

# Chemical and Volatile Composition of Mango Wines Fermented with Different *Saccharomyces cerevisiae* Yeast Strains

X. Li<sup>1</sup>, B. Yu<sup>2</sup>, P. Curran<sup>2</sup>, S.-Q. Liu<sup>1\*</sup>

(1) Food Science and Technology Programme, Department of Chemistry, National University of Singapore, 4 Science Drive 4, Singapore 117543

(2) Firmenich Asia Pte Ltd, Tuas, Singapore 638377

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**The aim of this study was to compare the chemical and volatile composition of mango wines fermented with *Saccharomyces cerevisiae* var. *bayanus* EC1118, *S. cerevisiae* var. *chevalieri* CICC1028 and *S. cerevisiae* var. *cerevisiae* MERIT.ferm. Strains EC1118 and MERIT.ferm showed similar growth patterns but strain CICC1028 grew slightly slowly. The ethanol level reached about 8% (v/v) for each mango wine and sugars (glucose, fructose and sucrose) were almost exhausted at the end of fermentation. There were only negligible changes in the concentrations of citric, succinic and tartaric acids, except for malic acid (decreased significantly). Different volatile compounds were produced, which were mainly fatty acids, alcohols and esters. Most volatiles that were present in the juice were consumed to trace amounts. The kinetic changes of volatiles were similar among the three yeasts but the concentrations of some volatiles varied with yeast. Strain MERIT.ferm produced higher amounts of higher alcohols, isoamyl and 2-phenylethyl acetates, whereas strain CICC1028 produced higher amounts of medium-chain fatty acids and ethyl esters of decanoate and dodecanoate. These results suggest that it may be possible to produce mango wines with differential characteristics using different *S. cerevisiae* strains.**

## INTRODUCTION

Mango (*Mangifera indica* L.) is commercially one of the most abundant tropical fruits in Southeast Asia, accounting for its large market share of the total mango produced worldwide (Tharanathan *et al.*, 2006). Over 30 different varieties of mango are grown and appreciated for its light to bright yellow colour, its sweet and delicious taste, high nutritive value (high amounts of amino acids, a good source of vitamin A and B6, and low in saturated fat, cholesterol, and sodium), as well as its affordable market price (Spreer *et al.*, 2009; Anonymous, n.d.).

The mango variety chosen for this study was *Mangifera indica* L. cv. *Chok Anan* (also called honey mango), which is mostly grown in Malaysia and Thailand. In contrast with most mango varieties, 'Chok Anan' mango has the ability to produce off-season flowering without chemical induction (Spreer *et al.*, 2009). Thus, apart from the main harvest in May, two more harvests follow in June and August. This characteristic enables 'Chok Anan' mangoes to have a large stock each year, which gives it an advantage to be a raw material for further processing, such as mango wine fermentation. Fermentation provides an alternative to selling 'Chok Anan' mango fruits, and further increases its value. Ripe 'Chok Anan' mangoes have a high content of sugar (16.70° Brix), especially sucrose, glucose and fructose. The sugar content of 'Chok Anan' mango is comparable to that of some grape varieties, making it even more suitable for wine fermentation.

The research on mango wine lacked intensive drive till recently although it started from 1960's. Czyhrinciwk (1966) reported the first study on mango wine production. Onkarayya and Singh (1984) screened twenty varieties of mangoes from India for wine production. Obisanya *et al.* (1987) studied the fermentation of mango juice into wine using locally isolated *Saccharomyces cerevisiae* and *Schizosaccharomyces* species of palm wine and they concluded that *Schizosaccharomyces* yeasts were suitable for the production of sweet, table mango wine and *Saccharomyces* yeasts were suitable for the production of dry mango wine with a higher ethanol level. Reddy and Reddy (2005) developed a method of mango juice extraction with pectinase and characterized ethanol and some volatile contents of mango wine. They concluded that the aromatic compounds of mango wine were comparable in concentration to those of grape wine. Reddy and Reddy (2009) published further results of characterizing kinetic changes of higher alcohols in mango wine and concluded that pectinase treatment could enhance the mango juice yield and increase the synthesis of higher alcohols (within a desirable range) as well as mango wine quality. Kumar *et al.* (2009) used response surface methodology (RSM) for the simultaneous analysis of the effects of fermentation conditions (temperature, pH and inoculum size) on the chemical characteristics of mango wine.

There is still no complete profiling of volatile compounds of mango wine although a complete profile of volatiles of fresh

\*Corresponding author: chmLsq@nus.edu.sg [Tel.: +65 6516 2687; fax: +65 6775 7895]

mango juice is available (Pino & Mesa, 2005; Pino *et al.*, 2005). Information is also lacking on the changes in the concentrations of sugars, organic acids and volatile compounds during mango wine fermentation. Further, selection of *Saccharomyces* yeasts plays a very important part in mango wine flavor modulation, because mango wines with different flavor profiles may result when fermenting the same mango juice with different strains or species of *Saccharomyces* yeasts. To the best of our knowledge, there are no comprehensive reports on the characteristics of mango wines fermented by different *Saccharomyces* yeast strains.

The aim of this study was to compare the fermentation performance of three *Saccharomyces cerevisiae* yeasts (MERIT.ferm, CICC1028, EC1118) and the chemical and volatile composition of the resultant mango wines. The outcome of this study would help select *Saccharomyces* yeasts for further investigations involving *Saccharomyces* and non-*Saccharomyces* to enhance mango wine flavor.

## MATERIALS AND METHODS

### Yeast strains and culture media

*Saccharomyces cerevisiae* var. *bayanus* Lalvin EC1118 (Lallemand Inc, Brooklyn Park, Australia) and *Saccharomyces cerevisiae* var. *chevalieri* CICC1028 (China Centre of Industrial Culture Collection, Beijing), and *Saccharomyces cerevisiae* MERIT.ferm (Chr.-Han., Denmark) were used in this study. Yeast strains were maintained in nutrient broth (pH 5.0) consisting of 2% (w/v) glucose, 0.25% (w/v) yeast extract, 0.25% (w/v) bacteriological peptone, 0.25% (w/v) malt extract and were incubated at 25°C for up to 48-72 hours. The yeasts with 20% glycerol were stored at -80°C before use.

### Preparation of mango juice

Mangoes ('Chok Anan' variety) from Malaysia were purchased from a local market in Singapore and were juiced, centrifuged at 21,000 rpm (41,415×g, Beckman Centrifuge, USA) for 15 min and stored at -50°C for further use. Pre-culture medium prepared from the mango juice (16.7°Brix, containing 4.9 g of fructose, 0.6 g of glucose and 12.4 g of sucrose per 100 mL juice; pH 4.63) was sterilized through a 0.45 µm polyethersulfone filter membrane (Sartorius Stedium Biotech, Germany), inoculated with 1% (v/v) of selected yeast strains and incubated for 48 hours until yeasts grew to at least 10<sup>7</sup> cfu/mL. The mango juice (pH adjusted to 3.5 with 50% w/v food grade D,L-malic acid from Suntop Ltd, Singapore) used for fermentation was sterilized with 100 ppm of potassium metabisulphite (The Goodlife Homebrew centre, Norfolk, England) and left overnight at 25°C before use. Potato dextrose agar (PDA) (39g/L, Oxoid, Basingstoke, Hampshire, England) was used for plating to monitor the growth of the three *Saccharomyces* yeasts.

### Fermentation

Replicate mango juice fermentations with each *Saccharomyces* yeast were carried out in 300 mL sterile Erlenmeyer conical flasks (plugged with cotton wool, then wrapped with aluminum foil) and each flask contained 250 mL mango juice. The juices were inoculated with 1% (v/v) pre-culture of the three *Saccharomyces* yeasts and fermentation was conducted at 20°C statically for 14 days. Samples were taken during fermentation (Day 0, 2, 4, 6, 11 and 14).

### Measurement of pH and Brix

The total soluble solids (Brix) and pH were measured at the

TABLE 1

Physicochemical properties, organic acid and sugar concentrations of mango wines before and after fermentation.

Yeast strains	Day 0			Day 14		
	MERIT.ferm	CICC1028	EC1118	MERIT.ferm	CICC1028	EC1118
<b>Physicochemical properties</b>						
pH	3.52±0.00 <sup>a</sup>	3.52±0.00 <sup>a</sup>	3.52±0.00 <sup>a</sup>	3.54±0.01 <sup>a</sup>	3.69±0.01 <sup>b</sup>	3.56±0.02 <sup>a</sup>
Brix	16.61±0.03 <sup>a</sup>	16.71±0.02 <sup>a</sup>	16.68±0.03 <sup>a</sup>	5.36±0.06 <sup>a</sup>	5.39±0.02 <sup>a</sup>	5.30±0.17 <sup>a</sup>
<b>Plate count (10<sup>5</sup> cfu/mL)</b>	5.22±3.12 <sup>a</sup>	4.64±2.46 <sup>a</sup>	8.34±4.99 <sup>b</sup>	8920±6921 <sup>a</sup>	547±122 <sup>b</sup>	9455±3297 <sup>a</sup>
<b>Organic acids (g/100mL)</b>						
Citric acid	0.27±0.04 <sup>a</sup>	0.34±0.01 <sup>b</sup>	0.23±0.01 <sup>a</sup>	0.21±0.03 <sup>a</sup>	0.20±0.02 <sup>a</sup>	0.24±0.03 <sup>a</sup>
Tartaric acid	0.12±0.03 <sup>a</sup>	0.09±0.02 <sup>a</sup>	0.13±0.04 <sup>a</sup>	0.14±0.02 <sup>a</sup>	0.11±0.03 <sup>a</sup>	0.14±0.01 <sup>a</sup>
Succinic acid	0.083±0.012 <sup>a</sup>	0.075±0.011 <sup>a</sup>	0.080±0.007 <sup>a</sup>	0.086±0.011 <sup>a</sup>	0.081±0.003 <sup>a</sup>	0.083±0.002 <sup>a</sup>
Malic acid	0.79±0.03 <sup>a</sup>	0.86±0.01 <sup>b</sup>	0.745±0.02 <sup>a</sup>	0.36±0.05 <sup>a</sup>	0.33±0.01 <sup>a</sup>	0.41±0.03 <sup>a</sup>
<b>Reducing sugars (g/100mL)</b>						
Fructose	4.96±0.08 <sup>a</sup>	4.87±0.06 <sup>a</sup>	5.05±0.07 <sup>a</sup>	N.D.*	N.D.	N.D.
Glucose	0.63±0.02 <sup>a</sup>	0.61±0.01 <sup>a</sup>	0.62±0.02 <sup>a</sup>	N.D.	N.D.	N.D.
Sucrose	12.25±0.31 <sup>a</sup>	12.44±0.11 <sup>a</sup>	13.85±0.07 <sup>b</sup>	0.013±0.00 <sup>a</sup>	0.013±0.00 <sup>a</sup>	0.013±0.00 <sup>a</sup>

