Effects of co-inoculation and sequential inoculation of *Wickerhamomyces anomalus* and *Saccharomyces cerevisiae* on the physicochemical properties and aromatic characteristics of longan (*Dimocarpus longan* Lour.) wine

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ORIGINAL ARTICLE

Abstract

Wickerhamomyces anomalus and *Saccharomyces cerevisiae* were mixed by co-inoculation or sequential inoculation, and the physicochemical properties, electronic sensory characteristics, and aromatic characteristics of longan (*Dimocarpus longan* Lour.) wine were evaluated to analyze the effects of mixed fermentation on wine quality. The results demonstrate that mixed fermentation obtained by co-inoculation or sequential inoculation decreases the alcohol content of longan wine. Furthermore, mixed fermentation also leads to the reduction of the electronic sensory acidity and richness of longan wine. Moreover, the two mixed inoculation methods resulted in different effects on the aromatic characteristics of longan wine. The varieties of aldehyde and ketone aromatic compounds increase in longan wine fermented by co-inoculation, with increasing amounts of acids, aldehydes, ketones, and other compounds, and a decrease in the amounts of ester compounds. However, the variety of ester aromatic compounds and the amounts of acids, aldehydes, and ketones increase when using sequential inoculation. Therefore, the application of mixed fermentation can regulate the physicochemical properties, as well as the electronic sensory characteristics and aromatic characteristics of longan wine, and this contributes to the enrichment of the different types of longan wine.

Practical Application

Two methods were used to ferment longan wine, consisting of sequential inoculation or co-inoculation with *Wickerhamomyces anomalus* and *Saccharomyces cerevisiae*. The basic physicochemical indexes, electronic sensory characteristics, and aromatic substances were then analyzed to determine the influence of the two inoculation methods on the qualities of the wine. Our study provides a theoretical basis for the application of both inoculation methods in the production of longan wine and a reference for the production of other fruit wines.

Keywords: non-*Saccharomyces* yeasts; *Wickerhamomyces anomalus*; longan wine; physicochemical properties; aromatic characteristics

Introduction

There have been numerous studies on *Wickerhamomyces anomalus*, also called *Pichia anomala* and *Hansenula anomala*, in recent years due to its unique physiological characteristics and metabolic features (Morales *et al.*, 2020; Schneider *et al.*, 2012; Ye *et al.*, 2014). They include the following aspects: (1) tolerance to various

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extreme environmental conditions such as high/low pH, high osmotic pressure, and anaerobic conditions; (2) capability of synthesizing many glycosidases that can hydrolyze flavor precursor compounds containing glucosidic bonds in raw materials, which results in the release of flavor compounds; (3) generation of numerous volatile ester compounds with floral and fruity aromas. Research results show that β-D-glucosidase, β-D-xylosidase, and α-D-rhamnosidase can be secreted by *W. anomalus*. When wine is fermented with *W. anomalus*, various amounts of ester compounds (such as ethyl acetate, isoamyl acetate, and 2-phenethyl acetate) with floral and fruity aromas can be added to improve the sensory characteristic scores of wines (Padilla *et al*., 2018). In apple ciders fermented using *W. anomalus*, the varieties and amounts of various flavor compounds (including acetate esters, ethyl esters, higher alcohols, aldehydes, and ketones) increase and result in a higher sensory evaluation score (Ye *et al*., 2014); In addition, the application of *W. anomalus* also can increase the ethyl acetate in *Prunus salicina* Lindl. cv. "Kongxinli" fruit wine when it is produced using mixed inoculation with *W. anomalus* and *Saccharomyces cerevisiae* (Liu *et al*., 2020a). Thus, *W. anomalus* delivers the capability of regulating the aromatic and sensory characteristics of wines, and has promising application prospects in wine production.

Longan (*Dimocarpus longan* Lour.) belongs to the Sapindaceae family (Han *et al*., 2020), whose fruits contain abundant nutrients and show medicinal value, including relieving uneasiness and enhancing the functions of the heart and spleen (Park *et al*., 2016). Longan fruit is considered to be medicine and food and has a favorable development value. As for fermentation of longan wine, Yuan *et al*. (2020) carried out research on fermentation with aroma-producing yeast and *S. cerevisiae*. They performed co-inoculation using active dry wine yeasts and aroma-producing yeast in the ratio of 1:1 to analyze the influence of different fermentation parameters (sugar content, acidity, fermentation temperature, and fermentation time) on basic physicochemical indexes and sensory characteristics of longan wine.

Sanoppa *et al*. (2019) probed the effects of inoculation of *Torulaspora delbrueckii* on the nonvolatile and volatile compounds in longan wines. They found that longan wine fermented with *T. delbrueckii* by co-inoculation produced the highest number of total volatile aromatic compounds. In addition, co-inoculation generated high levels of the main volatile compounds. However, the different influences of mixed fermentation with *W. anomalus* and *S. cerevisiae* on various qualities (such as basic physicochemical parameters, aromatic characteristics, and sensory characteristics) of longan wines have not yet been clarified.

In the present study, based on sequential inoculation and co-inoculation, longan wine was fermented by mixing *W. anomalus* with *S. cerevisiae* to analyze the influence of both inoculation methods on the quality of wine from different aspects. These included basic physicochemical indexes, electronic sensory characteristics, and aromatic substances. This provides a theoretical basis for the application of both yeasts in the production of longan wine and a reference for the production of other fruit wines.

Materials and Methods

Reagents

Glucose, sucrose, yeast extract, peptone, sulfur dioxide (SO_2), and pectinase were reagent pure grade and obtained from Sangon Biotech Co., Ltd. (Shanghai, China). Sodium hydroxide (NaOH), ethanol (95%), sodium chloride (NaCl), and cyclohexanone were analytically pure grade and purchased from Luoyang Chemical Co., Ltd. (Luoyang, China).

Yeast strains and culture conditions

Commercial *Saccharomyces cerevisiae* X16 obtained from the Laffort Company (France) was used as a reference strain. The *W. anomalus* C11 strain was isolated from the fruit of *Rosa roxburghii* Tratt. All yeast cells were cultured on yeast extract peptone dextrose medium (1% yeast extract, 2% peptone, 2% glucose, and 2% agar) containing 100 mg/L of chloromycetin at 28°C for 72 h, and then were maintained at 4°C for later use.

