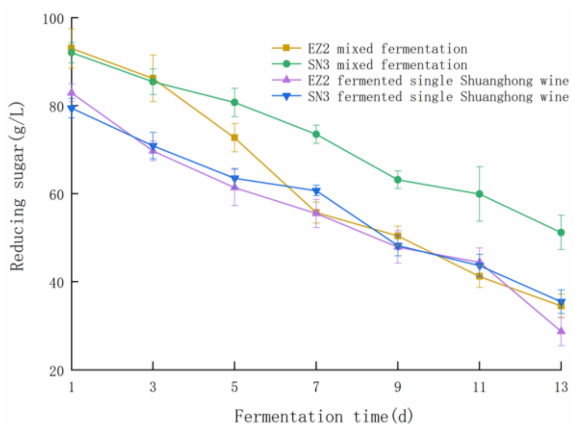


Fig. 4: Changes in reducing sugar content following different brewing methods

Analysis of the Changes in Reducing Sugar Content in Wine During Fermentation Using Different Brewing Methods

The EZ2 and SN3 strains of *S. cerevisiae* were used alone to ferment Shuanghong wine and Shuanghong mixed with Gongniang No. 1. Reducing sugar content was detected on days 1, 3, 5, 7, 9, 11, and 13 of fermentation. The results showed that the reduced sugar content decreased gradually during the fermentation process. The reduced sugar content following fermentation with strain EZ2 decreased rapidly from days 3-7 of mixed fermentation, indicating that yeast metabolism was relatively vigorous and converted a large amount of sugar into alcohol and other components. On day 13 of fermentation, the reducing sugar content of the mixed fermentation samples of strains EZ2 and SN3 were 34.5 and 51.2 g/L, respectively. The changes in reducing sugar content of wine samples are shown in Fig. 4.)

Analysis of the Changes in Soluble Solids Content of Wine During Fermentation Using Different Brewing Methods

Mixed with Gongniang No. 1. Soluble solid content of the sample was detected on days 1, 3, 5, 7, 9, 11, and 13 of fermentation. The results showed that the soluble solid content of samples decreased during the fermentation processes. On day 13 of fermentation, the soluble solids content of the mixed wine samples fermented using strains EZ2 and SN3 were 9.6 and 10.9% respectively. The changes in soluble solids content are shown in (Fig. 5).

Determination of Aroma Substances in Wine Samples Following Different Brewing Processes

The aroma substances in wines fermented using different methods (mixed fermentation with EZ2, single fermentation with EZ2, mixed fermentation with SN3, and single fermentation with SN3) were determined. A total of 30 aroma substances were detected, including 13 lipids, 4 alcohols, 7 acids, and 6 terpenes, as well as other components. The determination results of the types and contents of aroma components in the four different fermentation methods are shown in Table 1. The types of aroma components in the wine fermented using EZ2 were more abundant than those using SN3. The content of ester components, such as ethyl caproate, ethyl caproate, ethyl caproate, ethyl decanoate, ethyl undecanoate, and ethyl tetradecanoate in the mixed and single Shuanghong wines fermented using the EZ2 strain was high. The total content of esters in mixed brewing and single Shuanghong brewing using EZ2 was 19053.76 and 14928.74 µg/L respectively, indicating that the EZ2 strain was highly capable of synthesizing ester components during fermentation. The hexanoic acid content, which results in cheese and ester aromas, in the mixed brew fermented using EZ2 was 849.56 µg/L, higher than that of other brewing methods, indicating that this method can increase the ester aroma in wine. The phenyl ethyl alcohol content in the mixed and single Shuanghong brews fermented using the SN3 strain was 21087.45 and 24374.45 µg/L respectively, which is high.

The PCA was carried out for the 30 aroma components detected (Fig. 6). The total contribution rate of the two PCs was 85.0%, of which the contribution rate of PC1 was 45.6% and that of PC2 was 39.4%. It can be seen from the scatter diagram in Fig. 6a that there were differences between the two strains of *S. cerevisiae* in terms of aroma components produced by fermentation. Fig. 6b shows the load diagram of aroma components following different brewing methods. The load coefficient reflects the influence of various aroma substances on the principal components in the fermentation process. The higher the absolute value of the load coefficient, the greater the contribution of the aroma substances to the principal components. It can be seen from the figure that the characteristic aromas of the mixed wines fermented using the EZ2 strain were ethyl hexanoate, phenylethyl acetate, and octanoic, hexanoic, and decanoic acids. The characteristic aromas of the mixed wine fermented using the SN3 strain were isoamyl and phenyl ethyl alcohol, ethyl pelargonic, and benzaldehyde.

