Impact of Pulp on the Chemical Profile of Mango Wine

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The aim of this study was to assess the effect of mango pulp inclusion (which mimicked the maceration step in grape wine fermentation) on the fermentation dynamics and chemical profile of mango wine, especially the volatiles. The growth of *Saccharomyces cerevisiae* MERIT.ferm was slower in the mango juice with pulp (uncentrifuged juice), with a corresponding slower reduction in °Brix, relative to the juice without pulp (centrifuged juice). The utilisation of glucose, fructose and sucrose was similar in both uncentrifuged and centrifuged juices, with almost complete consumption. Citric, tartaric, malic, pyruvic and succinic acid were the major organic acids in the wines fermented from both the uncentrifuged and centrifuged juices. Citric acid decreased slightly in the macerated wine. Tartaric and malic acid decreased in both wines. Pyruvic acid increased slightly and succinic acid remained almost constant in both wines. Monoterpenes, as one of the signature aroma compounds in mango juice, decreased dramatically in both wines, but were ten times higher in the macerated wine. Terpenols were at least four times higher in the macerated wine. The macerated wine also produced higher levels of fusel alcohols and acetate esters compared to the non-macerated wine. On the other hand, the non-macerated wine possessed a higher concentration of medium-chain fatty acids and corresponding ethyl esters. This study indicates that the inclusion of pulp in mango wine fermentation would contribute to the aroma complexity.

INTRODUCTION

Mango (*Mango indica* L.) is an economically important fruit in South and Southeast Asia (Tharanathan *et al*., 2006). However, substantial quantities of mangoes are wasted because of its short shelf life. Therefore, postharvest quality preservation via various approaches and further processing of this fruit become essential (Sivakumar *et al*., 2011). A possible alternative to processing and preserving mango is to ferment its juice into wine, which is helpful in keeping mango flavour characteristics for a longer period. This is possible due to the high sugar content (16 to 18%) (with sucrose, glucose and fructose being the principal sugars) and other nutrients in fully-ripened mango fruits (Reddy & Reddy, 2005).

The production of mango wine was first reported in the 1960s (Czyhrinciwk, 1966). In recent years, analysis of the volatile compounds of mango wine has been done on several cultivars, such as *Banginapalli*, *Alphonso*, *Haden*, *Chok anan*, *Allampur baneshan*, *Neelam bangalora, Raspuri*, *R2E2* and *Harum manis* (Reddy & Reddy, 2005; Reddy *et al*., 2010; Li *et al*., 2011; Pino & Queris, 2011; Li *et al*., 2012). Reddy and Reddy (2005) used *Saccharomyces cerevisiae*

together with pectinase to ferment six local varieties of Indian mango. They concluded that the ethanol level and aromatic components in mango wine were comparable to those of grape wine. Reddy *et al*. (2010) found isoamyl alcohol and ethyl acetate to be in amounts comparable to that of grape wine. Pino and Queris (2011) reported the main aroma-active compounds in mango wine as aldehydes, esters and alcohols by using odour activity values. They also found ethyl butanoate and decanal to be the powerful contributors to the aroma of mango wine. Li *et al*. (2012) differentiated the characters of mango wines fermented from three mango varieties and suggested that the Thai *Nam Doc Mai* mango wine was similar to white wine from grapes due to its higher production of alcohols and esters, while the Australian *R2E2* wine was able to retain its original aroma due to its retention of lactones.

Wine aroma, widely considered to be a key aspect of wine quality, is the result of the interaction between fruit components and those produced during processing, fermentation and ageing. It is now well established that, apart from free aroma compounds, a significant part of several aroma compounds is accumulated in fruits as odourless non-

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volatile glycosides (Adedeji *et al*., 1992; Drider *et al*., 1994). The aglycone moieties of glycosides include monoterpenes, alcohols, aldehydes, acids, esters, C_{13}-norisoprenoids and other compounds. To date, various studies have been conducted on glycosidically-bound volatiles in different mango cultivars (Adedeji *et al*., 1992; Drider *et al*., 1994; Ollé *et al*., 1998; Lalel *et al*., 2003). Mango fruit possess glycosidases on their own and their activities are enhanced during the maturation process. The glycosidically-bound aroma compounds therefore continuously increase in the pulp as maturity progresses, but the activities of glycosidases in the fruit are greatly reduced at typical wine fermentation pH (Lalel *et al*., 2003). Thus, the mango pulp still contains glycosides that serve as aroma precursors and the aroma compounds can be released during fermentation, since *S. cerevisiae* glycosidases are able to release glycosidically-bound aroma compounds during wine fermentation (Mateo & Stefano, 1997; Palmeri & Spagna, 2007). However, this aspect has not been examined in mango wine fermentation and provides a potential method for enhancing the mango wine aroma profile by fermentation with the inclusion of pulp, which mimics the maceration process of red grape wine fermentation.

The aim of this study was to compare the fermentation dynamics and chemical profile of mango wine fermented with uncentrifuged (with pulp included) and centrifuged (with pulp removed) juices. Terpenes are significant contributors to the unique mango aroma (Macleod *et al*., 1988; Pino *et al*., 2005; Pino and Mesa, 2006), but are largely metabolised upon mango wine fermentation (Li *et al*., 2011, 2012). The hypothesis of the present study was that mango pulp contained more aroma compounds bound as glycosides than the centrifuged mango juice, and that wine yeast would release these bound aroma compounds from the glycosides present in the pulp, which would also compensate for the loss of the terpenes during fermentation. The ultimate aim was to enable the intensification and diversification of the mango wine flavour so as to achieve unique mango wine style and distinction.

MATERIALS AND METHODS
Yeast strain and media
Saccharomyces cerevisiae MERIT.ferm (Chr.-Han., Horsholm, Denmark) was used in this study because of its ability to produce more branched-chain alcohols and esters in 'Chok Anan' mango wine (Li *et al*., 2011). The yeast was 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 and 0.25% (w/v) malt extract and incubated at 25°C for up to 48 h. The yeast was stored at -80°C with 20% glycerol before use. Potato dextrose agar (PDA) (39 g/L, Oxoid, Basingstoke, Hampshire, England) was used for plating to monitor the growth of the *Saccharomyces* yeast at indicated time points.