Preparation of fermented longan wine

Fresh longan fruit produced in Thailand was purchased from a local fruit market in Guiyang, Guizhou Province, China. The longan fruit was selected, peeled, pitted, and squeezed to obtain its must. Sulfur dioxide (50 mg/L), pectinase (200 mg/L), and dimethyl dicarbonate (600 mg/L) were added to the must, which was maintained at room temperature for 12 h. After completing the treatment, the sugar was adjusted to 24° Brix. Then, the mixture was divided into four groups and poured into 2.5-L sterile Erlenmeyer flasks with rubber plugs and respirators, with three parallel repetitions for each. In terms of the first group (*S. cerevisiae*), only *S. cerevisiae* X16 was inoculated, at a concentration of 10^7 colony-forming units (cfu)/mL; in the second group (*W. anomalus*), only *W. anomalus* was inoculated, at a concentration of 10^8 cfu/mL. As for the third group (co-inoculation), *W. anomalus* and *S. cerevisiae* were co-inoculated, separately showing concentrations of 10^8 and 10^7 cfu/mL.

For the fourth group (sequential inoculation group), *W. anomalus* with a concentration of 10^8 cfu/mL was inoculated at the beginning of fermentation. On the 4th day of fermentation, *S. cerevisiae* with a concentration of 10^7 cfu/mL was inoculated. The samples in different groups were statically maintained and fermented at 22°C in an incubator with a thermostat until the fermentation was complete.

Detection of basic physiochemical parameters for longan wine

The determination of the alcohol content, total acidity, and volatile acid of longan wine was performed according to the method proposed by Xia *et al*. (2018). The total sugar content in longan wine was determined by applying the optimized anthrone method (Liu *et al*., 2020a). The pH value was measured by employing a pH meter (Model PT-10, Sartorius AG, Germany).

Detection of electronic sensory characteristics for longan wine

The electronic sensory indexes for longan wine were determined using an electronic tongue system (SA402B, Insent, Japan). Measurements were obtained according to the instrument instructions and the method proposed by Liu *et al*. (2020b). Each wine sample was measured in three parallel repetitions, and the data were collected four times in each parallel repetition.

Detection of the aromatic composition of longan wine

The aromatic composition of longan wine was determined by combining headspace solid phase microextraction (TQ8040, Agilent, USA) with gas chromatography-mass spectrometry (GC-MS) (Tandee *et al*., 2020). The qualitative analysis was conducted by searching the National Institute of Standards and Technology (NIST) standard library for matches with the GC-MS data. By using cyclohexanones as internal standards, the aromatic compositions were quantitatively analyzed by applying the internal standard method.

Data Analysis

Principal component analysis (PCA) and significance analysis were conducted on the data with the aid of SPSS 25 software. Excel 2010 software was used for data processing and plotting. Adobe Photoshop CS was applied to edit the figures. The data are expressed in the form of the mean ± standard deviation. $P < 0.05$ indicates that the difference shows statistical significance.

Results and Analysis

Influence of mixed fermentation of *W. anomalus* and *S. cerevisiae* on the basic physiochemical parameters of longan wine

The basic physiochemical parameters of fermented longan wine in the different groups are shown in Table 1. The longan wines fermented by inoculation with mixed *S. cerevisiae* with *W. anomalus* exhibited lower alcohol content than that fermented by inoculation with *S. cerevisiae* alone (control group). No significant difference was found in the total acidities, volatile acid, or pH values of the four groups of longan wine.

Influence of mixed fermentation of *W. anomalus* and *S. cerevisiae* on the electronic sensory characteristics of longan wine

Figure 1 shows the electronic sensory characteristics of fermented longan wines in different groups analyzed using the electronic tongue detection system. The groups of longan wine produced by co-inoculation and sequential inoculation delivered lower acidity and richness than the group produced by inoculation with *W. anomalus* or *S. cerevisiae* alone.

Table 1. Basic physiochemical parameters of longan wine.

Groups	Alcohol content % (v/v)	Total sugar (g/L)	Total acidities (g/L)	Volatile acid (g/L)	pH
S. cerevisiae	12.8 ± 0.34	0.79 ± 0.02	11.64 ± 0.27	0.80 ± 0.14	3.87 ± 0.01
W. anomalus	11.8 ± 0.00	1.35 ± 0.31	11.93 ± 0.11	1.18 ± 0.16	3.88 ± 0.01
Co-inoculation	11.8 ± 0.00*	0.97 ± 0.05	12.08 ± 0.51	1.00 ± 0.06	3.88 ± 0.01
Sequential inoculation	11.9 ± 0.12*	0.92 ± 0.00	12.03 ± 0.37	1.17 ± 0.06	3.86 ± 0.02

*$P < 0.05$, compared with the group in which *S. cerevisiae* is inoculated alone.

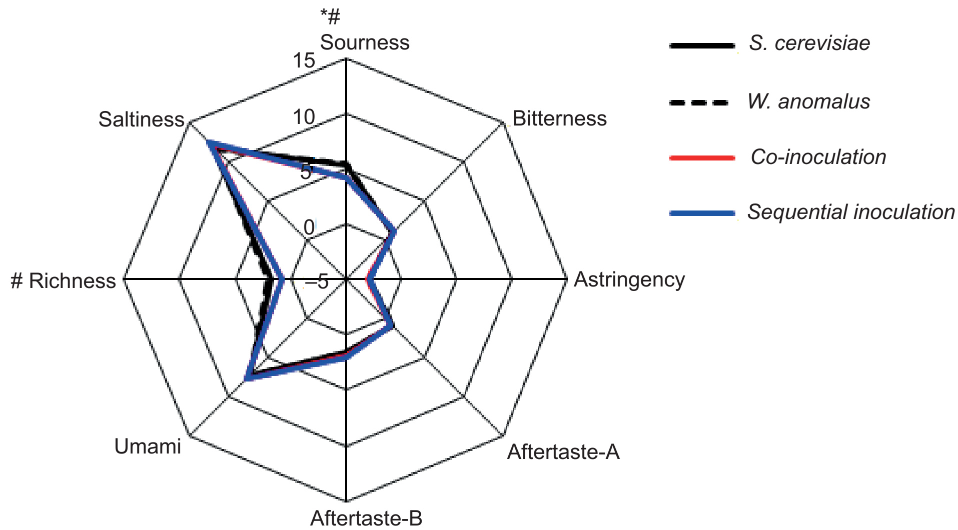

Figure 1. Results of electronic sensory characteristics of longan wine. Aftertaste-A and Aftertaste-B refer to the aftertastes of astringency and bitterness, respectively. *P < 0.05, compared with the *S. cerevisiae* group; #P < 0.05, compared with the *W. anomalus* group.