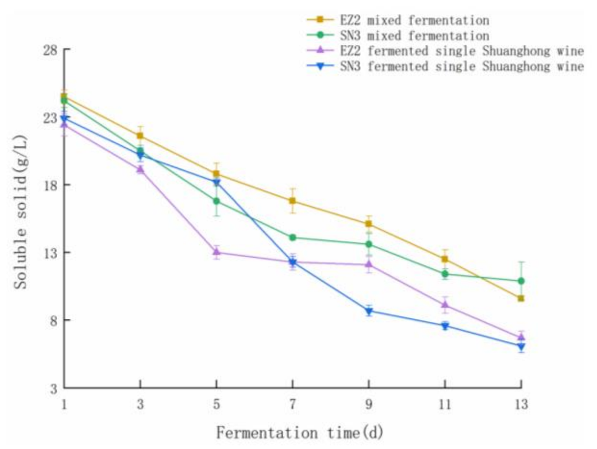
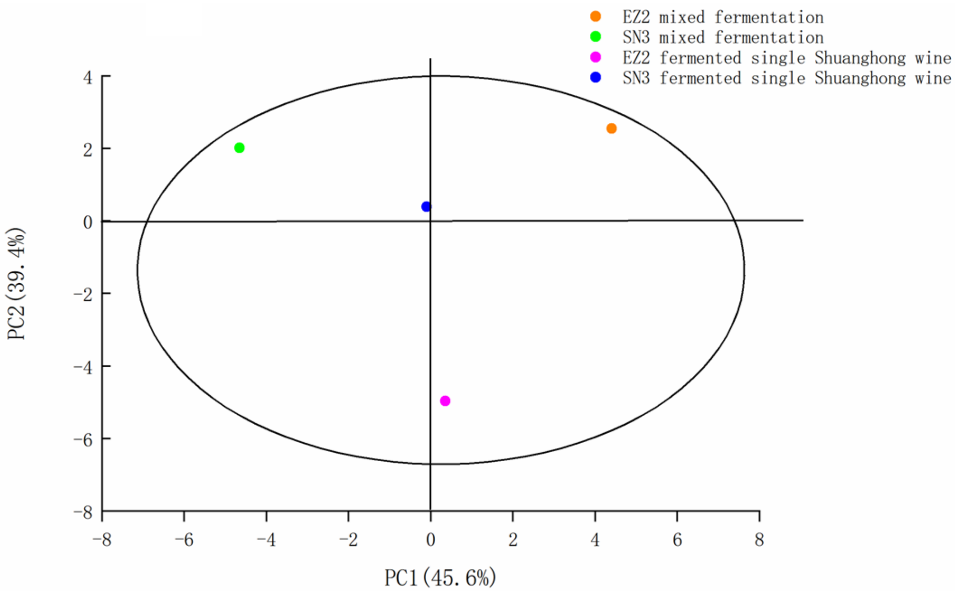


Fig. 5: Changes in soluble solid content following different brewing methods

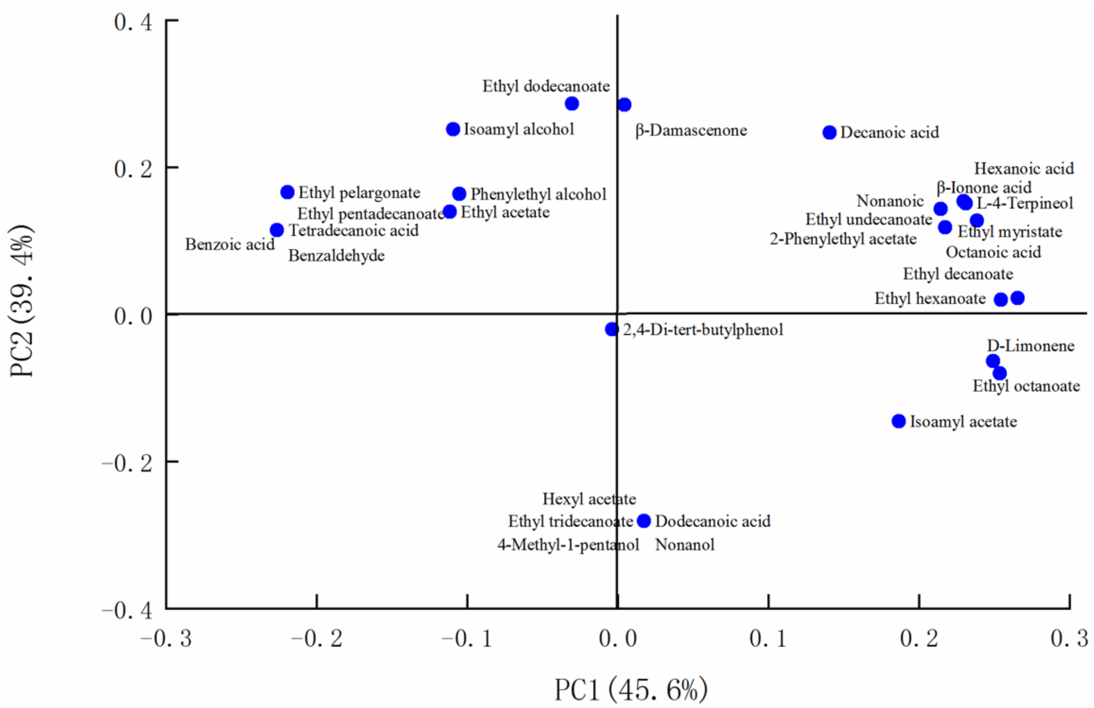
Table 1: Content of aroma components in wine prepared using different fermentation methods

