Effects of Different Inoculation Regimes of *Torulaspora delbrueckii* and *Oenococcus oeni* on Fermentation Kinetics and Chemical Constituents of Durian Wine

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This work evaluated the effects of inoculation time of *Oenococcus oeni* on the kinetics of fermentation and chemical constituents of durian wine produced using a non-*Saccharomyces* yeast, *Torulaspora delbrueckii*. The growth of *T. delbrueckii* in mixed-culture fermentations was significantly adversely affected by the presence of *O. oeni*, and the growth of malolactic bacteria was also affected by the metabolism of yeast during fermentation. The level of ethanol produced in simultaneous alcoholic and malolactic fermentation (SIM, 6.93%, v/v) was comparable to that in the *Saccharomyces cerevisiae* EC-1118 control (6.75%, v/v); both levels were relatively higher than that in the *T. delbrueckii* Biodiva control (6.39%, v/v) and the other two sequential fermentations (oenococci inoculated after four and seven days of alcoholic fermentation, SEQ 4th, 6.34% and SEQ 7th, 6.33% v/v respectively). The final concentrations of organic acids and esters in the mixed-culture wines were correlated with the inoculation time of *O. oeni*. SIM produced relatively higher levels of ethyl esters (ethyl esters of hexanoate, octanoate, decanoate and lactate) and acetate esters (ethyl acetate and isoamyl acetate) than those in SEQ 4th, SEQ 7th and the Biodiva control. This suggests that SIM would contribute fruity aroma properties to and modulate the mouthfeel of durian wine. The production of 3-(ethylthio)-1-propanol could compensate for the weak onion-like odour caused by the decrease in initial volatile sulphur compounds. Overall, this research suggests that SIM treatment is an effective way to produce durian wine with higher ester production.

INTRODUCTION

In recent years, an increasing number of researchers have been paying more attentions to wines from tropical fruit such as banana (Byarugaba-Bazirake *et al*., 2013), papaya (Lee *et al*., 2010) and lychee (Chen & Liu, 2016) due to their pleasant and characteristic flavours. Other tropical fruits, such as durian and mangosteen, are also promising for novel fruit wine production but have received much less research attention than other fruits. Durian (*Durio zibethinus* Murr.) is a unique and popular tropical fruit grown widely in Southeast Asia. Durian is not only a good source of carbohydrate, fat, fibre and protein, but also contains abundant phenolic compounds, and medium-chain saturated and unsaturated fatty acids (Haruenkit *et al*., 2010). In addition, there are more than 170 volatile aroma compounds found in the durian flavour compound array, including esters (e.g. ethyl 2-methylbutyrate) and volatile sulphur compounds (e.g. thiols and sulphides) (Ho & Bhat, 2015). Durian is normally consumed fresh and its availability is limited to its fruiting season from May to August, and short shelf life of two to five days at room temperature (Haruenkit *et al*., 2010). Therefore, alcoholic fermentation (AF) of durian pulp may provide an alternative way to preserve, extend shelf life and add value to this fruit.

Alcoholic fermentation is normally conducted by *Saccharomyces cerevisiae* strains with the conversion of sugars to ethanol, and yeasts play a significant role in the production and modulation of the wine aroma profile by releasing flavour compounds from fruit precursors or synthesising yeast-derived aroma compounds (Viana *et al*., 2008; Sun *et al*., 2013; Tristezza *et al.*, 2013, 2016). Recently, non-*Saccharomyces* yeasts like *Torulaspora delbrueckii* received more attention due to their potential positive roles in the organoleptic characteristics of wines, such as the production of low levels of volatile acidity and acetaldehyde,

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which are beneficial in terms of wine quality (Bely *et al*., 2008). Previous studies have reported that *T. delbrueckii* possessed good fermentation efficiencies with residual sugar levels of less than 2 g/L and produced comparable levels of ethanol to *S. cerevisiae* when fermented at 20°C (Lu *et al*., 2015, 2016a). Further, *T. delbrueckii* showed a better capacity for producing higher alcohols and ethyl esters, which could contribute to the fruity notes and aroma complexity of the wines (Lu *et al*., 2016a).

Malolactic fermentation (MLF) is an enzyme-mediated process in which L-malic acid is decarboxylated into L-lactic acid and carbon dioxide by lactic acid bacteria (LAB), mainly by O*enococcus oeni* (Nehme *et al*., 2010; Knoll *et al*., 2011). Malolactic fermentation is usually conducted after AF and could improve wine quality via deacidification, the production of desirable aroma compounds and the enhancement of microbial stability (Izquierdo *et al*., 2012). *O. oeni* strains (Viniflora, Enoferm Beta and PN4) are employed in MLF due to their better tolerance of the harsh physicochemical conditions, such as low pH, high ethanol content, presence of inhibitory metabolites (e.g. SO_2 and medium-chain fatty acids) and low nutritional status (Alexandre *et al*., 2004; Nehme *et al*., 2010).

Various studies have shown that simultaneous AF and MLF (SIM) using *S. cerevisiae* and *O. oeni* can be carried out successfully, especially in grape and lychee wines (Abrahamse & Bartowsky, 2012; Izquierdo *et al*., 2012; Garofalo *et al.*, 2015; Chen & Liu, 2016). Chen & Liu (2016) reported that simultaneous AF by *S. cerevisiae* MERIT. ferm and MLF by *O. oeni* Viniflora could improve the production of aroma compounds in lychee wine. However, Sun *et al*. (2013) reported that sequential inoculation (SEQ) of *S. cerevisiae* and *O. oeni* has great potential for the production of cherry wines. Therefore, it seems that the different inoculation strategies, namely SIM and SEQ, may have different effects on wine aroma composition (Mendoza *et al*., 2011). This is because the success or failure of MLF is closely associated with yeast and bacterial strain pairing, and the possible interactions between them (Alexandre *et al*., 2004; Nehme *et al*., 2010). In addition, little information is available on the mixed culture fermentation initiated by non-*Saccharomyces* yeasts and oenococci.

Our previous study assessed and compared the compatibility of three commercial strains of *O. oeni* (Viniflora, PN4 and Enoferm Beta) with *T. delbrueckii* Biodiva via simultaneous AF and MLF in durian wine fermentation and showed that *O. oeni* PN4 had better compatibility than the other two *O. oeni* strains (Lu *et al*., 2017). The present study was a further investigation, and the objective was to examine, for the first time, the kinetics of yeast and bacterial population, organic acids and aroma compounds related to the different inoculation regimes of *T. delbrueckii* Biodiva and *O. oeni* PN4 during durian wine fermentation.

MATERIALS AND METHODS

Microbial strains and growth media

Saccharomyces cerevisiae var. *bayanus* EC-1118, *Torulaspora delbrueckii* Biodiva and *Oenococcus oeni* PN4 were purchased from Lallemand Inc. (Brooklyn Park, Australia). A sterile broth was prepared by autoclaving (121°C, 15 min), which consisted of 2.5 g of yeast extract, 2.5 g of bacteriological peptone, 2.5 g of malt extract and 20 g of glucose per litre of water. The freeze-dried yeast strains were propagated in the sterile broth (pH 5.0) for 72 h at 20°C and stored at -80°C before use. The broth cultures (5%, v/v) were propagated in pasteurised durian pulp (60°C, 20 min) and incubated statically at 30°C for 72 h in order to achieve colony-forming units (CFU) of at least 1×10^7 per mL, which served as the pre-cultures for final inoculation. The freeze-dried *O. oeni* was propagated in a modified MRS (supplemented with 20% apple juice, v/v) broth (Sigma-Aldrich, Singapore) for five days at 30°C to obtain pure cultures with cell populations of at least 1×10^7 CFU/mL, and stored at -80°C until use. The bacteria pre-culture was prepared by inoculating 5% (v/v) broth culture (*O. oeni* PN4) into the modified MRS broth (the same as above) for five days at 30°C to obtain cell populations of over 1×10^7 CFU/mL.