Influence of mixed fermentation by *W. anomalus* and *S. cerevisiae* on the aromatic characteristics of longan wine

Ester compounds

Ester compounds are secondary metabolites generated during alcohol metabolism, most of which exhibit floral and fruity aromatic characteristics, and they are important aromatic compounds for various fermented wines (Song *et al.*, 2019). As shown in Table 2, a total of 19 ester compounds were detected from the four groups of fermented longan wine, in which 16, 14, 15, and 19 ester compounds were separately detected from the *S. cerevisiae* group, *W. anomalus* group, co-inoculation group, and sequential inoculation group, respectively. Ethyl 3-hydroxybutyrate was only detected from longan wine produced through mixed fermentation (co-inoculation and sequential inoculation), and ethyl 9-decenoate and isoamyl octanoate are specific to longan wine fermented through sequential inoculation. In addition, the amount of aromatic ester compounds in longan wine fermented by co-inoculation was significantly lower than that fermented by inoculation with only *S. cerevisiae* or *W. anomalus*. There was no difference between longan wines fermented through sequential inoculation with *S. cerevisiae* and *W. anomalus* in terms of the amounts of ester compounds.

Ethyl acetate provides aromas of pineapple and sweet fruits, and it is one of the most predominant ester aromatic compounds in fermented wine, with a threshold of approximately 150 mg/L (Yan *et al.*, 2020). Ethyl acetate at approximately 80 mg/L can favorably impart a fruity aroma to grape wine and increase the complexity of wines. The highest concentration ethyl acetate was measured in the longan wine fermented by sequential inoculation (66.87 ± 8.59 mg/L), while the ethyl acetate of the other three groups was approximately 30 mg/L (Table 2).

Alcohol compounds

Alcohol compounds are secondary metabolites generated through the metabolism of yeast cells during fermentation, and they contribute important regulatory effects on the aromatic type of fermented wines (Jolly *et al.*, 2014). As shown in Table 3, a total of nine alcohol compounds were detected from the four groups of fermented longan wine, in which there were high amounts of isobutanol and phenethyl alcohol. Compared with the *W. anomalus* group, the longan wines fermented by mixed fermentation (including co-inoculation and sequential inoculation) delivered a significantly greater amount of alcohol compounds. The concentration of alcohol compounds in the co-inoculation group was the highest (95.48 ± 5.01 mg/L).

Higher alcohols are commonly known as fusel oil, which is a general term for monovalent alcohols with at least three carbon atoms. A moderate amount of higher alcohols can endow wines with a unique aroma and flavor. If the amount of higher alcohols is too low, a light-bodied wine will result, which leads to insufficient aroma. However, a high amount of higher alcohols is likely to lead to headache and drunkenness after consumption (Gonzalez and Morales, 2017). Research shows that there are 80–540 mg/L of higher alcohols in grape wine (Yan *et al.*, 2020), and that the lowest amount (69.06 ± 5.03 mg/L) of higher alcohols was obtained in longan

Table 2. Ester compounds and their concentrations in longan wine from different fermentations.

Volatile compounds	Odor descriptor	Concentration (mg/L)			
		S. cerevisiae	*W. anomalus*	Co-inoculation	Sequential inoculation
Ethyl acetate	Pineapple, fruity, solvent, balsamic	31.81 ± 1.96	38.88 ± 0.98	30.77 ± 0.77	66.87 ± 8.59
Isoamyl acetate	Banana, fruity, sweet	40.23 ± 2.69	37.08 ± 3.82	17.73 ± 0.09	39.08 ± 4.15
Phenethyl acetate	Floral	9.76 ± 0.09	8.74 ± 0.44	3.44 ± 0.54	6.98 ± 0.15
Isobutyl acetate	Fruity	1.58 ± 0.18	—	—	1.75 ± 0.05
Citronellyl acetate	Fruity	0.89 ± 0.17	—	—	0.65 ± 0.04
Ethyl 9-decenoate		—	—	—	6.31 ± 0.60
Ethyl octanoate	Sweet, floral, fruity, banana, pear, brandy	112.41 ± 9.28	79.90 ± 3.58	45.26 ± 3.43	80.44 ± 9.29
Isoamyl octanoate		—	—	—	0.78 ± 0.08
Ethyl hexanoate	Fruity, green apple, banana, brandy, wine-like	10.22± 0.57	6.78 ± 1.29	4.93 ± 0.10	6.81 ± 0.19
Ethyl 3-hydroxybutyrate		—	—	0.73 ± 0.09	0.60 ± 0.00
Ethyl decanoate	Brandy, fruity, grape	57.80 ± 4.57	53.64 ± 4.65	31.12 ± 3.78	65.28 ± 5.23
Ethyl benzoate	Fruity	1.59 ± 0.02	1.50 ± 0.15	1.51 ± 0.08	1.63 ± 0.03
Diethyl succinate		0.85 ± 0.11	1.93 ± 0.40	1.29 ± 0.13	4.82 ± 0.28
Ethyl laurate	Fruity, fatty	19.51 ± 0.62	20.45 ± 0.39	12.23 ± 0.47	13.36 ± 2.04
Ethyl myristate	Fatty	2.44 ± 0.01	2.64 ± 0.32	3.71 ± 0.28	2.01 ± 0.03
Ethyl palmitate	Fatty	1.74 ± 0.40	2.76 ± 0.36	5.68 ± 0.07	2.21 ± 0.12
Methyl benzoate	Fatty	1.51 ± 0.09	1.38 ± 0.11	1.57 ± 0.11	1.24 ± 0.06
Methyl salicylate		1.52 ± 0.08	2.51 ± 0.14	2.49 ± 0.17	2.23 ± 0.10
Butyrolactone		2.27 ± 0.34	1.34 ± 0.11	1.56 ± 0.21	1.12 ± 0.13
∑		296.13 ± 21.18	259.53 ± 16.74	164.02 ± 10.32$^{*\#}$	304.17 ± 31.16

“—” represents a compound that is not detected; *$P < 0.05$, compared with the *S. cerevisiae* group; $^{\#}P < 0.05$, compared with the *W. anomalus* group.