<sup>a,b,c</sup> ANOVA (n=4) at 95% confidence level with same letters indicating no significant difference.

\*N.D.: not detected.

indicated time points by using a refractometer (ATAGO, Japan) and a pH meter (Metrohm, Switzerland), respectively. Samples were analyzed in duplicate for each wine replicate.

#### Analysis of reducing sugars and organic acids by HPLC

Wine samples after centrifugation and filtration (0.2µm) were stored at -50°C before analysis. The sugars (g/100mL) were measured by HPLC (Shimadzu HPLC, Class-VP software version 6.1) according to the method of Chávez-Servín *et al.* (2004), using a carbohydrate ES column (Prevail, 150×4.6 mm). The column was eluted at 25°C with a degassed mobile phase containing a mixture of acetonitrile and water (78:22) at a flow rate of 0.5 mL/min (isocratic mode). All the compounds were detected with an evaporative light scattering detector. Samples were analyzed in duplicate for each wine replicate (n=4). The identification and quantification of sugars were achieved by using retention time and standard curves of pure sugar compounds (Sigma-Aldrich, St. Louis, MO, USA).

The organic acids (tartaric, citric, succinic and malic acids) were determined by HPLC (Shimadzu) using a Supelcogel C-610H column (Supelco, Bellefonte, PA, USA) connected to a photodiode array detector. The column was eluted at 40°C with a degassed aqueous mobile phase containing 0.1% sulphuric acid at a flow rate of 0.4 mL/min (isocratic mode). Samples were analyzed in duplicate for each wine replicate. The identification and quantification of compounds were carried out by using retention time, UV spectrum (210 nm) and standard curves of pure organic acid compounds (Sigma-Aldrich, St. Louis, MO, USA).

#### Analysis of volatile compounds by HS-SPME-GC-MS/FID

The method was based on that described elsewhere (Lee *et al.*, 2010a; Trinh *et al.*, 2010) with some modifications. Volatile compounds of fresh juice and final fermented juice (samples after 14-day fermentation) were measured using headspace (HS) solid-phase microextraction (SPME) method coupled with gas chromatography (GC)-mass spectrometer (MS) and flame ionization detector (FID) (HS-SPME-GC-MS/ FID). Carboxen/PDMS fibre (85 µm) (Supelco, Sigma-Aldrich, Barcelona, Spain) was used for extraction. Five millilitres of mango wine sample was extracted by HS-SPME at 60°C for 40 min under 250 rpm agitation. The fibre was desorbed at 250°C for 3 min and the sample was injected into Agilent 7890A GC (Santa Clara, CA, USA), which was coupled to FID and Agilent 5975C triple-axis MS. Separation was achieved using capillary column (Agilent DB-FFAP) of 60 m × 0.25 mm I.D. coated with 0.25 µm film thickness of polyethylene glycol modified with nitroterephthalic acid. The carrier gas was helium. The operation conditions were as follows: the oven temperature was programmed from 50°C for 5 min, then increased with 5°C/min until 230°C, and kept at 230°C for 30 min. The FID temperature was set at 250°C, and the MSD was operated in the electron impact mode at 70 eV. The volatile compounds were identified by using Wiley mass spectrum library and comparison of linear retention index (LRI) of each volatile with the LRI in other reports (Tairu *et al.*, 1999; Lee *et al.*, 2010a; Trinh *et al.*, 2010). LRI was determined by using a series of alkanes (C5-C40) run under the same HS-SPME-GC-MS/ FID condition as sample

analysis and it was calculated according to the equation:

$$LRI=100\times[(t_1-t_2)/(t_{z+1}-t_2)+z]$$

where z is the number of carbon atoms of the n-alkane eluting before and (z + 1) is the number of carbon atoms of the n-alkane eluting after the peak of interest. FID peak area was used to calculate RPA of each volatile and it can help semi-quantitatively compare the relative difference of each volatile, minor or major, among three wines. The final fermented samples ("Day 14" sample) were analyzed in duplicate for each wine replicate, but fresh mango juice was analyzed in triplicate.

Major volatiles (high RPA in the FID chromatogram; which are important for wine quality) were quantified using individual external standards dissolved in 10% v/v mango juice diluted with water, except for ethanol dissolved in 100% v/v mango juice (Lee *et al.*, 2010b; Trinh *et al.*, 2010). Good linearity was obtained for all standard curves ( $R^2>0.97$ ). The kinetic changes of the concentration of these compounds were monitored throughout the whole fermentation. The HS-SPME-GC-MS/ FID condition used for quantification is the same as the above-mentioned conditions. Samples were analyzed in duplicate for each wine replicate (n=4). Thereafter, odor activity values (OAVs) of these quantified volatiles were calculated according to their established threshold levels (in synthetic wine base) in other published reports (Guth, 1997; Bartowsky & Pretorius, 2008).

#### Statistical analysis

ANOVA ( $P<0.05$ ) was used to determine the significance of the difference of each chemical or volatile factor among three fermentations.

## RESULTS AND DISCUSSION

### Brix, pH and yeast growth

The mango juice had a soluble solids content of 16.7°Brix. The three strains of *S. cerevisiae* yeasts had similar fermentation characteristics in terms of Brix change, pH changes and yeast growth. The pH values fluctuated from 3.50 to 3.69 and Brix values were reduced to 5.3°-5.4° for all three mango wines during the fermentation. The cell populations of all three yeasts increased from the initial  $5\times 10^5$  cfu/mL (MERIT.ferm),  $4.5\times 10^5$  cfu/mL (CICC1028),  $8.5\times 10^5$  cfu/mL (EC1118) and reached their respective maximum on day 14, where strain EC1118 showed the highest growth at  $9.46\times 10^8$ cfu/mL, followed by strain MERIT.ferm at  $8.92\times 10^8$ cfu/mL and strain CICC1028 at  $5.47\times 10^7$ cfu/mL (Table 1). Based on the plate counts, it seemed that strain CICC1028 was less stress-tolerant of stress than strains EC1118 and MERIT.ferm because its cell count was about 10 times less.

### Changes of sugars and organic acids

Fructose, glucose and sucrose were the three reducing sugars detected in the fresh mango juice. The sugar contents in the juices inoculated with the three *S. cerevisiae* displayed rapid reduction during fermentation. Strain CICC1028 showed the fastest consumption of fructose and glucose among the three yeasts (data not shown). In addition, the three strains showed a similar pattern of sucrose utilization. At day 14,

TABLE 2

Major volatile compounds (GC-FID peak area  $\times 10^6$ ) and their relative peak areas (RPA) in fresh 'Chok Anan' mango juice.

