Evaluation of the volatile profile of wax apple (*Syzygium samarangense*) wines fermented with different commercial *Saccharomyces cerevisiae* strains

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Abstract The effect of four commercial *Saccharomyces cerevisiae* strains (D254, VIC, BV818, and RV100) on the volatile profile of wax apple (*Syzygium samarangense*) wine was investigated in this study. Alcohols and esters were the most two abundant groups of identified volatiles in wax apple wines. However, different *S. cerevisiae* strains possess various capacities in releasing/synthesizing volatiles with varied mRNA levels of genes involved in volatiles metabolism during wax apple wine fermentation. VIC, which yielded the highest total concentration of volatiles and largest number of volatiles with odor activity value (OAV) > 1, could be used as a starter culture to produce wax apple wine characterized with intense aroma. D254 and RV100, which produced the greatest variety of volatiles and scored the highest in global aroma,

respectively, could be used to enhance the wine complexity. Four wax apple wines could be differentiated by their main volatile compounds.

Keywords *Saccharomyces cerevisiae* · Volatile compound · Fruit wine · Wax apple · Fermentation

Introduction

Wax apple (*Syzygium samarangense*), which belongs to the *Myrtaceae* family, is widely planted in Malaysia, India, and southern China (Hao et al., 2016). Wax apple fruits are typically pink or light red in color, contain a water content of up to 90% and have a juicy and refreshing taste. Moreover, wax apple is considered significantly beneficial to human health because it is a rich source of phytochemicals including ellagitannins, proanthocyanidins, flavanones, anthocyanidins, flavonol glycosides, triterpenoids, chalcones, and volatile terpenoids (Huang et al., 2016). Wax apple is ordinarily consumed when fresh, but its availability is limited by its seasonal fruiting characteristic and finite shelf-life. It is thus essential to develop wax apple production to add value to the wax apple fruit. One method is turning it into fruit wine. Fruit wines are popular for their strong fruit aroma. Developing wax apple wine can not only reduce post-harvest losses of excess fruits, but also meet the demand of diversity for wine market.

Aroma is an important factor that determines the flavor and quality of the final fruit wine product (Jiang and Zhang, 2010). It is well known that the fruit variety, maturity, yeast strain, brewing condition, etc. could affect the aroma components. Among the factors, yeast strain plays a central role in the formation of aroma and flavor of

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fruit wine through a series of biochemical mechanisms during alcoholic fermentation (Câmara et al., 2006; Takush and Osborne, 2012). Different yeast strains could bring about distinguishing flavor profiles that affect the sensory properties of wines despite the same fermentation (Xiao et al., 2015). Nowadays, a wide range of wine yeast strains are commercially available. Wine industry is particularly focus on the yeast strains that could produce the wine of interest. However, to the best of our knowledge, reports on aroma of wax apple wine and the effect of yeast strains on the profile of volatile components of wax apple wine have not appeared in international journals.

The objective of this study was to determine and comparatively analyze the volatile profile of wax apple wines yielded by four commercial *S. cerevisiae* strains (D254, VIC, BV818, and RV100).

Materials and methods

Yeast strains

Four commercial *S. cerevisiae* strains were used in this test. Strain D254 was purchased from LALVIN (Fredericia, Denmark). Strains VIC, BV818, and RV100 were purchased from Angel (Yichang, China).

Fruit juice preparation and fermentation conditions

Wax apple cv. KingBox was purchased from the local fruit market. The cleaned wax apple was pressed using a juice extractor (A9, SKG, Foshan, China) and added with 100 mg/L of sodium metabisulphite to avoid bacterial contamination.

The wax apple juice had a soluble solid level of 13°Brix, a pH value of 4.18 and total acidity 2.48 g/L (expressed as g/L of tartaric acid). The fruit juice was adjusted to a total soluble solid of 22°Brix and a final pH of 3.80 using sucrose and citric acid, respectively. 200 mg/L of activated (hydration in 5% of glucose solution at 37 °C for 30 min) commercial yeast strains were added to the juice, and the main fermentation was conducted at 22 °C without stirring. When the residual sugar was lower than 4 g/L, the wines were centrifuged at 1500×g for 20 min, and the supernatants were sampled. All fermentations were performed in triplicates.

Physicochemical properties analysis

The general physicochemical properties of the wines were analyzed according to Zhu et al. (2014). Total soluble solid was assessed using a hand refractometer (WYT, Hao-Chuang, ChengDu, China). Residual sugar and acidity

were determined by titration, and the results were expressed as glucose and tartaric acid, respectively. Volatile acidity was obtained by distillation combined with titration, and the results were expressed as acetic acid. Alcoholic content was obtained by distillation combined with a portable densimeter (DA-130 N, KEM, Tokyo, Japan). Similarity, total acid of wax apple juice was determined by titration, and the result was expressed as tartaric acid. pH of wax apple juice was determined by a pH meter (SevenEasy S20, METTLER TOLEDO, Zurich, Switzerland). Each measurement was conducted three times.