Preparation of centrifuged and uncentrifuged mango juices
'Chok Anan' mangoes from Malaysia were purchased from a local market in Singapore and stored at 25°C until fully ripened before use. The preparation of mango juice was done in accordance with the method of Li *et al*. (2011), with modifications. The ripened mangoes were juiced and divided into two lots. The first lot was centrifuged at 21 000 rpm (41 415 *g*, Beckman Centrifuge, MA, USA) and 4°C for 10 min to remove the pulp, and the centrifuged juice (supernatant) was stored at -50°C until further use. The second lot, containing the pulp, was stored directly at -50°C without being subjected to centrifugation (uncentrifuged juice). The pre-culture medium was prepared using the centrifuged mango juice (14.97 °Brix, containing 31 g/L of fructose, 15 g/L of glucose and 76 g/L of sucrose, pH 4.76), and the juice was heat-treated using a Hirayama HG-80 autoclave (Hirayama, Saitama, Japan) at 100°C for 3 min and cooled to room temperature before use. The medium was inoculated with 10% (v/v) of the yeast strain and incubated for 48 h at 25°C until yeasts grew to at least 10^7 cfu/mL. The centrifuged and uncentrifuged mango juices (pH adjusted to 3.5 with 50% w/v food grade d/l-malic acid from Suntop Ltd, Singapore) used for fermentation were treated with sulphur dioxide in the form of $K_2S_2O_5$, potassium metabisulphite (The Goodlife Homebrew Centre, Norfolk, England) at 100 ppm ($K_2S_2O_5$ was added just before heating), then heated at 60°C for 15 minutes and cooled to room temperature before use. The reason for the addition of sulphur dioxide was to prevent polyphenol oxidase from causing browning of the mango juice.

Fermentation
Mango juice fermentations were carried out in triplicate in 500 mL sterile Erlenmeyer conical flasks, with each flask containing 450 mL of centrifuged or uncentrifuged mango juice. All samples were inoculated with 1% (v/v) pre-culture of *S. cerevisiae* MERIT.ferm, and the fermentations were conducted statically at 20°C for 10 days. Sampling was done during fermentation (Day 0, 2, 4, 7, 10).

Sample analysis
Both pH and total soluble solids (°Brix) were measured in duplicate at the indicated time points by a pH meter and a refractometer respectively.

The concentration of sugars and organic acids was analysed in duplicate by Shimadzu ultrafast liquid chromatography (UFLC). Cell-free wine samples were obtained by centrifugation and filtration through a 0.2 μm regenerated cellulose filter membrane (Sartorius Stedium Biotech, Geottingen, Germany). The column used for sugar analysis was a Zorbax carbohydrate column (4.6 × 150 mm, 5-micron, Agilent, Santa Clara, CA, USA). The column was connected to an evaporative light scattering detector (40°C, 350 Kpa N_2, Gain 5). Organic acids were determined with a Supelcogel C-610H column (Supelco Bellefonte, PA, USA) connected to a photodiode array detector at UV 210 nm. Other UFLC running conditions were as described in Li *et al*. (2012).

The volatile compounds were measured in duplicate using a headspace (HS) solid-phase microextraction (SPME) method, coupled with a gas chromatography (GC)-mass spectrometer (MS) and flame ionisation detector (FID) (HS-SPME-GC-MS/FID). A carboxen/PDMS fibre (85 μm) (Supelco, Sigma-Aldrich, Barcelona, Spain) was used for

the extraction of volatiles. Sample volume was 5 mL and it was subjected to extraction by HS-SPME at 60°C for 40 min under 250 rpm agitation. The fibre was then desorbed at 250°C for 3 min, before the sample was injected into the Agilent 7890A GC (Santa Clara, CA, USA), which was coupled to the FID and Agilent 5975C triple-axis MS. The capillary column for separation was an Agilent DB-FFAP of 60 m x 0.25 mm I.D., coated with 0.25 μm film thickness of polyethylene glycol modified with nitroterephthalic acid. For quantitative analysis of the samples, major volatiles (major peaks in the FID chromatogram) were quantified by comparison with available external standards. The method was modified from Chen *et al*. (2006) and validated. The other details of the HS-SPME-GC-MS/FID are given in Li *et al*. (2012). All samples were clarified by centrifugation before GC analysis. Odour activity values of these quantified volatiles were calculated according to their established threshold levels in the literature (Guth, 1997; Lambrechts & Pretorius, 2000; Yamamoto *et al*., 2004; Zemni *et al*., 2007; Bartowsky & Pretorius, 2008).

Statistical analysis
The statistical differences of the chemical composition of mango wine fermented from centrifuged and uncentrifuged juices (before and after fermentation) were evaluated by one-way analysis of variance (ANOVA) or Student's T-test. All tests of significance were conducted at a probability level of $P < 0.05$. The means and standard deviations were obtained from triplicate fermentation samples.

RESULTS AND DISCUSSION
Yeast growth, °Brix and pH changes
The growth dynamics of *S. cerevisiae* MERIT.ferm in centrifuged and uncentrifuged juices (corresponding to non-macerated wine and macerated wine respectively) were similar, although with some differences during the first four days (Fig. 1). The viable cell count in the non-macerated wine increased more rapidly and reached a maximum of 1.26×10^8 cfu/mL on day 2, followed by a stationary phase until day 10. Rapid cell growth in the macerated wine was observed only after day 2, and reached a maximum of 8.35×10^7 cfu/mL on day 10. The final viable cell count in the macerated wine was lower than that in the non-macerated

wine (Table 1).