Groups	LRI <sup>(1)</sup>	CAS No. <sup>(2)</sup>	Compounds	Peak area	RPA (%)	Aroma descriptors of pure compounds <sup>(3)</sup>
Monoterpenes	1088	007785-70-8	Alpha-pinene	10.86 $\pm$ 1.23	0.87	Resinous, pine-like
	1127	000079-92-5	Camphene	1.55 $\pm$ 0.27	0.12	Harsh, camphoraceous, coniferous
	1206	013466-78-9	Delta-3-carene	78.91 $\pm$ 7.28	6.36	Harsh, terpene-like, coniferous
	1211	000471-84-1	Alpha-fenchene	3.52 $\pm$ 0.21	0.28	Camphor-like
	1219	002867-05-2	Alpha-thujene	15.87 $\pm$ 1.05	1.28	Woody, green herb
	1226	018172-67-3	Beta-pinene	1.08 $\pm$ 0.36	0.09	Sharp, terpenic, conifers
	1235	000099-86-5	Alpha-terpinene	71.43 $\pm$ 5.33	5.75	Sharp, terpenic, lemon
	1254	095327-98-3	Limonene	59.42 $\pm$ 4.28	4.79	Citric, terpenic, orange note
	1265	000555-10-2	Beta-phellandrene	5.71 $\pm$ 0.87	0.46	Mint, terpene-like
	1268	000508-32-7	Tricyclene	5.64 $\pm$ 0.79	0.45	-
	1290	027400-71-1	Cis-ocimene	2.28 $\pm$ 0.33	0.18	Citrus, green, lime
	1305	000099-85-4	Gamma-terpinene	40.2 $\pm$ 5.22	3.24	Fatty, terpenic, lime
	1339	000099-87-6	<i>p</i> -Cymene	12.56 $\pm$ 2.78	1.01	Citrus, terpenic, woody
	1343	000535-77-3	<i>m</i> -Cymene	123.33 $\pm$ 10.25	9.94	Citrus, terpenic, woody
	1352	000586-62-9	Alpha-terpinolene	560.55 $\pm$ 20.27	45.16	Citrus, lime, pine
	1450	000673-84-7	Allo-ocimene	1.87 $\pm$ 0.52	0.15	Floral, nutty, peppery
1529	001195-32-0	<i>p</i> -Cymenene	101.87 $\pm$ 7.22	8.21	Citrus, pine-like	
<b>Subtotal</b>				<b>1096.65</b>	<b>88.35</b>	
Sesquiterpenes	1695	000087-44-5	Trans-Caryophyllene	0.38 $\pm$ 0.07	0.03	Woody, clove note
	1778	028624-23-9	Delta-Selinene	0.42 $\pm$ 0.13	0.03	-
	1826	004630-07-3	Valencene	0.19 $\pm$ 0.02	0.02	Orange, citrus, woody
	1829	017066-67-0	Beta-selinene	2.52 $\pm$ 0.88	0.20	-
<b>Subtotal</b>				<b>3.51</b>	<b>0.28</b>	
Alcohols	1032	000064-17-5	Ethanol	7.1 $\pm$ 1.23	0.57	Alcoholic
	1476	000928-96-1	Cis-3-hexenol	87.69 $\pm$ 7.99	7.06	Green, leafy
	1578	000704-76-7	2-Ethyl-1-hexanol	2.55 $\pm$ 0.92	0.21	Oily, rose, sweet
	1794	000470-08-6	Beta-fenchol	0.18 $\pm$ 0.03	0.01	Camphor-like, woody
	1808	000464-43-7	Endo-borneol	0.16 $\pm$ 0.08	0.01	Camphor-like, woody
	1999	000078-70-6	Linalool	0.19 $\pm$ 0.03	0.02	Fresh floral, herbal, rosewood, petitgrain
	2035	000060-12-8	2-Phenylethyl alcohol	0.35 $\pm$ 0.12	0.03	Rose, honey, floral
<b>Subtotal</b>				<b>98.22</b>	<b>7.91</b>	
Esters	1284	000109-21-7	Butyl butanoate	2.14 $\pm$ 0.21	0.17	Fruity, pineapple, sweet
	1396	003681-71-8	3-Hexenyl acetate	10.71 $\pm$ 1.44	0.86	Sharp fruity-green, sweet, green banana-like
	1410	002497-18-9	Trans-2-hexenyl acetate	0.24 $\pm$ 0.01	0.02	Fruity, green, leafy
	1440	000629-33-4	Hexyl formate	5.31 $\pm$ 0.66	0.43	Green, ethereal, fruity
	1466	033467-74-2	Cis-3-hexenyl propionate	0.52 $\pm$ 0.04	0.04	Fresh, fruity, green
	1546	016491-36-4	Cis-3-hexenyl isobutyrate	1.20 $\pm$ 0.33	0.10	Apple, fruity, green
	1700	065405-80-3	(Z)-3-hexenyl (E)-2-butenate	0.17 $\pm$ 0.00	0.01	Green, sweet, fruity
	1948	000110-38-3	Ethyl dodecanoate	0.32 $\pm$ 0.03	0.03	Sweet, Wine, Brandy
<b>Subtotal</b>				<b>20.61</b>	<b>1.66</b>	

**TABLE 2 (CONTINUED)**Major volatile compounds (GC-FID peak area  $\times 10^6$ ) and their relative peak areas (RPA) in fresh 'Chok Anan' mango juice.

Groups	LRI <sup>(1)</sup>	CAS No. <sup>(2)</sup>	Compounds	Peak area	RPA (%)	Aroma descriptors of pure compounds <sup>(3)</sup>
Acids	1549	000064-19-7	Acetic acid	0.62 $\pm$ 0.03	0.05	Vinegar-like
	1728	000067-43-6	Butanoic acid	1.35 $\pm$ 0.09	0.11	Cheesy, rancid butter
	2171	000124-07-2	Octanoic acid	0.24 $\pm$ 0.01	0.02	Acidic, fatty, soapy
<b>Subtotal</b>			<b>2.21</b>	<b>0.18</b>		
Aldehydes	1152	000066-25-1	Hexanal	0.79 $\pm$ 0.05	0.06	Fatty, green, grassy
	1310	006728-26-3	Trans-2-hexenal	2.95 $\pm$ 0.21	0.24	Apple, strawberry
	1500	000142-83-6	Trans, trans-2,4-hexadienal	0.60 $\pm$ 0.04	0.05	Fatty, sweet, green
	1572	000098-01-1	Furfural	0.19 $\pm$ 0.01	0.02	Almond, caramel, sweet
	1633	000100-52-7	Benzaldehyde	0.10 $\pm$ 0.00	0.01	Bitter almond
	1723	000432-25-7	Beta-cyclocitral	0.17 $\pm$ 0.02	0.01	Fruity, green, minty
	1731	000620-23-5	3-Methyl-benzaldehyde	0.28 $\pm$ 0.04	0.02	Sweet fruity cherry
	1771	000104-87-0	<i>p</i> -Tolualdehyde	2.69 $\pm$ 0.76	0.22	Sweet aromatic, bitter almond and cherry notes
<b>Subtotal</b>			<b>7.77</b>	<b>0.63</b>		
Ketones	1281	006137-06-0	4-Methyl-2-heptanone	0.65 $\pm$ 0.02	0.05	-
	1701	004077-47-8	Mesifurane	0.16 $\pm$ 0.04	0.01	-
	1758	000096-48-0	Dihydro-2(3H)-furanone	0.53 $\pm$ 0.04	0.04	-
	1834	000695-06-7	5-Ethyl-dihydro-2(3H)-furanone	0.50 $\pm$ 0.11	0.04	Herbaceous, waxy, creamy note
	1938	023696-85-7	Beta-damascenone	1.31 $\pm$ 0.23	0.11	Sweet, floral, fruity
	2051	000104-50-7	Gamma-octalactone	0.97 $\pm$ 0.09	0.08	Coconut
<b>Subtotal</b>			<b>4.12</b>	<b>0.33</b>		
Furan	1044	003208-16-0	2-Ethyl furan	4.95 $\pm$ 0.53	0.40	Ethereal rum, cocoa note
	1514	001746-11-8	2,3-Dihydro-2-methyl-benzofuran	1.66 $\pm$ 0.02	0.13	-
<b>Subtotal</b>			<b>6.61</b>	<b>0.53</b>		
Ether	1434	016409-43-1	Cis-rose oxide	0.81 $\pm$ 0.09	0.07	Rose, geranium
	1537	068780-91-6	Trans-linalool oxide	0.42 $\pm$ 0.03	0.03	Sweet, lemon, cineol
	1563	001786-08-9	Nerol oxide	0.94 $\pm$ 0.08	0.08	Floral, orange blossom, green, sweet
<b>Subtotal</b>			<b>2.17</b>	<b>0.17</b>		
<b>Total</b>			<b>1241.87</b>	<b>100</b>		

<sup>(1)</sup> LRI of all the relative tables was determined on the DB-FFAP column, relative to C<sub>5</sub>-C<sub>40</sub> hydrocarbons.<sup>(2)</sup> CAS.number of all the relative tables was obtained from Wiley MS library.<sup>(3)</sup> Aroma descriptors obtained from <http://www.thegoodscentcompany.com>.

sugar consumption was almost complete in the fermentation process, with only about 0.013 g/100 mL of sucrose left in day 14 samples (Table 1). Compared with the study of Reddy and Reddy (2009), the residual sugar level in our study was even lower from similar starting concentrations, which might be due to different mango cultivars or yeasts used.

Organic acids showed different changes during fermentation (Table 1). Citric acid in all three mango wines stayed almost constant at 0.20-0.27 g/100 mL (except for strain CICC1028). In addition, tartaric and succinic acids did not change significantly for all three wines at 0.1 g/100 mL

and 0.08 g/100 mL, respectively. D,L-malic acid was spiked in mango juice at the beginning of the fermentation to adjust pH to 3.5. Therefore, the total malic acid increased from 0.3 g/100mL to about 0.8 g/100 mL after spiking. The total malic acid decreased by day 6 and remained constant afterwards (data for day 6 not shown). The decrease in total malic acid before day 6 might not be due to malic acid catabolism because *S. cerevisiae* is generally not capable of metabolizing malic acid. However, D- and L-malic acid molecules could enter the cells of *S. cerevisiae* strains by passive diffusion (Coloretti *et al.*, 2002). Furthermore, the decrease in malic acid was not

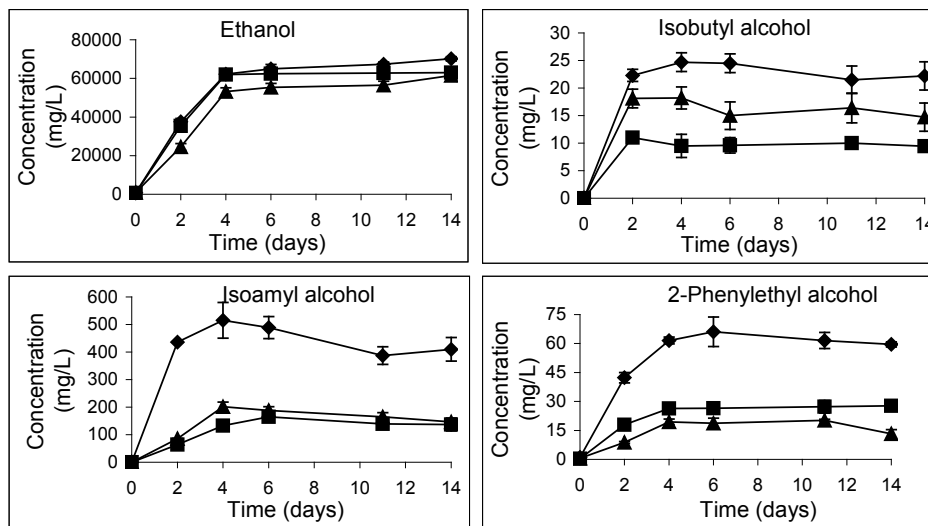


FIGURE 1

Changes of alcohols in mango wines during fermentation by *S. cerevisiae* MERIT.ferm (◆), *S. chevalieri* CICC-1028 (▲) and *S. bayanus* EC-1118 (■).

likely due to malolactic fermentation, given the lack of lactic acid (none detected) and the addition of 100 ppm of potassium metabisulphite to the juice.

