J Food Sci Technol (October 2018) 55(10):4001–4012
https://doi.org/10.1007/s13197-018-3325-5

ORIGINAL ARTICLE

Selection of non-*Saccharomyces* yeasts for orange wine fermentation based on their enological traits and volatile compounds formation

Lanlan Hu[1,2] · Jia Wang[1,2] · Xueao Ji[1,2] · Rui Liu[1,2] · Fusheng Chen[1,2] · Xiuyan Zhang[1,2]

Revised: 26 June 2018 / Accepted: 28 June 2018 / Published online: 16 July 2018

Abstract In order to select the non-*Saccharomyces* yeasts for orange wine fermentation, the enological traits and volatile compounds formation of ten non-*Saccharomyces* yeast strains were evaluated through physicochemical methods and solid-phase microextraction coupled to GC–MS, respectively. The results indicated that non-*Saccharomyces* yeast fermentation had lower maximum populations (7.8–8.0 Log cfu/mL), longer fermentation period (7–10 days), lower ethanol (4.13–7.79%), lower total acids (7.48–8.51 g/L) and higher volatile acids concentrations (0.08–0.23 g/L) when compared with those of *Saccharomyces cerevisiae* fermentation. *Hanseniaspora uvarum*, *Hanseniaspora opuntiae*, *Hanseniaspora occidentalis*, *Pichia kudriavzevii* and *Torulaspora delbrueckii* were selected as candidates for orange wine fermentation with higher volatile compounds concentration, odor active values and sensory evaluation scores. This study will provide a valuable selection method of non-*Saccharomyces* yeasts for orange wine fermentation, and an approach to improve the flavor of orange wine or other fruit wine.

Keywords Yeast · Volatile compounds · Odor active value · Sensory evaluation

✉ Xiuyan Zhang
xiuyanzhang73@mail.hzau.edu.cn

[1] College of Food Science and Technology, Huazhong Agricultural University, Wuhan 430070, Hubei Province, People's Republic of China

[2] Key Laboratory of Environment Correlative Dietology, Huazhong Agricultural University, Ministry of Education, Wuhan 430070, Hubei Province, People's Republic of China

Introduction

Orange is one of the most abundant fruit crops in China, with more than 36,790,000 tons produced in 2015 (Chen et al. 2016). High output of orange easily leads to excessive inventory and an increasing incidence of fruit decay. In addition to its consumption as fresh table fruit, orange can be processed into juice and wine (Ren et al. 2015). The added value of orange juice is lower than that of orange wine, so making orange wine is the best way to maintain the nutrient levels and increase the added value of orange. However, orange wines lack typical flavor characteristics, which will definitely decrease the competitiveness of the product in the wine market (Liu et al. 2015).

Commercial pure *Saccharomyces cerevisiae* has been used for orange wine fermentation (Kelebek et al. 2009). The fermentation process of commercial pure *S. cerevisiae* is easily controlled, but the organoleptic complexity of wine is poor when compared with those wines produced from successful spontaneous fermentation. Indigenous non-*Saccharomyces* yeast strains, which are naturally present on the surface of fruits or at the early stages of natural wine fermentation, may affect the flavor and quality of wine. However, some non-*Saccharomyces* yeast may have positive effect on the flavor and quality of fruit wines, while others may perform negatively. Additionally, some non-*Saccharomyces* yeast can't adapt to the wine fermentation environment (higher ethanol and SO_2 content) and have lower growth rate and fermentation capacity (Ciani and Comitini 2015; Medina-Trujillo et al. 2016; Wang et al. 2016; Padilla et al. 2017). Therefore, it is important to select the non-*Saccharomyces* yeast strains for wine fermentation. During the last 10 years, more and more selected non-*Saccharomyces* yeast strains have been used to improve the flavor and quality of different fruit wines

(Comitini et al. 2011; Domizio et al. 2011; Sun et al. 2014; Chen et al. 2015; Loira et al. 2015; Renault et al. 2015; Amorim et al. 2016; Canonico et al. 2016; Lu et al. 2016; Morales et al. 2017; Portugal et al. 2017; Puertas et al. 2017). However, the application of non-*Saccharomyces* yeast strains to improve the flavor and quality of orange wine has not been reported until now.

Ten non-*Saccharomyces* species, *Barnettozyma californica*, *Candida humilis*, *Candida tropicalis*, *Clavispora lusitaniae*, *Hanseniaspora occidentalis*, *Hanseniaspora opuntiae*, *Hanseniaspora uvarum*, *Pichia kudriavzevii* (*Issatchenkia orientalis*), *Pichia terricola* (*Issatchenkia terricola*) and *Torulaspora delbrueckii*, were isolated from the spontaneous processes of orange wine and orangeries. The tolerances to glucose, ethanol and SO_2 of ten non-*Saccharomyces* yeast strains were analyzed (Liu et al. 2015).

However, it is not known whether these non-*Saccharomyces* yeast strains can be used to improve the flavor and quality of orange wine. Therefore, the objective of this study is to select non-*Saccharomyces* yeasts for orange wine fermentation through analyzing their enological traits and volatile compounds formation. Research results will provide a valuable selection method of non-*Saccharomyces* yeasts for orange wine fermentation, and an approach to improve the flavor of orange wine or other fruit wine.

Materials and methods

Yeast strains and culture media

The non-*Saccharomyces* species including *B. californica*, *C. humilis*, *C. tropicalis*, *C. lusitaniae*, *H. occidentalis*, *H. opuntiae*, *H. uvarum*, *P. kudriavzevii*, *P. terricola* and *T. delbrueckii* were isolated from spontaneous processes of orange wine and orangeries in 2015 and kept in our lab. The commercial *S. cerevisiae* was purchased from Laffort group (ACTIFLORE CERVISIAE, Laffort Co., France).

YPD medium (10 g/L yeast extract, 20 g/L peptone, 20 g/L glucose) was used for the inocula preparation and yeast cells count.

Laboratory-scale fermentation of orange wine

The orange wine fermentation was performed as described by Tristezza et al. (2016) with some modifications. Fresh ponkan was peeled, crushed and deseeded to acquire its orange must. The must was then pasteurized for 10 min at 102 °C, cooled and poured into 250 mL bottle. The initial orange must contained 150 g/L total sugars at pH 3.36. After adding 50 mg/L SO_2 to the orange must, and 10^7 -cells/mL of yeast was inoculated into the orange must. The fermentation was carried out in 250 mL sterile glass bottles containing 180 mL of orange must at 25 °C without agitation.

Yeast counting

Samples were taken every day during orange wine fermentation and diluted onto YPD plates. The plates were then incubated at 28 °C for 2 days. Yeast cell numbers of each sample were determined by using the plate counting method (Suarez et al. 2007). Each sample was measured in duplicate.