Table 3. Alcohol compounds and their concentrations in longan wine from different fermentations.

Volatile compounds	Odor descriptor	Concentration (mg/L)			
		S. cerevisiae	*W. anomalus*	Co-inoculation	Sequential inoculation
Methanol	Alcohol-like	1.46 ± 0.15	1.76 ± 0.16	1.44 ± 0.05	2.09 ± 0.30
n-Propyl alcohol	Mellow, fruity	2.10 ± 0.26	1.95 ± 0.17	2.07 ± 0.04	2.51 ± 0.22
Isobutanol	Mellow, fruity	32.14 ± 1.85	21.31 ± 2.44	38.05 ± 0.81	32.36 ± 2.10
Hexyl alcohol	Grass-like	–	–	0.86 ± 0.04	-
Trans -2,4-hexadiene-1-ol		3.41 ± 0.35	1.82 ± 0.09	–	2.48 ± 0.18
2,3-Butanediol		4.67 ± 0.37	4.69 ± 0.48	7.14 ± 0.64	4.43 ± 0.29
Linalool	Floral, musk	3.35 ± 0.28	2.75 ± 0.03	3.07 ± 0.18	2.89 ± 0.20
Phenethyl alcohol	Sweet, floral, fruity	37.97 ± 2.78	35.69 ± 1.69	41.91 ± 3.11	39.82 ± 1.28
Citronellol	Grass-like, floral	1.33 ± 0.07	0.85 ± 0.13	0.94 ± 0.14	1.10 ± 0.08
∑		86.43 ± 6.11	70.82 ± 5.19	95.48 ± 5.01$^{\#}$	87.68 ± 4.65$^{\#}$

“—” represents a compound that is not detected; $^{\#}P < 0.05$, compared with the *W. anomalus* group.

wine fermented by inoculation with *W. anomalus* alone. By contrast, the concentrations of higher alcohols in the other three groups were all higher than 80 mg/L, and the concentration of higher alcohols in longan wine produced by co-inoculation was the highest, at 94.04 ± 4.96 mg/L (Table 3).

Linalool, phenethyl alcohol, and citronellol are higher alcohols with floral and fruity aromas that were present in the four groups of longan wines. The three higher alcohols reached the highest concentration (45.92 ± 3.43 mg/L) in the co-inoculation group, while the lowest (39.29 ± 1.85 mg/L) was measured in the *W. anomalus* group (Table 3).

Volatile acidity

Volatile acidity is also considered as an important aspect of aromatic compositions in fruit wines. As shown in Table 4, only two volatile acid compounds were found in longan wine, that is, acetic acid and caprylic acid. Acetic acid was detected in the four groups of fermented fruit wine, but caprylic acid was not found in the co-inoculation or the sequential inoculation groups. Compared with the *S. cerevisiae* group, the volatile acidity in longan wine increased based on mixed inoculation with *W. anomalus*, in which the co-inoculation group delivered the highest concentration (79.25 ± 4.41 mg/L) of volatile acidity.

Aldehyde and ketone compounds

The varieties and amounts of aldehyde and ketone compounds also contribute to the aromatic characteristics of fruit wines. As shown in Table 5, a total of three aldehyde and ketone compounds (i.e., acetaldehyde, acetone, and 5-hexen-2-one) were detected from the four groups of fermented longan wine. To be specific, the three aldehyde and ketone compounds were all present in the co-inoculation group, while in the other three groups, only acetaldehyde was detected. Relative to the *S. cerevisiae* and *W. anomalus* groups, the aldehyde and ketone compounds in longan wines significantly increased under co-inoculation and sequential inoculation. The application of co-inoculation increases the variety and amounts of aldehyde and ketone compounds.

Other compounds

Table 6 shows that among the four groups of fermented longan wine, a total of five other compounds were detected, in which two, five, four, and three compounds were separately found in the *S. cerevisiae*, *W. anomalus*, co-inoculation, and sequential inoculation groups, respectively. Compared with the *S. cerevisiae* and *W. anomalus* groups, the other compounds in the co-inoculation group significantly increased, while that in the sequential inoculation group did not vary. Therefore, the application of co-inoculation can effectively increase the varieties and amounts of other compounds in longan wine.

Above all, co-inoculation can enrich the variety of aldehyde and ketone aromatic compounds, while sequential

Table 4. Acid compounds and their concentrations in longan wine from different fermentations.

Volatile compounds	Odor descriptor	Concentration (mg/L)			
		S. cerevisiae	*W. anomalus*	Co-inoculation	Sequential inoculation
Acetic acid	Acetic acid taste	49.61 ± 5.39	71.02 ± 5.02	79.25 ± 4.41	78.75 ± 1.66
Octanoic acid	Cheese, fatty	6.18 ± 0.56	2.63 ± 0.11	–	–
∑		55.79 ± 5.95	73.65 ± 5.23	79.25 ± 4.41*	78.75 ± 1.66*

"—" represents a compound that is not detected; *P < 0.05, compared with the *S. cerevisiae* group.

Table 5. Aldehyde and ketone compounds and their concentrations in longan wine from different fermentations.