#### Volatile compounds in fresh mango juice

The isomers of monoterpenes ( $C_{10}H_{16}$ ) and sesquiterpenes ( $C_{15}H_{24}$ ) dominated the major volatiles of fresh mango juice, and their FID RPA reached 89% (Table 2). Further, several esters, acids, furanones, aldehydes and ketones were also important for the aroma of fresh 'Chok Anan' mangoes, such as butyl butanoate, 3-hexenyl acetate, hexyl formate, rose oxide, cis-3-hexenol, butanoic acid, beta-damascenone and trans-2-hexenal. Most of the volatiles identified in the mango juice were similar to those reported elsewhere (Pino *et al.*, 2005; Pino & Mesa, 2005). However, most of these volatiles (e.g. terpene hydrocarbons) were metabolized, although a few of them were still detectable after fermentation (e.g. beta-damascenone). The result is in contrast with some previous reports which claimed that fermentation would not affect the concentration of terpenes (Rapp, 1988; Ong & Acree, 1999; Alves, 2010). Nonetheless, Zoecklein *et al.* (1997) showed that some *Saccharomyces* strains would cause the decrease of terpenes, which is in agreement with our findings. The reason(s) for this discrepancy is not known and should be further investigated.

#### Volatile composition of mango wines after 14-day fermentation and kinetic changes of major volatiles

During the 14-day fermentation of mango juice, a number of volatiles were produced: 4 fatty acids, 5 alcohols, 23 esters, 5 ketones, 3 aldehydes and 1 sulfur compound [dihydro-2-methyl-3(2H)-thiophenone] (Table 3). The volatile composition of the three mango wines is almost the same, but the concentration of each volatile may be different. To compare the volatile compounds in the three wines, FID peak area and RPA were used and they can semi-quantitatively represent the concentration of different volatiles (Alves *et al.*, 2010; Lee *et al.*, 2010ab; Trinh *et al.*, 2010). For further accuracy, 12 major volatile compounds,

which are generally considered as important factors influencing fruit or grape wine quality (Gürbüz *et al.*, 2006; Alves *et al.*, 2010; Lee *et al.*, 2010ab; Trinh *et al.*, 2010), were quantified with external standards (Table 4).

Alcohols are quantitatively the largest group of all the volatiles, with RPA accounting for more than 60% for all three wines. In Tables 3 and 4, strain MERIT.ferm consistently produced the highest amounts of all major alcohols (ethanol, isobutyl alcohol, isoamyl alcohol and 2-phenylethyl alcohol). The kinetic changes of these major alcohols are consistent: constant after day 4 of fermentation (Fig. 1). Ethanol concentrations were 8.8%, 7.8%, 8.1% (v/v) for strains MERIT.ferm, CICC1028 and EC1118, respectively. The concentration of isoamyl alcohol was much higher than that of isobutyl and 2-phenylethyl alcohols in all three mango wines, with strain MERIT.ferm producing 409.9 mg/L, CICC1028 producing 146.4 mg/L and EC1118 producing 136.9 mg/L (Table 4). In addition, MERIT.ferm produced 22.2 mg/L of isobutyl alcohol and 59.6 mg/L of 2-phenylethyl alcohol, CICC1028 produced 9.4 and 24.5 mg/L, EC1118 produced 14.7 and 27.7 mg/L, respectively (Table 4). The levels of the three branched-chain higher alcohols except for isobutyl alcohol were higher than their published threshold levels for all three mango wines (Table 4).

These branched-chain higher alcohols are important components of the wine bouquet, which are released into the medium as secondary products of the metabolism of yeasts (Noguerol-Pato *et al.*, 2009). They are formed by transamination or deamination of the corresponding amino acids through the Ehrlich pathway (Myers *et al.*, 1970; Dickinson *et al.*, 1998; Etschmann *et al.*, 2002). The keto-acids formed from this pathway are decarboxylated to aldehydes and further reduced to branched-chain higher alcohols. Rapp and Mandery (1987) reported that the concentration of total higher alcohols in wine is in the range of 80–540 mg/L. High quantities of these compounds are considered to be undesirable in table wines, and concentrations below 350 mg/L can be considered

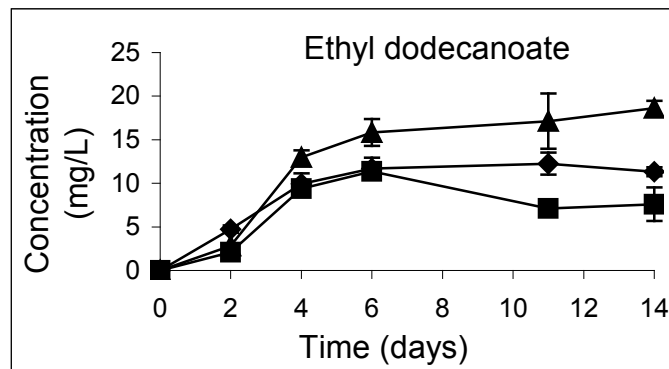
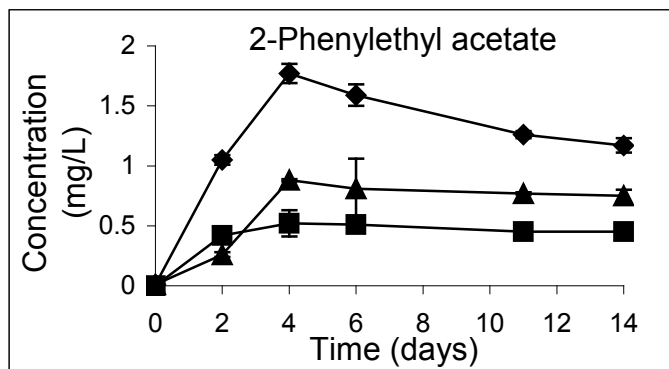
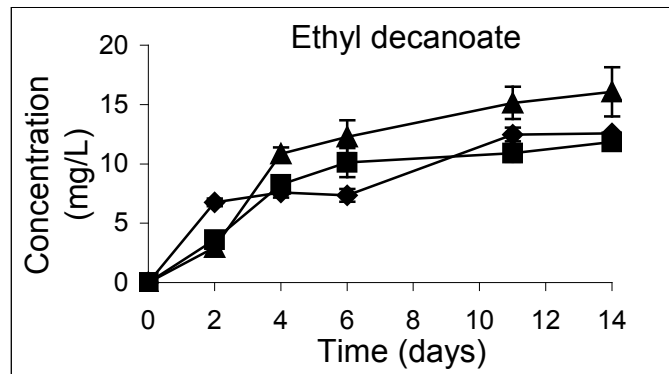
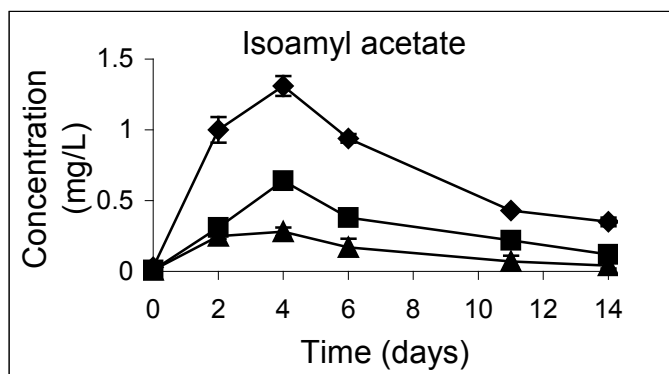
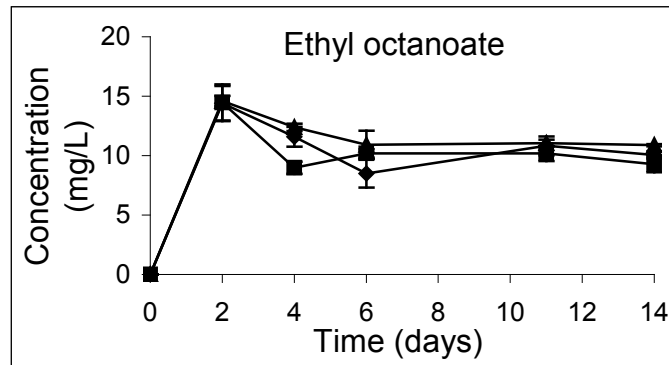
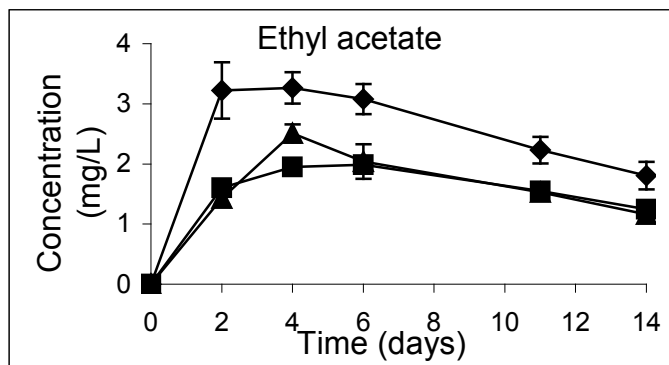


FIGURE 2

Changes of acetate esters in mango wines during fermentation by *S. cerevisiae* MERIT.ferm (◆), *S. chevalieri* CICC-1028 (▲) and *S. bayanus* EC-1118 (■).