Chemical compositions of orange wine

The concentrations of residual sugars, alcohols, total acidity, SO_2 and volatile acids were analyzed through methods recommended by the International Organization of the Vine and Wine (OIV 2005). Total acidity was expressed as malic acid (g/L), and the volatile acids were expressed as acetic acid (g/L). Each sample was measured in duplicate.

Volatile compound analysis of orange wine

Fermented orange wine was filtered through 0.45 μm absolute membranes, and its volatile compounds were then extracted by using a headspace solid-phase micro extraction (HS-SPME) method with 50/30 μm divinylbenzene/Carboxen/polydimethylsiloxane (DVB/CAR/PDMS) fiber (Supelco, Bellefonte, PA, USA). In total, 8 mL of filtered orange wine, 10 μL of the internal standard of cyclohexanone (0.946 mg/L in ethanol) and 3.0 g of NaCl were placed into a 20 mL headspace vial that was closed with a screwed cap and a 1.5 mm thick Teflon septum. The solution was agitated and equilibrated at 40 °C for 15 min, and a fiber was then inserted into the vial septum and exposed to the headspace for 40 min at 40 °C. An Agilent 6890N gas chromatograph (GC) on an HP-5 capillary column (length, 30 m; inside diameter, 0.32 mm; film thickness, 0.25 μm) coupled to an Agilent 5975B mass spectrometer was used to then analyze the sample. The flow rate of the carrier gas, helium, was held at 1.2 mL/min. The column temperature was programmed as follows: 40 °C for 2 min, increase to 230 °C at a rate of 5 °C/min, and then hold at 230 °C for 15 min. The temperatures for the injector and detector were set at 250 and 280 °C, respectively. The mass spectrometer was operated in electron impact mode at 70 eV. Volatile compounds of orange wine were identified by comparing their linear retention indices (RI) with those of pure standards, published data, or MS fragmentation patterns obtained from databases such as Wiley 7.0 and NIST05. Semiquantitation was conducted by using cyclohexanone as an internal

standard. Volatile compound contents were calculated from the GC peak areas relative to the GC peak area of the internal standard. The odor active value (OAV), as the ratio of the concentration of a flavor compound to its odor threshold (OT), is a parameter widely used to obtain odor patterns starting from quantitative compositions. In this research, compounds with a OAV $\geq$ 1 are considered to be responsible for aroma, and the higher their OAV, more they contribute to the aroma profile (Grosch 2001).

Sensory evaluation of orange wine

The sensory evaluation was performed as described by Belda et al. (2015). Orange wine was evaluated by nine trained assessors (five females and four males) from Huazhong Agricultural University. Samples (20 mL) were poured into wine glasses and presented in a random order. Potable water was provided for rinsing the palate during testing. The preferences for color, aroma and mouthfeel, taste lasting and overall acceptability were determined, and the total values of all the characteristics were calculated.

Statistical analyses

T test of yeast numbers, physicochemical parameters and sensory evaluation scores of different fermentations were analyzed in SPSS 19.0 for Windows (SPSS Inc., Chicago, IL, USA). Principal component analysis (PCA) was performed to identify the most influential volatile compounds in the different fermentations by Unscrambler X (CAMO ASA, Oslo, Norway).

Results and discussions

Growth kinetics of yeast strains during orange wine fermentation

The growth kinetic of each yeast strain during orange wine fermentation was shown in Fig. 1. The results indicated all the non-*Saccharomyces* yeast strains could grow in orange wine and had lower maximal populations (7.8–8.0 Log cfu/mL) than that of *S. cerevisiae* (8.3 Log cfu/mL) on the 4th day of fermentation. This result was contrary to other research reports (Sun et al. 2014; Luis et al. 2015). The differences in final population could be attributed to the strain factor or fermentation environment.

Sugar consumption kinetics of yeast strains during orange wine fermentation

Sugar consumption kinetic of each yeast strain during orange wine fermentation was shown in Fig. 1b. The results indicated that all the yeast strain could consume sugar in orange wine, and *S. cerevisiae* took shorter time (6 days) than non-*Saccharomyces* yeast did (7–10 days) to consume the same amount of sugar. This phenomenon had also been reported in other studies (Sun et al. 2014; Luis et al. 2015). This indicated that the non-*Saccharomyces* yeast strains had longer fermentation period than that of *S. cerevisiae*.

Chemical compositions of orange wine fermented by single yeast strain

The chemical compositions of orange wines fermented with different yeast strains were shown in Table 1. All the non-*Saccharomyces* yeast fermentations were characterized by lower ethanol concentration (4.13–7.79%), lower total acid content (7.48–8.51 g/L), higher volatile acid concentration (0.09–0.23 g/L), higher residual sugar concentration (5.6–6.0 g/L) except for *H. uvarum and H. opuntiae* fermentation, higher pH value (3.37–3.84) except for *H. uvarum* with significant difference from that of *S. cerevisiae* fermentation. Although the volatile acid concentration increased in non-*Saccharomyces* yeast fermentations, it was still lower than its threshold value (1.1 g/L). Therefore, it had no unpleasant effect on orange wine (Lambrechts and Pretorius 2000). The phenomenon about lower ethanol and higher residual sugar concentrations in non-*Saccharomyces* yeast fermentation was also found in previous reports (Comitini et al. 2011; Contreras et al. 2014; Belda et al. 2015). As for the production of total acids, volatile acids and pH value in non-*Saccharomyces* yeast fermentations, there were divergent opinions, and these significant differences might be correlated with the difference of yeast strain or fermentation environment, fruit and fermentation conditions (Comitini et al. 2011; Rantsiou et al. 2012; Contreras et al. 2014; Belda et al. 2015; Amorim et al. 2016; Canonico et al. 2016; Tristezza et al. 2016; Morales et al. 2017; Portugal et al. 2017; Puertas et al. 2017).

Volatile compounds of orange wine fermented with single yeast strain

The results (Tables 2, 3) indicated that different yeast strains produced different volatile compounds profiles and had different odor-active compounds (OAV $\geq$ 1) in orange wines, The findings were consistent with other research reports (Kim et al. 2008; Loira et al. 2015; Portugal et al. 2017).

