Selection of Indigenous *Saccharomyces cerevisiae* Strains from Spontaneous Fermentation of Vidal Icewine in Huanren Region and Evaluation of Their Oenological Properties

SHEN Jingyun, ZHANG Boqin, DUAN Changqing, YAN Guoliang*

(Centre for Viticulture and Enology, Key Laboratory of Viticulture and Enology, Ministry of Agriculture and Rural Affairs, College of Food Science and Nutritional Engineering, China Agricultural University, Beijing 100083, China)

Abstract: To obtain suitable yeast for Vidal icewine production, a rapid selection procedure was proposed in this study. Nine *Saccharomyces cerevisiae* strains were selected from spontaneous fermentation of Vidal icewine in Huanren region by determining their tolerance to ethanol and SO_2. The oenological evaluation in a flask and a fermenter showed that all the isolates could complete the alcoholic fermentation of Vidal icewine and produced different aroma profiles as compared to the commercial strain DV10 as evidenced by principal component analysis (PCA). SC42 and SC45, isolated at the final stage of spontaneous fermentation, were finally selected for their high fermentation activity. SC42 produced higher amounts of higher alcohol and esters along with a lower amount of acetic acid, while SC45 generated higher levels of glycerol, esters, *trans*-rose oxide and *β*-damascenone. Our present results proved the feasibility of this simple method to select suitable yeast strains for icewine industrial production, and also suggested that using indigenous *Saccharomyces* strains is a feasible way to improve the aroma quality and diversity of icewine products.

Keywords: Vidal icewine; *Saccharomyces cerevisiae*; spontaneous fermentation; strain selection; aroma compounds

桓仁产区优良冰酒酵母菌株的筛选及酿造特性评价

申静云，张博钦，段长青，燕国梁*

（中国农业大学食品科学与营养工程学院，葡萄与葡萄酒研究中心，农业农村部葡萄酒加工重点实验室，北京 100083）

摘 要：为获得可用于东北桓仁地区威代尔冰酒生产的酿酒酵母菌株，采用一种快速的酵母菌株筛选方法，通过测定菌株乙醇和二氧化硫耐受性，从威代尔冰酒自然发酵过程中筛选到9 株酿酒酵母菌株。进一步酿造实验结果显示，所筛选的酿酒酵母可以顺利完成冰酒发酵，产生的香气轮廓与商业酵母DV10相比也不同（主成分分析结果），最终获得了2 株具有高发酵活力且香气特征与商业酵母差异显著的酵母菌株SC42和SC45，其中SC42能够高产高级醇和酯类物质，并且低产乙酸，而SC45能够产生高含量的甘油、酯类物质以及反式玫瑰醚和*β*-大马士酮。结果表明，采用本研究的筛选方法能够快速有效地筛选到具有应用潜力的冰酒生产菌株，同时也证明了使用本土野生酵母菌株能够有效地改善冰酒香气品质，生产出与接种商业酵母不同风格的冰酒产品。

关键词：威代尔冰酒；酿酒酵母；自然发酵；菌株筛选；香气物质

DOI:10.7506/spkx1002-6630-20180903-022

中图分类号：TS261.1 文献标志码：A 文章编号：1002-6630（2020）02-0148-10

引文格式：

SHEN Jingyun, ZHANG Boqin, DUAN Changqing, et al. Selection of indigenous *Saccharomyces cerevisiae* strains from spontaneous fermentation of Vidal icewine in Huanren region and evaluation of their oenological properties[J]. 食品科学, 2020, 41(2): 148-157. DOI:10.7506/spkx1002-6630-20180903-022. http://www.spkx.net.cn

收稿日期：2018-09-03

基金项目：现代农业产业技术体系建设专项（CARS-29）；中央高校基本科研业务费专项（2017SP003）

第一作者简介：申静云（1993—）（ORCID: 000-0002-5311-1092），女，硕士研究生，研究方向为食品生物技术。

E-mail: shenjingyun813@163.com

*通信作者简介：燕国梁（1967—）（ORCID: 0000-0001-9936-4776），男，副教授，博士，研究方向为酿酒酵母分子生物学。

E-mail: glyan@cau.edu.cn

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Icewine is a dessert wine made from grapes that have been left on the vine until weather conditions are cold enough to freeze the grapes. During freezing, most of the water in grape berries is consequently frozen, and sugars, acids and nitrogenous compounds are concentrated[1]. To produce an authentic icewine, the entire harvesting and pressing process must be carried out below −8 ℃ and juice sugar content must be > 35 °Brix at the time of pressing[2]. Vidal and Riesling are the two typical cultivars used for the production of icewine due to the fact that their berries have relatively thick skins and the vines are of cold-resistance[3-4]. Icewine contains higher and distinct volatile compound relative to table wines[5]. *Saccharomyces cerevisiae* has a significant effect on the aroma quality of icewine because it produces thousands of aroma compounds, including higher alcohols, esters, fatty acids, and carbonyl compounds during alcoholic fermentation. The suitable icewine yeast could not only generate more desired aromatic compounds but also withstand the harsh fermentation conditions, because they are challenged by harsher stresses during alcoholic fermentation, including high sugar concentration (above 35 °Brix), low temperature (15−18 ℃), SO_2 and ethanol toxicity as compared to table wine yeast[6]. Some investigators evaluated the fermentation performances and oenological features of several commercial *S. cerevisiae* strains in icewine fermentation. Their results indicated that different yeast strains could produce diversified aromatic profiles and led to different sensory characteristics[7-9].

In recent years, there is an increasing interest in using indigenous wine yeasts for producing wines with distinctive quality. Indigenous yeasts can well adapt to the environmental conditions of wine production region and generate peculiar aromatic notes, which imparts the wine with typical sensory characteristics of each wine area[10-11]. However, little work has been done on icewine yeast strains. The screen of wine yeast is a time consuming and laborious procedures. The potential yeast strains were firstly isolated from grapes skin or spontaneous alcoholic fermentation. Their technological and oenological properties were further investigated under specific stress conditions and micro-fermentation trials, respectively. The desired technological features mainly included high fermentation performance, high resistance to ethanol, sulfur dioxide and low temperature, low production of hydrogen sulfide, among others. The oenological properties are expected to enhance volatile compounds, such as esters and higher alcohols with the scant production of off-flavors[12]. For simplifying the selection process, several simple and effective procedures have been proposed by focusing on the most important properties, including growth profiles, the resistance to SO_2 and ethanol, and the production of volatile acid[13-14]. Another improvement is to impart a particular stress challenge during spontaneous fermentation with the aim to enrich the strains that represent better adaptation to these specific conditions. This strategy has been successfully applied in the isolation of yeast strains with high tolerance to low temperature[15]. It is known that icewine yeast species endure severe stress during alcoholic fermentation, especially hyperosmotic stress and low temperature[6]. The simultaneous and sequential stresses led to very few yeast species persist in icewine fermentation. Therefore, it is assumed that it is relatively easy to screen out suitable yeast strains from spontaneous fermentation of icewine as compared to table wine fermentation.

Icewine production in China has developed rapidly in the recent years, and China has become an important icewine production country. Huanren is a typical icewine production region in the northeast of China with a suitable climate for icewine making[16-17]. At present, most Chinese wineries use the imported commercial yeast strains to inoculate icewine fermentation, which can reduce the variability of autochthonous yeast strains and thus, negatively influence the icewine aroma complexity and regional characteristics. Vidal is a typical cultivar used to produce Chinese icewine in China. To obtain potential indigenous *S. cerevisiae* strains for industrial production of Vidal icewine, we conducted the selection procedure of wine yeast in this work. For quick this procedure, a rapid selection procedure was proposed. By determining the tolerance to ethanol and SO_2, 9 potential *S. cerevisiae* strains were preselected from Vidal icewine spontaneous fermentation, and their oenological features were investigated in laboratory micro-fermentation with commercial strain DV10 as referenced strain.