The unfermented centrifuged juice had a total soluble solid content of 14.79 °Brix (Table 1). The total soluble solid content of the unfermented, uncentrifuged juice was determined to be 15.32 °Brix. A rapid reduction in °Brix was observed from day 0 in the non-macerated wine, whereas this rapid reduction in °Brix occurred only after day 2 in the macerated wine, being consistent with yeast growth dynamics. The total soluble solids reached a final value of around 5.45 °Brix for the non-macerated wine and 6.00 °Brix for the macerated wine. The lower yeast cell count in the macerated wine could be due to the more viscous nature of the uncentrifuged juice. A higher viscosity of the fermentation medium could result in less oxygen or nutrients being available, which may affect cell growth (Vaudano *et al*., 2004). The lower yeast count in the macerated wine could also be due to inhibitory substances contained in the uncentrifuged juice, or produced by yeast from substrates in the pulp, but this warrants further study. The pH in the non-macerated wine rose slightly, from 3.51 to 3.58, but the pH of the macerated wine increased more significantly, from 3.49 to 3.71, which could be ascribed to the differences in organic acid transformation in the uncentrifuged and centrifuged mango juices discussed below.

Sugar and organic acid contents before and after fermentation
The total sugar content of the centrifuged and uncentrifuged juices was 12.25% (w/v) and 12.46% (w/v) respectively (Table 1). The sugars were almost exhausted after fermentation. Fermentation was considered complete by day 10, with a residual amount of 0.41 to 0.47 g/L of glucose and sucrose. The content of the remaining fructose at day 10 was 0.18 g/L in the non-macerated wine and not detectable in the macerated wine.

The change in organic acids is shown in Table 1. Citric acid decreased significantly in the macerated wine. Tartaric acid decreased from about 0.5 g/L to about 0.2 g/L in both wines, likely due to precipitation of tartrate salts (e.g. potassium tartrate or calcium tartrate). Malic acid was the major organic acid in both the centrifuged and uncentrifuged juices (originally about 3 g/L), and increased to concentrations of 6.9 and 7.2 g/L after acidification with d/l-malic acid in the
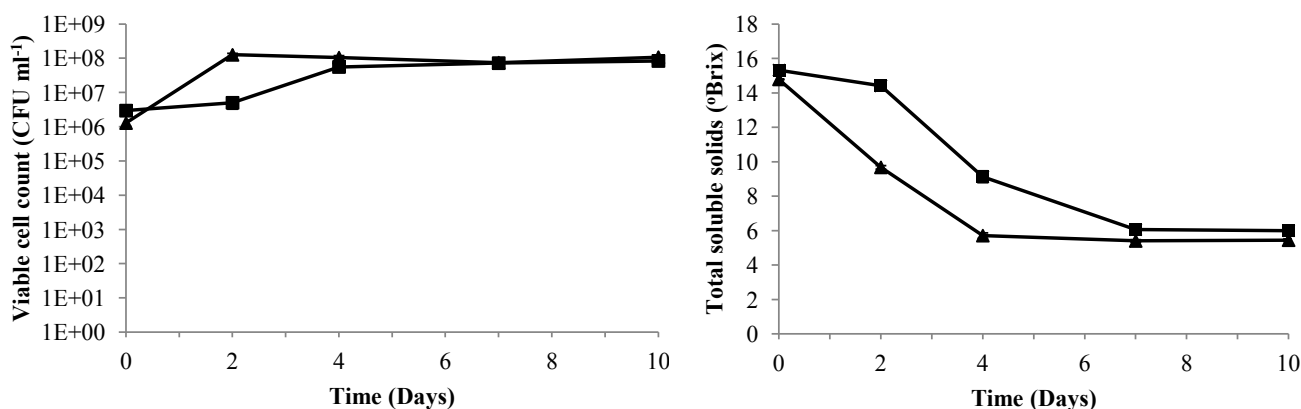

FIGURE 1
Yeast growth and changes in °Brix for mango wine fermentation with centrifuged (▲) and uncentrifuged juices (■).

TABLE 1
Physicochemical properties, alcohol, organic acid and sugar concentrations of mango wines with and without pulp.

Fermentation medium	Day 0		Day 10	
	Centrifuged juice	Uncentrifuged juice	Non-macerated wine	Macerated wine
Physicochemical properties				
pH	3.51 ± 0.02^a	3.49 ± 0.03^a	3.58 ± 0.00^b	3.71 ± 0.01^c
Total soluble solids (°Brix)	14.79 ± 0.03^a	15.32 ± 0.09^b	5.45 ± 0.07^c	6.00 ± 0.20^d
Cell count (10^6 cfu/mL)	1.28 ± 0.26^a	2.96 ± 0.84^a	107.8 ± 2.8^b	83.50 ± 8.88^c
Alcohol				
Ethanol (% v/v)	0.13 ± 0.03^a	0.15 ± 0.01^a	7.69 ± 0.06^b	7.99 ± 1.62^b
Glycerol (g/L)	N.D.	N.D.	5.43 ± 0.11^a	6.81 ± 0.14^b
Reducing sugars (g/L)				
Fructose	31.2 ± 0.8^a	34.7 ± 5.5^a	0.18 ± 0.00^b	N.D.
Glucose	15.7 ± 0.5^a	16.6 ± 0.7^a	0.42 ± 0.03^b	0.41 ± 0.01^b
Sucrose	75.6 ± 2.9^a	73.3 ± 3.3^a	0.43 ± 0.03^b	0.47 ± 0.03^b
Organic acid (g/L)				
Citric	1.93 ± 0.11^{ab}	2.01 ± 0.03^a	1.82 ± 0.07^{ab}	1.74 ± 0.09^b
Tartaric	0.44 ± 0.09^a	0.53 ± 0.05^a	0.23 ± 0.01^b	0.26 ± 0.14^b
Malic	6.92 ± 0.51^a	7.23 ± 0.03^a	3.82 ± 0.05^b	3.31 ± 0.21^b
Pyruvic	N.D.	N.D.	0.11 ± 0.00^a	0.11 ± 0.01^a
Succinic	1.02 ± 0.08^a	1.15 ± 0.07^a	0.98 ± 0.03^a	1.07 ± 0.05^a

[abcd] ANOVA (n = 6) at 95% confidence level with same letter indicating no significant difference (using ANOVA)
*N.D.: not detected

two juices respectively. Malic acid decreased significantly to 3.8 and 3.3 g/L respectively. As *S. cerevisiae* is known to be inefficient in metabolising malic acid, the decrease in malic acid concentration could be due mainly to the passive diffusion of d-malic acid into the yeast cells, which has been demonstrated in another study (Coloretti *et al*., 2002). Also, l-malic acid could be partially metabolised by *S. cerevisiae* (Rankine, 1966; Redzepovic *et al*., 2003). The decrease in malic acid was not likely due to malolactic fermentation, as no lactic acid was detected. There was no significant change in succinic acid before and after fermentation, or between the centrifuged and uncentrifuged juices. Pyruvic acid was not detected at day 0 and was produced in low concentrations of around 0.1 g/L after fermentation, with no significant difference between the centrifuged and uncentrifuged juices.