The results (Tables 2, 3) indicated that the *C. tropicalis* fermentation had the lowest volatile compounds concentration (16.96 mg/L) and was notably lack of odor-active compounds (OAV = 805) in all the yeast fermentations. Of

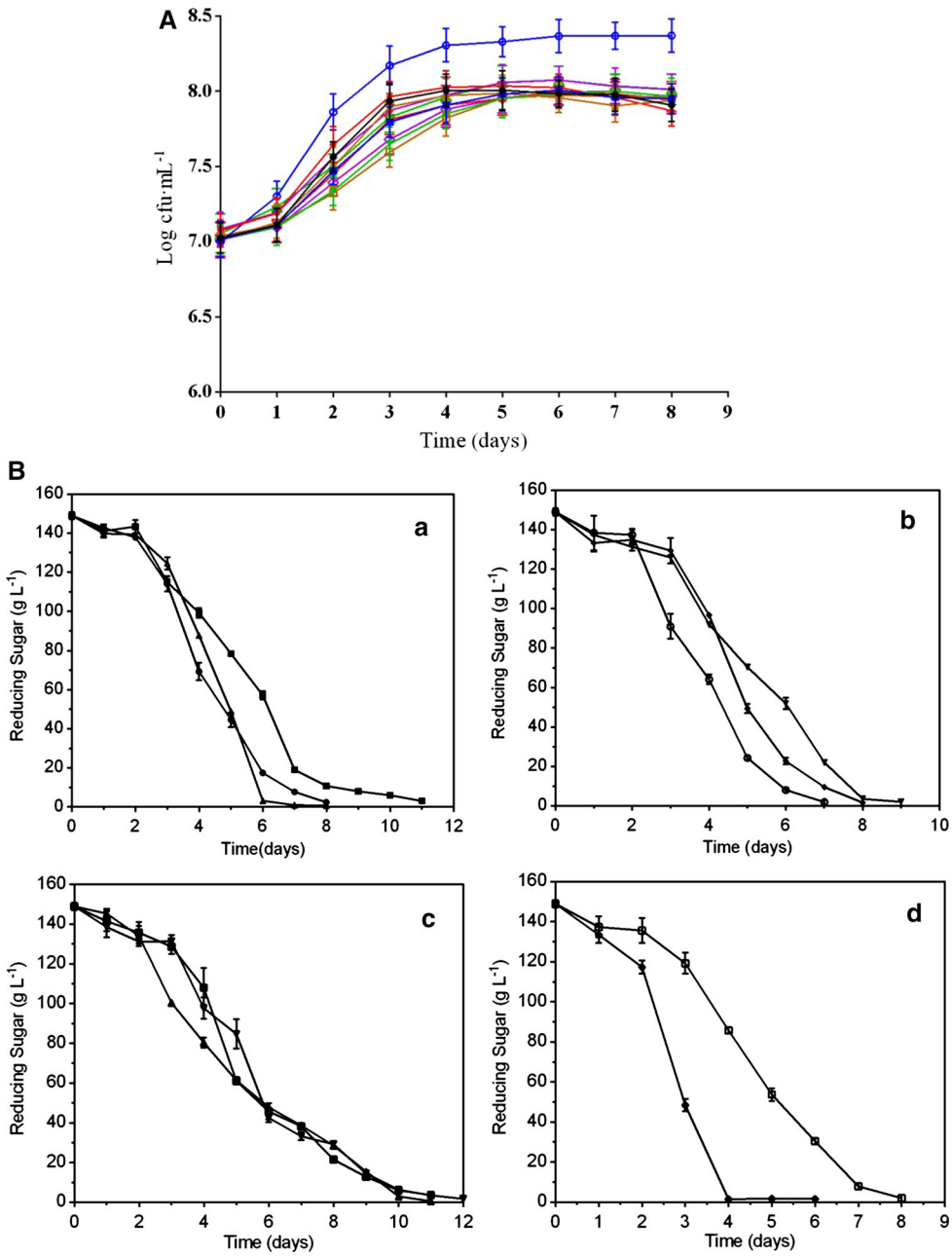

Fig. 1 Growth and sugar consumption kinetics of single non-*Saccharomyces* strains in orange juice. **a** Growth kinetics of non-*Saccharomyces*. (open circle) *H. occidentalis*, (filled square) *H. uvarum*, (filled triangle) *H. opuntiae*, (filled upward triangle) *P. kudriavzevii*, (filled diamond) *C. lusitaniae*, (open ballot box) *T. delbrueckii*, (filled circle) *S. cerevisiae*, (open square) *P. terricola*, (open triangle) *B. californica*, (open upward triangle) *C. tropicalis* and (open diamond) *C. humilis.* **b** Sugar consumption kinetics of non-*Saccharomyces*. *a* (filled circle) *C. tropicalis*, (filled square) *B. californica*, and (filled triangle) *H. uvarum*; *b* (filled upward triangle) *P. terricola*, (filled diamond) *H. opuntiae*, and (open circle) *C. humilis*; *c* (open ballot box) *H. occidentalis*, (open triangle) *P. kudriavzevii*, and (open upward triangle) *C. lusitaniae*; *d* (open square) *T. delbrueckii*, and (open diamond) *S. cerevisiae*

the 16 volatile compounds, ethyl caprylate concentration increased and two new compounds (neryl acetate, acetophenone) occurred with extremely significant differences from that of *S. cerevisiae* fermentation (Table 2). Its odor-active compounds were mainly composed of esters dominated by ethyl caprylate, ethyl hexanoate, isoamyl acetate, phenethyl acetate and ethyl caprate with significant lower than that of *S. cerevisiae* fermentation. The similar observation that *C. tropicalis* weakly contributed to the flavor of beer was reported by N'Guessan et al. (2010). Therefore, it is meaningless to use *C. tropicalis* to improve the flavor of orange wine for its poor ability to produce volatile compounds.

The results (Tables 2, 3) indicated that *B. californica* fermentation had the lowest volatile compounds concentration (117.14 mg/L) and OAV (892.5) except for the *Candia* spp. fermentations in all the yeast fermentations. Of the 11 volatile compounds, terpenes compounds with flower flavors, such as linalool, citronellol, terpineol and valencene, had extremely significant increase over that of *S. cerevisiae* fermentations (Table 2). Its' odor-active compounds were mainly composed of terpene compounds (citronellol, linalool and terpineol), esters (isoamyl acetate,

Table 1 Physicochemical parameters of orange wines fermented with single non-*Saccharomyces* strains (average ± SD)

Strains	Ethanol (%, v/v)	Residual sugars (g/L)	pH	Total acids[a] (g/L)	Volatile acids[b] (g/L)
C. tropicalis	6.54 ± 0.10**	9.0 ± 0.12**	3.48 ± 0.00**	7.61 ± 0.10**	0.10 ± 0.02**
B. californica	6.40 ± 0.12**	6.0 ± 0.13**	3.48 ± 0.00**	7.48 ± 0.08**	0.23 ± 0.02**
H. uvarum	7.79 ± 0.07**	5.00 ± 0.11	3.31 ± 0.01**	7.56 ± 0.05**	0.07 ± 0.01**
P. terricola	7.12 ± 0.08**	6.00 ± 0.1**	3.37 ± 0.01**	8.10 ± 0.04**	0.22 ± 0.02**
H. opuntiae	7.43 ± 0.07**	5.00 ± 0.09	3.84 ± 0.03**	7.79 ± 0.01**	0.08 ± 0.01
C. humilis	7.65 ± 0.17**	6.00 ± 0.12**	3.45 ± 0.01**	8.51 ± 0.06**	0.23 ± 0.01**
H. occidentalis	7.24 ± 0.13**	6.00 ± 0.14**	3.47 ± 0.01**	7.77 ± 0.06**	0.20 ± 0.02**
P. kudriavzevii	4.13 ± 0.08**	8.00 ± 0.14**	3.37 ± 0.06**	8.00 ± 0.07**	0.18 ± 0.01**
C. lusitaniae	6.41 ± 0.44**	6.0 ± 0.15**	3.50 ± 0.00**	8.15 ± 0.05**	0.09 ± 0.01**
T. delbrueckii	7.42 ± 0.16**	5.60 ± 0.12**	3.45 ± 0.00**	7.94 ± 0.07**	0.17 ± 0.02**
S. cerevisiae	8.68 ± 0.08	5.00 ± 0.1	3.33 ± 0.00	9.37 ± 0.03	0.08 ± 0.00