1 Materials and Methods

1.1 Materials and reagents

Vidal grapes were grown in Huanren region, Liaoning, China. Grapes were harvested, destemmed, crushed, and pressed at −8 ℃ to −9 ℃ to obtain grape juice (400 g/L of sugar, 13 g/L of total acidity and a pH of 3.35).

Pectinase HC was purchased from Lallemand, France.

1.2 Instruments and equipment

1200 High performance liquid chromatography (HPLC) and 7890 N gas chromatograph (GC) (equipped with a 5975BMS mass spectrum system) were purchased from Agilent Technologies (Santa Clara, CA, USA).

1.3 Methods

1.3.1 Yeast strains and isolation procedure

Alcoholic fermentation was allowed to proceed spontaneously under 15−17 ℃ for 40 d and the final alcohol concentration reached 11.8% (*V*/*V*). Samples for the isolation of yeasts were taken at different stages of fermentation (mid-exponential, early-stationary and late-stationary growth phases). Ninety five isolated strains were analyzed by the 5.8S-ITS-RFLP technology as described elsewhere[18], in which, 78 strains were identified to belong to *S. cerevisiae*. After determining the tolerance to ethanol and SO_2 and microsatellite PCR fingerprinting analysis, 9 *Saccharomyces* strains (SC26, SC28, SC33, SC36, SC39, SC40, SC41, SC42 and SC45) were obtained. A commercial *S. cerevisiae* strains Lalvin DV10 was used as reference strain, which is well known for its ability to ferment under stressful conditions of low pH, high total SO_2 and low temperature, and can ferment up to 18% (*V*/*V*) alcohol. It is widely used in the icewine production in Huanren region of China. All the strains were maintained in YPD (1 g/100 mL yeast extract; 2 g/100 mL peptone, 2 g/100 mL glucose) liquid media mixed with glycerol at −80 ℃.

1.3.2 Micro-fermentation experiments

The fermentation potentials of all strains were evaluated in shaking flask and fermenter. The fermentation was performed in triplicate and under static condition at 16 ℃ in 500 mL Erlenmeyer flasks equipped with fermentation locks and containing 350 mL of sterilized Vidal grape juice. *S. cerevisiae* strains were cultured in YPD media overnight on a rotary shaker at 180 r/min and 28 ℃, washed twice with sterile water, suspended in Vidal juice at a concentration of 1.0×10^6 CFU/mL and fermented for 30−32 d. Three better *S. cerevisiae* strains, SC33, SC42, and SC45, were selected for further investigation in duplicated 3-L glass fermenter with 2.5 L Vidal grape juice adding 60 mg/L SO_2. The inoculum size and the fermentation temperature were the same as the flasks experiment. Samples were taken in interval for the determination of viable cell counts. The final samples were centrifuged and stored at −20 ℃ for analysis of main products and volatile compounds.

1.3.3 Analytical techniques

The cell number of yeast was determined by plating on WL nutrient agar containing 100 mg/L chloramphenicol for inhibiting bacterial growth. To determine the tolerance to ethanol and SO_2, the isolated strains were inoculated in a simple synthetic must[19] with 15% ethanol and 100 mg/L SO_2 added, respectively. Their cell growth rates were compared with that of DV10 strain during 10 d cultivation. Their DNA samples were used as templates for microsatellite PCR fingerprinting, as described by Vaudano et al.[20]. The main products of final wines were determined by HPLC analysis using an Aminex HPX-87H column (300 mm × 7.8 mm, BioRad Laboratories, Hercules, CA, USA)[21]. The mobile phase flow rate of 0.6 mL/min was achieved with an elution gradient composed of solvent 5 nmol/L H_2SO_4 in water. Column temperature was 45 ℃ with refractive index detector (RID). Total analysis time was 30 min. Injection volume was 10 μL for malic acid, citrate acid and acetic acid analysis. Column temperature was 60 ℃ with diode array detector (210 nm, DAD). Total analysis time was 30 min. The volatile compounds of final wines were determined by headspace solid-phase micro-extraction coupled with gas chromatography-mass spectrometry (HS-SPME-GC-MS) according to our previous study[22]. Five milliliter of final fermentation sample, 1.00 g of NaCl and 10 μL of 4-methyl-2-pentanol (1.039 mg/mL water, internal standard) were blended in a 15 mL sample vial tightly capped with a PTFE silicon septum and containing a magnetic stirrer. Afterward the vial containing the sample was heated at 40 ℃ for 30 min on a heating platform agitation (80 r/min). A 7890N GC equipped with a 5975BMS mass spectrum system on a HP-INNOWAX column (60 m × 0.25 mm, 0.25 μm film thickness, J&W scientific, USA) was applied for aroma compounds analysis. The pretreated (conditioned at 270 ℃ for 1 h) SPME fiber (50/30 μm DVB/Carboxen/PDMS, Supelco, Bellefonte, PA) was then inserted into the headspace, extracted for 30 min with continued heating (40 ℃) and agitation (80 r/min). The fiber was instantly desorbed in the GC injector for 8 min at 250 ℃. GC inlet

was set in the splitless mode. The oven's starting temperature was 50 ℃ (held for 1 min), then raised to 220 ℃ (held for 5 min) at 3 ℃/min. The mass spectrometer was operated in the electron impact (EI) mode at 70 eV and in the scan and the selective ion mode (SIM) range of *m/z* 35–350. Analyses were performed in triplicate.

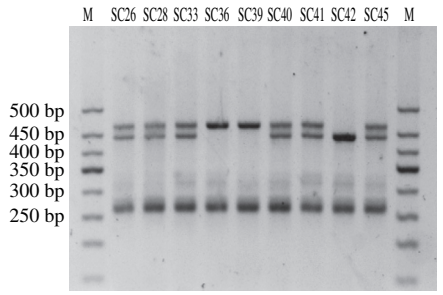
1.4 Statistical analysis

ANOVA of analysis on volatile and non-volatile compounds was done to establish signification of differences in different samples by SPSS version 24.0 Statistical Package (SPSS Inc., USA). The significant differences among these data were determined using Duncan's multiple range tests. $P < 0.05$ was regarded as significant difference.

2 Results and Analysis

2.1 The preselection of icewine yeast strains

The icewine spontaneous fermentation containing 80 mg/L SO_2 was conducted at 15–17 ℃. During 40 d fermentation process, 78 strains were isolated and identified as *S. cerevisiae*. Non-*Saccharomyces* strains, including *Hanseniaspora opuntiae*, *H. uvarum* and *Metschnikowia pulcherrima*, were only isolated before 10 d fermentations. By comparing their cell growth profiles with that of DV10 strain in a simple synthetic must containing 15% ethanol and 100 mg/L SO_2, respectively, 9 *Saccharomyces* strains (SC26, SC28, SC33, SC36, SC39, SC40, SC41, SC42 and SC45) were preselected from 78 strains (data not showed). Their genetic characterizations were further determined at strain level using microsatellite PCR fingerprinting with 2.5% agarose gel after microsatellite PCR of multiplex of locus SC8132X, YOR267C and SCPTSY7. The 3 primers generated microsatellite PCR fingerprints composed of 3 to 5 well distributed bands ranging from approximately 250 to 500 bp (Fig. 1). With the help of this molecular analysis, the 9 strains were roughly classified into 3 groups, 7 strains, including SC26, SC28, SC33, SC40, SC41 and SC 45, were classified in group I, SC36 and 39 belonged to group II, and SC42 located in group III alone.