Volatile compounds analysis

The volatile compounds of the wax apple wines (kept at 18 °C for 15 days) were extracted by headspace-solid phase microextraction (HS-SPME) and analyzed by gas chromatography-mass spectrometry (GC-MS) as previously described by Zhang et al. (2007). Each sample (5 g) was placed in a 20 mL vial sealed with silicone septa (Sigma Chemical Co., Saint Louis, MO, USA). Exactly 50 µL of 1,3-dichlorobenzene (100 mg/L) was used as the internal standard. Before extraction, the SPME fibre (75 µm CAR/PDMS, Supelco, Bellefonte, PA, USA) was inserted into the GC injector at 250 °C for 30 min. Then, the fibre was exposed to the headspace over the sample, where extraction was allowed to occur in a thermostatted bath adjusted to 60 °C with continued agitation by a magnetic stirrer for 30 min. Finally, the fibre was desorbed in the GC injector for 3 min.

Compound analysis was performed using an Agilent 7890A GC (Agilent Technologies Inc., Santa Clara, CA, USA) coupled with an Agilent 5975C mass spectrometer system equipped with a HP-5x capillary column (60 m × 0.25 mm inner diameter, 0.25 µm film thickness, Agilent Technologies Inc.). Helium (purity 99.999%) was used as the carrier gas at 1.0 mL/min. Desorption of analytes from the SPME fibre occurred at 250 °C for 3 min under a splitless mode. The oven was held at an initial temperature of 50 °C, and subsequently raised to 300 °C at a rate of 3 °C/min and held for 5 min. The mass spectra were obtained in an electron impact mode of 70 eV, and the range of mass (m/z) was scanned from 30 to 450 atomic mass units. The volatile compounds were identified by comparing retention indices and retention times with those obtained for authentic standards, or those of literature data, or with mass spectra in the Wiley7n Database (Agilent Technologies Inc.).

OAVs

The contribution of each aromatic compound to the overall flavor of the wines was evaluated by the odor activity

value, which was calculated by the ratio of the concentration of a compound to its odor threshold value (Velázquez et al., 2015).

Sensory analysis

The fermented wax apple wines were evaluated by a well-trained panel of 16 members (7 males and 9 females, among 20–50 years of age). 20 mL of wine sample was presented to panelists in plastic cups at room temperature of 22 ± 1 °C. Six sensory terms including fruity, sweet, sweaty, fatty, sulphury and global aroma were selected by the panelists during discussion to describe and differentiate the samples, and a scale from 0 to 5 was used to score each attribute's intensity, where 0 indicated that the descriptor was not perceived, and the intensity of values 1–5 was gradually enhanced.

Real-time quantitative PCR (qRT-PCR)

Cultures were sampled from wax apple juice at 16 h and the expression levels of genes *BAT1*, *BAT2*, *PDC1*, *PDC5*, *ADH2*, *ATF1*, *ATF2*, *EHT1*, *EEB1*, and *IAH1* were assessed. The total cellular RNA was extracted using a yeast RNA kit (R6870, Omega, Madison, WI, USA). By utilizing mRNA as a template, cDNA was synthesized using a FastQuant RT Kit (KR106, TIANGEN, Beijing, China). Changes in gene expression levels were assessed through qRT-PCR using an Ultra SYBR Two-Step qRT-PCR kit with ROX (reference dye for real-time PCR, TIANGEN). Actin was used as the loading control. The primers used for amplifying the target genes and the reference gene *ACT1* are listed in Table S1 in supplementary material. The expression level of the target genes in the strain D254 was normalized to the reference gene. Experiments were conducted thrice.

Statistical analysis

Statistical analyses were carried out using the SPSS version 18.0 software. ANOVA and Duncan's multiple range tests were applied to the data to determine significant differences. Differences at $P < 0.05$ were considered statistically significant. Principal component analysis (PCA) was applied to establish the relation between wine samples and volatile compounds using PLS_TOOLBOX 5.02 Version under Matlab 7.0 Version.