Data show the mean value of two replicates

**$p < 0.01$; *$p < 0.05$

[a]Expressed as g/L of tartaric acid

[b]Expressed as g/L of acetic acid

phenethyl acetate and ethyl caprate) and octanoic acid (Table 3) with significant difference from that of *S. cerevisiae*. While, it was never used to ferment fruit wine for the absence of enological traits. Therefore, *B. californica* may be used to produce orange wines with different flavor from that of *S. cerevisiae*, but its sensory evaluation should be carried out for the occurring of octanoic acid with sweat and cheese odors.

The *H. uvarum* fermentation had the relatively low volatile compounds concentration (226.18 mg/L), but it had the maximum OAV (21,221.4) in all the yeast fermentations (Tables 2, 3). Of 20 volatile compounds, the ethyl caprylate concentration (66.56 mg/L) had an extremely significant increase, followed by ethyl 3-phenylpropionate. Octyl formate, isopentyl isopentanoate, limonene, phenylethyl propionate and *p*-ethyl benzaldehyde were new compounds with significantly different concentration from that of *S. cerevisiae* (Table 2). Its odor-active compounds composition was significantly different from that of *S. cerevisiae* fermentation. It was mainly composed of esters (ethyl caprylate, phenylethyl propionate, ethyl hexanoate, octyl formate, ethyl caprate etc.). Additionally, terpene compounds (citronellol, limonene and linalool), guaiacol, phenethyl alcohol and octanoic acid also contributed to the orange wine's flavor (Table 3). These results indicated that the lower concentration of volatile compounds did not mean the lower OAV. *H. uvarum* considered to be floral yeast strain was widely studied since it could improve the flavor, reduce the ethanol content of wine (González-Robles et al. 2015; Tristezza et al. 2016; Hu et al. 2018). Based on the discussion, the *H. uvarum* could be used to ferment orange wine with different flavor from *S. cerevisiae* fermentations, but its sensory evaluation should be carried out.

The *P. terricola* fermentations had the relative high volatile compounds concentration (745.43 mg/L) and OAV (6654.23) in all the yeast fermentations (Tables 2, 3), Of the 22 compounds, the phenethyl alcohol, ethyl acetate, ethyl caprylate, phenethyl acetate, ethyl 3-phenylpropionate significantly increased over that of *S. cerevisiae* fermentation. Additionally, 1-pentanol, ethyl laurate, terpineol, citronellol, limonene, valencene, benzene acetaldehyde and *p*-ethyl benzaldehyde were new compounds with significantly different concentration over the *S. cerevisiae* fermentations (Table 2). Its odor-active compounds were abundant and mainly composed of esters (isoamyl acetate, ethyl acetate, ethyl hexanoate, ethyl caprylate, phenethyl acetate, ethyl 3-phenylpropionate 9-ethyl decadienoate and ethyl caprate), higher alcohols (1-pentanol and phenethyl alcohol), terpene compounds (citronellol, limonene, terpineol and linalool), benzene acetaldehyde and octanoic acid with significant difference from that of *S. cerevisiae* fermentation (Table 3). Moreover, González-Pombo et al. (2011) reported that addition of purified β-glucosidase from *P. terricola* could increase the amount of monoterpenes and norisoprenoids for aroma development in wines. Therefore, *P. terricola* could be used to produce orange wine with different flavor from that of *S. cerevisiae*. However, orange wine fermented by *P. terricola* contained excessive higher alcohol (451.18 mg/L) which might negatively affect the flavor of wines by

Table 2 Volatile compounds in orange wines fermented with single non-*Saccharomyces* strains (mg/L)