M. 50 bp DNA ladder.

Fig. 1 Electrophoretic patterns of isolated strains SC26, SC28, SC33, SC36, SC39, SC40, SC41, SC42, and SC45

2.2 Micro-vinifications in shaking flasks

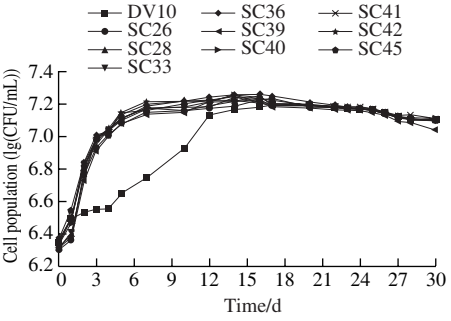


Fig. 2 Growth profiles of ten *S. cerevisiae* strains during alcoholic fermentation of Vidal icewine

The oenological performances of 9 strains were firstly investigated in the flasks using DV10 as referenced strain. Fig. 2 and Table 1 presented the cell growth rates (cell population) and the physiochemical compositions after alcoholic fermentation. All the fermentation process

Table 1 Physicochemical parameters of wines obtained with ten *S. cerevisiae* yeasts in flask fermentation

Compounds	DV10	SC26	SC28	SC33	SC36	SC39	SC40	SC41	SC42	SC45
Residual sugar/(g/L)	165.00 ± 7.26^{ab}	170.42 ± 1.60^{b}	170.06 ± 1.81^{b}	160.00 ± 4.34^{a}	165.16 ± 6.36^{ab}	168.13 ± 3.05^{ab}	160.49 ± 5.66^{a}	162.80 ± 2.30^{a}	160.12 ± 2.42^{a}	162.83 ± 2.66^{a}
Glycerol/(g/L)	10.44 ± 0.07^{b}	9.40 ± 0.30^{a}	9.66 ± 0.40^{a}	10.83 ± 0.08^{b}	10.26 ± 0.18^{ab}	10.02 ± 0.03^{a}	10.62 ± 0.99^{bc}	10.59 ± 0.74^{bc}	11.03 ± 0.81^{bc}	11.91 ± 0.17^{bc}
Ethanol/%	11.05 ± 0.01^{b}	11.03 ± 0.02^{b}	10.43 ± 0.02^{a}	11.08 ± 0.06^{b}	11.07 ± 0.09^{b}	11.05 ± 0.07^{b}	11.04 ± 0.03^{b}	10.31 ± 0.21^{a}	11.00 ± 0.11^{b}	11.04 ± 0.06^{b}
Acetic acid/(g/L)	1.70 ± 0.08^{bc}	1.86 ± 0.03^{d}	1.73 ± 0.01^{c}	1.66 ± 0.02^{b}	1.72 ± 0.03^{bc}	1.72 ± 0.01^{bc}	1.70 ± 0.01^{bc}	1.63 ± 0.02^{a}	1.63 ± 0.01^{a}	1.69 ± 0.05^{bc}
Oxalic acid/(g/L)	0.32 ± 0.01^{a}	0.46 ± 0.09^{a}	0.40 ± 0.01^{a}	0.45 ± 0.09^{a}	0.49 ± 0.06^{a}	0.44 ± 0.00^{a}	0.44 ± 0.02^{a}	0.48 ± 0.06^{a}	0.46 ± 0.03^{a}	0.44 ± 0.01^{a}
Citric acid/(g/L)	0.61 ± 0.03^{a}	0.78 ± 0.04^{d}	0.75 ± 0.04^{cd}	0.71 ± 0.01^{cd}	0.68 ± 0.01^{bc}	0.69 ± 0.01^{bc}	0.72 ± 0.02^{c}	0.66 ± 0.02^{b}	0.70 ± 0.03^{cd}	0.71 ± 0.02^{cd}
Malic acid/(g/L)	13.56 ± 0.40^{abc}	14.11 ± 0.09^{c}	13.72 ± 0.04^{bc}	13.30 ± 0.01^{ab}	13.62 ± 0.12^{abc}	13.45 ± 0.13^{ab}	13.17 ± 0.02^{ab}	13.09 ± 0.02^{a}	13.27 ± 0.04^{ab}	13.41 ± 0.00^{ab}
Succinic acid/(g/L)	3.23 ± 0.11^{a}	3.28 ± 0.16^{a}	3.16 ± 0.02^{a}	3.11 ± 0.04^{a}	3.17 ± 0.02^{a}	3.09 ± 0.01^{a}	3.15 ± 0.01^{a}	3.09 ± 0.01^{a}	3.14 ± 0.02^{a}	3.16 ± 0.04^{a}
Lactic acid/(g/L)	1.20 ± 0.01^{cd}	1.22 ± 0.01^{d}	1.15 ± 0.01^{bc}	1.17 ± 0.00^{c}	1.12 ± 0.03^{a}	1.14 ± 0.03^{ab}	1.12 ± 0.01^{a}	1.20 ± 0.02^{cd}	1.10 ± 0.02^{a}	1.15 ± 0.01^{bc}

Notes: Values are given as $\bar{x} \pm s$ deviation of three biological replicates and three HPLC detection runs. Data with different letters (a, b, c, d) within each column are different according to Duncan tests ($P < 0.05$). Same as follows.

completed after 30–32 d. The inoculated juice exhibited similar cell growth kinetics with the exception of DV10 which showed lower cell growth rate and cell population compared to indigenous strains. SC42 and SC45 strain, isolated at the final stage of spontaneous fermentation, presented higher cell growth rate and cell population. This was corresponding to the conclusion that indigenous strain can better adapt to grape must composition than commercial yeast with higher fermentation activity[23]. Most yeast strains consumed sugar concentration below 170 g/L with the exception of SC26 and SC28, and 8 strains formed considerable levels of ethanol (11.00%−11.08%) except for SC28 and SC41 with 10.43% and 10.31%, respectively. Glycerol is the major secondary compound that contributes to the viscosity and "softness" of wine with a positive effect on taste, SC45 produced the highest glycerol content (11.91 g/L). High-sugar grape must tends to yeast producing more acetic acid acting as a by-product[24]. The highest acceptable amount of acetic acid is 2.1 g/L in Canadian[25] and Chinese[26] icewine. The amounts of acetic acid in all samples were below this value (range in 1.63−1.86 g/L), with lowest level in SC41 and SC42 strain. No significant differences were observed in the amounts of other organic acids, including citric acid, malic acid, lactic acid, oxalic acid and succinic acid in different wines.