Results and discussion

Physicochemical properties of the wax apple wines

The time course of fermentations using four commercial *S. cerevisiae* strains is shown by the °Brix trend in wax apple wines (Fig. 1). The °Brix value of the four wax apple wines rapidly decreased in the initial stage, but differences in the fermentation time were observed. The shortest fermentation time was observed in RV100-fermented wine, which reached a stable °Brix value of 4%. Relatively long time (8 days) was taken by strain VIC, by which the °Brix value was constant at 5%. General physicochemical properties of the four wax apple wines are shown in Table 1. Strain D254 yielded the highest ethanol content and strain BV818 did the lowest. The highest amount of titratable acidity was observed in RV100-fermented wine, and the lowest content of volatile acidity was found in the wine produced by strain BV818. These results were corresponded with the previous reports for other fruit wines (Sun et al., 2011; Xiao et al., 2015; Zhu et al., 2014). Appropriate acidity and ethanol levels are advantageous to the sensory quality and stability of fruit wines (Iorizzo et al., 2014; Quéré et al., 2006). Some acids in wax apple wine are naturally present in wax apple fruit, while others are byproducts of fermentation (Venkatachalam et al., 2018). The different acidity contents could be resulted from the various metabolic abilities of *S. cerevisiae* strains used in this work in wax apple wine making.

Volatile components and odor activity values

The amount and type of the volatile compounds varied significantly in the wax apple wines produced by four *S.*

Fig. 1 °Brix changes in wax apple wines during fermentation

Table 1 General physicochemical properties of wax apple wines fermented by four *Saccharomyces cerevisiae* strains

	D254	VIC	BV818	RV100
Ethanol (%vol)	12.2 ± 0.05 ^a	12.1 ± 0.05 ^a	10.9 ± 0.04 ^b	12.1 ± 0.06 ^a
Titrateable acidity (g/L)	4.80 ± 0.11 ^b	4.91 ± 0.11 ^b	4.95 ± 0.15 ^b	5.34 ± 0.15 ^a
Volatile acidity (g/L)	0.81 ± 0.05 ^a	0.84 ± 0.05 ^a	0.66 ± 0.05 ^b	0.76 ± 0.04 ^a
Reducing sugar (g/L)	1.71 ± 0.08 ^b	1.34 ± 0.08 ^c	1.15 ± 0.10 ^d	2.27 ± 0.15 ^a

Values shown represent averages of triplicate samples (data are mean ± SD)

Values with different superscript roman letters (a, b, c, d) in the same row are significantly different according to the Duncan test ($p < 0.05$)

cerevisiae strains (Table 2). From the qualitative point of view, great differences of higher alcohols and esters were shown in the four wax apple wines, bringing out the variation in the sum of the volatiles (Fig. S1(A) in supplementary material). In the sight of quantity, alcohols and esters were the most two abundant groups of the identified volatile compounds. The maximum proportion of alcohols was yielded by strain BV818, whereas that of esters was produced by strain RV100 (Fig. S1(B) in supplementary material). The greatest variety and highest total content of volatiles was produced by strains D254 and VIC, respectively. These results demonstrated that different *S. cerevisiae* strains own different capacities in releasing or synthesizing volatiles in wax apple juice.

It is worth mentioning that the contribution of the compounds to the flavor of fruit wines depended on OAVs (Zhu et al., 2014). Sixteen types of compounds showed OAVs > 1 in this test (Table 3). The maximum sum of compounds with OAVs > 1 was produced by strain VIC, probably contributing to the wax apple wine with complex and intense aroma and flavor.

Alcohols were the most abundant group in terms of the amounts of the identified volatile compounds in the four wax apple wines. However, the four wax apple wines exhibited quantitative and qualitative differences in alcohol composition. The wine produced by strain VIC showed the highest concentration of 3-methyl-1-butanol, which produces a whiskey, malt, and burnt odor. 2-Phenylethanol, which gives a honey, spice, rose, and lilac aroma, was the most abundant alcohol in BV818-fermented wine. The concentration of 4-terpineol in the wine fermented with strain D254 was higher than its odor threshold, increasing oil and anise aromas of this wine. The greatest amount of higher alcohols was observed in VIC-fermented wine, in which the variety of higher alcohols was actually the lowest. Conversely, the wax apple wine produced by strain D254 showed the greatest variety (15 types) with the minimum of alcohols concentration. Higher alcohols are beneficial to wine flavor at a total concentration below of 300 mg/L (Duarte et al., 2010). Thus, higher alcohols could positively influence the aroma and flavor of the four wax apple wines in this study. Yeasts contribute

significantly to the profile of higher alcohol in wine (Vilanova and Sieiro, 2006). Higher alcohols produced by yeast can be synthesized by the Harris pathway from carbon source or from the degradation of branched-chain amino acids (BCAAs) via the so-called Ehrlich pathway (Ma et al., 2017). The significant differences in the total alcohol contents of the four wines could be caused by the variation in alcohol metabolic activities of yeast strains.