FIGURE 3

Changes of ethyl esters in mango wines during fermentation by *S. cerevisiae* MERIT.ferm (◆), *S. chevalieri* CICC-1028 (▲) and *S. bayanus* EC-1118 (■).

to contribute to the positive aromas of wines (Rapp & Mandery, 1986). Obviously, the higher alcohols (especially isoamyl alcohol) level of strain MERIT.ferm-fermented wine are in the “undesirable” range, however, they might be used as main precursors of branched-chain aromatic esters (e.g. isoamyl acetate, 2-phenylethyl acetate) and these esters can provide enhanced fruity and floral aroma for wine. Yilmaztekin *et al.* (2009) reported *Williopsis saturnus* is able to convert isoamyl alcohol into isoamyl acetate. If strain Merit.ferm could co-ferment mango juice with ester-producing *Williopsis* yeasts, it may probably promote the formation of branched-chain and aromatic esters.

Some quantitatively minor alcohols were also identified in mango wines, such as cis-3-hexenol, 1-octanol and citronellol (Table 3). They may impart sensory attributes such as “fruity” or “floral” flavor to mango wines. For example, citronellol is a

fragrant and flavourful compound that is of great interest to the wine making industry because it can be used to synthesize other aromatic compounds, e.g. rose oxide (lychee flavour) (Alves *et al.*, 2010). The occurrence of citronellol in mango wines but not in mango juice suggests that it was produced by yeasts during fermentation, likely as a result of hydrolysis of glycosides with bound citronellol as the aglycone (Ugliano *et al.*, 2006).

Esters are quantitatively the second largest group in the volatile profiles of the three fermented mango wines (over 25% RPA), including acetates, methyl esters, ethyl esters and other medium or long-chain esters.

According to RPA, the most significant acetates were ethyl acetate, isoamyl acetate and 2-phenylethyl acetate (Table 3). They showed similar modes of kinetic changes - reaching their maximum on day 4 and decreasing steadily thereafter (Fig. 2). The mango wine fermented with strain MERIT.ferm had higher

TABLE 3

Major volatile compounds (GC-FID peak area  $\times 10^6$ ) and their relative peak areas (RPA) in mango wine (day 14) fermented by three *S. cerevisiae* yeasts.

Groups	Compounds	LRI	CAS. No	MERIT.ferm		CICC1028		EC1118		Aroma descriptors of pure compounds <sup>(1)</sup>
				Peak area	RPA (%)	Peak area	RPA (%)	Peak area	RPA (%)	
Acids	Acetic acid	1549	000064-19-7	9.65 $\pm$ 0.14 <sup>a</sup>	0.118	3.01 $\pm$ 0.05 <sup>b</sup>	0.035	7.82 $\pm$ 0.7 <sup>c</sup>	0.102	Acidic, vinegar
	Octanoic acid	2170	000124-07-2	48.80 $\pm$ 1.4 <sup>a</sup>	0.605	65.1 $\pm$ 5.48 <sup>b</sup>	0.817	45.60 $\pm$ 0.13 <sup>a</sup>	0.57	Fatty, soapy, fruity, sour
	Decanoic acid	2390	000334-48-5	51.20 $\pm$ 0.856 <sup>a</sup>	0.635	75.21 $\pm$ 4.39 <sup>b</sup>	0.944	48.77 $\pm$ 2.67 <sup>a</sup>	0.635	Fatty, rancid, sour
	Dodecanoic acid	2607	000143-07-7	6.26 $\pm$ 0.40 <sup>a</sup>	0.078	11.31 $\pm$ 0.70 <sup>b</sup>	0.142	6.39 $\pm$ 0.20 <sup>a</sup>	0.083	Coconut, fatty
<b>Subtotal</b>				<b>111.91</b>	<b>1.436</b>	<b>154.43</b>	<b>1.938</b>	<b>105.2</b>	<b>1.37</b>	
Alcohol	Ethanol	1028	000064-17-5	5330 $\pm$ 109 <sup>a</sup>	66.08	4650 $\pm$ 347 <sup>b</sup>	58.34	5270 $\pm$ 208 <sup>b</sup>	69.57	Alcoholic
	Isobutyl alcohol	1172	000078-83-1	26.10 $\pm$ 0.52 <sup>a</sup>	0.324	20.5 $\pm$ 1.98 <sup>b</sup>	0.257	17.80 $\pm$ 0.52 <sup>c</sup>	0.232	Fruity, wine-like
	Isoamyl alcohol	1237	000123-51-3	201 $\pm$ 8.23 <sup>a</sup>	2.492	129 $\pm$ 16.4 <sup>b</sup>	1.619	120 $\pm$ 3.34 <sup>b</sup>	1.564	Alcoholic, fruity, banana
	Cis-3-hexenol	1475	000928-96-1	2.06 $\pm$ 0.12 <sup>a</sup>	0.026	2.14 $\pm$ 0.13 <sup>a</sup>	0.027	2.76 $\pm$ 0.12 <sup>b</sup>	0.036	Green, leafy
	1-Octanol	1650	000111-87-5	0.82 $\pm$ 0.12 <sup>a</sup>	0.01	0.28 $\pm$ 0.04 <sup>b</sup>	0.004	0.40 $\pm$ 0.05 <sup>b</sup>	0.005	Fatty, orange -like, citrus
	Citronellol	1867	000106-22-9	1.82 $\pm$ 0.16 <sup>a</sup>	0.023	1.02 $\pm$ 0.05 <sup>b</sup>	0.013	2.63 $\pm$ 0.48 <sup>a</sup>	0.034	Floral, rose, citrus, green
	2-Phenylethyl alcohol	1964	000060-12-8	118 $\pm$ 6.91 <sup>a</sup>	1.463	48.50 $\pm$ 4.45 <sup>b</sup>	0.609	64.70 $\pm$ 3.84 <sup>c</sup>	0.843	Sweet, rose, floral
<b>Subtotal</b>				<b>5679.8</b>	<b>70.418</b>	<b>4851.44</b>	<b>60.869</b>	<b>5578.29</b>	<b>72.684</b>	
Esters	Ethyl acetate	1009	000141-78-6	7.46 $\pm$ 0.24 <sup>a</sup>	0.09	5.73 $\pm$ 0.37 <sup>b</sup>	0.071	6.18 $\pm$ 0.73 <sup>b</sup>	0.081	Ethereal, fruity, sweet
	Isoamyl acetate	1112	000123-92-2	5.94 $\pm$ 0.40 <sup>a</sup>	0.074	1.12 $\pm$ 0.40 <sup>b</sup>	0.014	3.19 $\pm$ 0.24 <sup>c</sup>	0.042	Fruity, banana, pear
	n-Octyl acetate	1576	000112-14-1	0.99 $\pm$ 0.07 <sup>a</sup>	0.012	0.81 $\pm$ 0.04 <sup>b</sup>	0.01	0.80 $\pm$ 0.06 <sup>b</sup>	0.01	Floral, orange, jasmine-like
	Decyl acetate	1778	000112-17-4	1.95 $\pm$ 0.25 <sup>a</sup>	0.024	1.93 $\pm$ 0.15 <sup>a</sup>	0.024	1.64 $\pm$ 0.16 <sup>a</sup>	0.021	Fatty, waxy, soapy, fruity
	2-Phenylethyl acetate	1862	000103-45-7	31 $\pm$ 0.91 <sup>a</sup>	0.384	19.3 $\pm$ 0.41 <sup>b</sup>	0.242	12.5 $\pm$ 0.32 <sup>c</sup>	0.163	Floral, rose, sweet
	Ethyl hexanoate	1297	000123-66-0	10.31 $\pm$ 1.59 <sup>a</sup>	0.127	11.72 $\pm$ 1.23 <sup>a</sup>	0.147	9.69 $\pm$ 1.93 <sup>a</sup>	0.126	Banana, fruity, floral
	Ethyl octanoate	1453	000106-32-1	278 $\pm$ 2.87 <sup>a</sup>	3.446	298 $\pm$ 6.35 <sup>b</sup>	3.733	254 $\pm$ 18.7 <sup>a</sup>	3.31	Soapy, brandy, apple
	Ethyl nonanoate	1624	000123-29-5	0.58 $\pm$ 0.01 <sup>a</sup>	0.007	0.44 $\pm$ 0.04 <sup>b</sup>	0.006	0.48 $\pm$ 0.01 <sup>b</sup>	0.006	Fruity, nutty, waxy
	Ethyl decanoate	1746	000110-38-3	1400 $\pm$ 52.88 <sup>a</sup>	17.36	1910 $\pm$ 170 <sup>b</sup>	23.96	1360 $\pm$ 40.11 <sup>a</sup>	17.72	Waxy, sweet, apple
	Ethyl dodecanoate	1887	000106-33-2	370 $\pm$ 12.77 <sup>a</sup>	4.587	553 $\pm$ 102 <sup>b</sup>	6.938	239 $\pm$ 11.2 <sup>c</sup>	3.114	Soapy, waxy, floral
	Ethyl tetradecanoate	2161	000124-06-1	11.90 $\pm$ 1.86 <sup>a</sup>	0.148	17.80 $\pm$ 1.15 <sup>b</sup>	0.223	8.46 $\pm$ 0.20 <sup>c</sup>	0.11	--
	Ethyl hexadecanoate	2373	000628-97-7	20.80 $\pm$ 0.34 <sup>a</sup>	0.258	15.60 $\pm$ 0.34 <sup>b</sup>	0.196	11.00 $\pm$ 0.80 <sup>c</sup>	0.143	--
	Ethyl 9-hexadecenoate	2402	054546-22-4	24.50 $\pm$ 2.94 <sup>a</sup>	0.304	15.30 $\pm$ 2.01 <sup>b</sup>	0.192	8.65 $\pm$ 1.05 <sup>c</sup>	0.113	--
	Methyl octanoate	1470	000111-11-5	0.40 $\pm$ 0.02 <sup>a</sup>	0.005	0.51 $\pm$ 0.01 <sup>b</sup>	0.006	0.35 $\pm$ 0.01 <sup>c</sup>	0.005	Fruity, orange-like
	Methyl decanoate	1687	000110-42-9	2.18 $\pm$ 0.12 <sup>a</sup>	0.027	3.25 $\pm$ 0.09 <sup>b</sup>	0.041	1.94 $\pm$ 0.04 <sup>c</sup>	0.025	Oily, fruity, wine-like
	Methyl dodecanoate	1907	000111-82-0	0.86 $\pm$ 0.14 <sup>a</sup>	0.011	1.67 $\pm$ 0.14 <sup>b</sup>	0.021	0.63 $\pm$ 0.02 <sup>a</sup>	0.008	Waxy, soapy, creamy
	Isobutyl octanoate	1642	005461-06-3	5.82 $\pm$ 0.13 <sup>a</sup>	0.072	7.08 $\pm$ 0.30 <sup>b</sup>	0.089	4.24 $\pm$ 0.18 <sup>c</sup>	0.055	Fruity, green, oily
	Isobutyl decanoate	1859	030673-38-2	12.34 $\pm$ 1.06 <sup>a</sup>	0.153	16.43 $\pm$ 0.56 <sup>b</sup>	0.206	8.01 $\pm$ 0.38 <sup>c</sup>	0.104	Oily, brandy, apricot
	Isobutyl dodecanoate	2068	037811-72-6	1.24 $\pm$ 0.21 <sup>a</sup>	0.015	2.04 $\pm$ 0.13 <sup>b</sup>	0.026	0.68 $\pm$ 0.06 <sup>c</sup>	0.009	Oily, floral, waxy
	Isoamyl hexanoate	1543	002198-61-0	1.93 $\pm$ 0.04 <sup>a</sup>	0.024	1.77 $\pm$ 0.16 <sup>a</sup>	0.022	1.33 $\pm$ 0.02 <sup>b</sup>	0.017	Apple, pineapple, sweet
Isoamyl octanoate	1762	002035-99-6	42.82 $\pm$ 1.09 <sup>a</sup>	0.531	45.82 $\pm$ 1.72 <sup>a</sup>	0.575	31.68 $\pm$ 1.54 <sup>b</sup>	0.413	Fruity, sweet, waxy	
Isoamyl decanoate	1973	002306-91-4	35.95 $\pm$ 1.31 <sup>a</sup>	0.446	40.61 $\pm$ 2.69 <sup>b</sup>	0.51	20.97 $\pm$ 0.56 <sup>c</sup>	0.273	Brandy, rum, coconut	
Isoamyl dodecanoate	2180	006309-51-9	3.91 $\pm$ 0.13 <sup>a</sup>	0.048	3.95 $\pm$ 0.02 <sup>a</sup>	0.05	1.50 $\pm$ 0.03 <sup>b</sup>	0.02	Mild, waxy, peach	
<b>Subtotal</b>				<b>2270.21</b>	<b>28.147</b>	<b>2959.34</b>	<b>37.1</b>	<b>1985.92</b>	<b>25.877</b>	