Volatile compounds	Odor descriptor	Concentration (mg/L)			
		S. cerevisiae	*W. anomalus*	Co-inoculation	Sequential inoculation
Acetaldehyde	Pungent smell	2.94 ± 0.10	2.92 ± 0.11	2.98 ± 0.02	3.51 ± 0.25
Acetone	Solvent smell	–	–	0.31 ± 0.03	–
5-Hexene-2-ketone		–	–	1.13 ± 0.06	–
∑		2.94 ± 0.10	2.92 ± 0.11	4.42 ± 0.11*#	3.51 ± 0.25*#

"—" represents a compound that is not detected;*P < 0.05, compared with the *S. cerevisiae* group; #P < 0.05, compared with the *W. anomalus* group.

Table 6. Other compounds and their concentrations in longan wine from different fermentations.

Volatile compounds	Odor descriptor	Concentration (mg/L)			
		S. cerevisiae	*W. anomalus*	Co-inoculation	Sequential inoculation
2,6-Di-tert-butylphenol		—	2.73 ± 0.42	1.69 ± 0.21	7.75 ± 0.12
Ocimene	Floral	—	1.05 ± 0.23	1.25 ± 0.01	—
1,3-Dioxolane, 2,4,5-trimethyl-		3.59 ± 0.01	1.74 ± 0.14	21.77 ± 1.96	13.06 ± 2.34
2-Methyl-1,5-dioxyl-helico-[5,5] undecane		29.30 ± 4.33	19.42 ± 0.16	40.65 ± 1.48	11.08 ± 1.38
Benzothiazole		—	0.88 ± 0.18	—	—
∑		32.89 ± 4.34	25.82 ± 1.13	65.36 ± 3.66*#	31.89 ± 3.84

"—" represents a compound that is not detected; *P < 0.05, compared with the *S. cerevisiae* group; #P < 0.05, compared with the *W. anomalus* group.

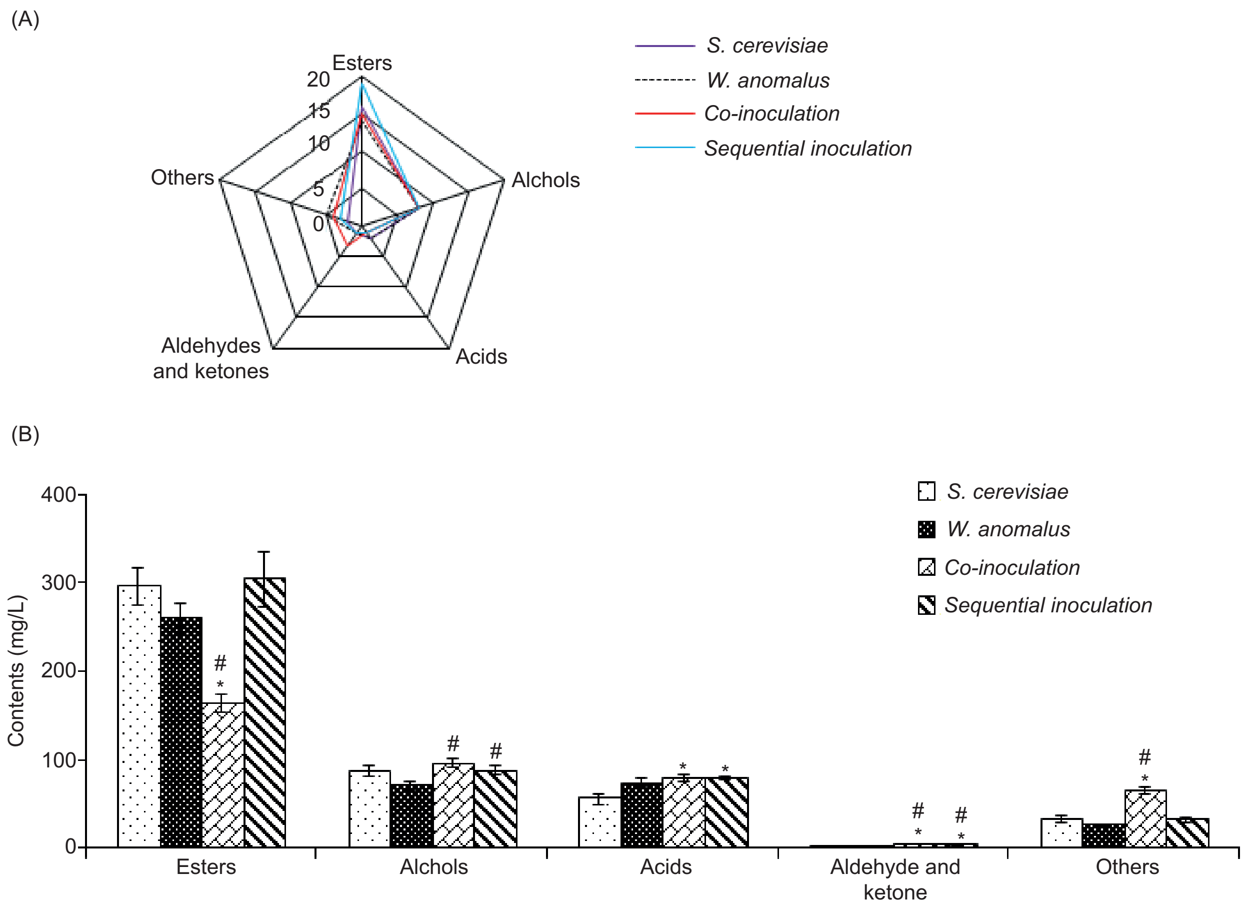

Figure 2. Types and amounts of aromatic compounds in longan wine. (A) Types of aromatic compounds. (B) Amounts of aromatic compounds. *P < 0.05, compared with the *S. cerevisiae* group; #P < 0.05, compared with the *W. anomalus* group.

inoculation can enhance the variety of aromatic ester compounds in longan wine (Figure 2A). Relative to the *S. cerevisiae* group, the amount of ester compounds significantly decreased, while those of acid, aldehyde, ketone, and other compounds in fermented longan wine significantly increased in the co-inoculation group (Figure 2B). Moreover, the acid, aldehyde, and ketone compounds in the longan wine fermented by sequential inoculation significantly increased. Compared with inoculation using *W. anomalus* alone, co-inoculation significantly decreased the ester compounds while increasing the alcohol, aldehyde, ketone, and other compounds in longan wine. Similarly, sequential inoculation enhanced the amounts of alcohol, aldehyde, and ketone compounds. Thus, mixed inoculation (co-inoculation and sequential inoculation) can regulate the aromatic

Table 7. The OAV of the main aromatic compounds in longan wine.