A total of 47 aroma compounds were identified in final wines, including 15 higher alcohols, 3 fatty acids, 14 esters, 4 aldehydes and 6 terpenes and 5 other compounds. 13 compounds that odor activity value (OAV) exceeds one and some characteristic aroma compounds of Vidal icewine were presented in Table 2. There were no enough references gave the thresholds of these aroma compounds in icewine matrix; some compounds thresholds were therefore obtained either from the system of table wine or ethanol solution. Generally, 10 yeast strains generated different aroma profiles in final wines. Higher alcohols are the largest group of aroma constituents in icewine. The concentration of 300−400 mg/L is acceptable, whereas the optimal level (below 300 mg/L) imparts a pleasant character[27]. The contents of higher alcohol in all samples were in an appropriate concentration range (< 300 mg/L) with the highest production by SC42 (226 mg/L), followed by SC36 and SC45. In Vidal icewine, isobutanol (pine note), isopentanol (organic reagent note), 2-phenylethanol (flowery and honey note), 1-octene-3-ol (fruit and flora note), and (*Z*)-3-hexen-1-ol (pine note) are the characteristic aroma compounds[28]. Except for (*Z*)-3-hexen-1-ol, other 4 compounds exceeded the individual sensory threshold in this study. SC42 and SC28 generated higher contents of phenylethyl alcohol, isopentanol and 1-octene-3-ol than other strains. SC42 was featured with the highest content of 2,3-butanediol (cream note). SC33 produced higher contents of (*Z*)-3-hexen-1-ol, isobutanol and 1-hexanol but with more fatty acids (octanoic acid and hexanoic acid). Fatty acids are described with fruity, cheese, fatty, and rancid notes. Volatile fatty acids can contribute to the complexity of wine at sub-sensory threshold levels, while they cause negative effect on wine aroma when above their thresholds[29]. Our data showed that all indigenous strains produced more fatty acids than commercial strain DV10.

Esters including acetate esters and fatty acid ethyl esters had a major impact on icewine aroma quality[7,28]. Their formation is largely dependent on inoculated strains[7,9]. Ethyl hexanoate, ethyl isobutyrate, ethyl 2-methylbutyrate, ethyl isovalerate and ethyl butyrate are major esters, and give Vidal icewine fruit note[28]. In comparison, Crandles et al.[7] identified 5 esters (ethyl isobutyrate, ethyl 2-methylbutyrate, isoamyl acetate, ethyl benzoate, and *β*-phenethyl acetate) as the characteristic esters in Canada Vidal icewine, with the highest production by the commercial strain EC1118. In this study, 14 esters were identified in all wines and 4 exceeded the individual threshold, including ethyl butanoate (banana and strawberry smell), ethyl decanoate (fruity smell), ethyl hexanoate (apple peel smell) and ethyl octanoate (banana and pear smell). The total contents of ester in 10 wines were ranges in 2 600 to 3 100 μg/L with the highest content by SC45 (3 076.83 μg/L), followed by SC42 (2 888.74 μg/L), SC33 (2 839.87 μg/L), SC26 (2 782.87 μg/L) and DV10 (2 756.35 μg/L). Different strains were featured with desired specific ester, confirming the conclusion that the formation of esters by wine *S. cerevisiae* is strain specific[7,9].

Aldehydes with low sensory threshold and apple-like odors are important to wine aroma. Phenylacetaldehyde reached the threshold in all wines and generate floral and honey note to Vidal icewine. SC36 produced the highest value (513.16 μg/L), followed by SC26 (489.47 μg/L) and SC39 (488.19 μg/L). The contents of terpenes and norisoprenoids significantly determined the aroma quality of icewine[7,28]. 4 Terpenes and 1 norisoprenoids (*β*-damascenone) were identified, in which *β*-damascenone (honey note) and *trans*-rose oxide (lychee note) reached their

Table 2 28 Main volatile aroma compounds identified in wines obtained with ten *S. cerevisiae* yeasts used after alcoholic fermentation