Esters contained the greatest diversity of the identified volatiles. Ethyl acetate, which provides a pineapple aroma, was the most abundant esters in VIC-fermented wine. Ethyl isobutyrate presenting OAV > 1 was only found in the wine fermented with strain VIC. Therefore, a sweet and rubber aroma could be expected in VIC-fermented wine. Ethyl decanoate, which can generate a grape aroma, uniquely exhibited OAV > 1 in D254-fermented wine. The concentrations of isoamyl acetate, ethyl hexanoate, and ethyl octanoate in the four wax apple wines were higher than their odor thresholds, thereby contributing an intense fruity odor to wax apple wines. Esters can affect wine flavor not only directly but also via complex synergistic interactions (Lytra et al., 2016). The greatest variety of esters was observed in D254-fermented wine, but the maximum concentration of esters was produced by strain VIC. This result suggested that strain VIC had a strong ability to accumulate esters.

Fatty acid is mainly formed during fermentation. At low levels, this group of volatiles could actively contribute to wine flavor. However, it is detrimental to the taste of wine whereby it confers a sweat, sour, and thin taste at high concentrations (Hernanz et al., 2009). The concentrations of acids in D254- and BV818-fermented wines were lower than their odor thresholds, so that this group contributed actively to the flavor of the two wax apple wines. The concentration of isovaleric acid in wines produced by strains VIC and RV100 reached its threshold value, contributing unfavorable sweat, acid, and rancid odors to VIC- and RV100-fermented wines. Fatty acid can be synthesized by malonyl-CoA and acetyl-CoA precursors, and the carbon chain is shortened through β -oxidation. It has been reported that the production of fatty acids is dependent on the yeast strains, the composition of juices, and the

Table 2 Mean concentration ($\mu\text{g/L}$) of volatile compounds detected in wax apple wines fermented by four *S. cerevisiae* strains