Durian pulp preparation and fermentation

The pulp was prepared in our laboratory from durian fruits (D666 cultivar) that were imported from Malaysia; it was homogenised and diluted with deionised water at a 3:7 (w/w) ratio to form a puree. The pH of the puree (pH 6.85) was adjusted to pH 4.0 using 1 mol/L of DL-malic acid, and the soluble solids content (8.25°Brix) was adjusted to 20°Brix with sucrose. The adjusted pulp was then pasteurised at 60°C for 20 min. Following that, spread plating was performed to verify the efficiency of pasteurisation. Triplicate laboratory fermentations were conducted using pasteurised durian pulp (300 mL each) in 500 mL sterile Erlenmeyer flasks. SIM was conducted by simultaneously inoculating *T. delbrueckii* Biodiva (6.02×10^5 CFU/mL) and *O. oeni* PN4 (6.61×10^5 CFU/mL). The same amount of yeast and bacteria was inoculated in the SEQ treatments (SEQ 4th and SEQ 7th days). SEQ 4th and SEQ 7th were conducted by inoculating oenococci after four and seven days of AF by *T. delbrueckii* Biodiva. In addition, the same amount of *T. delbrueckii* Biodiva monoculture was inoculated as the Biodiva control. The commercial *S. cerevisiae* EC-1118 (8.91×10^5 CFU/mL) was also inoculated as the EC-1118 control. The inoculated pulp was allowed to ferment statically at 30°C for 14 days, based on previous studies (Lu *et al*., 2016b, 2017). Samples were taken periodically at days 0, 2, 4, 5, 7, 8, 10 and 14 to assess the yeast and bacteria growth, °Brix, pH, sugars, organic acids and volatile compounds. All samples were stored at -20°C before analysis.

Analytical determinations

Yeast growth was monitored by spread-plating onto potato dextrose agar (PDA), and the plates were incubated at 30°C for two days (Lu *et al.*, 2017). *O. oeni* was monitored on modified MRS agar and incubated at 30°C for five days under anaerobic conditions. The MRS agar plates were prepared by dissolving MRS powder (49.6 g) in deionised water (800 mL) with Natamax® (a natural antimicrobial with natamycin as its active ingredient, 0.1 g, Danisco Singapore Pte Ltd, Singapore), followed by autoclaving at 121°C for 15 min, after which sterile apple juice (200 mL, pH 5.5) was added before dispensing. Natamax® was used

to inhibit the growth of yeasts. °Brix and pH were measured using a refractometer (ATAGO, Tokyo, Japan) and pH meter (Metrohm, Herisau, Switzerland) respectively.

Samples for sugar (glucose, fructose and sucrose) and organic acid determination were centrifuged twice (× 10 000 *g*, 4°C) for 10 min and filtered through 0.20 μm filters (Sartorius, Gottingen, Germany). Analysis was conducted using high-performance liquid chromatography (HPLC, Shimadzu, Kyoto, Japan) coupled with LC-10AT system. Sugars were determined using an evaporative light-scattering detector (ELSD) connected to a Zorbax carbohydrate column (150 mm × 4.6 mm; Agilent, Santa Clara, CA, USA), eluting at 30°C with a mobile phase of acetonitrile/water (80:20, v/v) at a flow rate of 1.4 mL/min. As for organic acids, a photodiode array detector set at 210 nm was connected to a Supelcogel C-610H column (300 mm × 7.8 mm; Supelco/Sigma-Aldrich, Barcelona, Spain). The mobile phase was set to flow at 0.4 mL/min and the column was eluted at 40°C with 0.1% (v/v) sulphuric acid. The identification of the compounds was based on the comparison of retention times of the samples against those of pure standards (Sigma-Aldrich, St Louis, MO, USA) run under the same conditions. The concentration was calculated based on standard curves ($R^2 > 0.99$).

Volatile compounds were performed using a gas chromatograph (GC)/mass spectrometer (MS) with a flame ionisation detector (FID) using headspace (HS) solid-phase microextraction (SPME) with a carboxen-poly (dimethylsiloxane) fibre (85 μm coating; Supelco/Sigma-Aldrich, Barcelona, Spain). Before analysis, an aliquot of durian wine was adjusted to pH 2.5 using 1 mol/L HCl. The aliquot of the durian wine (5 mL) was extracted at 60°C for 50 min by a HS-SPME fibre at a rotational speed of 250 rpm. The SPME fibre was then desorbed at 250°C for 3 min at the injection port of an Agilent 7890A GC coupled to an Agilent 5975C triple-axis MS and FID. With helium at 1.2 mL/min, The separation of the volatile compounds occurred in the Agilent DB-FFAP capillary column (60 m × 0.25 mm i.d), coated with a 0.25 mm thick film of polyethylene glycol modified with nitroterephthalic acid. The temperature of the GC was programmed to increase from 50°C to 230°C at a rate of 5°C/min within 30 min. The eluate was passed through the FID and MS, where ionisation was produced with 70 eV electron impact at 230°C. The identification of volatiles was carried out by matching the mass spectra with those in the Wiley MS library, and was confirmed with the linear retention index (LRI) values. External standards (Firmenich Asia Ltd, Singapore) were used to quantify the selected volatiles that were considered as significant for durian wine flavour according to the literature. The R^2 values of the standard curves were at least 0.98. The contribution of a volatile compound to overall fruit wine flavour was evaluated by its odour activity value (OAV) (Guth, 1997).

Statistical analysis

All samples were analysed in triplicate and the data were expressed as the mean ± standard deviation. A one-way analysis of variance (ANOVA) and Tukey's test (SPSS Corporation, Chicago, IL, USA, version 17.0) were performed on the experimental data obtained to compare the statistical differences between the durian pulp and final wines. The difference was considered statistically significant if $p < 0.05$. Principal component analysis (PCA) was carried out on selected volatile compounds using MATLAB R2008a (Mathworks, Massachusetts, USA).

RESULTS AND DISCUSSION

Evolution of microbial populations and fermentation kinetics

The evolution of yeasts and malolactic bacteria populations, and the kinetic changes in °Brix and pH, are shown in Fig. 1. Viable yeast populations (Fig. 1a) in all fermentations reached their maximum, ranging from 8.3×10^7 CFU/mL (*S. cerevisiae* EC-1118) to 2.2×10^8 CFU/mL (SIM), on day 2, and then followed a significantly different pattern of evolution, except for the Biodiva control and SEQ 7th (which followed the same trend). *T. delbrueckii* Biodiva in SIM, SEQ 4th and SEQ 7th was undetectable on day 7, day 10 and day 14 respectively (Fig. 1a). *T. delbrueckii* Biodiva in the control was undetectable by day 14, while *S. cerevisiae* EC-1118 in the control declined to around 1.0×10^5 CFU/mL at the end of the fermentation (Fig. 1a). The growth of *T. delbrueckii* in mixed-culture fermentations (SIM and both SEQ), especially in SIM and SEQ 4th, was significantly adversely affected by the presence of *O. oeni* PN4. This is consistent with a previous study, which showed that the growth of *S. cerevisiae* MERIT could be interfered with by *O. oeni* Viniflora in simultaneous AF and MLF in lychee wine (Chen & Liu, 2016). In contrast, a previous study showed that the presence of *O. oeni* C22L9 did not influence the growth of *S. cerevisiae* VRB and VN (Izquierdo *et al*., 2012), or that *S. cerevisiae* EC-1118 could adapt to the presence of *O. oeni* Viniflora (Taniasuri *et al*., 2016). It is important to note that the interaction between yeast and *O. oeni* can vary with strains; the outcome may differ even with the same strain but different matrices.