μg/L

Compounds	DV10	SC26	SC28	SC33	SC36	SC39	SC40	SC41	SC42	SC45
Isobutanol	**44 895.30 ± 0.55^{a}**	**41 823.37 ± 0.89^{b}**	**48 686.59 ± 4.82^{h}**	**49 868.93 ± 0.10^{i}**	**43 526.41 ± 0.84^{c}**	**47 496.75 ± 1.77^{g}**	**44 551.46 ± 0.77^{d}**	**47 136.11 ± 1.26^{f}**	**45 488.37 ± 0.89^{e}**	**36 370.67 ± 0.95^{a}**
Isopentanol	**48 991.89 ± 1.81^{d}**	**49 217.28 ± 3.22^{e}**	**57 792.22 ± 3.14^{i}**	**50 821.56 ± 2.04^{f}**	**47 683.32 ± 2.38^{b}**	**55 468.03 ± 1.37^{h}**	**52 263.29 ± 5.25^{g}**	**47 997.58 ± 2.01^{c}**	**54 834.52 ± 0.68^{h}**	**44 022.27 ± 1.8^{a}**
2-Phenylethanol	**11 371.42 ± 1.92^{b}**	**12 111.77 ± 1.74^{c}**	**13 135.74 ± 0.37^{f}**	**12 868.35 ± 0.92^{d}**	**10 465.51 ± 2.11^{a}**	**13 223.12 ± 1.24^{g}**	**13 452.98 ± 1.44^{h}**	**12 955.72 ± 1.82^{e}**	**13 774.35 ± 0.93^{i}**	**11 362.23 ± 1.09^{b}**
1-Octen-3-ol	**126.41 ± 0.78^{c}**	**114.23 ± 1.09^{a}**	**158.14 ± 1.22^{f}**	**123.27 ± 1.04^{c}**	**124.42 ± 0.83^{c}**	**132.58 ± 2.01^{d}**	**113.74 ± 0.37^{a}**	**118.85 ± 0.22^{b}**	**137.85 ± 0.21^{e}**	**125.31 ± 0.98^{c}**
(*Z*)-3-Hexen-1-ol	271.78 ± 0.27^{h}	242.10 ± 1.38^{b}	249.04 ± 1.09^{c}	281.22 ± 0.76^{i}	269.55 ± 0.35^{g}	252.15 ± 0.49^{d}	260.87 ± 0.96^{e}	235.62 ± 0.65^{a}	264.08 ± 0.99^{f}	271.96 ± 0.67^{h}
1-Octanol	12.00 ± 0.10^{bc}	11.34 ± 0.93^{ab}	9.91 ± 0.13^{a}	14.27 ± 1.03^{c}	11.29 ± 0.38^{ab}	10.92 ± 0.11^{ab}	11.55 ± 0.50^{ab}	10.66 ± 0.35^{ab}	10.88 ± 0.18^{ab}	12.69 ± 0.45^{bc}
2-Octanol	11.90 ± 0.10^{ab}	8.40 ± 0.56^{a}	8.61 ± 0.55^{a}	12.13 ± 1.24^{b}	9.23 ± 0.39^{a}	10.01 ± 1.40^{ab}	9.67 ± 0.33^{ab}	8.69 ± 0.45^{a}	8.24 ± 0.33^{a}	9.94 ± 0.08^{ab}
2,3-Butanediol	40 904.25 ± 4.32^{f}	37 992.54 ± 2.07^{d}	40 809.44 ± 0.79^{e}	30 733.92 ± 0.12^{a}	70 986.81 ± 3.10^{h}	36 528.44 ± 0.80^{c}	40 906.34 ± 0.93^{f}	43 485.83 ± 4.48^{g}	110 913.62 ± 1.95^{i}	32 735.72 ± 0.40^{b}
1-Hexanol	495.18 ± 8.01^{h}	414.72 ± 1.01^{b}	403.64 ± 1.92^{a}	525.28 ± 1.03^{i}	450.96 ± 1.47^{e}	439.69 ± 1.86^{d}	467.74 ± 1.79^{f}	406.77 ± 1.75^{a}	425.03 ± 1.37^{c}	476.09 ± 2.71^{g}
Total of higher alcohols	147 238.3 ± 0.2^{bc}	142 089.7 ± 4.5^{b}	161 404.7 ± 10.5^{d}	145 424.4 ± 15.9^{bc}	173 715.5 ± 14.6^{de}	153 724.8 ± 18.7^{c}	152 204.5 ± 17.4^{c}	152 510.9 ± 17.1^{c}	226 043.8 ± 13.6^{e}	125 575.5 ± 12.3^{a}
Ethyl butanoate	**334.85 ± 3.21^{h}**	**308.18 ± 0.25^{f}**	**232.48 ± 0.67^{a}**	**293.35 ± 0.49^{d}**	**336.49 ± 0.69^{h}**	**290.10 ± 0.14^{c}**	**297.09 ± 0.13^{e}**	**270.06 ± 0.08^{b}**	**293.16 ± 0.22^{d}**	**327.33 ± 0.47^{g}**
Ethyl decanoate	**277.97 ± 0.62^{a}**	**401.11 ± 0.16^{i}**	**280.02 ± 0.03^{b}**	**324.35 ± 0.49^{d}**	**331.44 ± 0.62^{e}**	**374.44 ± 0.62^{g}**	**378.10 ± 0.13^{h}**	**285.25 ± 0.35^{b}**	**313.44 ± 0.62^{c}**	**354.45 ± 0.64^{f}**
Ethyl hexanoate	**182.23 ± 0.18^{d}**	**181.50 ± 0.70^{d}**	**366.17 ± 0.23^{i}**	**160.36 ± 0.50^{b}**	**219.33 ± 0.04^{c}**	**81.05 ± 0.06^{a}**	**164.22 ± 0.31^{c}**	**293.17 ± 0.24^{h}**	**266.36 ± 0.50^{g}**	**256.2 ± 0.28^{f}**
Ethyl octanoate	**88.67 ± 2.32^{d}**	**90.64 ± 5.32^{de}**	**25.11 ± 1.22^{b}**	**94.21 ± 5.32^{de}**	**15.09 ± 6.34^{a}**	**31.11 ± 3.22^{c}**	**20.07 ± 3.12^{a}**	**18.98 ± 4.22^{a}**	**91.45 ± 4.55d^{e}**	**95.80 ± 1.23^{e}**
Hexyl acetate	33.07 ± 0.42^{a}	36.27 ± 0.37^{b}	98.06 ± 0.08^{e}	36.14 ± 0.19^{b}	44.15 ± 0.21^{d}	35.09 ± 0.13^{b}	44.41 ± 0.58^{d}	43.04 ± 0.05^{cd}	42.36 ± 0.50^{c}	39.37 ± 0.52^{b}
Ethyl acetate	1 375.88 ± 1.03^{c}	1 293.28 ± 0.39^{c}	1 264.03 ± 0.04^{b}	1 417.22 ± 0.31^{g}	1 317.05 ± 0.07^{d}	1 374.13 ± 0.18^{e}	1 238.08 ± 0.11^{a}	1 263.32 ± 0.45^{b}	1 383.15 ± 0.21^{f}	1 463.20 ± 0.28^{h}
Phenethyl acetate	78.35 ± 0.08^{a}	100.05 ± 0.07^{f}	81.31 ± 0.44^{b}	98.30 ± 0.42^{e}	99.36 ± 0.50^{ef}	78.01 ± 0.01^{a}	108.19 ± 0.27^{g}	87.36 ± 0.51^{c}	89.31 ± 0.43^{d}	117.04 ± 0.05^{h}
Diethyl succinate	363.37 ± 0.35^{h}	302.14 ± 0.20^{a}	353.09 ± 0.13^{g}	349.20 ± 0.28^{f}	332.16 ± 0.22^{c}	320.09 ± 0.12^{b}	347.13 ± 0.18^{e}	363.25 ± 0.35^{h}	346.19 ± 0.26^{d}	365.21 ± 0.30^{i}
Total of esters	2 756.35 ± 0.66^{b}	2 782.87 ± 3.20^{b}	2 772.32 ± 3.53^{b}	2 839.87 ± 2.32^{c}	2 757.14 ± 4.97^{b}	2 648.45 ± 2.41^{a}	2 666.89 ± 2.91^{a}	2 695.45 ± 3.93^{a}	2 888.74 ± 2.98^{c}	3 076.83 ± 3.97^{d}
Octanoic acid	**655.37 ± 0.02^{e}**	**412.17 ± 0.24^{a}**	**537.59 ± 0.59^{f}**	**977.95 ± 1.34^{h}**	**430.31 ± 0.44^{b}**	**483.23 ± 0.33^{d}**	**464.14 ± 0.19^{c}**	**1 221.05 ± 0.07^{i}**	**667.5 ± 0.70^{g}**	**517.37 ± 0.52^{e}**
Hexanoic acid	**2 615.07 ± 0.09^{a}**	**2 671.46 ± 0.65^{b}**	**3 182.35 ± 0.49^{e}**	**5 688.32 ± 0.45^{h}**	**2 617.18 ± 0.25^{a}**	**3 063.48 ± 0.67^{d}**	**2 958.31 ± 0.44^{c}**	**6 735.44 ± 0.62^{i}**	**3 808.55 ± 0.78^{g}**	**3 298.07 ± 0.09^{f}**
Decanoic acid	532.20 ± 1.01^{c}	471.47 ± 0.76^{a}	485.42 ± 5.59^{b}	533.26 ± 2.37^{c}	591.01 ± 2.41^{e}	482.10 ± 3.13^{b}	594.06 ± 4.18^{e}	640.52 ± 0.74^{f}	646.18 ± 0.25^{g}	543.34 ± 6.48^{d}
Total of fatty acids	3 802.61 ± 0.05^{b}	3 555.16 ± 0.12^{a}	4 205.45 ± 1.54^{c}	7 199.52 ± 0.32^{e}	3 638.54 ± 0.40^{ab}	4 028.80 ± 0.80^{c}	4 016.52 ± 1.10^{c}	8 597.01 ± 1.40^{f}	5 122.21 ± 0.90^{d}	4 358.82 ± 2.00^{c}
Phenylacetaldehyde	**447.33 ± 1.01^{b}**	**489.47 ± 0.66^{f}**	**448.2 ± 1.13^{b}**	**442.95 ± 0.07^{a}**	**513.16 ± 0.22^{g}**	**488.19 ± 0.26^{f}**	**468.96 ± 1.36^{d}**	**473.24 ± 0.33^{e}**	**460.37 ± 0.52^{c}**	**471.21 ± 0.30^{de}**
Nonanal	9.16 ± 0.01^{a}	11.14 ± 0.2^{bc}	11.18 ± 0.25^{bc}	9.15 ± 0.21^{a}	9.36 ± 0.5^{a}	10.37 ± 0.52^{ab}	11.11 ± 0.15^{bc}	10.37 ± 0.52^{ab}	11.41 ± 0.58^{bc}	12.17 ± 0.23^{c}
Acetoin	6.29 ± 0.19^{a}	6.3 ± 0.42^{a}	10.26 ± 0.37^{c}	9.14 ± 0.20^{bc}	39.24 ± 0.34^{e}	12.82 ± 0.26^{d}	12.79 ± 0.3^{d}	9.09 ± 0.12^{b}	44.86 ± 0.21^{f}	6.28 ± 0.35^{a}
Total of aldehydes	604.95 ± 0.25^{ab}	656.21 ± 1.37^{cd}	610.69 ± 2.2^{b}	589.56 ± 0.82^{a}	708.87 ± 0.95^{e}	667.41 ± 0.82^{d}	639.32 ± 2.01^{c}	634.71 ± 0.95^{c}	652.09 ± 1.78^{cd}	620.96 ± 1.01^{bc}
Trans-rose oxide	**146.31 ± 0.34^{c}**	**143.62 ± 0.87^{b}**	**160.56 ± 0.78^{e}**	**136.83 ± 0.25^{a}**	**147.86 ± 0.21^{c}**	**153.24 ± 0.33^{d}**	**160.98 ± 0.04^{e}**	**142.01 ± 0.01^{b}**	**143.53 ± 0.74^{b}**	**168.14 ± 0.19^{f}**
β-Citronellol	14.17 ± 0.13^{a}	14.17 ± 0.23^{a}	14.16 ± 0.23^{a}	14.18 ± 0.25^{a}	14.18 ± 0.25^{a}	14.18 ± 0.25^{a}	14.17 ± 0.24^{a}	14.16 ± 0.23^{a}	14.18 ± 0.25^{a}	14.18 ± 0.25^{a}
1-Terpinen-4-ol	153.09 ± 0.42^{d}	140.44 ± 0.62^{a}	185.11 ± 0.16^{h}	145.17 ± 0.24^{b}	155.87 ± 0.19^{e}	157.9 ± 0.14^{f}	146.02 ± 0.03^{b}	149.85 ± 0.21^{c}	174.97 ± 0.04^{g}	186.59 ± 0.12^{i}
Linalool	0.53 ± 0.06^{c}	0.51 ± 0.01^{c}	0.17 ± 0.02^{b}	1.09 ± 0.02^{e}	0.82 ± 0.06^{d}	0.06 ± 0.08^{a}	0.13 ± 0.08^{b}	0.07 ± 0.01^{a}	0.22 ± 0.01^{b}	0.94 ± 0.08^{d}
Total of terpenes	321.62 ± 0.30^{a}	310.98 ± 3.64^{a}	360.65 ± 3.61^{b}	312.32 ± 3.94^{a}	319.69 ± 0.02^{a}	337.22 ± 0.30^{ab}	334.39 ± 0.28^{ab}	320.08 ± 1.13^{a}	450.68 ± 0.33^{c}	370.71 ± 0.29^{b}
β-Damascenone	**97.46 ± 0.14^{c}**	**86.04 ± 0.06^{a}**	**109.08 ± 0.11^{h}**	**104.11 ± 0.15^{g}**	**88.85 ± 0.22^{b}**	**92.77 ± 0.33^{d}**	**90.15 ± 0.21^{c}**	**99.97 ± 0.05^{f}**	**107.96 ± 0.94^{h}**	**113.92 ± 0.12^{i}**