Volatile compounds	Ct	Bc	Hu	Pt	Hop	Ch
Alcohols						
1-Pentanol	–	3.48 ± 0.11**	–	345.89 ± 11.22**	–	40.78 ± 3.43**
1-Decanol	–	–	–	–	–	–
Phenethyl alcohol	6.60 ± 0.23*	1.36 ± 0.12*	62.60 ± 3.21	105.29 ± 2.32*	51.14 ± 3.67	10.74 ± 0.32*
Subtotal	6.60 ± 0.23*	4.84 ± 0.23**	62.60 ± 3.21	451.18 ± 13.54**	52.04 ± 3.70	51.52 ± 3.75*
Carboxylic acids						
Hexanoic acid	0.38 ± 0.08*	–	4.60 ± 0.73	–	3.96 ± 0.58	–
Methyl pentanoic acid	–	–	–	–	–	–
Octanoic acid	2.14 ± 0.03**	5.22 ± 0.45	13.14 ± 2.12	5.55 ± 0.23	13.57 ± 1.32	0.12 ± 0.07
Capric acid	0.05 ± 0.01**	–	0.38 ± 0.11*	0.29 ± 0.03**	0.84 ± 0.12*	–
Subtotal	2.57 ± 0.12**	5.22 ± 0.45**	18.12 ± 2.96*	5.84 ± 0.26**	18.37 ± 2.02*	0.12 ± 0.07**
Esters						
Isoamyl acetate	1.59 ± 0.12**	9.07 ± 1.01**	28.19 ± 1.23**	16.34 ± 1.02**	62.77 ± 5.34*	0.63 ± 0.05**
Ethyl acetate	–	–	–	49.89 ± 2.01**	–	–
Ethyl hexanoate	1.41 ± 0.05**	–	13.58 ± 1.00*	4.28 ± 0.29**	16.51 ± 1.43	–
Octyl formate	–	–	1.04 ± 0.12**	–	–	–
Ethyl benzoate	–	–	–	–	–	–
Ethyl caprylate	2.32 ± 0.07**	0.55 ± 0.10	66.56 ± 0.12**	20.89 ± 1.43**	73.47 ± 6.85**	0.94 ± 0.13
Neryl acetate	0.08 ± 0.02**	–	–	–	–	–
Isopentyl isopentanoate	–	–	0.22 ± 0.05**	–	–	–
Ethyl phenylacetate	–	–	–	–	–	–
Phenylethyl propionate	–	–	19.69 ± 3.21**	–	–	–
Phenethyl acetate	0.84 ± 0.02**	0.45 ± 0.07**	–	178.45 ± 16.34**	34.11 ± 1.34	8.40 ± 1.02**
Ethyl formate	–	–	–	–	–	–
Ethyl pelargonate	–	–	–	0.29 ± 0.04	0.21 ± 0.05	–
Ethyl 3-phenylpropionate	–	–	0.31 ± 0.04*	0.86 ± 0.07**	0.62 ± 0.04**	–
Ethyl 9-decenoate	0.07 ± 0.02**	–	0.45 ± 0.08**	0.44 ± 0.05**	2.08 ± 0.11**	0.03 ± 0.01**
Ethyl caprate	0.58 ± 0.04**	0.78 ± 0.13**	9.53 ± 0.79*	6.38 ± 0.11**	13.51 ± 0.58*	0.32 ± 0.08**
Isoamyl caprylate	–	–	–	–	0.28 ± 0.03	–
Ethyl laurate	0.04 ± 0.01**	–	0.59 ± 0.13**	0.42 ± 0.08**	0.73 ± 0.07**	0.02 ± 0.01
Ethyl palmitate	–	–	–	–	–	–
Subtotal	6.93 ± 0.35**	10.85 ± 1.31**	140.16 ± 6.77*	278.24 ± 21.44	204.29 ± 15.84	10.34 ± 1.30**
Terpene						
Linalool	0.27 ± 0.06**	63.30 ± 3.56**	1.38 ± 0.12	2.08 ± 0.11	1.43 ± 0.03	0.28 ± 0.04**
Terpineol	–	1.96 ± 0.32**	–	1.66 ± 0.23**	–	0.15 ± 0.03**
Citronellol	–	30.64 ± 1.01**	1.09 ± 0.09	1.59 ± 0.12*	0.99 ± 0.11	0.11 ± 0.01**
Limonene	0.48 ± 0.09**	–	1.37 ± 0.11**	1.84 ± 0.21**	2.04 ± 0.04**	0.33 ± 0.02**
Valencene	–	0.33 ± 0.02**	–	0.47 ± 0.06**	–	0.03 ± 0.01*
Subtotal	0.75 ± 0.11**	96.23 ± 0.11**	3.84 ± 0.11*	7.64 ± 0.11**	4.46 ± 0.11**	0.90 ± 0.11
Aldehydes						
4-Ethylbenzaldehyde	–	–	0.51 ± 0.11**	0.93 ± 0.11**	–	0.05 ± 0.01*
Benzeneacetaldehyde	–	–	–	1.09 ± 0.08**	0.59 ± 0.08**	–
Subtotal	–	–	0.51 ± 0.11**	2.02 ± 0.19**	0.59 ± 0.08**	0.05 ± 0.01*
Ketones						
Acetophenone	0.09 ± 0.02**	–	–	–	–	0.16 ± 0.03**
Subtotal	0.09 ± 0.02**	–	–	–	–	0.16 ± 0.03**
Phenols						
Vinyl guaiacol	0.02 ± 0.01**	–	0.95 ± 0.08*	0.51 ± 0.07*	–	–
Subtotal	0.02 ± 0.01*	–	0.95 ± 0.08*	0.51 ± 0.07*	–	–

Table 2 continued

Volatile compounds	Ct	Bc	Hu	Pt	Hop	Ch
Total	16.96 ± 0.85**	117.14 ± 2.10**	226.18 ± 13.24*	745.43 ± 41.61**	279.75 ± 21.75	63.09 ± 5.27**
Volatile compounds	Hoc	Pk	Cl	Td	Sc	
Alcohols						
1-Pentanol	–	–	36.80 ± 1.02**	466.65 ± 32.32**	–	
1-Decanol	–	–	–	–	0.91 ± 0.02	
Phenethyl alcohol	75.55 ± 4.32	226.47 ± 6.32**	13.19 ± 1.21*	78.91 ± 4.64	67.63 ± 9.32	
Subtotal	75.55 ± 4.32	226.47 ± 6.32**	49.99 ± 2.23	545.56 ± 36.96**	68.54 ± 9.34	
Carboxylic acids						
Hexanoic acid	–	–	–	–	7.21 ± 1.01	
Methyl pentanoic acid	0.83 ± 0.01**	–	–	–	–	
Octanoic acid	1.02 ± 0.11	1.45 ± 0.11	0.13 ± 0.04	–	33.33 ± 1.21	
Capric acid	–	–	–	–	1.45 ± 0.13	
Subtotal	1.85 ± 0.12**	1.45 ± 0.11**	0.13 ± 0.04**	–	41.99 ± 2.35	
Esters						
Isoamyl acetate	32.22 ± 2.12**	32.99 ± 3.53*	1.48 ± 0.02**	13.59 ± 1.43**	101.83 ± 9.43	
Ethyl acetate	148.06 ± 7.34**	577.90 ± 21.32**	46.63 ± 0.32**	–	25.28 ± 1.34	
Ethyl hexanoate	–	–	0.40 ± 0.01**	–	22.99 ± 2.53	
Octyl formate	–	–	–	–	–	
Ethyl benzoate	–	–	–	0.38 ± 0.07**	–	
Ethyl caprylate	7.97 ± 1.01**	7.74 ± 0.32**	0.48 ± 0.02	4.80 ± 0.11**	0.58 ± 0.11	
Neryl acetate	–	–	–	–	–	
Isopentyl isopentanoate	–	–	–	–	–	
Ethyl phenylacetate	–	1.34 ± 0.04**	0.08 ± 0.01**	0.55 ± 0.11**	–	
Phenylethyl propionate	–	–	–	–	–	
Phenethyl acetate	148.95 ± 9.87**	50.58 ± 2.42	8.19 ± 0.24**	167.88 ± 15.34**	40.89 ± 2.43	
Ethyl formate	–	–	–	0.49 ± 0.05**	–	
Ethyl pelargonate	–	0.32 ± 0.05	–	–	0.40 ± 0.06	
Ethyl 3-phenylpropionate	0.24 ± 0.04	0.33 ± 0.06	0.04 ± 0.01*	0.46 ± 0.08*	0.16 ± 0.02	
Ethyl 9-decenoate	0.54 ± 0.04**	0.23 ± 0.02**	0.02 ± 0.01**	0.02 ± 0.01**	5.40 ± 0.45	
Ethyl caprate	4.93 ± 0.34**	2.95 ± 0.12**	0.30 ± 0.02**	2.05 ± 0.14**	19.03 ± 1.34	
Isoamyl caprylate	–	–	–	–	0.40 ± 0.06	
Ethyl laurate	0.57 ± 0.05**	–	–	–	–	
Ethyl palmitate	–	–	0.04 ± 0.01**	–	–	
Subtotal	343.48 ± 20.81*	674.38 ± 27.88**	57.66 ± 0.67**	190.22 ± 17.34	216.96 ± 17.77	
Terpene						
Linalool	2.34 ± 0.23	1.46 ± 0.12	0.13 ± 0.02**	1.06 ± 0.11*	1.92 ± 0.16	
Terpineol	1.22 ± 0.11**	1.43 ± 0.24**	0.13 ± 0.01**	0.80 ± 0.08**	–	
Citronellol	0.79 ± 0.10	0.58 ± 0.09*	0.10 ± 0.02**	1.01 ± 0.06	1.01 ± 0.07	
Limonene	3.02 ± 0.11**	–	0.16 ± 0.02	–	–	
Valencene	–	–	–	–	–	
Subtotal	7.37 ± 0.11**	3.47 ± 0.11**	0.52 ± 0.11**	2.87 ± 0.11	2.93 ± 0.11	
Aldehydes						
4-Ethylbenzaldehyde	0.54 ± 0.09**	–	0.07 ± 0.01**	0.55 ± 0.09**	–	
Benzeneacetaldehyde	–	0.87 ± 0.11**	0.06 ± 0.01**	–	–	
Subtotal	0.54 ± 0.09**	0.87 ± 0.11**	0.13 ± 0.02**	0.55 ± 0.09**	–	
Ketones						
Acetophenone	–	–	–	–	–	
Subtotal	–	–	–	–	–	