Number	Volatile compounds	Odor threshold (μg/L)	OAV			
			S. cerevisiae	*W. anomalus*	Co-inoculation	Sequential inoculation
A1	Ethyl acetate	750.00	4.24	5.18	4.10	15.77
A2	Isoamyl acetate	93.93	432.58	398.71	190.65	420.22
A3	Phenethyl acetate	909	10.73	9.60	3.78	7.67
A4	Isobutyl acetate	8	0.20	—	—	0.22
A5	Ethyl octanoate	12.87	8.73	6.21	3.52	6.25
A6	Ethyl hexanoate	50	0.20	0.14	0.01	0.14
A7	Ethyl decanoate	1120	0.05	0.05	0.03	0.06
A8	Ethyl laurate	640	30.48	31.95	19.11	20.88
B1	Isobutanol	1600	0.02	0.01	0.02	0.02
B2	n-Propyl alcohol	94	22.34	20.74	22.02	26.70
B3	Linalool	15	223.33	183.33	204.67	192.67
B4	Phenethyl alcohol	10000	3.80	3.57	4.19	3.98
B5	Citronellol	180	7.39	4.72	5.22	6.11
C1	Octanoic acid	500	12.36	5.26	–	–

"—" represents a compound that is not detected; OAV: odor activity value.

characteristics of longan wine, including the variety and amount of aromatic compounds.

Main aromatic compounds

The odor activity value (OAV) can be used to further evaluate the influence of the main aromatic compounds on the aromatic characteristics of fruit wines. An OAV larger than 1 indicates that the compounds make a great contribution to the aroma of wines. On the contrary, an OAV lower than one implies that the compounds contribute little to the aroma (Borren and Tian, 2020). The OAVs of 14 main aromatic compounds in longan wine are displayed in Table 7, and the OAVs of 10 compounds were greater than 1, while those of four compounds were less than 1. The OAVs of isoamyl acetate, linalool, and ethyl laurate were large among the four groups of fermented longan wine, which indicates that they greatly contribute to the aroma of longan wine.

The influence of the main aromatic compounds in longan wine on the characteristics of wines was further evaluated by applying PCA. As shown in Figure 3A), a total of three principal components (PC1 [48.05%], PC2 [26.91%], and PC3 [25.04%]) were extracted, and their cumulative contribution rate was 100%.

The four groups of fermented longan wines show a large distribution difference in PCA. The sequential inoculation group is more closely related to many ethyl ester aromatic compounds (such as ethyl laurate, ethyl caprylate, ethyl caproate, and phenethyl acetate) (Figure 3B). The aromatic characteristics of the *W. anomalus* group are related to some acetic ester compounds (ethyl acetate and isobutyl acetate), and the characteristic aroma of the co-inoculation group is possibly derived from linalool. There was a lack of typical aromatic compositions in the *S. cerevisiae* group.

Discussion

Yeasts play an important role in the fermentation of fruit wines. The non-*Saccharomyces* yeasts generally refer to a class of yeasts that includes *W. anomalus*, *Hanseniaspora uvarum*, and *Metschnikowia pulcherrima*, etc., and does not include *S. cerevisiae* (Wang *et al*., 2016). Many non-*Saccharomyces* yeasts secrete multiple glycosidases, can improve various qualities (e.g., aroma, color, and taste) of fermented fruit wines, and contribute to increasing the richness and specificity of fruit wines (Morata *et al*., 2019). Therefore, the wines fermented by mixing *S. cerevisiae* with selected non-*Saccharomyces* yeasts not only retain a certain alcohol content but also exude improved quality characteristics (aromatic and sensory characteristics).

Mixed fermentation has become a commonly accepted production mode (Johnson, 2013). There have been reports on fermenting and producing various wines based on fruits—*Vitis vinifera* L. (Rossouw and Bauer, 2016), *Diospyros kaki* L.f (Jing *et al*., 2018), *Lycium barbarum* L. (Ju *et al*., 2017), and *Rosa roxburghii* (Zhao *et al*., 2020)—by mixing non-*Saccharomyces* yeasts with *S. cerevisiae*. However, research on the influence of

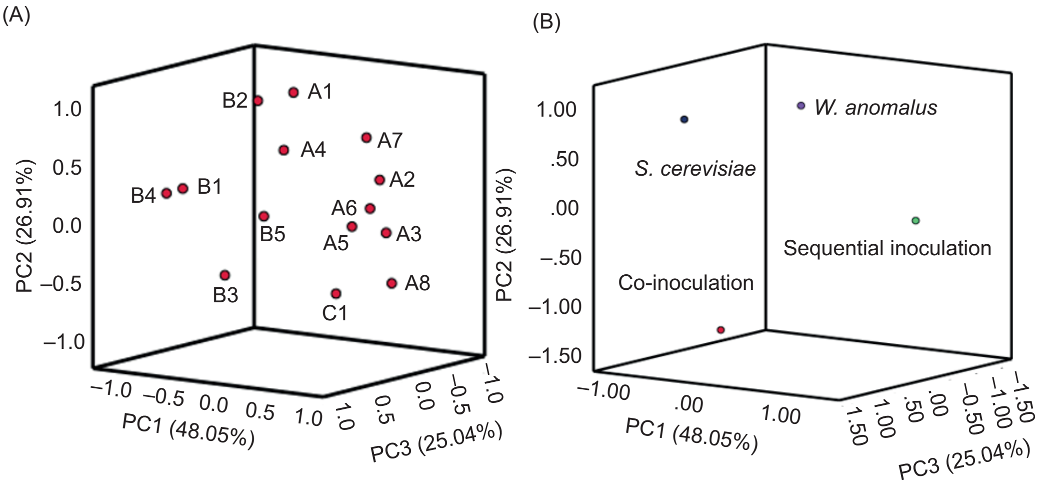

Figure 3. PCA of aromatic compounds in longan wine. (A) Principal component load plot of volatile aromatic compounds. (B) Principal component score of volatile aromatic compounds. Abbreviations: A1: Ethyl acetate, A2: Isoamyl acetate, A3: Phenethyl acetate, A4: Isobutyl acetate, A5: Ethyl octanoate, A6: Ethyl hexanoate, A7: Ethyl decanoate, A8: Ethyl laurate, B1: Isobutanol, B2: n-propyl alcohol, B3: Linalool, B4: Phenethyl alcohol, B5: Citronellol, C1: Octanoic acid.

non-*Saccharomyces* yeasts on the characteristics of longan wine is somewhat limited. Using sequential inoculation and co-inoculation based on *W. anomalus*, the influences of mixed fermentation on basic physiochemical parameters, electronic sensory characteristics, and aromatic characteristics of longan wine can be analyzed.