Code	Compounds	Odor description*	D254	VIC	BV818	RV100
<i>Alcohols</i>						
A1	Propanol	Alcohol, pungent	ND	17 $\pm$ 1	ND	ND
A2	Isobutanol	Wine, solvent, bitter	1369 $\pm$ 7 ^c	1743 $\pm$ 12 ^a	178 $\pm$ 4 ^d	1618 $\pm$ 6 ^b
A3	1-Butanol	Medicine, fruit	16 $\pm$ 1	ND	ND	ND
A4	3-Methyl-1-butanol	Whiskey, malt, burnt	6866 $\pm$ 54 ^b	17,464 $\pm$ 28 ^a	6545 $\pm$ 15 ^c	5590 $\pm$ 48 ^d
A5	2-Methylbutan-1-ol	Wine, onion	ND	3732 $\pm$ 61 ^a	875 $\pm$ 9 ^c	2769 $\pm$ 37 ^b
A6	(3S)-3-methylpentan-1-ol		8 $\pm$ 0 ^a	ND	8 $\pm$ 1 ^a	ND
A7	2,3-Butanediol		ND	119 $\pm$ 7 ^c	1177 $\pm$ 27 ^a	145 $\pm$ 4 ^b
A8	1-Hexanol	Resin, flower, green	11 $\pm$ 1 ^d	409 $\pm$ 5 ^b	163 $\pm$ 8 ^c	805 $\pm$ 8 ^a
A9	Trans-3-hexen-1-ol	Moss, fresh	21 $\pm$ 1 ^c	ND	30 $\pm$ 1 ^b	269 $\pm$ 7 ^a
A10	cis-4-Hexen-1-ol		87 $\pm$ 6	ND	ND	ND
A11	2-Ethylhexan-1-ol	Rose, green	909 $\pm$ 7 ^a	ND	8 $\pm$ 1 ^c	11 $\pm$ 0 ^b
A12	3,7-Dimethyloct-1,6-dien-3-ol	Flower, lavender	18 $\pm$ 1	ND	ND	ND
A13	1-Octanol	Chemical, metal, burnt	101 $\pm$ 7 ^a	ND	10 $\pm$ 0 ^b	9 $\pm$ 1 ^b
A14	cis-3-Nonen-1-ol		10 $\pm$ 0 ^a	ND	ND	6 $\pm$ 0 ^b
A15	(R)-(+)-citronellol		ND	ND	ND	6 $\pm$ 1
A16	2-Phenylethanol	Honey, spice, rose, lilac	40 $\pm$ 1 ^d	1797 $\pm$ 27 ^b	6659 $\pm$ 54 ^a	415 $\pm$ 6 ^c
A17	L-Menthol	Peppermint	ND	ND	8 $\pm$ 0	ND
A18	α -Cadinol	Herb, wood	8 $\pm$ 1	ND	ND	ND
A19	4-Terpineol	Turpentine, nutmeg, must	148 $\pm$ 5 ^a	39 $\pm$ 2 ^c	33 $\pm$ 2 ^d	46 $\pm$ 1 ^b
A20	α -Terpineol	Oil, anise	21 $\pm$ 1	ND	ND	ND
	Subtotal		9633 $\pm$ 67 ^d	25,320 $\pm$ 103 ^a	15,693 $\pm$ 97 ^b	11,689 $\pm$ 95 ^c
<i>Esters</i>						
E1	Ethyl acetate	Pineapple	736 $\pm$ 6 ^c	5072 $\pm$ 33 ^a	38 $\pm$ 3 ^d	2256 $\pm$ 14 ^b
E2	Ethyl propionate	Fruit	ND	26 $\pm$ 2 ^b	6 $\pm$ 0 ^c	42 $\pm$ 3 ^a
E3	Ethyl isobutyrate	Sweet, rubber	ND	147 $\pm$ 5	ND	ND
E4	Isobutyl acetate	Fruit, apple, banana	ND	7 $\pm$ 0 ^b	ND	13 $\pm$ 1 ^a
E5	Ethyl butyrate	Apple	14 $\pm$ 1 ^d	153 $\pm$ 3 ^b	67 $\pm$ 3 ^c	188 $\pm$ 4 ^a
E6	Butyl acetate	Pear	ND	ND	ND	5 $\pm$ 0
E7	Ethyl lactate	Fruit	15 $\pm$ 0 ^b	19 $\pm$ 1 ^a	ND	ND
E8	Ethyl 2-methylbutyrate	Apple	ND	31 $\pm$ 2	ND	ND
E9	Ethyl isovalerate	Fruit	ND	12 $\pm$ 1 ^b	ND	18 $\pm$ 0 ^a
E10	Isoamyl acetate	Banana	356 $\pm$ 5 ^c	837 $\pm$ 8 ^a	643 $\pm$ 6 ^b	636 $\pm$ 5 ^b
E11	Ethyl hexanoate	Apple peel, fruit	63 $\pm$ 2 ^d	467 $\pm$ 7 ^b	234 $\pm$ 3 ^c	482 $\pm$ 4 ^a
E12	Ethyl trans-4-decenoate		35 $\pm$ 1	ND	ND	ND
E13	cis-3-Hexenyl acetate	Green, banana	ND	ND	ND	9 $\pm$ 0
E14	Hexyl acetate	Fruit, herb	10 $\pm$ 0 ^c	ND	14 $\pm$ 1 ^b	77 $\pm$ 1 ^a
E15	2-Ethylhexyl acetate		9 $\pm$ 0	ND	ND	ND
E16	Methyl octanoate	Orange	8 $\pm$ 0	ND	ND	ND
E17	Ethyl octanoate	Fruit, fat	447 $\pm$ 6 ^c	39 $\pm$ 2 ^d	605 $\pm$ 7 ^b	823 $\pm$ 8 ^a
E18	Ethyl 3-hydroxybutyrate	Marshmallow	5 $\pm$ 0 ^b	ND	5 $\pm$ 0 ^b	12 $\pm$ 0 ^a
E19	Ethyl nonanoate		9 $\pm$ 0	ND	ND	ND
E20	Ethyl decanoate	Grape	267 $\pm$ 3	ND	ND	ND
E21	3-Methylbutyl octanoate		27 $\pm$ 1	ND	ND	ND
E22	Ethyl benzoate	Camomile, flower, celery, fruit	12 $\pm$ 0	ND	ND	ND
E23	Diethyl succinate	Wine, fruit	112 $\pm$ 7 ^a	103 $\pm$ 7 ^a	6 $\pm$ 0 ^b	10 $\pm$ 0 ^b
E24	Ethyl phenylacetate	Fruit, sweet	ND	ND	8 $\pm$ 0 ^a	8 $\pm$ 0 ^a
E25	2-Phenethyl acetate	Rose, honey	133 $\pm$ 7 ^c	35 $\pm$ 0 ^d	216 $\pm$ 5 ^b	346 $\pm$ 4 ^a