Table 2 continued

Volatile compounds	Hoc	Pk	Cl	Td	Sc
Phenols					
Vinyl guaiacol	–	–	–	–	1.80 ± 0.23
Subtotal	–	–	–	–	1.80 ± 0.23
Total	428.79 ± 25.45	906.64 ± 34.53**	108.43 ± 3.07**	739.20 ± 54.50**	332.22 ± 29.80

Data show the mean value of two replicates

–, not detected; Ct, *C. tropicalis*; Bc, *B. californica*; Hu, *H. uvarum*; Pt, *P. terricola*; Ho, *H. opuntiae*; Ch, *C. humilis*; Hoc, *H. occidentalis*; Pk, *P. kudriavzevii*; Cl, *C. lusitaniae*; Td, *T. delbrueckii*; S, *S. cerevisiae*

$^{**}p < 0.01$; $^{*}p < 0.05$

causing a pungent and unpleasant flavor at levels above 400 mg/L, so its sensory evaluation should be carried out.

The *H. opuntiae* fermentations had lower volatile compounds concentration (279.75 mg/L) and the second maximum OAV (20,458.3) in all the yeast fermentations (Tables 2, 3). Of the 20 volatile compounds, ethyl caprylate and ethyl 3-phenylpropionate significantly increased over that of *S. cerevisiae*. Limonene, ethyl laurate and benzene acetaldehyde were new compounds with significantly different concentration over the *S. cerevisiae* fermentations (Table 2). Its odor-active compounds were mainly composed of esters (isoamyl acetate, ethyl hexanoate, ethyl caprylate, phenethyl acetate, ethyl 3-phenylpropionate, 9-ethyl decadienoate, ethyl caprate), benzene acetaldehyde, higher alcohols (phenethyl alcohol and 1-decanol) and terpene compounds (limonene, citronellol and linalool) (Table 3). *S. cerevisiae*/*H. opuntiae* mixed fermentation could generate higher amount of higher alcohol, phenylacetaldehyde and intensify the floral and sweet attributes of wine (Luana et al. 2018). Therefore, *H. opuntiae* could produce orange wine with different flavor from that of *S. cerevisiae*, but its sensory evaluation should be carried out.

The *C. humilis* fermentations contained the second lowest volatile compounds concentration (63.09 mg/L) and the lowest OAV (252.1) in all the yeast fermentations. Of the 17 volatile compounds, no volatile compounds had significant increase over that of *S. cerevisiae* fermentations, but six new compounds occurred with significant concentration difference from that of *S. cerevisiae* fermentations (Table 2). Its odor-active compounds mainly composed of esters (ethyl caprylate, phenethyl propionate, isoamyl acetate and ethyl caprate), higher alcohols (1-pentanol and phenethyl alcohol) and terpene compounds (limonene and citronellol) with significant difference from that of *S. cerevisiae* fermentations (Table 3). While, there were no reports about using of *C. humilis* for fermenting fruit wine. Therefore, it is meaningless to use *C. humilis* to improve the flavor of orange wine for its poor ability to produce volatile compounds.

The *H. occidentalis* fermentations contained a relative high volatile compounds concentration (428.79 mg/L) and OAV (3359) in all the yeast fermentations (Tables 2, 3). Of the 16 volatile compounds, the concentration of ethyl acetate with pineapple flavor and phenethyl acetate with rose, honey and tobacco odors had a significant increase over *S. cerevisiae* fermentations (Table 2). Its odor-active compounds were mainly composed of esters (isoamyl acetate, ethyl acetate ethyl caprylate, phenethyl acetate, 3-phenylpropionate, 9-ethyl decadienoate and ethyl caprate), phenethyl alcohol, terpene compounds (limonene, citronellol, linalool and terpineol) and methyl pentatonic acid with significant difference from that of *S. cerevisiae* fermentation (Table 3). While, there were also no reports about using *H. occidentalis* to improve flavor and quality of fruit wine. Based on this, *H. occidentalis* could be used to produce orange wine with different flavor from that of *S. cerevisiae* fermentations, but the sensory evaluation of orange wine should be carried out for the occurring of methyl pentatonic acid (Table 3).

Pichia kudriavzevii fermentations contained the maximum concentration of volatile compounds (906.64 mg/L) and the relative low OAV (3156.2) in all the yeast fermentations (Tables 2, 3). Of the 15 volatile compounds, ethyl acetate, ethyl caprylate and phenethyl alcohol had significant increases over *S. cerevisiae* fermentations (Table 2). Its odor-active compounds were mainly composed of esters (isoamyl acetate, ethyl acetate, ethyl caprylate, phenethyl acetate, ethyl 3-phenylpropionate and ethyl caprate), phenethyl alcohol and terpene compounds (citronellol, terpineol and linalool). Benzene acetaldehyde with floral, green and hyacinth flavor also contributed to the flavor of the *P. kudriavzevii* fermentations (Table 3). *P. kudriavzevii* can be used to reduce malic acid content and

Table 3 OAV of odor-active components in orange wines fermented with single non-*Saccharomyces* strains