Ethanol and glycerol contents of mango wine
The ethanol content of the mango wine that was produced was 7.69% (v/v) for the non-macerated wine and 7.99% (v/v) for the macerated wine (Table 1). These figures are comparable to those in other studies on mango wine, which reported ethanol contents of between 6.5 and 10.7% (v/v) (Reddy & Reddy, 2005; Reddy *et al*., 2010; Pino & Queris 2011). Glycerol is a simple alcohol and a by-product formed by *S. cerevisiae* in order to maintain the cytosolic redox balance, especially under anaerobic conditions (Wang *et al*., 2001). It is known to impart a sweet taste to wines and contributes to mouthfeel (Suárez-Lepe & Morata, 2012). Although no glycerol was detected at day 0, its concentration increased significantly to 5.4 g/L for the non-macerated wine and 6.8 g/L for the macerated wine. The higher concentration of glycerol in the macerated wine was probably due to the

higher osmotic pressure in the uncentrifuged juice (Scanes *et al*., 1998) and the resultant impact on redox imbalance. These concentrations were consistent with the common contents of glycerol in wines of between 5 and 8 g/L (Scanes *et al*., 1998).

Volatile profile of mango juice and wine
Major volatiles in juice
The dominant class of compounds in the mango juice belonged to the terpene group, in which the most abundant compound was α-terpinolene. This was consistent with previous studies that showed that terpene hydrocarbons were the major volatiles of fresh mango juice, with cultivars such as *Kaew, Obispo, Corazon, Huovo de Toro* and *Chok Anan* containing high α-terpinolene contents (Macleod *et al*., 1988; Pino & Mesa, 2006; Li *et al*., 2011). Alcohols were the second dominant class of compounds, and (*Z*)-3-hexenol was the major one contributing a green and grassy odour (Pino *et al*., 2005; Li *et al*., 2011). However, ethanol was low in concentration in the fresh mango juice.

Evolution of terpenes
Terpenes decreased significantly in the first four days of fermentation in both the non-macerated and the macerated wine (Fig. 2). This was observed for all monoterpenes, including α-terpinolene and limonene. This decrease in terpene content has also been reported in litchi wine (Wu *et al*., 2011). The loss of terpenes could be due to the biotransformation and biodegradation of terpenes by yeast (Marais, 1984; Misra *et al*., 1996). For example, under the initial aerobic conditions of fermentation, *S. cerevisiae* was able to utilise terpenes as a biosynthetic intermediate
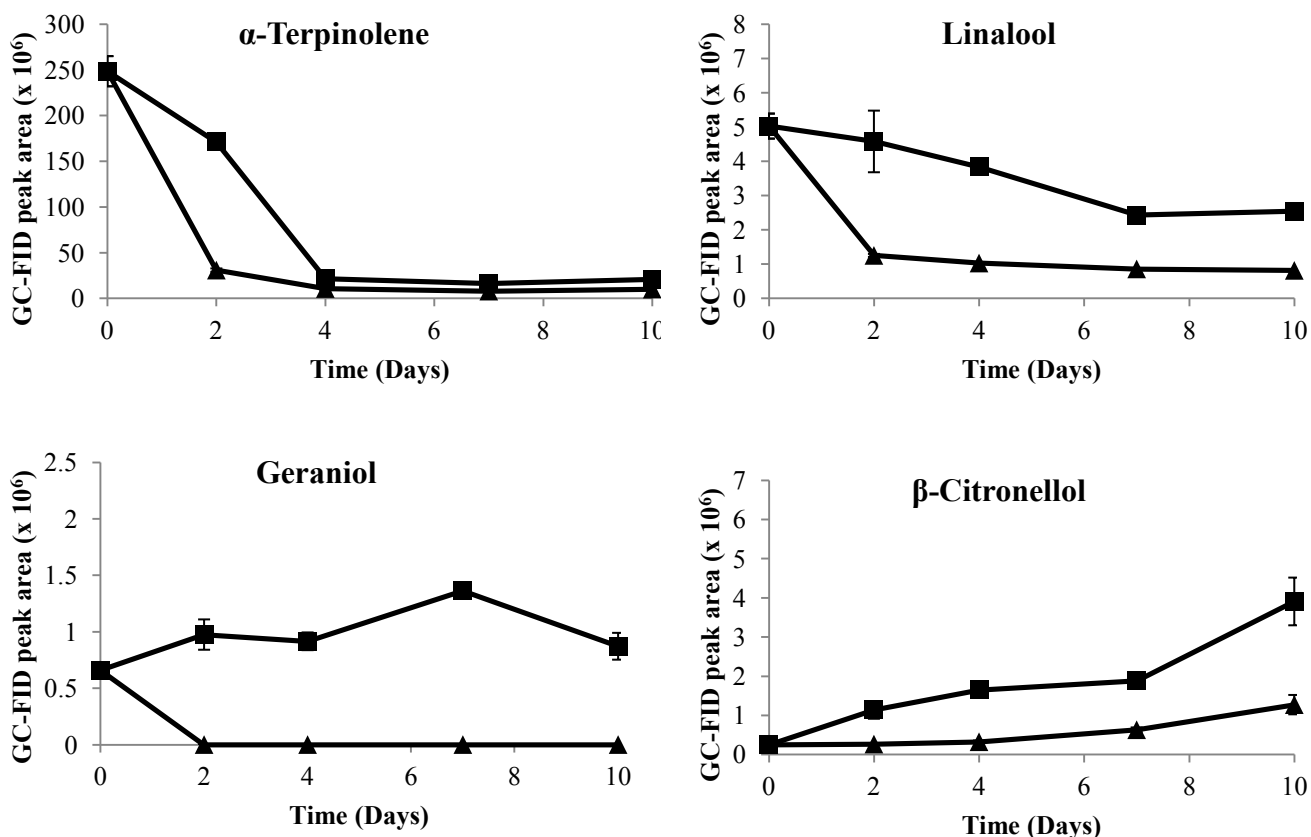
FIGURE 2
Evolution trends of terpenes and terpene alcohols during mango wine fermentation with centrifuged (▲) and uncentrifuged juices (■).