	Aroma type	EZ2 mixed fermentation(ug/L)	SN3 mixed fermentation(ug/L)	EZ2 fermented single Shuanghong wine(ug/L)	SN3 fermented single Shuanghong wine(ug/L)
Esters	Ethyl acetate	153.49±12.60	261.68±18.25	73.98±2.74	nd
	Hexyl acetate	nd	nd	165.25±28.36	nd
	Isoamyl acetate	378.60±26.00	nd	440.63±43.77	nd
	Ethyl hexanoate	848.01±16.78	379.21±50.45	612.92±63.46	675.34±46.82
	Ethyl octanoate	4230.56±186.14	2441.90±226.91	3865.73±465.67	3116.72±691.19
	2-Phenylethyl acetate	2706.80±263.60	1481.91±298.44	1589.89±289.03	1422.41±317.70
	Ethyl pelargonate	202.98±18.03	604.63±47.28	123.19±21.08	286.97±57.37
	Ethyl decanoate	8518.39±435.83	5670.39±727.14	7132.85±801.23	7894.26±467.86
	Ethyl undecanoate	802.52±4.35	nd	nd	nd
	Ethyl dodecanoate	498.06±11.55	514.99±19.97	222.01±28.93	469.74±61.09
	Ethyl tridecanoate	nd	nd	702.29±57.16	nd
	Ethyl myristate	714.35±57.77	nd	nd	nd
	Ethyl pentadecanoate	nd	565.14±52.30	nd	nd
Higher alcohols	Isoamyl alcohol	2018.45±311.02	2792.18±545.55	691.90±71.80	1440.41±295.67
	Phenylethyl alcohol	17416.18±1794.62	21087.45±2035.40	14398.01±2809.60	24374.45±4029.95
	4-Methyl-1-pentanol	nd	nd	276.43±54.16	nd
	Nonanol	nd	nd	287.29±43.44	nd
Acids	Hexanoic acid	849.56±34.62	487.47±49.55	498.40±27.72	613.50±80.66
	Octanoic acid	14507.82±1712.51	5750.83±573.25	6803.02±569.88	7756.93±586.10
	Nonanoic acid	136.79±21.72	nd	nd	nd
	Decanoic acid	10564.59±1356.44	6556.25±758.27	3666.39±457.19	7761.33±596.89
	Dodecanoic acid	nd	nd	366.68±45.27	nd
	Tetradecanoic acid	nd	34.65±4.24	nd	nd
	Benzoic acid	nd	3489.37±381.78	nd	nd
Terpene and others	L-4-Terpineol	798.55±42.49	508.72±20.31	521.36±64.55	607.93±51.02
	D-Limonene	198.98±19.80	nd	156.07±42.45	170.01±62.09
	β-Ionone	227.44±24.18	nd	nd	nd
	Benzaldehyde	nd	37.66±2.87	nd	nd
	2,4-Di-tert-butylphenol	36.53±3.14	38.15±1.71	74.91±3.20	289.26±20.60
	β-Damascenone	500.33±79.61	492.31±51.42	411.88±37.13	490.97±12.03

Data are presented as mean values ± standard deviation of triplicate samples



(a)



(b)

Fig. 6: Results of Principal Component Analysis (PCA) of aroma components following different brewing methods; (a) Scatter plot of PCA of aroma; (b) Load diagram of PCA of aroma