Volatile compounds	Ct	Bc	Hu	Pt	Hop	Ch
Alcohols						
1-Pentanol	–	–	–	34.6 ± 1.3**	–	4.1 ± 0.3**
1-Decanol	–	–	–	–	2.3 ± 0.2	–
Phenethyl alcohol	–	–	6.3 ± 0.1	10.5 ± 0.2*	5.1 ± 0.2*	1.1 ± 0.2**
Carboxylic acids				–		
Methyl pentanoic acid	–	–	–	–	–	–
Octanoic acid	–	1.9 ± 0.2**	4.9 ± 0.2**	2.1 ± 0.2**	5.0 ± 0.2**	–
Esters				–		
Isoamyl acetate	53.0 ± 2.0**	302.3 ± 1.5**	939.7 ± 21.7**	544.7 ± 7.3**	2092.3 ± 31.4**	21.0 ± 0.2**
Ethyl acetate	–	–	–	6.7 ± 0.1**	–	–
Ethyl hexanoate	282.0 ± 9.0**	–	2716.0 ± 15.3**	856.0 ± 9.3**	3302.0 ± 20.2**	–
Octyl formate	–	–	208.0 ± 3.4**	–	–	–
Ethyl caprylate	464.0 ± 11.1**	110.0 ± 1.2	13,312.0 ± 35.3**	4178.0 ± 23.5**	14,694.0 ± 35.2**	188.0 ± 4.2**
Phenylethyl propionate	–	–	3938.0 ± 14.2**	–	–	–
Phenethyl acetate	3.4 ± 0.5**	1.8 ± 0.1**	–	713.8 ± 23.1**	136.4 ± 6.4*	33.6 ± 1.4**
Ethyl 3-phenylpropionate	–	–	2.5 ± 0.2*	6.9 ± 0.1**	5.0 ± 0.3**	–
9-Ethyl decadienoate	–	–	1.5 ± 0.1**	1.5 ± 0.1**	6.9 ± 0.3**	–
Ethyl caprate	2.9 ± 0.1**	3.9 ± 0.2**	47.7 ± 1.1**	31.9 ± 1.2**	67.6 ± 2.9**	1.6 ± 0.1**
Terpene						
Linalool	–	158.3 ± 0.8**	3.5 ± 0.2*	5.2 ± 0.2	3.6 ± 0.2*	–
Terpineol	–	7.8 ± 0.4**	–	6.6 ± 0.3**	–	–
Citronellol	–	306.4 ± 2.1**	10.9 ± 0.4	15.9 ± 0.3**	9.9 ± 0.4	1.1 ± 0.1**
Limonene	–	–	6.9 ± 0.3**	9.2 ± 0.6**	10.2 ± 0.8**	1.7 ± 0.1**
Aldehydes						
Benzeneacetaldehyde	–	–	–	218.0 ± 2.4**	118.0 ± 4.5**	–
Phenols						
Vinyl guaiacol	–	–	23.8 ± 0.4**	12.8 ± 0.6**	–	–
Total OAV of compounds	805.0 ± 22.7**	892.5 ± 6.5**	21,221.4 ± 92.9**	6654.2 ± 70.9**	20,458.3 ± 103.2**	252.1 ± 6.6**

Volatile compounds	Hoc	Pk	Cl	Td	S
Alcohols					
1-Pentanol	–	–	3.7 ± 0.2**	46.7 ± 2.1**	–
1-Decanol	–	–	–	–	2.3 ± 0.1
Phenethyl alcohol	7.6 ± 0.3	22.6 ± 0.3**	1.3 ± 0.2**	7.9 ± 0.8	6.8 ± 0.5
Carboxylic acids					
Methyl pentanoic acid	5.8 ± 0.3**	–	–	–	–
Octanoic acid	–	–	–	–	12.3 ± 0.2
Esters					
Isoamyl acetate	1074.0 ± 7.3**	1099.7 ± 25.8**	49.3 ± 1.2**	453.0 ± 7.3**	3394.3 ± 29.4
Ethyl acetate	19.7 ± 0.2**	77.1 ± 2.1**	6.2 ± 0.2**	–	3.4 ± 0.1
Ethyl hexanoate	–	–	80.0 ± 2.4**	–	4598.0 ± 20.4
Octyl formate	–	–	–	–	–
Ethyl caprylate	1594.0 ± 15.7**	1548.0 ± 11.3**	96.0 ± 3.1*	960.0 ± 9.2**	116.0 ± 3.2
Phenylethyl propionate	–	–	–	–	–
Phenethyl acetate	595.8 ± 9.5**	202.3 ± 6.3*	32.8 ± 0.3**	672.0 ± 4.3**	163.6 ± 2.5
Ethyl 3-phenylpropionate	1.9 ± 0.1*	2.6 ± 0.0**	–	3.7 ± 0.5**	1.3 ± 0.1
9-Ethyl decadienoate	1.8 ± 0.1**	–	–	–	18.0 ± 0.2
Ethyl caprate	24.7 ± 0.4**	14.8 ± 0.3**	1.5 ± 0.1**	10.3 ± 0.7**	95.2 ± 1.2
Terpene					

Table 3 continued

Volatile compounds	Hoc	Pk	Cl	Td	S
Linalool	5.9 ± 0.3	3.7 ± 0.4	–	2.7 ± 0.1*	4.8 ± 0.3
Terpineol	4.9 ± 0.3**	5.7 ± 0.5**	–	3.2 ± 0.1**	–
Citronellol	7.9 ± 1.0	5.8 ± 0.3**	1.0 ± 0.1	10.1 ± 0.1	10.1 ± 0.5
Limonene	15.1 ± 1.1**	–	–	–	–
Aldehydes					
Benzeneacetaldehyde	–	174.0 ± 3.1**	12.0 ± 0.5**	–	–
Phenols					
Vinyl guaiacol	–	–	–	–	45.0 ± 1.3
Total OAV of compounds	3359.0 ± 36.6**	3156.2 ± 50.5**	283.8 ± 8.3**	2169.0 ± 25.2**	8471.0 ± 60.0

"–", OAV < 1; Ct, *C. tropicalis*; Bc, *B. californica*; Hu, *H. uvarum*; Pt, *P. terricola*; Ho, *H. opuntiae*; Ch, *C. humilis*; Hoc, *H. occidentalis*; Pk, *P. kudriavzevii*; Cl, *C. lusitaniae*; Td, *T. delbrueckii*; S, *S. cerevisiae*

**$p < 0.01$; *$p < 0.05$

improve the quality of wine (Kim et al. 2008). Therefore, *P. kudriavzevii* could be used to produce orange wine with different flavor from that of *S. cerevisiae*.