for sterol synthesis (Zea *et al*., 1995). Terpenes could also be volatilised and drawn off by the CO_2 produced, and lost during the metabolite exchange taking place between the medium and the atmosphere (Arévalo Villena *et al*., 2006). Generally, the decrease in terpenes in the macerated wine was less compared to that in the non-macerated wine, and was at slightly higher levels at day 10 (Fig. 2). The concentration of α-terpinolene was about ten times higher in the macerated wine than in the non-macerated wine (Table 2). This could be due to more terpenes being extracted into wine from the pulpy materials by the ethanol produced and/or due to the release of glycosidically-bound terpenes in the mango pulp through the β-glucosidase activity of *S. cerevisiae* (Mateo & Stefano, 1997). The decrease in terpene concentration would cause the loss of the original mango aroma, as terpenes quantitatively represent a large share of mango volatiles and contain some of the main contributors to mango aroma (Pino *et al*., 2005). From our result, the inclusion of pulp could, to some extent, help compensate for terpene losses.

Terpene alcohols (terpenols) have previously been reported to be present as free volatiles in some mango varieties (Ollé *et al*., 1998). Linalool, geraniol, β-citronellol, nerol and α-terpineol were identified in 'Chok Anan' mango juice (data not shown), with β-citronellol and geraniol being the major ones. These terpenols are also known to be important to the aroma of wine from grape varieties of Muscat, Riesling and other aromatic grape varieties (Marais, 1984; Swiegers *et al*., 2005). The biotransformation of terpenols by *S. cerevisiae* has been characterised in different

Muscat wines and beers (Marais 1984; King & Dickinson, 2000; Takoi *et al*., 2010; Gamero *et al*., 2011). It was found that geraniol can be converted into β-citronellol and other terpenols by yeast metabolism, while nerol and linalool can also be converted into α-terpineol, terpinolene and limonene. The production of terpenols can also occur and this has previously been hypothesised to be a result of *de novo* synthesis by *S. cerevisiae* through an alternative pathway involving the metabolism of leucine (Carrau *et al*., 2005). In the current study, linalool decreased in both wines throughout the fermentation (Fig. 2), but the rate of decrease was higher in the non-macerated wine. β-Citronellol increased throughout fermentation in both the non-macerated and the macerated wine, but the rate of increase was higher in the latter (Fig. 2). Geraniol could have been converted into β-citronellol, resulting in its depletion in the non-macerated wine at day 2 of fermentation (Fig. 2).

An opposite trend was observed for geraniol in the macerated wine, where it initially increased, followed by a slight decrease after day 7, and the final amount of geraniol in the macerated wine was higher than its initial concentration at day 0 (Fig. 2). In addition to yeast transformation, β-glucosidase activity is another factor that could contribute to the changes in terpene and terpenol contents. Previous studies on mango showed that terpenols also existed as bound fractions in mango (Drider *et al*., 1994; Ollé *et al*., 1998). Furthermore, β-glucosidase activity is also known to be present in *S. cerevisiae*, with higher activities in the early stages of fermentation (Mateo & Stefano, 1997).

TABLE 2

Volatiles (mg/L) and their odour activity values (OAVs) of non-macerated and macerated mango wines.

Group	Compound	LRI[a]	Non-macerated wine (mg/L)	OAV[b]	Macerated wine (mg/L)	OAV	Odour threshold (mg/L)	Odour descriptors
Acid	Acetic acid	1408	61.61 ± 5.65^a	0.31	165 ± 12.35^b	0.82	200[c]	Acidic, vinegar
	Hexanoic acid	1780	N.D.	0.00	0.36 ± 0.09^a	1.20	0.3[c]	Sour, fatty, sweaty
	Octanoic acid	1990	6.04 ± 0.53^a	0.69	1.75 ± 0.29^b	0.20	8.8[d]	Fatty, soapy, sour
	Decanoic acid	2188	1.09 ± 0.21^a	0.07	0.24 ± 0.02^b	0.02	15[c]	Fatty, rancid, sour
	Dodecanoic acid	2393	0.736 ± 0.099^a	0.07	0.13 ± 0.01^b	0.01	10[e]	Coconut, fatty
Alcohol	Isobutyl alcohol	981	34.1 ± 6.1^a	0.85	65.7 ± 11.2^b	1.6	40[c]	Fruity, wine-like
	Active amyl alcohol	1173	9.63 ± 0.80^a	0.15	17.97 ± 2.33^b	0.28	65[f]	Winey, fruity
	Isoamyl alcohol	1173	171 ± 10.08^a	5.7	182 ± 6.29^a	6.07	30[c]	Alcoholic, banana
	(Z)-3-Hexenol	1350	0.599 ± 0.074^a	1.50	0.90 ± 0.08^b	2.26	0.4[c]	Green, leafy
	2-Phenylethyl alcohol	1944	72.78 ± 7.97^a	7.28	86.90 ± 12.50^a	8.69	10[c]	Sweet, rose, floral
Ester	Ethyl acetate	858	0.13 ± 0.02^a	0.02	0.17 ± 0.02^a	0.02	7.5[c]	Ethereal, fruity, sweet
	Isobutyl acetate	979	0.066 ± 0.008^a	0.04	0.10 ± 0.02^b	0.06	1.6[d]	Sweet, fruity, apple
	Isoamyl acetate	1061	0.648 ± 0.120^a	216	1.24 ± 0.01^b	414	0.003[c]	Fruity, banana
	Ethyl hexanoate	1158	0.338 ± 0.041^a	67.6	0.18 ± 0.03^b	36.5	0.005[c]	Banana, fruity, floral
	(Z)-3-Hexenyl acetate	1206	0.044 ± 0.001^a	5.46	0.046 ± 0.002^a	5.80	0.008[e]	Fresh, green, fruity
	Ethyl octanoate	1374	4.25 ± 0.73^a	2123	1.12 ± 0.02^b	559	0.002[c]	Waxy, pineapple, fruity
	Ethyl decanoate	1575	15.09 ± 1.87^a	75.45	2.09 ± 0.44^b	10.44	0.2[d]	Waxy, sweet, apple
	Citronellyl acetate	1595	0.024 ± 0.001^a	0.10	N.D.	0.00	0.25[g]	Floral, green, fruity, citrus
	2-Phenylethyl acetate	1773	2.38 ± 0.54^a	9.52	2.46 ± 0.31^a	9.84	0.25[c]	Floral, rose, sweet
	Ethyl dodecanoate	1785	6.22 ± 0.39^a	5.19	0.69 ± 0.10^b	0.58	1.2[d]	Soapy, waxy, floral
Terpene	α-Terpinolene	1264	0.03 ± 0.00^a	-	0.39 ± 0.04^b	-	Not applicable	Woody, citrus, pine, green
	β-Citronellol	1770	0.008 ± 0.003^a	0.08	0.034 ± 0.001^b	0.34	0.1[c]	Rose, citrus, green
	Geraniol	1861	N.D.	0	0.005 ± 0.000^a	0.17	0.03[c]	Sweet, floral, fruity, citrus