TABLE 3 (CONTINUED)

Major volatile compounds (GC-FID peak area  $\times 10^6$ ) and their relative peak areas (RPA) in mango wine (day 14) fermented by three *S. cerevisiae* yeasts.

Groups	Compounds	LRI	CAS. No	MERIT.ferm		CICC1028		EC1118		Aroma descriptors of pure compounds <sup>(1)</sup>
				Peak area	RPA (%)	Peak area	RPA (%)	Peak area	RPA (%)	
Ketones	Acetoin	1401	000513-86-0	0.12 $\pm$ 0.01 <sup>a</sup>	0.001	0.02 $\pm$ 0.00 <sup>b</sup>	0	0.36 $\pm$ 0.04 <sup>c</sup>	0.005	Butter-like
	2-Undecanone	1695	000112-12-9	0.09 $\pm$ 0.02 <sup>a</sup>	0.001	0.80 $\pm$ 0.04 <sup>b</sup>	0.01	1.35 $\pm$ 0.09 <sup>c</sup>	0.017	Rose, citrus, orris-like
	1-(4-Methylphenyl)-ethanone	1903	000122-00-9	0.17 $\pm$ 0.02 <sup>a</sup>	0.002	0.21 $\pm$ 0.00 <sup>b</sup>	0.003	0.18 $\pm$ 0.00 <sup>a</sup>	0.002	Floral
	Beta-damascenone	1938	023696-85-7	0.44 $\pm$ 0.00 <sup>a</sup>	0.005	0.56 $\pm$ 0.02 <sup>b</sup>	0.007	0.38 $\pm$ 0.02 <sup>c</sup>	0.005	Berry, woody, floral
	Gamma-decalactone	2281	000706-14-9	0.16 $\pm$ 0.02 <sup>a</sup>	0.002	0.16 $\pm$ 0.00 <sup>a</sup>	0.002	0.17 $\pm$ 0.02 <sup>a</sup>	0.002	Creamy, fruity, peach
<b>Subtotal</b>				<b>0.98</b>	<b>0.011</b>	<b>1.75</b>	<b>0.022</b>	<b>2.44</b>	<b>0.031</b>	
Aldehydes	Acetaldehyde	939	000075-07-0	5.20 $\pm$ 0.32 <sup>a</sup>	0.064	2.60 $\pm$ 1.04 <sup>b</sup>	0.032	6.01 $\pm$ 1.91 <sup>a</sup>	0.078	Pungent, green
	Benzaldehyde	1637	000100-52-7	0.31 $\pm$ 0.02 <sup>a</sup>	0.004	0.28 $\pm$ 0.00 <sup>a</sup>	0.004	0.23 $\pm$ 0.02 <sup>b</sup>	0.003	Bitter almond
	<i>p</i> -Tolualdehyde	1773	000104-87-0	1.38 $\pm$ 0.16 <sup>a</sup>	0.017	2.92 $\pm$ 0.16 <sup>b</sup>	0.037	1.33 $\pm$ 0.09 <sup>a</sup>	0.017	Cherry, sweet
<b>Subtotal</b>				<b>6.89</b>	<b>0.085</b>	<b>5.8</b>	<b>0.073</b>	<b>7.57</b>	<b>0.098</b>	
Miscellaneous	Dihydro-2-methyl-3(2H)-thiophenone	1637	013679-85-1	0.58 $\pm$ 0.13 <sup>a</sup>	0.007	0.59 $\pm$ 0.02 <sup>a</sup>	0.007	0.48 $\pm$ 0.02 <sup>b</sup>	0.006	Sulfur, fruity, berry
<b>Subtotal</b>				<b>0.58</b>	<b>0.007</b>	<b>0.59</b>	<b>0.007</b>	<b>0.48</b>	<b>0.006</b>	
<b>Total</b>				<b>8510.37</b>		<b>7973.35</b>		<b>7579.9</b>		

<sup>abc</sup>ANOVA (n=4) at 95% confidence level with same letters indicating no significant difference.

<sup>(1)</sup> Descriptors were retrieved from <http://www.thegoodscentscompany.com>.

concentrations of acetate esters than the other two (Table 4). Acetates are produced from the reaction of acetyl-CoA with alcohols (Perestrelo *et al.*, 2006) and thus, the higher production of acetates by strain MERIT.ferm-fermented wine may be due to the higher quantities of ethanol and branched-chain higher alcohols that strain MERIT.ferm produced (i.e. increased substrate availability). Additionally, the concentrations of 2-phenylethyl acetate and isoamyl acetate for all three wines were higher than their threshold levels for all three wines (Table 4), but ethyl acetate was slightly lower than its threshold level (Table 4). The esters of this group have a positive contribution to the overall quality of the wine and most produce moderate "floral" or "fruity" flavours (Table 3)

Ethyl esters are produced enzymatically during the synthesis or degradation of fatty acids (Alves *et al.*, 2010). The concentration of these esters is dependent on several factors, including: yeast strain, fermentation temperature, aeration and sugar content (Perestrelo *et al.*, 2006). Ethyl esters can add moderate notes of ripe fruits to fermented wine if they are in the desirable range (Alves *et al.*, 2010). The major ethyl esters in our fermented wines were ethyl octanoate, ethyl decanoate and ethyl dodecanoate (Table 3), and the concentrations of these esters were higher than their threshold levels for all three wines (Table 4). The kinetic changes of the three esters are shown in Fig. 3. In addition, strain CICC1028-fermented wine had significantly higher concentrations of the three ethyl esters than the other two wines, which could be linked to its high production of medium-chain fatty acids (Table 4). This is supported by a recent study that demonstrates the crucial role of the fatty acid precursor level in ethyl ester production by *S.*

*cerevisiae* (Saerens *et al.*, 2008).