Notes: The aroma compounds (OVA > 1) were highlighted, other volatile compounds (OVA > 0.1) were underlined. Values are given as $\bar{x} \pm s$ deviation of two biological replicates and three HPLC detection runs. Same as Table 4.

threshold. *β*-Damascenone is the key odorant in Vidal icewine, its content varied with the inoculated yeast strains. SC45 produced the highest value (113. 92 μg/L), which was 16.9% higher than that of DV10, followed by SC28 (109.08 μg/L), SC42 (107.96 μg/L) and SC33 (104.11 μg/L) strain. *Trans*-rose oxide and *cis*-rose oxide can generate lychee and rose smell to Vidal icewine[28]. The commercial strains EC1118 and V1116 produced around 46 μg/L *cis*-rose oxide in Canada Vidal icewine[7]. In this study, 168.14 μg/L *trans*-rose oxide was produced by SC45, followed by SC28 (160.56 μg/L), which were higher than those of commercial strains. These results were in disagreement with the data of Synos et al.[9], who proposed that native yeasts produced less monoterpenes than commercial strains in Cabernet franc icewine fermentation. The inconsistency might be due to the differences in grape variety and the inoculated yeast strains.

To further highlight the important role of different yeast strains on aroma quality, principal component analysis (PCA) was applied using glycerol, acetic acid and the aromatic compound that OAV exceeding one (Table 1 and 2). As showed in Fig. 3, the first and second PCs explained 33.78% (PC1) and 21.81% (PC2) of the variance, which grouped ten strains roughly into 4 clusters. The wines fermented by SC28, SC33, SC41 and SC42 were located in the positive part of PC1, which were positive with isobutanol, isopentanol, 2-phenylethanol, 1-octen-3-ol, ethyl hexanoate, octanoic acid, hexanoic acid, *β*-damascenone and glycerol. Other strains were placed in the negative part of PC1 and were associated with ethyl butanoate, ethyl decanoate, phenylacetaldehyde, *trans*-rose oxide and acetic acid. PC2 (21.81%) differentiated SC33, SC41, SC42, SC45 and DV10 with other strains. The wines produced by SC45 and

DV10 located together in the upper left quadrant. The wines of SC33, SC41 and SC42 were located in the upper right quadrant. The main principal components responsible for the differences were ethyl butanoate, ethyl octanoate, octanoic acid, β-damascenone and glycerol. These analyses allowed us to identify SC33, SC42 and SC45 as suitable strains based on their diversified aromatic profiles. SC33 generated high level of higher alcohol, fatty acid and β-damascenone, SC42 formed the highest contents of higher alcohol with the lowest acetic acid, while SC45 strain produced the highest levels of esters, phenylacetaldehyde, β-damascenone and *trans*-rose oxide. In the analysis of microsatellite PCR fingerprinting, SC33 and SC45 strains were clustered into genotype group I, while SC42 was located in III group alone. These results suggested that genetic diversity is extensively presented in wine strains of *S. cerevisiae*[30]. Using three microsatellite loci might be not enough to accurately determine the genetic background of wine yeast, other analysis such as restriction analysis of mitochondrial DNA and comparative analysis of the karyotype should be simultaneously applied.

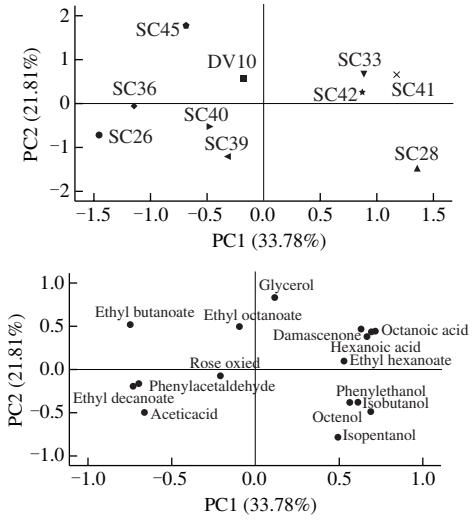


Fig. 3 PCA of wines resulting from glycerol, acetic acid and aromatic compounds with OAV exceeding one produced by ten *S. cerevisiae* yeasts

2.3 Micro-vinifications in 3-L fermenter

The data of flask fermentation allowed us to select SC33, SC42 and SC45 as the potential strains for the next round of evaluation. Their oenological traits were investigated in 3 L glass fermenter with DV10 as reference strain. The parameters related to cell growth, chemical composition and aroma compounds were shown in Table 3 and Table 4, respectively. It was interesting to find that the increments of fermentation volume had a significant effect on these parameters. Different from flask trails, indigenous strains in fermenter showed higher cell growth rate but had no significant difference in maximum biomass compared to DV10. 150.26–155.57 g/L sugar were left in fermenter wines, while these values in flask wines were ranged in 160.0–165.0 g/L. Glycerol contents were increased to 12.91 (DV10) to 13.92 g/L (SC45), and acetic acid level was decreased to 1.27 g/L (SC42) to 1.63 g/L (SC33) in fermenter wines compared to 10.44–11.91 g/L glycerol and 1.63–1.70 g/L acetic acid in flask wines, respectively. The increased concentrations were also found in ethanol.