Table 2 continued

Code	Compounds	Odor description*	D254	VIC	BV818	RV100
E26	Ethyl stearate		9 ± 0	ND	ND	ND
E27	Ethyl laurate	Leaf	ND	ND	29 ± 1 ^b	39 ± 1 ^a
E28	Ethyl myristate	Ether	6 ± 1 ^a	ND	5 ± 0 ^b	5 ± 0 ^b
E29	Ethyl hexadecanoate	Wax	25 ± 0	ND	ND	ND
	Subtotal		2298 ± 32 ^c	6949 ± 70 ^a	1875 ± 27 ^d	4970 ± 41 ^b
<i>Acids</i>						
AC1	Acetic acid	Sour	31 ± 2 ^c	1337 ± 22 ^a	495 ± 7 ^b	ND
AC2	Isobutyric acid	Rancid, butter, cheese	21 ± 1 ^c	51 ± 2 ^a	ND	45 ± 1 ^b
AC3	Butyric acid	Rancid, cheese, sweat	ND	ND	ND	11 ± 1
AC4	Isovaleric acid	Sweat, acid, rancid	9 ± 0 ^c	48 ± 2 ^b	ND	90 ± 3 ^a
AC5	2-Methylbutyric acid	Cheese, sweat	ND	27 ± 1 ^b	ND	51 ± 3 ^a
AC6	Hexanoic acid	Sweat	16 ± 0 ^d	40 ± 2 ^c	245 ± 3 ^b	295 ± 3 ^a
AC7	Octanoic acid	Sweat, cheese	21 ± 1 ^c	10 ± 0 ^d	268 ± 4 ^b	333 ± 6 ^a
AC8	Decanoic acid	Rancid, fat	40 ± 1 ^a	ND	17 ± 1 ^b	12 ± 0 ^c
	Subtotal		137 ± 4 ^d	1512 ± 18 ^a	1026 ± 20 ^b	837 ± 12 ^c
<i>Aldehydes and ketones</i>						
AK1	Acetaldehyde	Pungent, ether	51 ± 2 ^c	61 ± 2 ^b	23 ± 2 ^d	83 ± 3 ^a
AK2	Acetal	Fruit, cream	35 ± 1 ^b	35 ± 2 ^b	56 ± 1 ^a	29 ± 1 ^c
AK3	Furfural	Bread, almond, sweet	86 ± 4 ^a	76 ± 1 ^b	ND	ND
AK4	Decanal	Soap, orange peel, tallow	ND	ND	14 ± 1 ^a	5 ± 0 ^b
AK5	Phenylacetaldehyde	Honey, sweet	ND	ND	13 ± 1 ^a	12 ± 0 ^a
AK6	2-Nonanone	Hot milk, soap, green	ND	ND	6 ± 1 ^b	8 ± 0 ^a
AK7	Nonanal	Fat, citrus, green	ND	ND	21 ± 1 ^a	13 ± 1 ^b
	Subtotal		172 ± 4 ^a	172 ± 2 ^a	134 ± 2 ^c	151 ± 2 ^b
<i>Sulphur compounds</i>						
S1	Dimethyl sulfide	Cabbage, sulfur, gasoline	103 ± 5 ^b	133 ± 8 ^a	ND	ND
S2	3-Methylthiopropanol	Sweet, potato	ND	ND	18 ± 2 ^a	19 ± 3 ^a
S3	Dihydro-2-methyl-3(2H)-thiophenone	Cabbage, onion, must	ND	ND	ND	8 ± 1
	Subtotal		103 ± 5 ^b	133 ± 8 ^a	18 ± 2 ^d	27 ± 1 ^c
<i>Styryl derivatives</i>						
SD1	Styrene	Balsamic, gasoline	66 ± 2 ^c	66 ± 1 ^c	157 ± 2 ^b	183 ± 4 ^a
	Subtotal		66 ± 2 ^c	66 ± 1 ^c	157 ± 2 ^b	183 ± 4 ^a
<i>Lactones</i>						
L1	γ-Butyrolactone	Caramel, sweet	ND	ND	105 ± 4 ^a	52 ± 3 ^b
	Subtotal		0	0	105 ± 4 ^a	52 ± 3 ^b
<i>Terpenes</i>						
T1	Limonene	Lemon, orange	ND	ND	43 ± 3 ^a	8 ± 1 ^b
T2	p-Cymene		8 ± 1 ^c	59 ± 3 ^a	9 ± 1 ^c	19 ± 2 ^b
	Subtotal		8 ± 1 ^d	59 ± 3 ^a	52 ± 1 ^b	27 ± 1 ^c
<i>Furan</i>						
F1	2-Ethylfuran		40 ± 2 ^a	40 ± 1 ^a	ND	ND
F2	2,3-Dihydrobenzo		7 ± 1	ND	ND	ND
	Subtotal		47 ± 1 ^a	40 ± 1 ^b	0	0
	Total		12,465 ± 37 ^d	34,252 ± 54 ^a	19,060 ± 48 ^b	17,935 ± 42 ^c

Values shown represent averages of triplicate samples (data are mean ± SD). Values with different superscript roman letters (a, b, c, d) in the same row are significantly different according to the Duncan test ($p < 0.05$)

ND not detected

*<http://www.flavornet.org/flavornet.html>

Table 3 OAVs (> 1) for the main volatile compounds in wax apple wines fermented by four *S. cerevisiae* strains