[ab] ANOVA (n = 6) at 95% confidence level with same letter indicating no significant difference (using T-test).

*N.D.: not detected, ± standard deviation

[a] Linear retention index

[b] Odour activity values (OAV) were calculated by dividing concentration by the odour threshold value of the compound.

[c] The odour threshold obtained from Guth (1997).

[d] The odour threshold obtained from Bartowsky and Pretorius (2008).

[e] The odour threshold obtained from Lambrechts and Pretorius (2000).

[f] The odour threshold obtained from Zemni *et al*. (2007).

[g] The odour threshold obtained from Yamamoto *et al*. (2004).

Thus, it could be that the higher geraniol and β-citronellol concentrations in the macerated wine (Fig. 2, Table 2) were also due to more terpenol-containing glycosides in the mango pulp being hydrolysed by the β-glucosidase from yeast. More geraniol could lead to more β-citronellol production, and the reduction in geraniol correlated with the increase in β-citronellol production (Fig. 2). The concentrations of β-citronellol and geraniol were below their threshold (Table 2), but they may contribute to the wine bouquet synergistically (Styger *et al*., 2011). Other glycosidically-bound aroma compounds in mango, such as geranial and 1,8-menthadien-4-ol, were also increased (data not shown), being consistent with the result in Lalel *et al*. (2003).

Evolution of alcohols

Ethanol, isobutyl alcohol (2-methyl-1-propanol), isoamyl alcohol (3-methyl-1-butanol), active amyl alcohol (2-methyl-1-butanol) and 2-phenylethyl alcohol were identified as the major alcohols present in the mango wines. Their dynamic changes were similar in both the centrifuged and uncentrifuged juice fermentations, with rapid formation of these compounds initially, followed by a plateau at around day 4 (Fig. 3). This trend was similar to that found in our previous study (Li *et al*., 2011). Isobutyl alcohol, isoamyl alcohol, active amyl alcohol and 2-phenylethyl alcohol are also classified as higher alcohols or fusel alcohols, which are known to have a significant impact on the character of wine. These alcohols are produced mainly by yeasts during alcoholic fermentation. This takes place through the Ehrlich pathway, involving branched-chain and aromatic amino acids (Lambrechts & Pretorius, 2000). Amino acids such as l-valine, l-isoleucine, l-leucine and l-phenylalanine are known to be responsible for the synthesis of isoamyl alcohol, active amyl alcohol, isobutyl alcohol and 2-phenylethyl alcohol (Styger
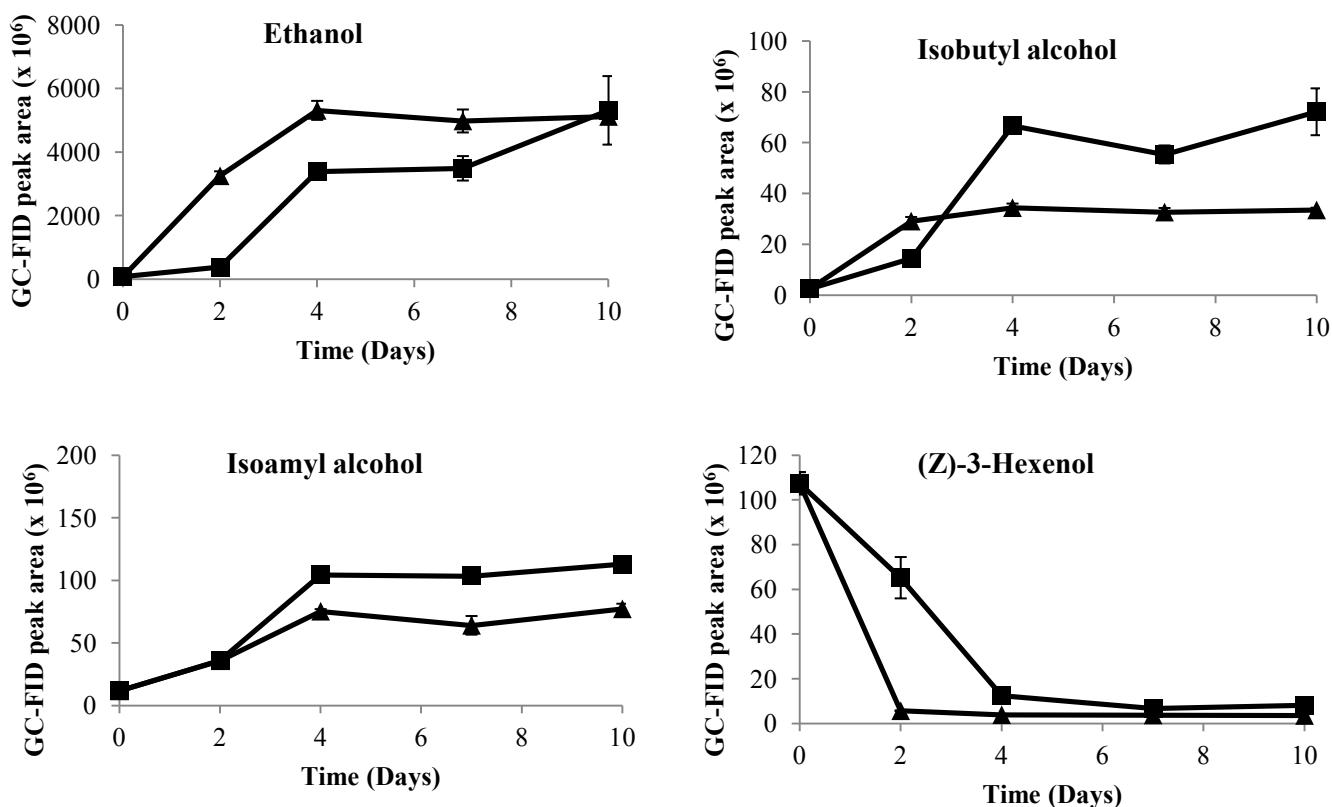
FIGURE 3