Other esters, such as ethyl hexanoate, isobutyl octanoate, isoamyl hexanoate, isoamyl octanoate, were also identified in mango wines (Table 3). Strains CICC1028 and MERIT.ferm were better at producing these esters than strain EC1118.

Acetic, octanoic, decanoic and dodecanoic acids were the major fatty acids detected in mango wines. Acetic acid was highest in the MERIT.ferm-fermented wine, and it reached 0.034, 0.01, 0.025 g/100 mL for strains MERIT.ferm, CICC1028 and EC1118 on day 14, respectively (Table 4). The kinetic change of acetic acid is shown in Fig. 4. Acetic acid in high concentrations is undesirable in alcoholic beverages, which may impart a vinegar off-odor. Acetic acid in the MERIT.ferm and EC1118 fermented mango wine was slightly higher than the threshold level (Table 4), but whether this would affect wine quality needs sensory evaluation. In the study of Lambrechts and Pretorius (2000), acetic acid between 0.02-0.07 g/100mL was considered optimal depending on the style of wine, therefore, acetic acid in Merit.ferm and EC1118 fermented mango wine may not bring about a negative flavour note. In addition, strain CICC1028 produced the highest levels of medium-chain fatty acids such as octanoic acid, decanoic acid and dodecanoic acid (Table 3). The kinetic change of octanoic acid is shown in Fig. 1, and it increased initially, and then decreased slightly and remained constant after day 6. Decanoic and dodecanoic acids showed similar kinetic changes to those of octanoic acid (data not shown). The concentration of octanoic acid was also quantified in Table 4, and it was just at the threshold level for the three wines. These medium-chain fatty acids may impart fatty, rancid and soapy off-odours, so they must be controlled

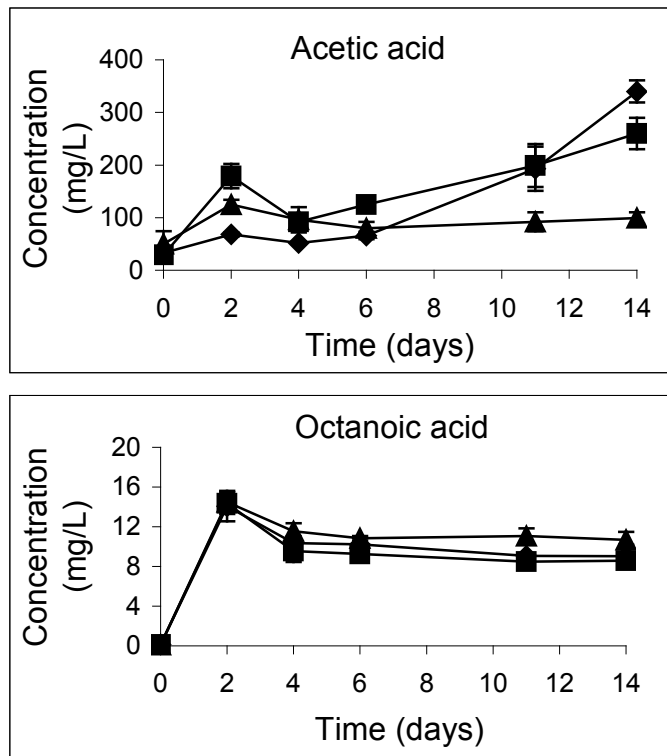


FIGURE 4

Changes of fatty acids in mango wines during fermentation by *S. cerevisiae* MERIT.ferm (◆), *S. chevalieri* CICC-1028 (▲) and *S. bayanus* EC-1118 (■).

TABLE 4.

Concentrations of selected volatile compounds (mg/L) and the corresponding odor activity values (OAVs) in mango wines fermented with culture of three *S. cerevisiae* yeasts on Day 14.

Compounds	CAS No.	Retention index	MERIT.ferm (mg/L)	OAV <sup>(1)</sup>	CICC1028 (mg/L)	OAV	EC1118 (mg/L)	OAV	Odor threshold (mg/L)
Ethyl acetate	000141-78-6	1009	1.81±0.23 <sup>a</sup>	0.24	1.16±0.08 <sup>b</sup>	0.15	1.25±0.11 <sup>b</sup>	0.17	7.5 <sup>(2)</sup>
Ethanol	000064-17-5	1028	70136±1080 <sup>a</sup>	--	61543±663 <sup>b</sup>	--	63027±464 <sup>b</sup>	--	Not applicable
Isobutyl alcohol	000078-83-1	1097	22.20±2.60 <sup>a</sup>	0.56	14.72±2.54 <sup>b</sup>	0.37	9.43±0.83 <sup>c</sup>	0.24	40 <sup>(2)</sup>
Isoamyl acetate	000123-92-2	1112	0.35±0.03 <sup>a</sup>	11.67	0.04±0.02 <sup>b</sup>	1.33	0.12±0.01 <sup>c</sup>	4	0.03 <sup>(2)</sup>
Isoamyl alcohol	000123-51-3	1237	409.85±42.66 <sup>a</sup>	13.67	146.43±6.71 <sup>b</sup>	4.88	136.91±23.18 <sup>b</sup>	4.56	300 <sup>(2)</sup>
Ethyl octanoate	000106-32-1	1453	10.06±0.31 <sup>a</sup>	5030	10.88±0.10 <sup>b</sup>	5440	9.27±0.68 <sup>a</sup>	4635	0.002 <sup>(2)</sup>
Acetic acid	000064-19-7	1549	340±21 <sup>a</sup>	1.7	99±11 <sup>b</sup>	0.5	260±30 <sup>c</sup>	1.3	200 <sup>(2)</sup>
Ethyl decanoate	000110-38-3	1746	12.56±0.06 <sup>a</sup>	62.80	16.07±2.06 <sup>b</sup>	80.35	11.85±0.31 <sup>a</sup>	59.25	0.2 <sup>(3)</sup>
2-Phenylethyl acetate	000103-45-7	1862	1.17±0.06 <sup>a</sup>	4.68	0.75±0.05 <sup>b</sup>	3	0.45±0.01 <sup>c</sup>	1.8	0.25 <sup>(2)</sup>
Ethyl dodecanoate	000106-33-2	1887	11.32±0.51 <sup>a</sup>	9.43	18.61±0.84 <sup>b</sup>	15.51	7.60±1.92 <sup>c</sup>	6.33	1.2 <sup>(3)</sup>
2-Phenylethyl alcohol	000060-12-8	1964	59.56±1.30 <sup>a</sup>	5.96	13.27±2.19 <sup>b</sup>	2.45	27.72±0.69 <sup>c</sup>	2.77	10 <sup>(2)</sup>
Octanoic acid	000124-07-2	2100	9.04±1.16 <sup>a</sup>	1.03	10.67±0.81 <sup>b</sup>	1.21	8.58±0.41 <sup>a</sup>	0.975	8.8 <sup>(3)</sup>

<sup>abc</sup>ANOVA (n=4) at 95% confidence level with the same letters indicating no significant difference.

<sup>(1)</sup>Odour activity values (OAV) were calculated by dividing concentration by the odour threshold value of the compound.

<sup>(2)</sup>The odor threshold was obtained from Guth (1997).

<sup>(3)</sup>The odor threshold was obtained from Bartowsky & Pretorius (2008).

at low levels or at least not higher than their threshold levels. Furthermore, they could also act as potential inhibitors of alcoholic fermentation (Lambrechts & Pretorius, 2000). This may explain why the cell count of strain CICC1028 was 10 times **lower** than those of strains Merit.ferm and EC1118.

Acetaldehyde, benzaldehyde, *p*-tolualdehyde were identified in mango wines and acetaldehyde was the major aldehyde (Table 3). Compared with other volatiles, aldehydes were only a minor group with less than 0.1% RPA. At low levels, acetaldehyde gives a pleasant fruity aroma to wines, but in higher concentrations, it has a pungent, irritating odor (Miyake & Shibamoto, 1993). In addition, acetaldehyde originated as an intermediary product of yeast metabolism from pyruvate through the glycolytic pathway and it is also a precursor for acetate, acetoin as well as ethanol (Collins, 1972).

Five ketones were identified in mango wines. Beta-damascenone concentration decreased during fermentation, whereas other ketones such as 2-undecanone, acetoin almost kept constant after day 4 (data not shown). Beta-damascenone was one of a few compounds which were identified in both fresh mango juice and wine. A sulfur ketone [dihydro-2-methyl-3(2H)-thiophenone] was found in all three mango wines (Table 3) but not in the fresh mango juice, which was probably produced by yeasts during fermentation. This sulfur compound is usually found in malt whiskey (Masuda & Nishimura, 1982) and it may contribute to blackberry flavor. This is the first time that dihydro-2-methyl-3(2H)-thiophenone was found in mango wine from the best of our knowledge. Although these ketones

had low concentrations, they may contribute to “floral” or “fruity” aroma to mango wines synergistically.

It will be valuable to correlate the data obtained from instrumental analysis with the result of sensory evaluation. It is recognised that sensory analysis was not performed in this study due to a lack of trained panel. Further studies will include sensory evaluation to compare the sensory and chemical profiles of mango wines fermented with different yeasts on a larger-scale.