Our results showed that the longan wine fermented either through co-inoculation or sequential inoculation exhibited lower alcohol content than the control group, that is, the *S. cerevisiae* group (Table 1). The possible reason for this is that the two fungi interact during the fermentation, which influences their metabolism for carbohydrates, thus leading to the reduction in the alcohol content. Research results indicate that *W. anomalus*, which secretes the virulence factor with broad-spectrum resistance, can effectively inhibit the pollution caused by infectious microbes (Padilla *et al*., 2018). Nevertheless, the influence of *W. anomalus* on *S. cerevisiae* during the production of longan wine has not yet been clarified, and remains to be further explored.

Our research results also reveal that mixed fermentation can regulate the aromatic characteristics of longan wine. As for the variety of aromatic compounds, co-inoculation can increase the variety of aromatic aldehyde and ketone compounds in longan wine, while sequential inoculation can enrich the variety of aromatic ester compounds (Figure 2A). In terms of aromatic compounds, ester compounds significantly decreased, while those of acids, aldehydes, ketones, and other compounds in longan wine fermented through co-inoculation significantly increased (Figure 2B). The longan wine produced based on sequential inoculation delivered significantly greater amounts of acid, aldehyde, and ketone compounds. Therefore, the longan wine produced by different methods of mixed fermentation presents diverse aromatic characteristics. Hence, it is feasible to apply different mixed inoculation methods according to different production demands.

The proportion of yeasts in mixed inoculation also influences the quality characteristics of fruit wines. Ju *et al.* (2019) suggested that wolfberry wine fermented by mixing *H. uvarum* with *S. cerevisiae* in the inoculation ratio of 3:1 presents the highest sensory score, and its aromatic characteristics greatly differ. Wine with a low alcohol content fermented by mixing *H. uvarum* with *S. cerevisiae* in a 2:1 ratio exhibits suitable acidity and sweetness, complex aroma and flavor, and highly stable microbes (Cui *et al*., 2020). Only the influence of different inoculation methods (co-inoculation and sequential inoculation) on basic physiochemical properties and aromatic characteristics of longan were analyzed in this study. Although the influence of different inoculation ratios on the quality of longan wine was not analyzed, it will be further examined in subsequent research.

Above all, the influences of mixed fermentation (co-inoculation and sequential inoculation) of *W. anomalus* and *S. cerevisiae* on basic physiochemical properties, electronic sensory characteristics, and aromatic characteristics of longan wine were analyzed. Our results indicate that mixed fermentation can reduce the alcohol content in longan wine, and it also can decrease the acidity and the electronic sensory richness of the wine. Thus, different inoculation methods greatly affect the aromatic characteristics of longan wine. Co-inoculation can increase the variety of aldehyde and ketone aromatic compounds, decrease the amounts of esters, and increase the amounts of acids, aldehydes, ketones, and other compounds. Sequential inoculation can increase the variety of ester aromatic compounds and amounts of acid, aldehyde, and ketone compounds. Therefore, mixed fermentation can regulate the quality characteristics (including basic physiochemical parameters, electronic sensory characteristics, and aromatic characteristics) and enrich the variety of longan wine.

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References

Borren, E. and Tian, B., 2020. The important contribution of non-*Saccharomyces* yeasts to the aroma complexity of wine: a review. Foods 10(1): E13. https://doi.org/10.3390/foods10010013

Cui, Y., Liu, S., Deng, Q.Y., Shi, L. and Lv, W. 2020. Mixed fermentation of *Hanseniaspora uvarum* and *Saccharomyces cerevisiae* for low alcohol white wine. The Food Industry 41(08): 60–64.

Gonzalez, R. and Morales, P., 2017. Wine secondary aroma: understanding yeast production of higher alcohols. Microb Biotechnol 10(6): 1449–1450. https://doi.org/10.1111/1751-7915.12770

Han, D. M., Luo, T., Zhang, L., Wu, J. Q., Wu, H. T., Wu, Z. X., *et al.* 2020. Optimized precooling combined with SO_2-released paper treatment improves the storability of longan (*Dimocarpus longan* Lour.) fruits stored at room temperature. Food Science of Nutrition 8(6): 2827–2838. https://doi.org/10.1002/fsn3.1577

Jing, X., Yang, H., Su, W., Dong, T. D. and Huang, S. S., 2018. Fermentation performances of persimmon wine with mixed yeasts of non-*Saccharomyces* and *Saccharomyces cerevisiae*. China Brewing 37(12): 52–56. https://doi.org/10.11882/j.issn.0254-5071.2018.12.010

Johnson, E.A., 2013, Biotechnology of non-*Saccharomyces* yeasts-the basidiomycetes. Application in Microbiology Biotechnology 97(17): 7563–7577. https://doi.org/10.1007/s00253-013-5046-z

Jolly, N.P., Varela, C. and Pretorius, I.S., 2014. Not your ordinary yeast: non-*Saccharomyces* yeasts in wine production uncovered. FEMS Yeast Research 14(2): 215–237. https://doi.org/10.1111/1567-1364.12111

Ju, N., Hu, J., Zhao, M.M., Ke, Y. and Chen, L., 2019. Effects of mixed cultures with *Hanseniaspora uvarum* and *Saccharomyces cerevisiae* on fermented wolfberry wine. Science and Technology of Food Industry 40(06): 106–113. https://doi.org/10.13386/j.issn1002-0306.2019.06.018