The possible reasons for the different fermentation performance could be the different yeasts (non-*Saccharomyces* used in the present study) or bacteria used in the different fermentations. The role played by the yeasts and bacterial strains and the fermentation condition would affect their compatibility in wine fermentation (Taniasuri *et al*., 2016). In addition, malolactic bacteria may deplete certain nutrients or survival factors required by yeasts (Alexandre *et al*., 2004). Furthermore, the higher levels of acetic acid produced in SIM and SEQ (Table 1; Fig. 2) might be another reason for the early yeast cell death (Jussier *et al*., 2006; Taniasuri *et al*., 2016). Acetic acid (undissociated form) could pass through the yeast cell membrane via passive diffusion, leading to intracellular acidification by protonation and resulting in the reduction of cell biomass due to the ATP channelling pH homeostasis (Graves *et al*., 2007). Moreover, it is important to note that the production of lactic acid was inversely correlated with the kinetics of *T. delbrueckii* in SIM and SEQ (Fig. 2); therefore, lactic acid may have resulted in the decline in *T. delbrueckii*, just like acetic acid.

The cell populations of *O. oeni* PN4 in SIM increased gradually to 1.5×10^7 CFU/mL by day 2 and then decreased slightly to 5.8×10^6 CFU/mL by day 14. In SEQ 4th and SEQ 7th, on the other hand, the bacterial populations first decreased slightly (by day 5 and 8 respectively), and then

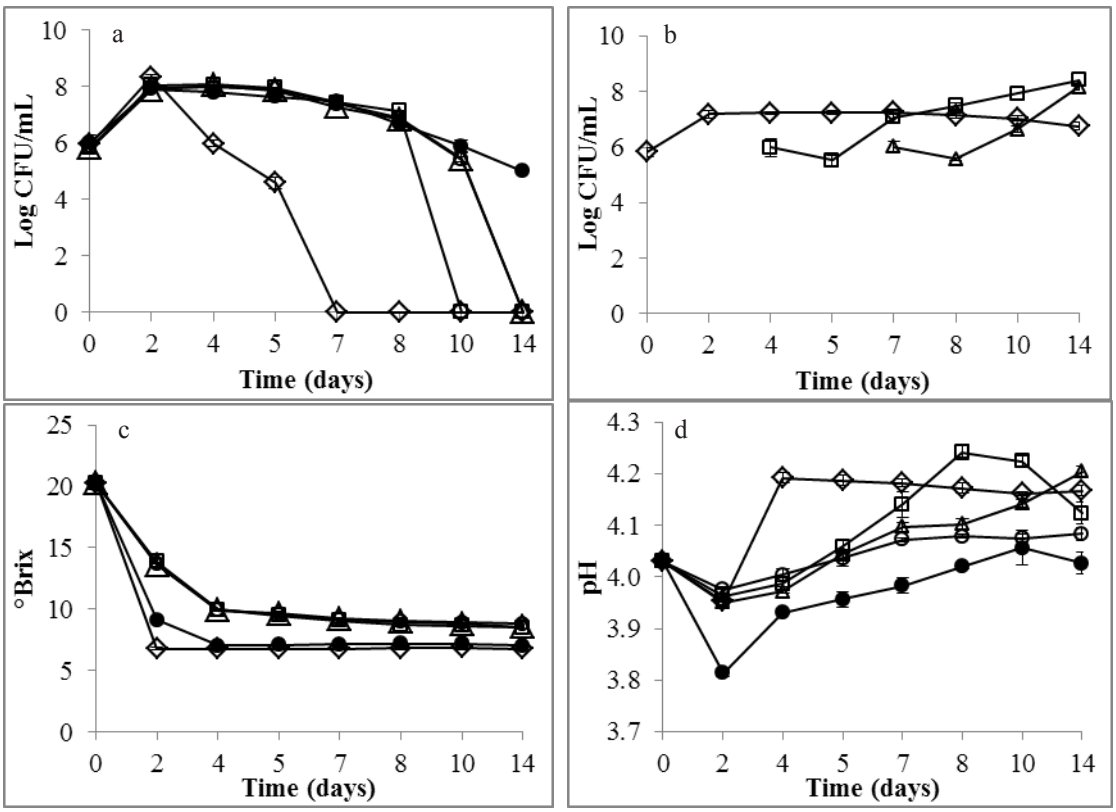

FIGURE 1

(a) Viable cell count of yeast; (b) viable cell count of *O. oeni* PN4 during durian wine fermentation; (c) changes in ºBrix; (d) changes in pH during durian wine fermentation. (●) *S. cerevisiae* EC-1118 monoculture fermentation; (○) *T. delbrueckii* Biodiva monoculture fermentation; (◇) simultaneous inoculation (SIM) of *T. delbrueckii* Biodiva and *O. oeni* PN4; (□) sequential inoculation (SEQ 4th): *O. oeni* PN4 inoculated after four days of fermentation with *T. delbrueckii* Biodiva; (△) sequential inoculation (SEQ 7th): *O. oeni* PN4 inoculated after seven days of fermentation with *T. delbrueckii* Biodiva. The values are the means of triplicate fermentations ± standard deviation.

increased to 2.6×10^8 and 1.5×10^8 CFU/mL respectively (Fig. 1b). Our results agree with those reported by Mendoza *et al*. (2011) and Lu *et al*. (2016b), who found that bacterial populations did not decrease significantly after simultaneous AF and MLF. Izquierdo *et al*. (2012) also observed that there was an early bacterial cell death in SEQ fermentations. This could be due to the duration of adaption by *O. oeni* to the environmental stresses (e.g. pH and ethanol content) within the first few days (Fig. 1b), after which their survival and performance improved as the fermentation progressed. At the end of the fermentation (day 14), the bacterial viability in SEQ 4th and SEQ 7th was similar, but significantly higher than that in SIM. This might be due to the fact that bacteria were stimulated in SEQ, or maybe they did not grow more in SIM because of lactic acid formation or because they had already consumed all the available nutrients (Alexandre *et al*., 2004).

All treatments showed similar trends in total soluble solids, with the °Brix values rapidly decreasing within the first four days and remaining stable onwards (Fig. 1c). The °Brix value in SIM decreased rapidly from 20.2% to around 6.8% by day 2, while in the *T. delbrueckii* Biodiva control, SEQ 4th and SEQ 7th the °Brix values decreased to around 8.6% by day 5 (Fig. 1c). It is important to highlight that the final °Brix value in SIM was comparable to that in the *S. cerevisiae* EC-1118 control, but was significantly lower than that in the *T. delbrueckii* Biodiva control, SEQ 4th and SEQ 7th (Table 1, Fig. 1c). In SIM, sucrose, glucose and fructose were depleted in the first four days (data not shown), while in SEQ 4th and SEQ 7th, the utilisation of sugars followed the same trend as the *T. delbrueckii* Biodiva control, with high amounts of residual fructose (13 g/L) and glucose (6 g/L) respectively (Table 1). This may indicate that the bacteria in SIM could improve the utilisation of sugars during fermentation, corresponding to its lower levels of residual sugars (sum of fructose, glucose and sucrose, < 2 g/L). The residual sugars in the *T. delbrueckii* Biodiva control, SEQ 4th and SEQ 7th were around 18 g/L at day 14 (Table 1), which was significantly higher than that in the *S. cerevisiae* EC-1118 control (< 1 g/L).