## CONCLUSION

The presence of volatile compounds and their concentrations during mango juice fermentation were dependent on the yeast strain used as the starter culture. The results obtained by the HS-SPME-GC-MS/FID technique showed differences in the volatile profiles of three mango wines fermented with different *S. cerevisiae* yeast strains. The mango wine from the fermentation with strain MERIT.ferm produced more branched-chain higher alcohols and aromatic branched-chain esters when compared to the other two wines fermented with strains EC1118 and CICC1028, so it can be selected as a good candidate for mixed-culture fermentation with one *Williopsis saturnus* yeast in subsequent studies.

## LITERATURE CITED

- Anonymous, [http://www.liancome.com/liancome\\_mango.htm](http://www.liancome.com/liancome_mango.htm).
- Anonymous, <http://www.thegoodscentscompany.com>.
- Alves, J. A., Lima, L. C. O., Dias, D. R., Nunes, C. A. & Schwan, R. F., 2010. Effects of spontaneous and inoculated fermentation on the volatile profile of lychee (*Litchi chinensis* Sonn) fermented beverages. *Int. J. Food Sci. Tech.* 45, 2358-2365
- Bartowsky, E. J. & Pretorius, I. S., 2008. Microbial formation and modification of flavor and off-flavor compounds in wine. In: König, H., Uden, G. & Frohlich, J. (eds). *Biology of microorganisms on grapes, in must and in wine*. Springer-Verlag, Berlin. pp 215-217.
- Chávez-Servín, J. L., Castellote, A. I. & López-Sabater, M. C., 2004. Analysis of mono- and disaccharides in milk-based formulae by high-performance liquid chromatography with refractive index detection. *J. Chromatogr. A.* 1043, 211-215.
- Collins, E. B., 1972. Biosynthesis of flavor compounds by microorganisms. *J. Dairy Sci.* 55(7), 1022-1028.
- Coloretti, F., Zambonelli, C., Castellari, L., Tini, V. & Rainieri, S., 2002. The effect of DL-malic acid on the metabolism of L-malic acid during wine alcoholic fermentation. *Food Technol. Biotech.* 40(4), 317-320.
- Czyrnciuk, N., 1966. The technology of passion fruit and mango wines. *Am. J. Enol. Viticult.* 17, 27-30.
- Dickinson, J. R., Harrison, S. J. & Michael, J. E., 1998. An investigation of the metabolism of valine to isobutyl alcohol in *Saccharomyces cerevisiae*. *J. Biol. Chem.* 273(40), 25751-25756.
- Etschmann, M., Bluemke, W., Sell, D. & Schrader, J., 2002. Biotechnological production of 2-phenylethanol. *Appl. Microbiol. Biot.* 59(1), 1-8.
- Gürbüz, O., Rouseff, J. M. & Rouseff, R. L., 2006. Comparison of aroma volatiles in commercial Merlot and Cabernet Sauvignon wines using gas chromatography – olfactory and gas chromatography – mass spectrometry. *Int. J. Food Sci. Tech.* 41, 3990-3996.
- Guth, H., 1997. Quantitation and sensory studies of character impact odorants of different white wine varieties. *J. Agric. Food Chem.* 45, 3027-3032.
- Kumar, Y. S., Prakasam, R. S. & Reddy, O. V. S., 2009. Optimisation of fermentation conditions for mango (*Mangifera indica* L.) wine production by employing response surface methodology. *Int. J. Food Sci. Tech.* 44, 2320-2327.
- Lambrechts, M. G. & Pretorius, I. S. (2000). Yeast and its importance to wine aroma – a review. *S. Afr. J. Enol. Vitic.* 21, 97-129.
- Lee, P. R., Ong, Y. L., Yu, B., Curran, P. & Liu, S. Q., 2010a. Profile of volatile compounds during papaya juice fermentation by a mixed culture of *Saccharomyces cerevisiae* and *Williopsis saturnus*. *Food Microbiol.* 22(7), 853-861.
- Lee, P. R., Ong, Y. L., Yu, B., Curran, P. & Liu, S. Q., 2010b. Evolution of volatile compounds in papaya wine fermented with three *Williopsis saturnus* yeasts. *Int. J. Food Sci. Tech.* 45(10), 2032-2041.
- Masuda, M. & Nishimura, K., 1982. Changes in volatile sulfur compounds of whisky during aging. *J. Food Sci.* 47(1), 101-105.
- Miyake, T., & Shibamoto, T., 1993. Quantitative analysis of acetaldehyde in foods and beverages. *J. Agric. Food Chem.* 41(11), 1968-1970.
- Myers, M. J., Issenberg, P. & Wick, E. L., 1970. L-leucine as a precursor of isoamyl alcohol and isoamyl acetate, volatile aroma constituents of banana fruit discs. *Phytochemistry* 9(8), 1693-1700.
- Noguerol-Pato, R., González-Barreiro, B., Cancho-Grande, B. & Simal-Gándara, B., 2009. Quantitative determination and characterization of the main odorants of Mencia monovarietal red wines. *Food Chem.* 117(3), 473-484.
- Obisanya, M. O., Aina, J. O. & Oguntime, G. B., 1987. Production of wine from mango (*Mangifera indica* L.) using *Saccharomyces* and *Schizosaccharomyces* species isolated from palm wine. *J. Appl. Bacteriol.* 63, 191-196.
- Ong, P.K.C. & Acree, T.E., 1999. Similarities in the aroma chemistry of Gewürztraminer variety wines and lychee (*Litchi chinensis* Sonn.) fruit. *J. Agric. Food Chem.* 47, 665-670.
- Onkarayya, H. & Singh, H., 1984. Screening of mango varieties for dessert and mandeira-style wine. *Am. J. Enol. Viticult.* 35, 63-65.
- Perestrelo, R., Fernandes, A., Albuquerque, F., Marques, J. & Camara, J., 2006. Analytical characterization of the aroma of Tinta Negra Mole red wine: identification of the main odorants compounds. *Anal. Chim. Acta* 563(1-2), 154-164.
- Pino, J. A., & Mesa, J., 2005. Contribution of volatile compounds to mango (*Mangifera indica* L.) aroma. *Flavour Frag. J.* 21, 207-213.
- Pino, J. A., Mesa, J., Munoz, Y., Marti, M. P. & Marbot, R., 2005. Volatile components from mango (*Mangifera indica* L.) cultivars. *J. Agric. Food Chem.* 53, 2213-2223.
- Rapp, A. & Mandery, H., 1986. Wine aroma. *Experientia* 42, 873-884.
- Rapp, A. & Mandery, H., 1987. New progress in wine and wine research *Experientia* 45, 873-884.
- Rapp, A., 1988. Wine aroma substances from gas chromatographic analysis. In: Liskens, H. & Jackson, J. (eds). *Modern methods of plant analysis*. Springer-Verlag, Berlin. pp. 29-65.
- Reddy, L. V. A. & Reddy, O. V. S., 2005. Production and characterization of wine from mango fruit (*Mangifera indica* L.). *World J. Microb. Biot.* 21, 1345-1350.

- Reddy, L. V. A. & Reddy, O. V. S., 2009. Effect of enzyme maceration on synthesis of higher alcohols during mango wine fermentation. *J. Food Quality* 32, 34-47.
- Saerens, S. M. G., Delvaux, F., Verstrepen, K. J., van Dijck, P., Thevelein, J. M. & Delvaux, F.R., 2008. Parameters affecting ethyl ester production by *Saccharomyces cerevisiae* during fermentation. *Appl. Environ. Microbiol.* 74(2):454-461.
- Spreer, W., Ongprasert, S., Hegele, M., Wunsche, J. N. & Muller, J., 2009. Yield and fruit development in mango (*Mangifera indica* L. cv. *Chok Anan*) under different irrigation regimes. *Agr. Water Manage.* 96, 574-584.
- Tairu, A. O., Hofmann, T. & Schieberle, P., 1999. Characterization of the key aroma compounds in dried fruits of the west african peppertree *Xylopiya aethiopica* (Dunal) A. Rich (*Annonaceae*) using aroma extract dilution analysis. *J. Agri. Food Chem.* 47, 3285-3287.
- Tharanathan, R. N., Yashoda, H. M. & Prabha, T. N., 2006. Mango (*Mangifera indica* L.), "the king of fruits"—an overview. *Food Rev. Int.* 22, 95-123.
- Trinh, T. T. T., Woon, W. Y., Yu, B., Curran, P. & Liu, S. Q., 2010. Effect of L-isoleucine and L-phenylalanine addition on aroma compound formation during longan juice fermentation by a co-culture of *Saccharomyces cerevisiae* and *Williopsis saturnus*. *S. Afr. J. Enol. Vitic.* 31(2), 116-124.
- Ugliano, M., Bartowsky, E.J., McCarthy, J., Moio, L. & Henschke, P.A., 2006. Hydrolysis and transformation of grape glycosidically bound volatile compounds during fermentation with three *Saccharomyces* yeast strains. *J. Agric. Food Chem.* 54, 6322-6331.
- Yilmaztekin, M., Erten, H. & Cabaroglu, T., 2009. Enhanced production of isoamyl acetate from beet molasses with addition of fusel oil by *Williopsis saturnus* var. *saturnus*. *Food Chem.* 112, 290-294.
- Zoecklein, B. W., Marcy, J. E., Williams, J. M. & Jasinski, Y., 1997. Effect of native yeasts and selected strains of *Saccharomyces cerevisiae* on glycosyl glucose, potential volatile terpenes, and selected aglycones of white Riesling (*Vitis vinifera* L.) wines. *J. Food Compos. Anal.* 10, 55-65.