Table 3 Cell growths and physicochemical parameters of wines fermented by three selected *S. cerevisiae* yeasts and commercial yeast DV10

Index	DV10	SC33	SC42	SC45
V_{max}/(g/(L·h))	0.207	0.323	0.316	0.262
Maxmium biomass/(CFU/mL)	2.44×10^7	2.35×10^7	2.31×10^7	2.38×10^7
Residual reducing sugar/(g/L)	150.26 ± 0.12^a	154.49 ± 0.13^b	151.43 ± 0.76^a	155.57 ± 0.92^b
Glycerol/(g/L)	12.91 ± 0.11^a	13.04 ± 0.01^a	13.4 ± 0.06^b	13.92 ± 0.01^c
Ethanol/%	12.08 ± 0.03^a	12.07 ± 0.05^a	12.10 ± 0.09^a	11.97 ± 0.12^a
Acetic acid/(g/L)	1.40 ± 0.03^b	1.63 ± 0.03^d	1.27 ± 0.02^a	1.54 ± 0.03^c
Oxalic acid/(g/L)	0.22 ± 0.01^a	0.23 ± 0.01^a	0.23 ± 0.02^a	0.22 ± 0.01^a
Citric acid/(g/L)	0.62 ± 0.06^a	0.63 ± 0.05^a	0.68 ± 0.03^a	0.65 ± 0.03^a
Malic acid/(g/L)	11.16 ± 0.31^a	11.18 ± 0.11^a	11.63 ± 0.19^a	11.35 ± 0.42^a
Succinic acid/(g/L)	3.63 ± 0.19^a	3.69 ± 0.20^a	3.71 ± 0.26^a	3.69 ± 0.13^a
Lactic acid/(g/L)	1.25 ± 0.08^a	1.21 ± 0.07^a	1.22 ± 0.08^a	1.23 ± 0.05^a

The aroma quality was improved by the increment of fermentation volume. Most volatiles including higher alcohol, esters, fatty acids, terpenes and β-damascenone in fermenter wines were higher than those of flask trials, especially higher alcohols in SC45 fermentation, the total content was around 1.5 folds higher than that of flask wine. Geraniol, an important desired terpene, reached its threshold in fermenter wine, and gives Vidal icewine the floral characteristic with rose smell[28]. With regards to the impact of individual stain, SC45 was still featured with higher desired aroma quality with higher contents of higher alcohol, esters, *trans*-rose oxide and β-damascenone, which was followed by SC42 that generating relatively higher content of esters, terpene and β-damascenone with the lowest amount of acetic acid. DV10 was characteristic with higher contents of higher alcohol, especially isobutanol, isopentanol and (*Z*)-3-hexen-1-ol. PCA was applied to classify the 4 strains into 3 groups using glycerol, acetic acid and the aromatic compound that OAV exceed 1. SC33 and SC42 are positioned together at the low left quadrant on the PCA plot, while SC45 and DV10 were separated and located in the upper right and left quadrant, respectively. These data were different from the results of flask fermentation, in which SC45 and DV10 strains were

clustered together. This confirmed that the alteration of fermentation volume has a comprehensive effect on aroma compound formation.

Table 4 Contents of 28 main volatile compounds in wines fermented by 3 selected *S. cerevisiae* yeasts and commercial yeast DV10

μg/L

Compounds	DV10	SC33	SC42	SC45
Isobutanol	**115 516.65 ± 0.66^{d}**	**804 448.95 ± 1.13^{a}**	**89 379.19 ± 0.50^{b}**	**96 635.02 ± 0.83^{c}**
Isopentanol	**76 161.02 ± 4.20^{d}**	**61 938.13 ± 4.94^{a}**	**66 522.15 ± 1.87^{b}**	**70 134.74 ± 0.95^{c}**
2-Phenylethanol	**14 546.69 ± 0.49^{a}**	**15 464.80 ± 5.67^{b}**	**15 806.28 ± 0.26^{c}**	**21 916.14 ± 4.36^{d}**
1-Octen-3-ol	**94.83 ± 2.74^{a}**	**113.83 ± 0.05^{b}**	**116.42 ± 0.36^{b}**	**116.52 ± 0.27^{b}**
(*Z*)-3-Hexen-1-ol	315.71 ± 3.76^{c}	274.35 ± 0.56^{a}	272.12 ± 0.06^{a}	293.63 ± 0.47^{b}
1-Octanol	9.86 ± 0.56^{a}	10.90 ± 0.01^{b}	10.52 ± 0.32^{ab}	10.36 ± 0.32^{ab}
2-Octanol	10.80 ± 0.12^{a}	11.32 ± 0.23^{ab}	11.39 ± 0.26^{ab}	11.62 ± 0.40^{b}
2,3-Butanediol	69 016.62 ± 2.80^{c}	66 384.75 ± 5.04^{a}	66 897.77 ± 1.55^{b}	122 911.48 ± 1.79^{d}
1-Hexanol	580.55 ± 0.16^{d}	538.90 ± 1.92^{b}	520.55 ± 0.47^{a}	553.42 ± 1.57^{c}
Total of higher alcohols	276 105.67 ± 12.65^{c}	225 353.25 ± 1.95^{a}	239 705.58 ± 6.75^{b}	312 794.40 ± 2.80^{d}
Ethyl butanoate	**282.12 ± 0.41^{c}**	**259.45 ± 0.49^{b}**	**247.45 ± 0.54^{a}**	**284.05 ± 1.14^{c}**
Ethyl decanoate	**344.93 ± 0.08^{c}**	**263.85 ± 1.33^{b}**	**255.01 ± 0.45^{a}**	**482.63 ± 0.33^{d}**
Ethyl hexanoate	**118.15 ± 0.60^{b}**	**129.12 ± 0.51^{c}**	**100.73 ± 0.34^{a}**	**139.43 ± 0.02^{d}**
Ethyl octanoate	**915.44 ± 0.43^{c}**	**821.70 ± 0.65^{b}**	**615.39 ± 0.10^{a}**	**966.97 ± 0.28^{d}**
Hexyl acetate	56.87 ± 0.02^{a}	68.82 ± 0.20^{d}	61.71 ± 0.37^{b}	67.39 ± 0.32^{c}
Ethyl acetate	1 467.35 ± 0.98^{d}	1 151.74 ± 0.92^{a}	1 158.53 ± 0.35^{b}	1 350.49 ± 0.33^{c}
Phenethyl acetate	70.07 ± 0.04^{b}	67.53 ± 0.42^{a}	78.95 ± 1.19^{c}	84.55 ± 0.32^{d}
Diethyl succinate	363.80 ± 0.59^{b}	358.39 ± 0.96^{a}	362.60 ± 0.15^{b}	455.69 ± 0.55^{c}
Total of esters	37 13.58 ± 1.00^{c}	3 221.15 ± 2.41^{b}	2 986.29 ± 0.34^{a}	3 925.84 ± 0.84^{d}
Octanoic acid	**918.89 ± 0.34^{a}**	**1 183.53 ± 4.58^{c}**	**1 084.56 ± 0.44^{b}**	**1 414.98 ± 0.41^{d}**
Hexanoic acid	**2 358.38 ± 5.87^{b}**	**2 321.13 ± 10.79^{a}**	**2 467.59 ± 0.54^{c}**	**3 066.11 ± 6.20^{d}**
Decanoic acid	278.38 ± 0.25^{a}	688.86 ± 9.81^{c}	347.85 ± 1.24^{b}	776.91 ± 4.20^{d}
Total of fatty acids	3 555.65 ± 6.06^{d}	4 193.52 ± 16.03^{b}	3 900.12 ± 1.19^{c}	5 258.00 ± 2.42^{a}
Phenylacetaldehyde	**468.66 ± 0.48^{b}**	**474.83 ± 4.10^{c}**	**463.72 ± 3.68^{ab}**	**453.62 ± 1.67^{a}**
Nonanal	10.46 ± 0.46^{a}	10.18 ± 0.39^{a}	10.64 ± 0.47^{a}	11.21 ± 0.35^{a}
Acetoin	25.59 ± 0.51^{b}	22.29 ± 0.38^{a}	22.37 ± 0.50^{a}	24.75 ± 0.12^{b}
Total of aldehydes	661.67 ± 0.64^{a}	681.70 ± 4.07^{a}	671.83 ± 7.28^{a}	657.65 ± 2.64^{a}
Trans-rose oxide	**155.01 ± 0.15^{c}**	**131.21 ± 1.48^{a}**	**148.15 ± 1.1^{b}**	**208.26 ± 2.61^{d}**
Geraniol	**271.43 ± 0.59^{b}**	**271.85 ± 0.01^{b}**	**253.29 ± 0.79^{a}**	**273.52 ± 2.37^{b}**
β-Citronellol	14.39 ± 0.03^{a}	14.40 ± 0.03^{a}	14.42 ± 0.01^{a}	14.35 ± 0.15^{a}
1-Terpinen-4-ol	603.83 ± 0.24^{b}	578.14 ± 0.65^{a}	584.63 ± 1.49^{a}	625.37 ± 5.69^{c}
Linalool	0.33 ± 0.01^{a}	0.34 ± 0.07^{a}	0.26 ± 0.02^{a}	0.65 ± 0.01^{b}
Total of terpenes	1 071.80 ± 1.08^{c}	1 022.69 ± 4.48^{b}	1 014.27 ± 2.65^{a}	1 130.85 ± 6.46^{d}
β-Damascenone	**87.38 ± 0.53^{a}**	**112.78 ± 2.97^{b}**	**109.40 ± 0.50^{b}**	**128.31 ± 1.41^{c}**