Our results are in line with the findings of Chen and Liu (2016), who reported that *O. oeni* did not affect the sugar utilisation of yeasts (*S. cerevisiae* MERIT. ferm) in simultaneous AF and MLF in lychee wine fermentation. Jussier *et al*. (2006) reported that *O. oeni* could metabolise sugars, especially fructose and glucose, to produce ethanol, carbon dioxide and organic acids (e.g. lactic acid and acetic acid) in SIM. In addition, the level of ethanol produced in SIM (6.93%, v/v) was comparable to that in the *S. cerevisiae* EC-1118 control (6.75%, v/v), which was relatively higher

TABLE 1
Oenological parameters of durian wines fermented simultaneously and sequentially with *T. delbrueckii* and *O. oeni* [#]

		Durian wines (day 14)				
Parameters	**Durian puree**	**EC-1118**	**Biodiva**	**SIM**	**SEQ 4th**	**SEQ 7th**
pH	4.03 ± 0.00a	4.03 ± 0.02a	4.08 ± 0.01b	4.17 ± 0.01c	4.12 ± 0.02d	4.20 ± 0.01c
°Brix (%)	20.23 ± 0.18a	7.04 ± 0.15b	8.80 ± 0.19c	6.75 ± 0.06b	8.50 ± 0.14c	8.55 ± 0.17c
Ethanol (%)	0.08 ± 0.01a	6.75 ± 0.47b	6.39 ± 0.28b	6.93 ± 0.40b	6.34 ± 0.43b	6.33 ± 0.36b
Sugars (g/L)						
Fructose	9.51 ± 0.32a	0.10 ± 0.00b	13.02 ± 0.81c	0.16 ± 0.02b	12.07 ± 0.75c	12.98 ± 0.58c
Glucose	14.86 ± 0.30a	0.29 ± 0.01b	5.59 ± 0.36c	0.79 ± 0.05b	6.20 ± 0.50c	5.68 ± 0.21c
Sucrose	218.47 ± 2.19a	0.21 ± 0.01b	0.43 ± 0.05b	0.48 ± 0.02b	0.38 ± 0.05b	0.44 ± 0.04b
Organic acid (g/L)						
Acetic acid	0.725 ± 0.011a	1.173 ± 0.021b	1.033 ± 0.026c	1.275 ± 0.005d	1.966 ± 0.004e	1.727 ± 0.062f
α-Ketoglutaric acid	0.078 ± 0.004a	0.140 ± 0.006b	0.135 ± 0.007b	0.100 ± 0.015ab	0.075 ± 0.007a	0.100 ± 0.004ab
Citric acid	0.485 ± 0.017a	0.204 ± 0.006b	0.158 ± 0.009c	0.101 ± 0.005d	nd	nd
Lactic acid	0.228 ± 0.016a	1.124 ± 0.012b	0.591 ± 0.019c	3.667 ± 0.040d	3.933 ± 0.229d	3.357 ± 0.067e
Malic acid	6.356 ± 0.072a	3.817 ± 0.026b	5.670 ± 0.074c	2.529 ± 0.389d	3.582 ± 0.049b	3.830 ± 0.012b
Oxalic acid	0.008 ± 0.000a	0.009 ± 0.001a	0.008 ± 0.000a	0.009 ± 0.001a	0.008 ± 0.002a	0.013 ± 0.002a
Succinic acid	0.539 ± 0.004a	1.964 ± 0.021b	1.423 ± 0.037c	3.809 ± 0.057d	1.041 ± 0.003e	1.162 ± 0.026f
Tartaric acid	0.831 ± 0.183a	0.115 ± 0.006b	0.218 ± 0.002b	0.218 ± 0.022b	0.104 ± 0.008b	0.114 ± 0.015b
Pyruvic acid	Nd	0.124 ± 0.010a	0.248 ± 0.004b	nd	nd	nd

[#] EC-1118 = *S. cerevisiae* EC-1118 monoculture fermentation; Biodiva = *T. delbrueckii* Biodiva monoculture fermentation; SIM = simultaneous inoculation of *O. oeni* PN4 and *T. delbrueckii* Biodiva; SEQ 4th = inoculation of *O. oeni* PN4 after four days of fermentation of *T. delbrueckii* Biodiva; SEQ 7th = inoculation of *O. oeni* PN4 after seven days of fermentation of *T. delbrueckii* Biodiva.
nd: not detected.
[a,b,c,d,e,f] Statistical analysis ANOVA (n = 3) at 95% confidence level, with same letters indicating no significant difference; the values are the means of triplicate fermentations ± standard deviation.

than in the *T. delbrueckii* Biodiva control (6.39%, v/v), SEQ 4th (6.34%, v/v) and SEQ 7th (6.33%, v/v), as shown in Table 1, but no significant difference was found among all treatments. This corresponded with the results of Lu *et al*. (2017), who reported that even though the bacteria accelerated yeast death they did not affect the progress of AF.

The pH of all five fermentations decreased from 4.03 to around 3.95 (*T. delbrueckii* Biodiva control) and 3.81 (*S. cerevisiae* EC-1118 control) by day 2 (Fig. 1d), and then the pH in the two control groups increased gradually to 4.08 (*T. delbrueckii* Biodiva control) and 4.03 (*S. cerevisiae* EC-1118 control) by day 14. The decline in pH could be ascribed to the production of organic acids (α-ketoglutaric, pyruvic, succinic and lactic acids) during yeast growth (Rosi & Canuti, 2003). In SIM, the pH rapidly increased to around 4.20 by day 4 and then decreased slightly to 4.17 (Fig. 1d). The pH in SEQ 4th increased sharply to 4.24 by day 8 after the inoculation of *O. oeni* and then decreased gradually to 4.12 (Fig. 1d). In SEQ 7th, the pH gradually increased to 4.20 (day 14) after inoculation of *O. oeni* at day 7 (Fig. 1d). Compared with the control groups (two AFs only), larger increases in pH were observed in both SIM and SEQ (Fig. 1d), which agreed with previous studies (Chen & Liu, 2016). This was likely due to the metabolism of L-malic acid by *O. oeni* in SIM and SEQ (Table 1, Fig. 2).

Changes in organic acids

The kinetics of organic acids are shown in Fig. 2. Most of the biochemical characteristics of SIM were different from SEQ 4th and SEQ 7th (Table 1), and the latter two SEQ were also different from each other in changes of acetic, lactic and succinic acids (Table 1, Fig. 2). It seems that the bacterial inoculation time significantly affected the concentrations of organic acids. This was consistent with several previous studies (Izquierdo *et al*., 2012; Nehme *et al*., 2010).

The largest utilisation of malic acid was found in SIM, with a residual level of 2.53 g/L, which was significantly lower than that in SEQ 4th (3.53 g/L), SEQ 7th (3.83 g/L) and the two control groups, with concentrations of 3.82 g/L (EC-1118 control) and 5.67 g/L (Biodiva control) respectively (Table 1, Fig. 2).

Our results are consistent with previous studies (Chen & Liu, 2016; Taniasuri *et al*., 2016), where the concentration of malic acid remained at 3.1 g/L or 3.8 g/L with simultaneous AF (*S. cerevisiae* EC-1118 or MERIT) and MLF (*O. oeni* Viniflora) after 28 and 20 days fermentation in durian and lychee wine respectively. As DL-malic acid was used to adjust the pH of durian pulp in this work, it therefore is important to note that only L-malic acid could be metabolised by *O. oeni* through decarboxylation to L-lactic acid and CO_2 (Nehme *et al*., 2010). D-malic acid remained in the final wine, or was partially absorbed by the yeasts via passive diffusion (Chen & Liu, 2016; Lu *et al*., 2016a, 2016b; Taniasuri *et al*., 2016).

Ideally, enzymatic analysis of D- and L-malic acid should be carried out to ascertain their changes.