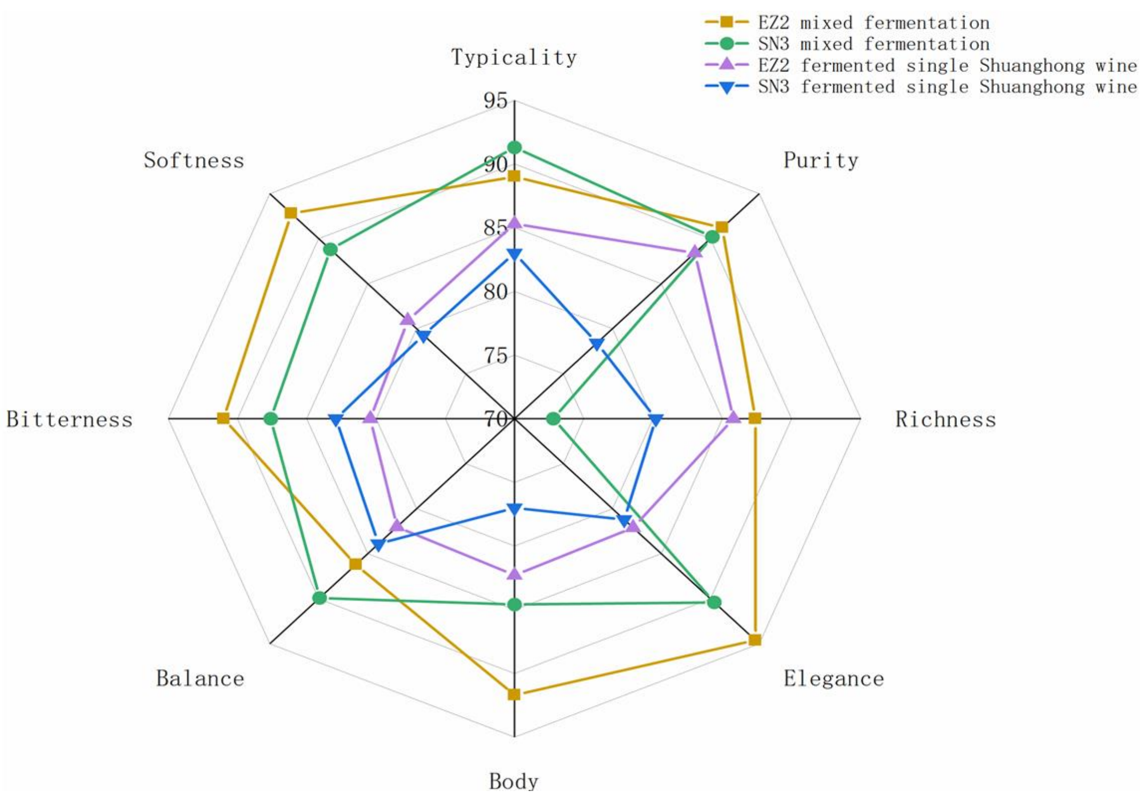


Fig. 7: Sensory evaluation radar diagram of wine made using different brewing methods

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Comprehensive Sensory Evaluation Results of Wine Brewed by Different Brewing Methods

The sensory evaluation radar of the four types of wine obtained by different brewing methods in Fig. 7. We found that the mixed wine sample fermented using the EZ2 strain had a high purity, elegance and smoothness, low bitterness, a rich taste, good typicality, and the highest evaluation score. Researchers used the mixed brewing method to brew peach red wine, mixing C*hardonnay* and *Cabernet Sauvignon* at a ratio of 1:1. The sensory score of the wine reached 91 points, with a strong aroma and a refreshing taste (Huang *et al*., 2021). Mixed wine brewing has the advantages of both raw materials. Compared with single-variety *V. amurensis* wine, the aroma is more elegant, the taste is softer, the complexity of the wine is increased and it is more easily favored by consumers.

Discussion

Acid affects the flavor of wine only after ester. In the process of wine brewing, acids play a very important role, affecting the quality and taste of the wine (Wang *et al*., 2019). To improve the sensory quality of wines, in the process of brewing, it is necessary to reduce the high acidity of grape juice (Li *et al*., 2019). *V. amurensis* is high in sugar and acid and its acidity is significantly higher than that of Eurasian grape populations (Niu *et al*., 2022). Thus, the proper deacidification fermentation of the grapes is an important way to improve the flavor and increase the brewing quality of *V. amurensis* wine.