Clavispora lusitaniae fermentations contained the lower concentration of volatile compounds (108.43 mg/L) and the second lowest OAV (283.8) in all the yeast fermentations (Tables 2, 3). Of the 20 volatile compounds, ethyl acetate had a significant increase over that of *S. cerevisiae* fermentation. 1-pentanol, ethyl phenylacetate, ethyl palmitate, terpineol, valencene, 4-ethylbenzaldehyde and benzene acetaldehyde occurred as new compounds with a significant difference from that of *S. cerevisiae* fermentations (Table 2). Its odor-active compounds mainly composed of esters (isoamyl acetate, ethyl acetate, ethyl hexanoate, ethyl caprylate, phenethyl acetate and ethyl caprate), higher alcohol (1-pentanol, phenethyl alcohol), citronellol and benzene acetaldehyde with significant lower OAV than that of *S. cerevisiae* fermentation (Table 3). There were no reports about using of *C. lusitaniae* for fermenting fruit wine. Therefore, it's not significant to use *C. lusitaniae* to produce different flavor of orange wine.

Torulaspora delbrueckii fermentations contained lower concentration of volatile compounds (2169.0 mg/L) and lower OAV (2169) in all the yeast fermentations (Tables 2, 3). Of the 16 volatile compounds, ethyl caprylate, phenethyl acetate and 3-phenylpropionate had a significant increase over that of *S. cerevisiae* fermentation. 1-pentanol, ethyl benzoate, ethyl phenylacetate, ethyl formate, terpineol and 4-ethylbenzaldehyde occurred as new compounds with a significant difference from that of *S. cerevisiae* fermentations (Table 2). Its odor-active compounds mainly composed of esters (isoamyl acetate, ethyl caprylate, phenethyl acetate, ethyl 3-phenylpropionate and ethyl caprate), higher alcohol (1-pentanol, phenethyl alcohol) and terpenes (linalool, terpineol and citronellol) with significant lower OAV than that of *S. cerevisiae* fermentations (Table 3). *T. delbrueckii* had been used to ferment wine with enhancing bioflavour or reducing ethanol content (Canonico et al. 2016). Therefore, *P. kudriavzevii* could be used to produce orange wine with different flavor from that of *S. cerevisiae*, but sensory evaluation of its fermentation should be carried out for excessive higher alcohol (545.56 mg/L).

PCA of volatile compounds in orange wines fermented with a single yeast strain

A PCA analysis was performed to determine the correlation and segregation of volatile compounds in different yeast strain fermentations. Here 99.84% of variance was explained by seven different components, and PC1 and PC2 accounted for 49 and 40% of variance respectively. An interesting finding in the PCA plot (data not shown) was that *P. kudriavzevii* and *H. occidentalis* were grouped in the upper right quadrant, and *P. terricola* and *T. delbrueckii* were grouped in the upper left quadrant, while other non-*Saccharomyces* yeast strains were clustered centrally, near the intersection of PC1 and PC2. The *P. kudriavzevii* and *H. occidentalis* had a correlation with high concentration of ethyl acetate and phenethyl alcohol. High concentration of pentanol and phenethyl acetate were correlated with *P. terricola* and *T. delbrueckii*. Other non-*Saccharomyces* yeast strains were not segregated completely, indicating these yeast strains had similar volatile compound profiles in orange wine.

Table 4 Sensory evaluation of orange wines fermented with non-*Saccharomyces* mono-cultures (average ± SD)

Species	Color (3)	Aroma (6)	Mouthfeel (6)	Taste lasting (3)	Overall acceptability (2)	Total (20)
C. tropicalis	1.83 ± 0.41	2.33 ± 0.52	2.6 ± 0.75	1.67 ± 0.52	1.00 ± 0.00	9.44 ± 0.89
H. uvarum	2.33 ± 0.52	4.50 ± 0.84	3.33 ± 0.52	1.67 ± 0.52	1.00 ± 0.00	12.83 ± 0.75**
P. terricola	2.33 ± 0.52	2.67 ± 0.52	2.17 ± 0.75	1.17 ± 0.41	1.00 ± 0.00	9.33 ± 0.82
H. opuntiae	2.0 ± 0.63	3.33 ± 0.52	2.83 ± 0.75	1.33 ± 0.52	1.17 ± 0.41	10.66 ± 0.82
C. humilis	2.5 ± 0.55	2.67 ± 0.52	2.0 ± 0.63	1.17 ± 0.41	1.17 ± 0.41	9.50 ± 0.84
H. occidentalis	2.67 ± 0.52	3.5 ± 0.55	3.0 ± 0.63	2.0 ± 0.41**	1.00 ± 0.00	12.33 ± 0.82**
P. kudriavzevii	2.5 ± 0.55	4.5 ± 0.55	4.83 ± 0.41**	2.17 ± 0.41**	1.67 ± 0.52	15.66 ± 0.82**
C. lusitaniae	2.17 ± 0.41	2.5 ± 0.52	2.8 ± 0.55	1.07 ± 0.52	1.0 ± 0.41	9.54 ± 0.75
T. delbrueckii	2.0 ± 0.63	3.83 ± 0.75	3.83 ± 0.75**	1.83 ± 0.41*	1.17 ± 0.41	12.67 ± 0.82**
S. cerevisiae	2.0 ± 0.63	3.17 ± 0.41	2.5 ± 0.55	1.17 ± 0.41	1.17 ± 0.41	10.00 ± 0.89

Data show the mean value of scores from nine assessors

**$p < 0.01$; *$p < 0.05$

Sensory evaluation of orange wines fermented with a single yeast strain

Table 4 showed that orange wine fermented by *P. kudriavzevii* had the highest sensory evaluation score (15.66) among the orange wine samples, followed by fermentation of *H. uvarum* (12.83), *T. delbrueckii* (12.67) and *H. occidentalis* (12.33) with significant difference from that of *S. cerevisiae* fermentations. The sensory evaluation score *of H. opuntiae* fermentation was higher than that of *S. cerevisiae* fermentation (10.66) without no significant difference. The sensory evaluation of *B. californica* fermentation was not carried out for its unpleasant smell. Therefore, the *P. kudriavzevii*, *H. uvarum*, *T. delbrueckii*, *H. occidentalis* and *H. opuntiae* could be used to improve the flavor of orange wine.

Conclusion

Based on the enological traits and the volatile compounds profiles of ten non-*Saccharomyces* yeast strains, *C. tropicalis*, *C. humilis*, *C. lusitaniae*, *B. californica* and *P. terricola* could not be used to ferment orange wine for the poor flavor and quality of their fermentations, while *H. uvarum*, *H. opuntiae*, *H. occidentalis*, *P. kudriavzevii* and *T. delbrueckii* could be candidates to improve the flavor of orange wine. The research results will provide a valuable selection method of non-*Saccharomyces* yeasts for orange wine fermentation and an approach to improve the flavor of orange wine or other fruit wine. However, further studies should be carried out to evaluate whether these differences among the non-*Saccharomyces* yeast strains will persist after inoculation with *S. cerevisiae* to complete alcoholic fermentation of orange wine.

Acknowledgements This work was supported by the Major Science and Technology Program of Ningxia Hui Autonomous Region (2016BZ0601/02/03) and the Fundamental Research Funds Project for the Central Universities (2662015PY068).

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