Ju, N., Zhao, M.M., Ke, Y., Chen, L., Zhao, Z. H. and Ma, Y., 2017. Isolation, screening and analysis of aroma components of non-*Saccharomyces* for wolfberry wine. Food and Fermentation Industries 43(11): 125–131. https://doi.org/10.13995/j.cnki.11-1802/ts.014953

Liu, X.Z., Li, H., Li, Y.F., Li, T. T. and Huang, M. M., 2020a. Effects of inoculation of *Wickerhamomyces anomalus* on the physicochemical property and aroma profile of *Prunus salicina* Lindl. cv 'Kongxinli' fruit wine. Food Science and Technology 45(11): 21–27. https://doi.org/10.13684/j.cnki.spkj.2020.11.005

Liu, X.Z., Zhao, H.B., Li, Y.F., Yu, Z. H., Liu, X. H. and Huang, M, Z., 2020b. Identification and oenological properties analysis of a strain of *Hanseniaspora uvarum* from *Rosa roxburghii*. Food and Fermentation Industries 46(08): 97–104. https://doi.org/10.13995/j.cnki.11-1802/ts.022942

Morata, A., Escott, C., Loira, I., Del Fresno, J. M., González, C. and Suárez-Lepe, J. A., 2019. Influence of *Saccharomyces* and non-*Saccharomyces* yeasts in the formation of pyranoanthocyanins and polymeric pigments during red wine making. Molecules 24(24): 4490. https://doi.org/10.3390/molecules24244490

Morales, M.L., Ochoa, M., Valdivia, M., Ubeda, C., Romero-Sanchez, S., Ibeas, J. I., *et al.* 2020. Volatile metabolites produced by different flor yeast strains during wine biological ageing. Food Research International 128: 108771. https://doi.org/10.1016/j.foodres.2019.108771

Padilla, B., Gil, J.V. and Manzanares, P., 2018. Challenges of the non-conventional yeast *Wickerhamomyces anomalus* in winemaking. Fermentation 4(3): 1–14. https://doi.org/10.3390/fermentation4030068

Park, S., Kim, J.H., Son, Y., Goh, S. H. and Oh, S., 2016. Longan (*Dimocarpus longan* Lour.) fruit extract stimulates osteoblast differentiation via Erk1/2-Dependent RUNX2 activation. Journal of Microbiology Biotechnology 26(6): 1063–1066. https://doi.org/10.4014/jmb.1601.01092

Rossouw, D. and Bauer, F.F., 2016. Exploring the phenotypic space of non-*Saccharomyces* wine yeast biodiversity. Food Microbiology 55: 32–46. https://doi.org/10.1016/j.fm.2015.11.017

Sanoppa, K., Huang, T.C. and Wu, M.C., 2019. Effects of *Saccharomyces cerevisiae* in association with *Torulaspora delbrueckii* on the aroma and amino acids in longan wines. Food Science of Nutrition 7(9): 2817–2826. https://doi.org/10.1002/fsn3.1076

Schneider, J., Rupp, O. and Trost, E., et al. 2012. Genome sequence of *Wickerhamomyces anomalus* DSM 6766 reveals genetic basis of biotechnologically important antimicrobial activities. FEMS Yeast Research 12(3): 382–386. https://doi.org/10.1111/j.1567-1364.2012.00791.x

Song, R. R., Duan, W. P., Zhu, X., Liu, Q., Yang, X. S. and Han, S. Y., 2019. Effects of sequential inoculation with *Torulaspora delbrueckii* and *Saccharomyces cerevisiae on* aroma profile of dry red wine. Food and Fermentation Industries 45(24): 1–9. https://doi.org/10.1007/s00217-016-2781-2

Tandee, K., Kittiwachana, S. and Mahatheeranont, S., 2020. Antioxidant activities and volatile compounds in longan (*Dimocarpus longan* Lour.) wine produced by incorporating longan seeds. Food Chemistry 348: 128921. https://doi.org/10.1016/j.foodchem.2020.128921

Wang, C., Mas, A. and Esteve-Zarzoso, B., 2016. The interaction between *Saccharomyces cerevisiae* and non-*Saccharomyces* yeast during alcoholic fermentation is species and strain specific. Frontier in Microbiology 13(7): 502. https://doi.org/10.3389/fmicb.2016.00502

Xia, TQ., Gao, X.Y., Liu, X.L., Wang, Q. and Wang J. L., 2018. Optimization of clarification process and determination of physical and chemical indexes of red raspberry fruit wine. China Brewing 37(08), 138–142. https://doi.org/10.11882/j.issn.0254-5071.2018.08.027

Yan, H. J., Zhang, M. Y., Sun, K., Yang, X. K., Ge, C., Zhou, J. F., *et al*. 2020. Effects of the co-fermentation of *Saccharomyces cerevisiae* and *Hanseniaspora uvarum* on aroma of Muscat wine. Food and Fermentation Industries 46(11): 165–171. https://doi.org/10.13995/j.cnki.11-1802/ts.023634

Ye, M., Yue, T. and Yuan, Y., 2014. Effects of sequential mixed cultures of *Wickerhamomyces anomalus* and *Saccharomyces cerevisiae* on apple cider fermentation. FEMS Yeast Research 14(6): 873–882. https://doi.org/10.1111/1567–1364.12175

Yuan, X.R., Yu, X.C., Yang, F., Zhang, L., Chi, Y. L.,Wang, Y., *et al*. 2020. Fermentation of longan fruit wine with aroma-producing yeast and *Saccharomyces cerevisiae*. China Brewing 39(07): 74–77. https://doi.org/10.11882/j.issn.0254-5071.2020.07.015

Zhao, H.B., Li, H., Tian, Y., Yu, Z. H., Liu X. H., Huang, M. Z., *et al*. 2020. Isolation, identification, physiological characteristics and mixed fermentation of a non-*Saccharomyces cerevisiae* from *Rosa roxburghii*. Science and Technology of Food Industry 41(16), 114–120. https://doi.org/10.13386/j.issn1002-0306.2020.16.019