In addition, the rate of L-malic acid degradation was related to the bacterial activity, with the highest malolactic activity during day 0 to day 2 in SIM, day 5 to day 10 in SEQ 4th and day 8 to day 14 in SEQ 7th (Fig. 2). The influence of inoculation time of *O. oeni* on malolactic activity seems to be strain specific for each yeast-bacteria combination in wine fermentations. Our results are in line with the findings of Herrero *et al*. (2003), who demonstrated that the maximum malolactic activity of *O. oeni* was found in its early growth stage. However, Ugliano and Moio (2005) reported that the

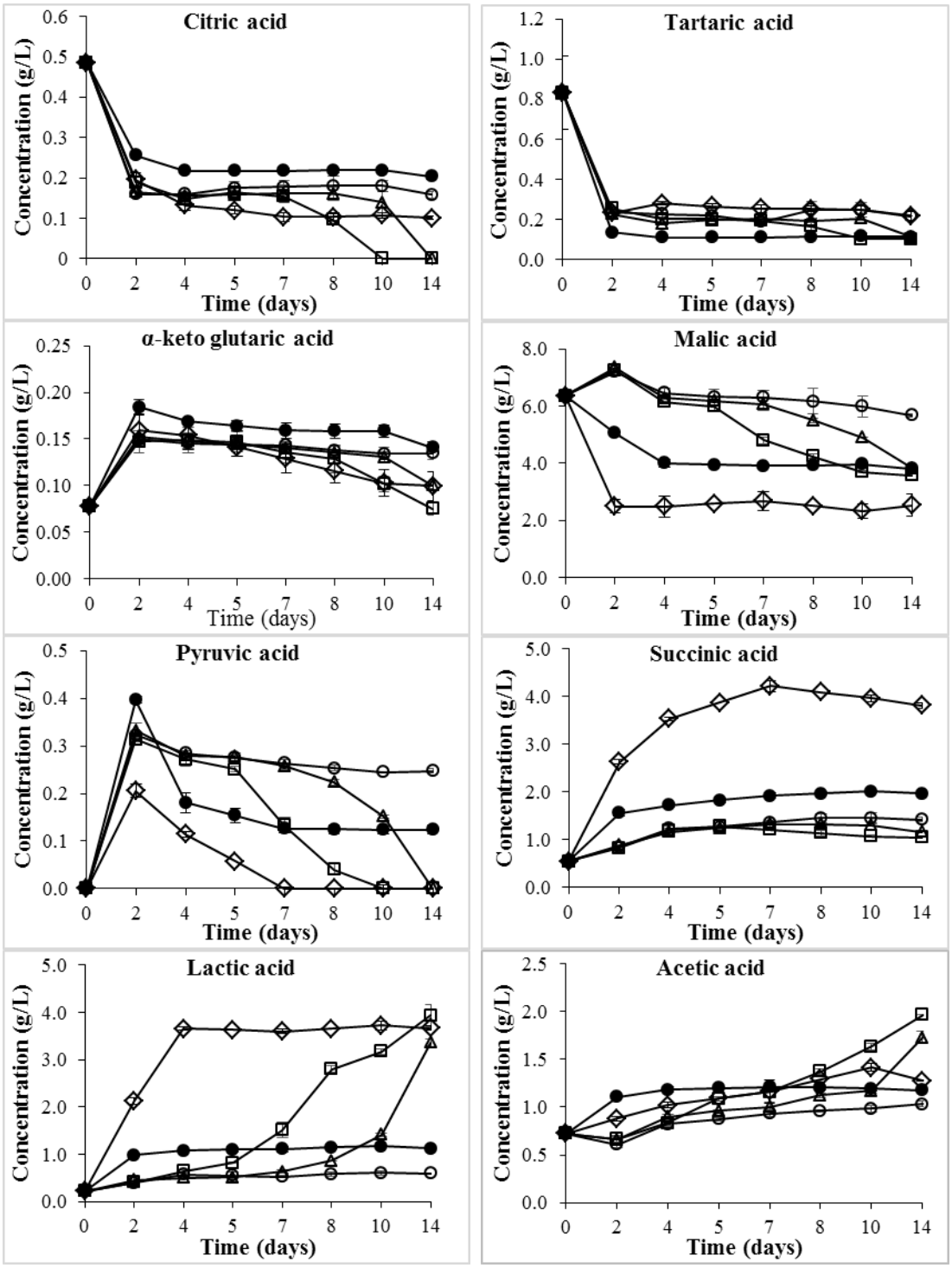

FIGURE 2

Kinetics of organic acids during durian wine fermentation. (●) *S. cerevisiae* EC-1118 monoculture fermentation; (○) *T. delbrueckii* Biodiva monoculture fermentation; (◇) simultaneous inoculation (SIM) of *T. delbrueckii* Biodiva and *O. oeni* PN4; (□) sequential inoculation (SEQ 4th): *O. oeni* PN4 inoculated after four days of fermentation with *T. delbrueckii* Biodiva; (△) sequential inoculation (SEQ 7th): *O. oeni* PN4 inoculated after seven days of fermentation with *T. delbrueckii* Biodiva. The values are the means of triplicate fermentations ± standard deviation.

TABLE 2
Concentrations of major volatile compounds (mg/L) in durian wine (day 14) fermented with *T. delbrueckii* and *O. oeni* [#]

Compounds quantified	Day 0		EC-1118		Biodiva		SIM		SEQ 4th		SEQ 7th		Odour threshold[f]	LRI
	Mean	OAV[i]	Mean	OAV[i]	Mean	OAV[i]	Mean	OAV[i]	Mean	OAV[i]	Mean	OAV[i]		
Hexanoic acid	1.00 ± 0.05a	0.13	0.29 ± 0.02b	0.04	0.21 ± 0.01cd	0.03	0.16 ± 0.01d	0.02	0.27 ± 0.01bc	0.03	0.24 ± 0.01bc	0.03	8.00	1845
Octanoic acid	0.43 ± 0.01a	0.05	0.31 ± 0.02b	0.04	0.22 ± 0.01c	0.03	0.24 ± 0.01c	0.03	0.22 ± 0.00c	0.03	0.22 ± 0.00c	0.03	8.80	2058
1-Octanol	nd	0.00	0.001 ± 0.000a	0.00	0.001 ± 0.000a	0.00	0.001 ± 0.000a	0.00	nd	0.00	0.001 ± 0.000a	0.00	0.9	1567
Isobutyl alcohol	nd	0.00	23.56 ± 1.28a	0.59	18.00 ± 2.87b	0.45	22.96 ± 1.98a	0.57	18.15 ± 1.31b	0.45	14.97 ± 0.98b	0.37	40.00	1103
Isoamyl alcohol	nd	0.00	1484.53 ± 33.56a	49.48	1347.65 ± 85.95ab	44.92	1419.81 ± 35.56ab	47.33	1147.48 ± 190.52bc	38.25	1019.19 ± 169.94c	33.97	30.00	1216
2-Phenylethyl alcohol	nd	0.00	31.13 ± 2.18a	3.11	36.33 ± 1.82ab	3.63	64.20 ± 3.07c	6.42	37.01 ± 2.08b	3.70	36.41 ± 1.37ab	3.64	10.00	1931
Ethyl acetate	0.22 ± 0.02a	0.70	5.27 ± 0.39b	0.70	2.92 ± 0.50c	0.39	3.69 ± 0.13c	0.49	3.43 ± 0.04c	0.46	3.33 ± 0.07c	0.44	7.50	-
Isoamyl acetate	nd	0.00	0.12 ± 0.01a	4.00	0.01 ± 0.00b	0.33	0.06 ± 0.02c	3.00	0.02 ± 0.00b	0.67	0.02 ± 0.00b	0.67	0.03	1114
Methyl octanoate	0.092 ± 0.006a	-	0.014 ± 0.001b	-	0.008 ± 0.001bc	-	0.004 ± 0.001c	0.00	0.005 ± 0.000c	-	0.006 ± 0.000c	-	-	1384
Ethyl 2-methylbutyrate	2.00 ± 0.21a	111.11	0.22 ± 0.01b	12.22	0.16 ± 0.04b	8.89	0.07 ± 0.01b	3.89	0.13 ± 0.02b	7.22	0.12 ± 0.01b	6.67	0.018[&]	1049
Ethyl hexanoate	nd	0.00	0.48 ± 0.02a	9.60	0.23 ± 0.02b	4.60	0.41 ± 0.03c	8.20	0.22 ± 0.01b	4.40	0.24 ± 0.01b	4.80	0.05	1224
Ethyl octanoate	0.12 ± 0.01a	6.00	0.57 ± 0.02b	28.50	0.17 ± 0.01a	8.50	0.19 ± 0.00a	9.50	0.17 ± 0.01a	8.50	0.17 ± 0.02a	8.50	0.02	1430
Ethyl nonanoate	nd	0.00	0.005 ± 0.001a	0.00	0.003 ± 0.001a	0.00	0.014 ± 0.001b	0.01	0.005 ± 0.000a	0.00	0.004 ± 0.000a	0.00	1.3	1529
Ethyl decanoate	0.07 ± 0.00a	0.34	0.56 ± 0.02b	2.80	0.15 ± 0.01c	0.75	0.53 ± 0.04b	2.65	0.13 ± 0.01c	0.65	0.14 ± 0.00c	0.70	0.20	1634
Ethyl dodecanoate	nd	0.00	0.20 ± 0.01ab	0.17	0.17 ± 0.00bc	0.14	0.16 ± 0.00c	0.13	0.19 ± 0.00d	0.16	0.18 ± 0.00bd	0.15	1.2	1837
Isoamyl octanoate	nd	0.00	0.060 ± 0.000a	0.48	0.050 ± 0.000b	0.40	0.052 ± 0.001c	0.42	0.050 ± 0.001b	0.40	0.050 ± 0.000b	0.40	0.125	1452
Benzaldehyde	0.031 ± 0.000a	0.01	0.002 ± 0.000bd	0.00	0.001 ± 0.000b	0.00	0.004 ± 0.001c	0.00	0.001 ± 0.000b	0.00	0.003 ± 0.000cd	0.00	3.50[g]	1537
Diethyl disulphide	0.91 ± 0.07a	211.40	0.010 ± 0.001b	2.33	0.015 ± 0.001b	3.49	0.012 ± 0.002b	2.79	0.014 ± 0.001b	3.26	0.015 ± 0.001b	3.49	0.0043	1205
1-Propanethiol	0.64 ± 0.09a	856.00	0.00 ± 0.00b	0.00	0.00 ± 0.00b	0.00	0.00 ± 0.00b	0.00	0.00 ± 0.00b	0.00	0.00 ± 0.00b	0.00	0.00075	-