Code	Compounds	Odor threshold (µg/L)	D254	VIC	BV818	RV100
A19	4-Terpineol	120	1.2	0.3	0.3	0.4
E3	Ethyl isobutyrate	15	0.0	9.8	0.0	0.0
E5	Ethyl butyrate	20	0.7	7.6	3.4	9.4
E9	Ethyl isovalerate	3	0.0	3.9	0.0	5.9
E10	Isoamyl acetate	30	11.9	27.9	21.4	21.2
E11	Ethyl hexanoate	22	2.8	21.2	10.6	21.9
E17	Ethyl octanoate	22	20.3	1.8	27.5	37.4
E20	Ethyl decanoate	200	1.3	0.0	0.0	0.0
E23	Diethyl succinate	70	1.6	1.5	0.1	0.1
AC4	Isovaleric acid	33	0.1	1.4	0.0	2.7
AK5	Phenylacetaldehyde	1	0.0	0.0	13.2	12.3
AK7	Nonanal	15	0.0	0.0	1.4	0.9
S1	Dimethyl sulfide	27	3.8	4.9	0.0	0.0
SD1	Styrene	26	2.5	2.5	6.0	7.0
L1	γ -Butyrolactone	35	0.0	0.0	3.0	1.5
T1	Limonene	25	0.0	0.0	1.7	0.3

Fig. 2 Principal components analysis for the main volatiles(OAVs > 1) in wax apple wines fermented by four *S. cerevisiae* strains. (A) distinction between the samples (scores); (B) relation between the volatile compounds (loadings)

fermentation conditions (Joyeux et al., 1984). The varied abilities in synthesizing/converting precursors or breaking carbon chain could lead to the differences in acids.

Carbonyl compounds mainly including aldehydes and ketones could confer a richer, more elegant, and unique aroma to wine (Nyanga et al., 2013). The contents of phenylacetaldehyde and nonanal accumulated by strain BV818 were higher than their odor thresholds, enhancing honey, sweet, fat, citrus, and green odors of BV818-fermented wine. Similar level but with lower content of phenylacetaldehyde was found in strain RV100-fermented wine. Although three types of aldehydes were observed in

D254- and VIC-produced wines, the total contents of carbonyl compounds accumulated by strains D254 and VIC were higher than those by strains BV818 and RV100.

Sulphur compounds can be produced through sulfate assimilatory or dissimilatory reduction pathways in microorganisms (Kappler and Dahl, 2001; Sun et al., 2011). D254- and VIC-fermented wines contained high concentration (higher than its odor threshold) of dimethyl sulphide, which was absent in wax apple wines produced by strains BV818 and RV100. Dimethyl sulphide contributes an undesirable sense to wine at a high concentration. Dimethyl sulphide could be synthesized by

Fig. 3 The expression levels of genes involved in alcohols and esters metabolism. The expression levels of (A) *BAT1*, (B) *BAT2*, (C) *PDC1*, (D) *PDC5*, (E) *ADH2* involved in alcohols metabolism. The expression levels of (F) *ATF1*, (G) *ATF2*, (H) *EHT1*, (I) *EEB1*, (J) *IAH1* involved in esters metabolism. Significant difference (indicated with * upon the columns) of wines fermented by strains VIC, BV818, and RV100 from that by strain D254 was confirmed for $p < 0.05$ ($n = 3$)

homocysteine precursor, which is an intermediate for methionine synthesis in yeast (López Del Castillo-Lozano et al., 2007; Perpète et al., 2006). 3-Methylthiopropanol,

which produces a sweet- and potato-like odor, was found in wines yielded by strains BV818 and RV100 but was not present in other two wines. 3-Methylthiopropanol could be

produced by methionine metabolism in yeast (Steingass et al., 2014). Different productions of dimethyl sulphide and 3-methylthiopropanol could be attributed to the varied amino acid metabolism of yeasts. Dihydro-2-methyl-3(2H)-thiophenone was only present in RV100-fermented wine. These results were agreement with that *S. cerevisiae* is responsible for the metabolism of volatile sulphur compounds (Swiegers and Pretorius, 2007).

Styrene showing OAVs > 1 was found in the four wax apple wines, so that unpopular balsamic and gasoline aromas might be generated in the four wines. A high level of γ -butyrolactone was observed in wax apple wines produced by strains BV818 and RV100. This component could contribute an intense caramel and sweet odor to BV818- and RV100-produced wines. Limonene could contribute to the fruit wine with a pleasant lemon and orange odor. The wine fermented with strain BV818 exhibited the highest concentration of limonene, enhancing the fruity odor of this wine. Two furans were identified. 2-Ethylfuran was found in D254- and VIC-fermented wines, whereas 2,3-dihydrobenzofuran was unique to the wine produced by strain D254.