Evolution trends of alcohols during mango wine fermentation with centrifuged (▲) and uncentrifuged juices (■).

et al., 2011). The macerated wine consistently showed a higher concentration of the major higher alcohols towards day 10 (Fig. 3). While various fermentation conditions, such as yeast growth and fermentation temperature, are also known to affect the production of higher alcohols, the amino acid content is still the major influential factor (Lambrechts & Pretorius, 2000).

In contrast to the trend of higher alcohols, the concentration of (*Z*)-3-hexenol was drastically reduced (Fig. 3). (*Z*)-3-Hexenol has not been reported as a fermentation product of yeast and might have been transformed to esters or reduced to 1-hexanol. 2-Phenylethyl alcohol, isoamyl alcohol and (*Z*)-3-hexenol were at levels higher than their threshold levels in both the non-macerated and macerated wines, and the isobutyl alcohol level was higher than its threshold in the macerated wine only (Table 2). Isoamyl alcohol is known to be a major constituent of mango wine and is responsible for the winey odour (Reddy *et al*., 2010). Isobutyl alcohol is responsible for the fruity and winey odours, whereas (*Z*)-3-hexenol can contribute an intense green grassy odour. On the other hand, 2-phenylethyl alcohol is one of the few aromatic alcohols responsible for the rose and floral odours (Swiegers *et al*., 2005).

Evolution of fatty acids
The major volatile fatty acids in the mango wines were acetic, octanoic, decanoic and dodecanoic acids. The pattern of their dynamic changes was similar to that of alcohols (Fig. 4). Acetic acid was higher in the macerated wine (165 mg/L) than in the non-macerated wine (61.61 mg/L), whereas the non-

macerated wine showed significantly higher concentrations of octanoic, decanoic and dodecanoic acids. Acetic acid fulfils important metabolic roles in yeast and can be produced in *S. cerevisiae* through the oxidation of acetaldehyde. The medium-chain volatile fatty acids are known as intermediates produced by yeasts in the biosynthesis of long-chain fatty acids, which are the essential precursors for lipid components in yeast (Lambrechts & Pretorius, 2000). These compounds are commonly associated with soapy, rancid and fatty odours and could adversely affect the aroma of wine. On the other hand, acetic acid may impart a vinegar-like character to wine when present at high concentrations (Swiegers *et al*., 2005). The quantitative differences in fatty acids between the non-macerated and macerated wines are shown in Table 2. All fatty acids (except for hexanoic acid) were at levels below their threshold levels. The reason for the differences in fatty acids between the non-macerated and macerated wines merits further research.