[#] EC-1118 = *S. cerevisiae* EC-1118 monoculture fermentation; Biodiva = *T. delbrueckii* Biodiva monoculture fermentation; SIM = simultaneous inoculation of *O. oeni* PN4 and *T. delbrueckii* Biodiva; SEQ 4th = inoculation of *O. oeni* PN4 after four days of fermentation of *T. delbrueckii* Biodiva; SEQ 7th = inoculation of *O. oeni* PN4 after seven days of fermentation of *T. delbrueckii* Biodiva;
[a,b,c,d] Statistical analysis ANOVA (n = 3) at 95% confidence level, with same letters indicating no significant differences; the values are the means of triplicate fermentations ± standard deviation.
nd: not detected.
[f] From Bartowsky and Pretorius (2009); [g] From Buttery *et al.* (1990); [&] Ferreira *et al.* (2000)
[i] Odour activity values calculated by dividing the concentration by the odour threshold value of the compound.

degradation of L-malic acid by *O. oeni* was quite slow in the first two weeks after inoculation. This could be due to the different treatments (inducing MLF after AF completed) or different bacteria (*O. oeni* Lalvin 31, EQ 54, Lalvin O.S.U. and Uvaferm Alpha) used in different fermentations.

The production of lactic acid in SIM, SEQ 4th and SEQ 7th correlated with the degradation of L-malic acid (Fig. 2). The transformation of L-malic acid to L-lactic acid and CO_2 is beneficial to the taste and the quality of the wine due to the reduced acidity (Ugliano & Moio, 2005). Although the degradation of malic acid in SEQ 4th and SEQ 7th was less than that in SIM, comparable levels of lactic acid were produced (Table 1, Fig. 2). Maicas *et al*. (2002) reported that the lactic acid could be from sugars catabolised by *O. oeni* via the hetero-fermentative pathway. Lactic acid could also be produced via the metabolism of citric acid by *O. oeni* (Swiegers *et al*., 2005), which was consistent with the lower levels of citric acid in SEQ 4th and SEQ 7th (Table 1, Fig. 2).

Compared with the two control groups, more acetic acid was produced in SIM, SEQ 4th and SEQ 7th (Table 1, Fig. 2). Acetic acid could be generated from sugars via the hetero-fermentative pathway (Bartowsky & Henschke, 2004), or from citric acid metabolism by *O. oeni* under the catalysis of citrate lyase (Swiegers *et al*., 2005; Abrahamse & Bartowsky, 2012). This is in line with lower residual levels of citric acid (Table 1, Fig. 2). The time point of bacterial inoculation significantly affected the production of acetic acid, with the highest concentration in SEQ 4th (1.97 g/L), followed by SEQ 7th (1.73 g/L) and SIM (1.28 g/L). Several studies have shown that simultaneous inoculation of malolactic bacteria with yeast could lead to relatively higher amounts of acetic acid (Abrahamse & Bartowsky, 2012; Massera *et al*., 2009; Taniasuri *et al*., 2016), which could be ascribed to the availability of sugars in the fermented medium (Massera *et al*., 2009). Although the concentrations of acetic acid in all MLF treatments (SIM and SEQ) exceeded the optimum value (0.7 g/L) in grape wines (Viana *et al*., 2008), it must be noted that this optimum value may not be suitable for durian wines fermented from durian pulp, which has a different composition. In addition, acetic acid is the key precursor of fruity acetate esters produced during fermentation (Swiegers *et al*., 2005; Chen & Liu, 2016), and relatively higher levels of ethyl and isoamyl acetates were found in SIM and SEQ than in the *T. delbrueckii* Biodiva control (Table 2, Fig. 3).

The production of succinic acid varied among the different treatments (Table 1). Marked increases in succinic acid were observed in the *S. cerevisiae* EC-1118 and *T. delbrueckii* Biodiva controls, from 0.54 g/L to 1.96 g/L and 1.42 g/L respectively (Table 1, Fig. 2). This agrees with previous studies, which found that *S. cerevisiae* EC-1118 (Taniasuri *et al*., 2016) and *T. delbrueckii* Biodiva (Lu *et al*., 2016a, 2016b) are good producers of succinic acid. A significantly higher level of succinic acid was produced in SIM (3.81 g/L) than in the two control groups (Table 1, Fig. 2). Our results are in line with the findings of Zhang and Gänzle (2010), who reported that succinic acid could be produced and contributed by *Lactobacillus sp.* via metabolising α-ketoglutarate. This is consistent with the decrease in α-ketoglutaric acid in SIM and SEQ (Fig. 2). Relatively lower levels of succinic acid were observed in SEQ 4th (1.04 g/L) and SEQ 7th (1.16 g/L) than in the *T. delbrueckii* Biodiva control (Table 1, Fig. 2). Taniasuri *et al*. (2016) reported that the decline in succinic acid in MLF could be ascribed to the transformation to fumaric acid (which could further transform to malic acid) or the corresponding ester, namely diethyl succinate. However, it must be indicated that succinic acid has an unusual bitter-salty taste, and succinic acid in excess may be undesirable for the taste of the durian wine.