PCA of the main volatiles with OAVs(> 1)

PCA was applied to the sixteen volatile compounds with OAVs of > 1 in Table 3. The percentage of the cumulative contribution of the variance of PCs was 86.53%. As shown in Fig. 2(A), the four wines were differentiated by their main aroma profiles. The relative importance of each volatile was established in Fig. 2(B). The compounds 4-terpineol (A19) and ethyl decanoate (E20) were more related to D254-fermented wine. The wine yielded by strain VIC was associated with ethyl isobutyrate (E3) and dimethyl sulfide (S1). The compounds nonanal (AK7), γ -butyrolactone (L1), and limonene (T1) were mainly related to BV818-fermented wine. The RV100-produced wine was related to ethyl butyrate (E5), isovaleric acid (AC4), and styrene (SD1). Although three groups of compounds (aldehydes, lactones, and terpenes for BV818-fermented wine and esters, acids, and styryl derivatives for RV100-fermented wine) strongly affected the aroma characteristic of BV818- and RV100-fermented wines, the unpopular odors were included. It is thus essential to improve the fermentation technology or inoculation model when strains BV818/RV100 were used during the wax apple wine making.

Sensory analysis

Sensory properties of the wax apple wines produced by different *S. cerevisiae* strains were shown in Fig. S2 in supplementary material. The highest “fruity” score was

yielded by strain RV100. Fruity nose is a fundamental trait of fruit wine and usually considered an important indicator of fruit wine quality. The intense fruity aroma of RV100-fermented wine could be attributed to the great amounts of ethyl butyrate, ethyl isovalerate, ethyl hexanoate, and ethyl octanoate. The wax apple wine fermented with strain BV818 was characterized by an intense sweet note, which could be attributed to the large quantities of phenylacetaldehyde and γ -butyrolactone. Although the strongest sweaty and fatty senses were yielded by RV100, it did not affect the global aroma of RV100-fermented wine. Wines produced by D254 and VIC presented the undesirable sulphury odor, which could affect the global aroma of the two wines.

mRNA levels

Alcohols and esters accounted for more than 90% of the total volatiles content of the identified volatiles. To explore the metabolic difference of the four *S. cerevisiae* strains in wax apple juice, the mRNA levels of ten genes involved in higher alcohol and ester metabolism were investigated.

For higher alcohols biosynthesis, BCAAs are converted to their corresponding alcohols via transamination by aminotransferases (encoded by *BAT* genes), decarboxylation by decarboxylase (encoded by *PDC* genes), and reduction by alcohol dehydrogenase (encoded by *ADH* genes), successively (Ma et al., 2017). Acetate ester and ethyl ester synthesis in yeasts is catalyzed by alcohol *O*-acetyltransferases, which are mainly encoded by *ATF1* and *ATF2*, and *EHT1* and *EEB1*, respectively (Sumby et al., 2010). Isoamyl acetate-hydrolyzing esterase encoded by *IAH1* is responsible for the hydrolysis of isoamyl acetate to isoamyl alcohol and acetic acid (Li et al., 2017).

As shown in Fig. 3, the expression levels of the tested genes changed in the four yeast strains to varying levels, suggesting that the four yeast strains had different metabolic activities of alcohol and ester in wax apple juice. The mRNA levels of *BAT1*, *BAT2*, *PDC1*, *PDC5*, *ADH2*, *ATF2*, and *EEB1* were the highest in strain VIC, resulting in the high contents of higher alcohols and esters. The highest expression level of *IAH1* was observed in strain BV818. This result revealed that strain BV818 might own strong ability of decomposing esters, thereby accumulating less content of esters. The expression levels of the ten genes in strain RV100 was higher than that in strain D254 at varied degrees. This finding was corresponded with the result of total content of alcohols and esters. Therefore, modification on the key genes involved in volatiles metabolism could be effective to the formation of different characteristic aromas.

The present study revealed that the profile of volatile components was distinguishing in wax apple wines

produced by different *S. cerevisiae* strains. It is mainly attributed to the various capacities of *S. cerevisiae* strains to release or synthesize volatiles during wax apple wine making. Strain VIC yielded the highest total concentration of volatiles and largest number of volatiles with OAVs > 1. These characteristics allowed to produce wax apple wine characterized with intense aroma. Strains D254 and RV100, which produced the greatest variety of volatile components and scored the highest in global aroma, respectively, could be used to enhance the complexity of wax apple wine. These findings provided comprehensive knowledge on the aroma character of wax apple wine and laid a foundation for making of wax apple wine with the desired flavor profile.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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