In one study, malolactic fermentation and mixed brewing methods were used to reduce the acidity of Gongniang No.1 *V. amurensis* wine. After separately using two different methods of brewing, the acidity of wine was decreased to 8.84 g/L and 8.65 g/L (Yuan *et al*., 2019). Researchers studied the effect of deacidification yeast on the acidity of “Fox” wine and found that alcohol fermentation started faster under these conditions. Under optimal conditions, the acidity of wine samples can be reduced to 7.69 g/L (Zhong *et al*., 2019). Research reports show that most strains with deacidification effects are non-S. *cerevisiae* and *Lactobacillus* (Olaoye *et al*., 2022). In this study, the mixed wine brewing method, comprising fermentation using the EZ2 strain, effectively reduced the acidity of the wine. This finding could help to improve the brewing process of *V. amurensis* wine. However, the mechanism underlying the deacidification effect of the EZ2 strain requires further in-depth research.

One of the most important attributes that influence the quality of wines is the aroma and it can account for the differences between a lot of wines (Francis and Newton, 2005). The aroma of wine is the result of a biological and technological sequence that is also influenced by the fermentation process (Belda *et al*., 2017; Hernandez-Orte *et al*., 2015). Ethyl ester is an important ester compound in wine, which can enhance the fruity flavor of wine (Noguerol-Pato *et al*., 2012). Most esters in wine can produce pleasant aromas, such as lemon, apple, pineapple, and apple fruit (Tao *et al*., 2008; Jiang and Zhang, 2018), and are mainly synthesized through the action of microorganisms and esterification reactions during aging. The content and composition of esters in wine fermented by different yeast strains are different (Capozzi *et al*., 2019). Phenylethyl alcohol is produced by the metabolism of phenylalanine and has unique fragrances of violet, clove, fennel, and rose. It is one of the main compounds that give the wine a rich and elegant flavor characteristic (Styger *et al*., 2011). The fatty acid components mainly resulted from lipid metabolism and long-chain fatty acid decomposition and were generally related to the cheese and fat flavors displayed in wine (Olivero and Trujillo, 2011; Molina *et al*., 2009). Higher alcohols are important aroma substances in wine, increasing the complexity of its flavor (González-Robles *et al*., 2015). The formation of higher alcohols in wine is affected by many factors, including the sugar content in grape juice, the type of yeast inoculated, and fermentation temperature (Vilanova *et al*., 2007).

Conclusion

In this study, the effects of four different brewing methods based on two local *S. cerevisiae* strains on Shuanghong wine were analyzed. The results showed that the mixed brew containing Shuanghong and Gongniang No.1 fermented using the EZ2 strain produced the best taste and richest aromas. In terms of brewing methods, the mixture of two raw grape materials produced a better wine flavor and taste, with good typicality and balance and mellow thickness. In terms of the fermentation efficiency of *S. cerevisiae*, the EZ2 strain had a good deacidification effect and the two types of wine samples obtained through fermentation with this strain had lower acidity. The contents and types of ester aroma components in wine samples fermented using the EZ2 strain were also more diverse. The local *S. cerevisiae* EZ2 strain, originating from a *V. amurensis*-producing area, can be used as a new type of ferment in *V. amurensis* wine brewing. At present, most *V. amurensis* wines are fermented using commercial yeasts, which do not effectively highlight their characteristics (Romano *et al*., 2003). Therefore, it is of great practical significance to utilize novel brewing yeasts with excellent characteristics, such as the EZ2 strain.

Based on the results of this study, a novel brewing process for *V. amurensis* wine has been established. Here, the raw materials of Shuanghong and Gongniang No.1 are mixed at a ratio of 2:1 and fermented with local *S. cerevisiae* EZ2. The obtained wine has low acidity, a rich aroma, and good taste. However, the wine brewed with the aforementioned four methods should be compared in terms of color, organic acid types and contents, and polyphenol content. In subsequent research, the mixed brewing method can be further optimized, including the blending ratio, brewing time, and brewing temperature. Further in-depth analysis should also be conducted on the mechanism of deacidification by the local EZ2 strain and its impact on aroma components. This study provides a theoretical basis for solving the problem of high acidity in *V. amurensis* and designing scientific mixed brewing techniques. Moreover, the mixed brewing process and the selection of local *S. cerevisiae* have resulted in the proposal of new methods and ideas for the production of *V. amurensis* wine with high-quality characteristics. The results and application of this study will have a profound impact on improving the quality of *V. amurensis* wine with regional characteristics and the development of high-end wine products.

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Author's Contributions

Xiaochun Yu: Responsible for methodology and original drafted preparation.

Jing Xu: Participated in the experimental designed.

Zixuan Wang: Contributed to the properties analysis of wine with different brewed methods.

Ming Li: Responsible for data analysis and manuscript preparation.

Xue Chen: Contributed to the analysis of aroma components.

Shuying Li: Participated in the experimental designed.

Yongping Xu: Responsible for reviewed and edited of the manuscript.

Ethics

All authors read and approved the final version and are responsible for any ethical issue that may arise after the publication of this manuscript.

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