Pyruvic acid was produced by yeast but mainly metabolised by malolactic bacteria (Table 1, Fig. 2). The early degradation in AF only (day 2 to day 7) could be caused by the biochemical metabolism related to the production of ATP and the regeneration of $NAD(P)^+$ in energy conservation (Jackowetz & Mira de Orduña, 2012). A relatively lower level of pyruvic acid (0.12 g/L) was accumulated in the *S. cerevisiae* EC-1118 control than in the *T. delbrueckii* Biodiva control (0.25 g/L), which agrees with our previous results (Lu *et al*., 2015). In addition, pyruvic acid could be metabolised by *S. cerevisiae* into acetaldehyde and further to ethanol (Wang *et al*., 2015). The pyruvic acid intermediate in SIM, SEQ 4th and SEQ 7th was completely consumed by *O. oeni* (Table 1, Fig. 2). This is consistent with several previous studies (Chen & Liu, 2016; Jackowetz & Mira de Orduña, 2012). However, Taniasuri *et al*. (2016) reported that similar kinetic changes and residual levels of pyruvic acid were found in AF, SIM and SEQ fermented by *S. cerevisiae* EC-1118 and *O. oeni* Viniflora. Malolactic bacteria can gain energy from the metabolism of pyruvate via the generation of acetyl-P, then ATP (Liu, 2003).

The decrease in tartaric acid in all treatments is most likely due to the precipitation of potassium bitartrate (Table 1, Fig. 2). A previous study has shown that neither yeast nor *O. oeni* could metabolise this acid during fermentation (Taniasuri *et al*., 2016). The small increases in oxalic acid in all fermentation could be released from durian pulp, not produced by yeast nor *O. oeni* (Lu *et al*., 2016a; Taniasuri *et al*., 2016).

Impact of inoculation time of malolactic bacteria on volatile profiles of durian wine

Hexanoic and octanoic acids were significantly metabolised in all fermentations, possibly to form the corresponding ethyl esters (Table 2) or to be absorbed by yeast mannoproteins released during AF or autolysis (Alexandre *et al*., 2004). A relatively lower level of hexanoic acid (0.16 mg/L) was detected in SIM than that in the other treatments, which is in line with the higher amount of ethyl hexanoate in SIM (Table 2, Fig. 3). Comparable levels of octanoic acid were found in all treatments, although significantly lower than that in durian pulp (Table 2). This may indicate that the different MLF inoculation regimes did not significantly affect the metabolism of octanoic acid. Our results differ from several previous studies (Pozo-Bayon *et al*., 2005; Taniasuri *et al*., 2016), in which significant increases in fatty acids (octanoic and decanoic acids) were reported after MLF. The reason might be the different yeast and bacteria used in the different fermentations. The reduction in medium-chain fatty acids in this study is desirable, as large amounts of fatty acids could inhibit the growth of both yeast and bacteria and hence could result in stuck fermentation (Alexandre *et al*., 2004; Viana

et al., 2008).

Similar to ethanol, SIM produced the highest amounts of higher alcohols except for 1-octanol, while SEQ 4th and SEQ 7th produced comparable levels to the *T. delbrueckii* Biodiva control (Table 2, Fig. 3). Isoamyl alcohol and 2-phenylethyl alcohol increased rapidly in the first few days of fermentation and then kept stable or gradually reduced (Fig. 3). The increases in these higher alcohols could contribute to the

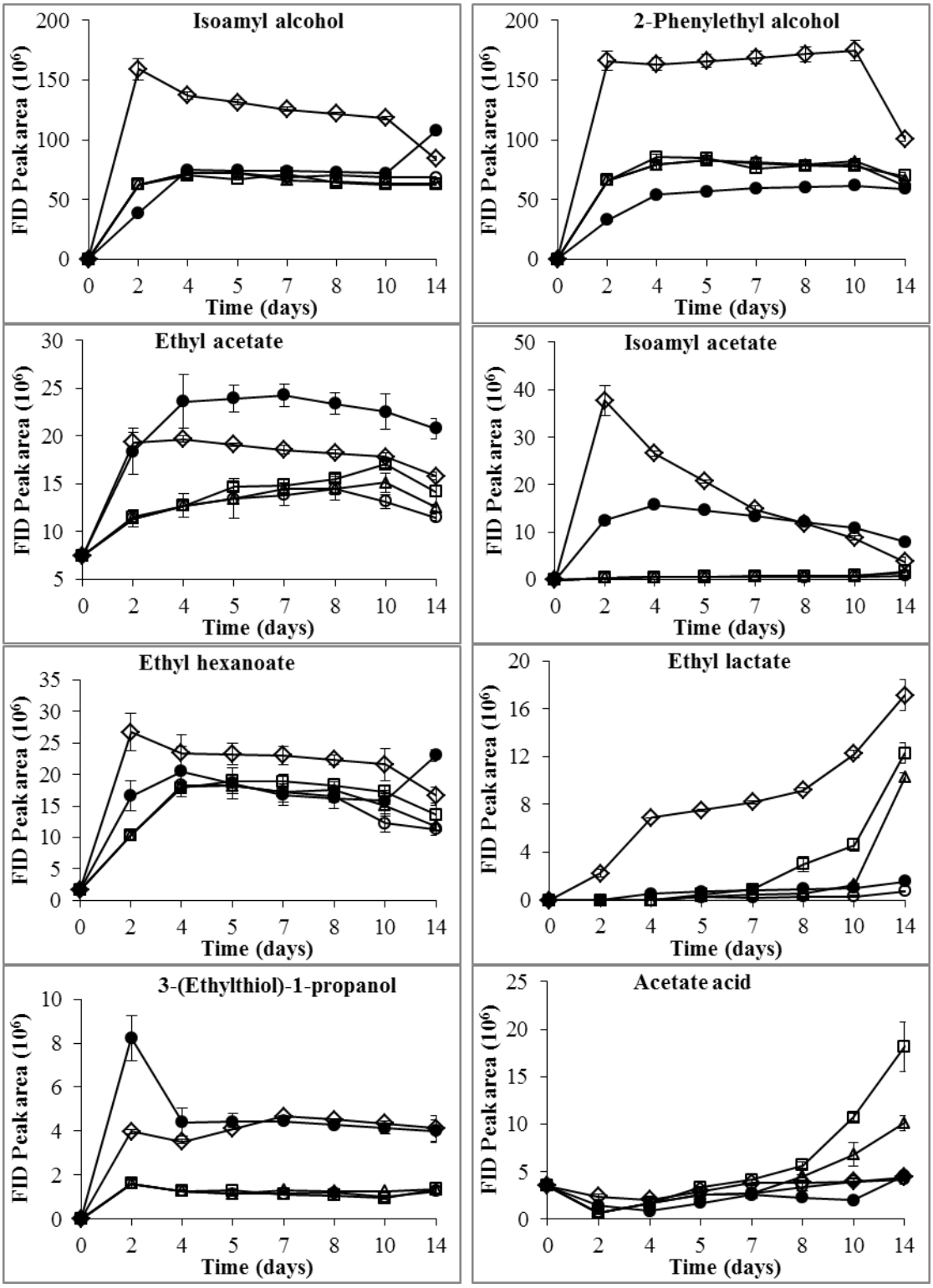

FIGURE 3

Kinetics of ethanol, acetic acid, isoamyl acetate, ethyl acetate, ethyl hexanoate and ethyl lactate during durian wine fermentation. (●) *S. cerevisiae* EC-1118 monoculture fermentation; (○) *T. delbrueckii* Biodiva monoculture fermentation; (◇) simultaneous inoculation (SIM) of *T. delbrueckii* Biodiva and *O. oeni* PN4; (□) sequential inoculation (SEQ 4th): *O. oeni* PN4 inoculated after four days of fermentation with *T. delbrueckii* Biodiva; (△) sequential inoculation (SEQ 7th): *O. oeni* PN4 inoculated after seven days of fermentation with *T. delbrueckii* Biodiva. The values are the means of triplicate fermentations ± standard deviation.