An aromatic series could be defined as a group of volatile compounds with similar odor descriptors[31]. In the fermenter trials, 14 aroma compounds showed an OAV above 1 (Table 4). According to previous researches[22,32-34], these compounds associated with the attributes 'banana', 'green apple', 'citrus', 'sweet', 'pear', 'pineapple', 'lemon', 'rose', 'fruity', 'fatty', 'rancid', 'nail polish', 'alcohol', 'balsamic', 'floral' and 'green'. To better understand the influence of different strains on wine odor profile, an aromatic series was established by combination of OAVs of different volatiles with similar odor descriptions[31,33]. 6 Aromatic series of volatile compounds were established, including fruity, floral, sweet, herbaceous, chemical and fatty (Fig. 4). Aroma compounds (OVA > 1) are calculated for floral series: ethyl octanoate, 2-phenylethanol, phenylacetaldehyde, *trans*-rose oxide, geraniol, *β*-damascenone; for fruity series: ethyl butyrate, ethyl decanoate, ethyl hexanoate, ethyl octanoate, *β*-damascenone; for sweet series: ethyl octanoate, 2-phenylethanol, phenylacetaldehyde, *β*-damascenone; for herbaceous series: isobutanol, (*Z*)-3-hexen-1-ol; for chemical series: isobutanol, isopentanol, 1-octen-3-ol; for fatty series: ethyl decanoate, isopentanol, (*Z*)-3-hexen-1-ol, hexanoic acid, octanoic acid. Of these, the floral, fruity and sweet series were prominent, followed by the fatty, chemical and herbaceous series. Generally, indigenous strains produced higher values of 6 aroma series than those of DV10, especially SC45 strain, which produced the highest floral, fruity and sweet series due to generating more fatty acid ethyl esters, 2-phenylethanol, phenylacetaldehyde, *trans*-rose oxide, geraniol and *β*-damascenone. SC42 also achieved higher floral and sweet series. Higher fatty series was always achieved in isolated strains fermentation due to their higher ability to form fatty acid compared to DV10.

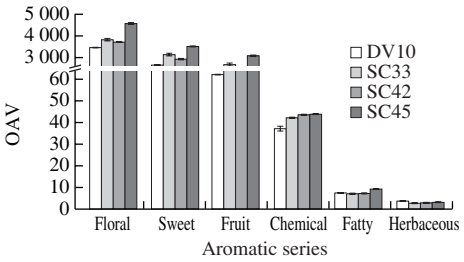


Fig. 4 Aroma series in wines produced by DV10, SC33, SC42 and SC45

3 Discussions

To produce the wine product with unique regional character, in recent years, there is an increasing trend to use native strains as oenological starter cultures for wine production. In this study, to select suitable indigenous yeast for Vidal icewine production in Huanren region of China, we conducted spontaneous icewine fermentation and isolated nine potential *Saccharomyces* strains. The results showed that all the isolated strains can successfully finish the alcoholic fermentation and produced desired and different aroma profiles comparable to that of commercial strain DV10. Finally, the strain SC42 and SC45, featured with an interesting fermentation kinetics, good fermentative vigor, low acetic acid and higher production of desired aroma compounds, were selected as the potential strain for the possible industrial application in the future work.

It should be mentioned that in the determination of oenological traits, we found that the chemical compositions (sugar, glycerol, acetic acid and ethanol contents) and most volatile compounds in flask fermented wines by the same yeast strain were not correspondingly consistent with those of fermenter wines. This suggested that the variation of fermentation volume have a major effect on the formation of aromatic compounds in wine. The similar result has also been reported by Regodón et al.[14], who ascribed the inconsistent results between 2 L fermenter and 50 mL fermentations to the frequent manipulation of the flasks for the sampling. The conditions in flask trial favor oxidative metabolism, which leads to greater volatile acid production. Considering the data that less sugar left in fermenter, we preferred to the assumption that the dissolved oxygen concentration in juice was improved in fermenter, which led to increased fermentation activity and alters the formations of chemical composition and aroma compounds. The detailed mechanism needs to be further investigated.

Importantly, our data proved the assumption that the extreme stress (high sugar concentration 400 g/L and low temperature 15–17 ℃ combined with ethanol and SO_2 toxicity) in icewine spontaneous fermentation can provide a good selective pressure on natural microflora, which can increase the possibility of selecting suitable wine strains. Obviously, compared to the conventional procedure, this method is relatively simple. Screening suitable wine yeast is a time consuming and laborious procedures, sometimes needed sophisticated instruments, which is inconvenient for winemakers in wine enterprises. Our validated data provided a simple and feasible selection procedure for winemaker to screen potential icewine yeast strains for industrial production, at least, could simplify the screening process. Certainly, the oenological features of the selected suitable strain needed to be further extensively investigated in large fermenter before the industrial application.

4 Conclusions

A rapid selection procedure was proposed in this study. By determining the tolerance to ethanol (15%) and SO_2 (100 mg/L), respectively, nine candidate *Saccharomyces* strains were preselected from 78 strains isolated from different stages of Vidal icewine spontaneous fermentation. The determination of oenological traits in flask and fermenter indicated that all isolated strains can complete the alcoholic fermentation and showed diversified and desired aroma profiles compared to commercial strain DV10. 2 Strains SC45 and SC42 isolated at the final stage of spontaneous fermentation and featured with high fermentation activity and diversified aromatic qualities were finally selected as potential industrial strains. Collectively, our results suggested that selection and application of indigenous *Saccharomyces* strains is a feasible way to improve aroma quality and diversity of icewines, and produce the icewine products with unique regional characters.

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