Evolution of esters
Esters are generally associated with fruity and floral aromas in wine (Lambrechts & Pretorius, 2000). The dominant esters identified were ethyl esters of octanoate, decanoate, 9-decenoate and dodecanoate, as exemplified by ethyl octanoate and ethyl decanoate in Fig. 5. Ethyl esters increased rapidly initially and their concentrations were higher in the non-macerated wine. This trend was similar to that of the corresponding fatty acids (Fig. 4), and was also demonstrated in another study on the importance of the fatty acid precursor levels for the production of ethyl esters
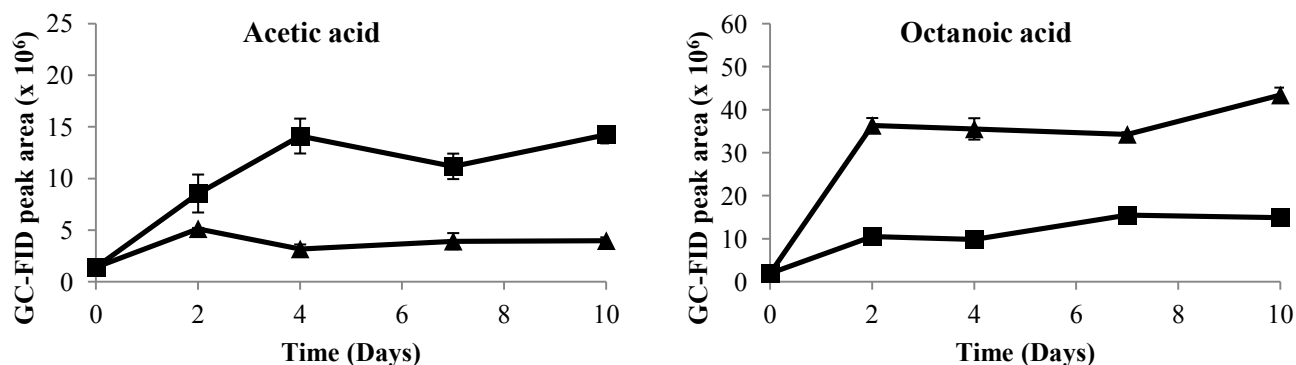
FIGURE 4
Evolution trends of volatile fatty acids during mango wine fermentation with centrifuged (▲) and uncentrifuged juices (■).
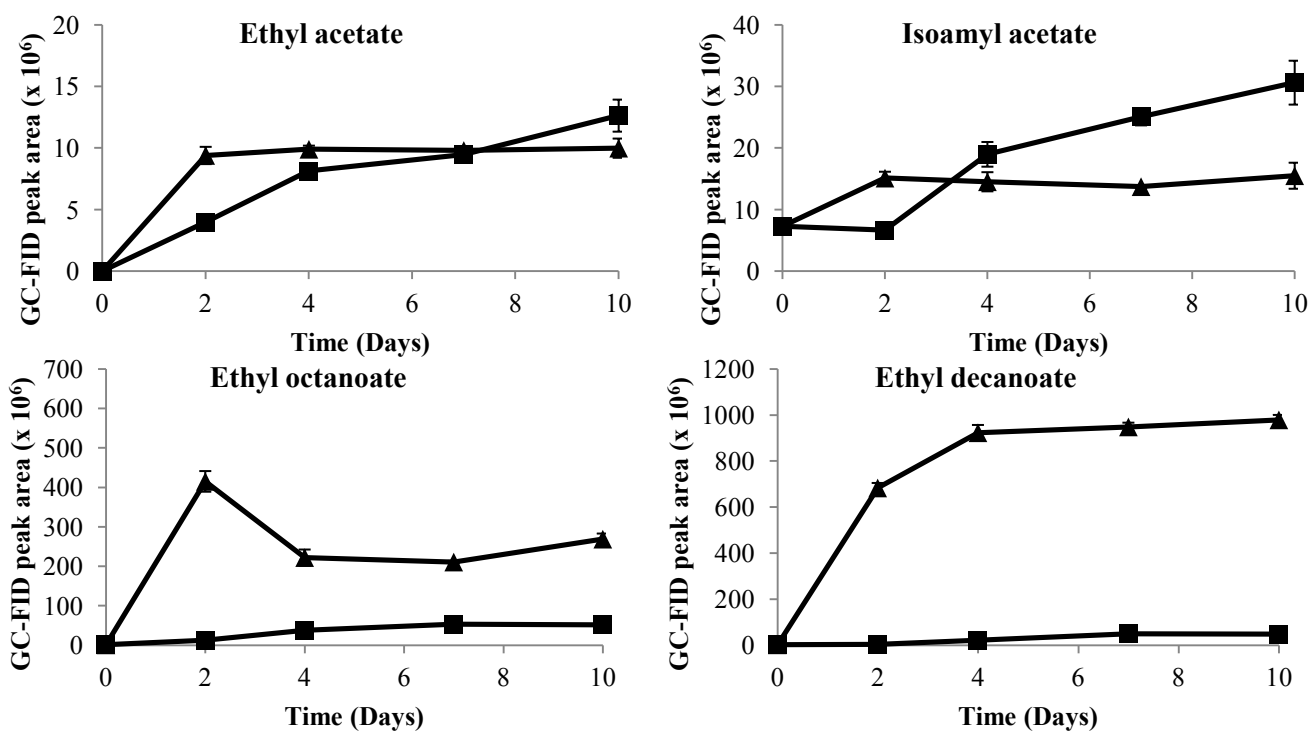

FIGURE 5
Evolution trends of esters during mango wine fermentation with centrifuged (▲) and uncentrifuged juices (■).

(Saerens *et al.*, 2008). The concentrations of ethyl esters were higher than their threshold levels (Table 2).

Acetate esters such as isoamyl acetate, isobutyl acetate, ethyl acetate and 2-phenylethyl acetate increased (exemplified by ethyl acetate and isoamyl acetate in Fig. 5). These esters are very important for wine fruitiness. Acetate esters were higher in the macerated wine (Table 2). Isoamyl acetate was found even at twice the concentration of that in the non-macerated wine at day 10 (Table 2). The higher concentrations of acetate esters in the macerated wine could be due to the higher concentrations of their respective higher alcohols in the same wine. This is because higher alcohols and acetyl-CoA are known as the main precursors for the formation of acetate esters (Suárez-Lepe & Morata, 2012).

Other esters and ethers identified in the mango wine included citronellyl acetate, neryl acetate and geranyl

ethyl ether, derived from the corresponding terpenols, demonstrating the further role of terpenols in mango wine aroma. These compounds have not yet been reported in other studies on mango wine. However, they have been suggested to be formed in litchi wine through the partial esterification of or etherification between the corresponding terpenols and acetyl-CoA (Wu *et al.*, 2011).

Evolution of sulphur compounds
Two sulphur compounds, dihydro-2-methyl-3(2H)-thiophenone and methionol, were also detected in both wines (data not shown). These compounds were not found in the centrifuged and uncentrifuged juices and most likely were produced by the yeast through the Ehrlich pathway during fermentation (Karagiannis & Lanaridis, 1999). For instance, methionol is known to be produced from the catabolism of

methionine and is responsible for potato, cooked cabbage and meat-like odours, while dihydro-2-methyl-3(2H)-thiophenone is associated with blackberry odour (Mestres *et al.*, 2000).

CONCLUSIONS

The mango wines fermented from centrifuged and uncentrifuged juices showed significant differences, especially in the volatile profile. The macerated wine contained more terpenes, terpenols, higher alcohols and fruity acetate esters than the non-macerated wine. The non-macerated wine possessed a higher concentration of medium-chain fatty acids and corresponding ethyl esters. The inclusion of pulp in mango wine fermentation, which is similar to the maceration process in red grape wine fermentation, was beneficial for retaining the original terpenic character of mango, and also improved other mango wine aroma characters (i.e. complexity) such as fruitiness.

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