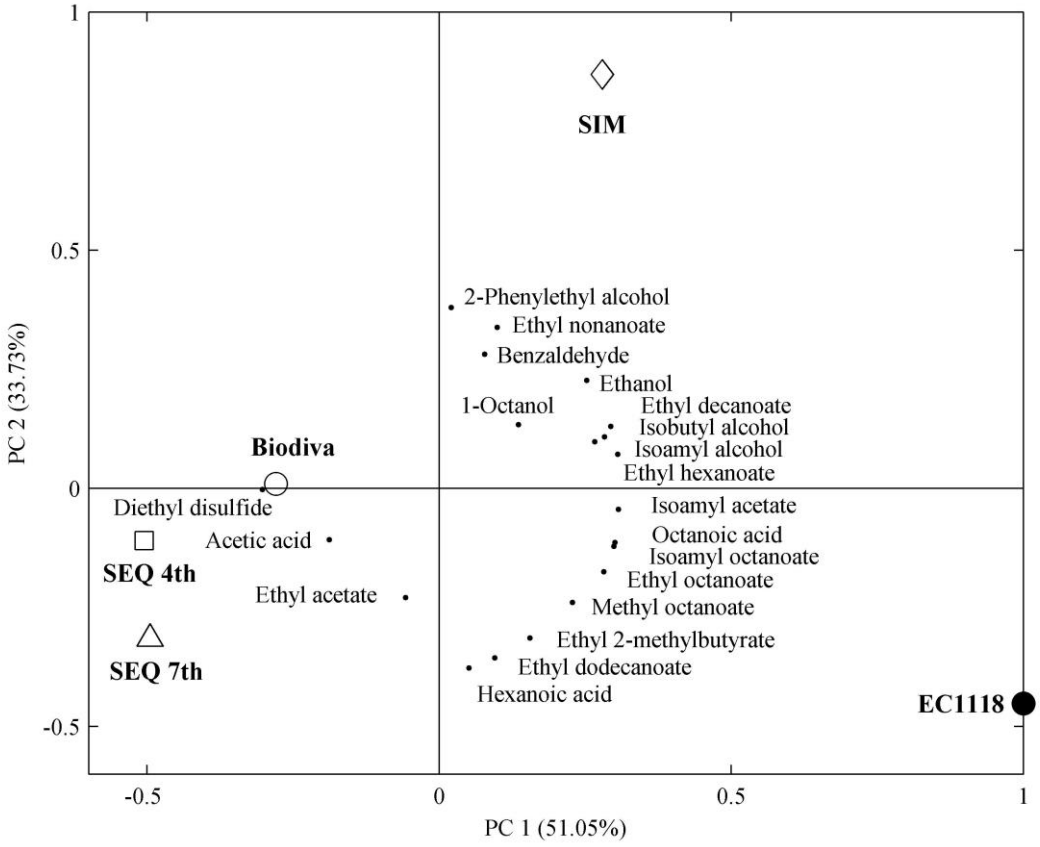

FIGURE 4

Bi-plot of principal component analysis of durian wine fermented with *S. cerevisiae* EC-1118, *T. delbrueckii* Biodiva and *O. oeni* PN4. SIM: Simultaneous inoculation of *T. delbrueckii* Biodiva and *O. oeni* PN4; SEQ 4th: *O. oeni* PN4 sequentially inoculated after four days of fermentation with *T. delbrueckii* Biodiva; SEQ 7th: *O. oeni* PN4 sequentially inoculated after seven days of fermentation with *T. delbrueckii* Biodiva.

fruity notes and aroma complexity of durian wines. De Revel *et al*. (1999) observed the increase in isoamyl alcohol after simultaneous MLF, while Jeromel *et al*. (2008) found increases in isobutyl alcohol and 2-phenylethyl alcohol after simultaneous MLF. In contrast, several studies have shown that similar levels of higher alcohols are observed in simultaneous MLF as compared to AF only (Abrahamse & Bartowsky, 2012; Chen & Liu, 2016; Taniasuri *et al*., 2016). This discrepancy could be due to the different yeasts used (*S. cerevisiae* and *O. oeni*). The reduction in these higher alcohols at the later stage of fermentation could be ascribed to the production of corresponding esters (Table 2). The concentrations of isoamyl alcohol (30 mg/L, in 10% ethanol v/v) and 2-phenylethyl alcohol (10 mg/L, in 10% ethanol v/v) were higher than their odour thresholds and could impart alcoholic and whiskey, floral and rose aroma notes to the final wines (Table 2).

Most esters that are endogenously present in durian pulp, such as methyl 2-methylbutyrate and ethyl 2-methylbutyrate, were catabolised during fermentation (Table 2). Various esters, such as ethyl esters (ethyl esters of hexanoate, octanoate, nonanoate, decanoate and dodecanoate) and acetate esters (ethyl acetate and isoamyl acetate), were produced by yeast during AF (Table 2). The production of these esters would compensate for the loss of the endogenous fruity esters. The final levels of esters in MLF wines seemed to be related to the inoculation time of *O. oeni*. SIM produced relatively higher levels of ethyl esters than those in SEQ 4th, SEQ 7th and the *T. delbrueckii* Biodiva control (Table 2). Ethyl esters of hexanoate, octanoate and decanoate are associated with fruity notes with concentrations well above their odour detection thresholds (Table 2). This would contribute fruity sensory properties to durian wines (Francis & Newton, 2005).

Ethyl lactate is quantitatively the most important ester produced during MLF (Pozo-Bayon *et al*., 2005). SIM produced the highest amount of ethyl lactate, followed by SEQ 4th and SEQ 7th (Fig. 3). The ethyl lactate in SIM, SEQ 4th and SEQ 7th were approximately 22, 16 and 13 times higher than that produced by the *T. delbrueckii* Biodiva control respectively (Fig. 3). All MLF treatments also produced more ethyl lactate than the *S. cerevisiae* EC-1118 control (Fig. 3). The production of ethyl lactate was significantly associated with its precursor (lactic acid) produced during MLF (Fig. 2, Table 2), and this was consistent with previous studies (Abrahamse & Bartowsky, 2012; Chen & Liu, 2016). Other than imparting fruity notes to durian wines, ethyl lactate could also increase mouthfeel (Ugliano & Moio, 2005), while its odour detection threshold (around 14 mg/L) was relatively higher than that of the other ethyl esters (Francis & Newton, 2005).

Acetate esters are formed from the corresponding higher

alcohols and acetyl-CoA (Swiegers *et al*., 2005). The amounts of ethyl acetate and isoamyl acetate in SIM were higher than that in the *T. delbrueckii* Biodiva control and SEQ, but less than in the *S. cerevisiae* EC-1118 control (Table 2, Fig. 3). Ethyl acetate in all treatments was lower than its odour detection threshold (7.50 mg/L, in 10% ethanol v/v) and may not significantly influence the sensory properties of the wine, while isoamyl acetate in SIM was higher than its odour detection threshold (0.03 mg/L, in 10% ethanol v/v). The concentrations of acetate esters in SIM increased rapidly by day 2 and then remained stable or declined gradually (Fig. 3). Acetate esters are also associated with fruity aroma, and isoamyl acetate could contribute a banana-like note. Our results agree with several previous findings (Abrahamse & Bartowsky, 2012; Massera *et al*., 2009; Taniasuri *et al*., 2016), in which SIM wines scored higher in fruity feature than SEQ wines.

Diacetyl is normally formed after MLF, but it was not found in durian pulp or in durian wines. It might have been reduced to acetoin or 2,3-butanediol (Chen & Liu, 2016; Knoll *et al*., 2011). Regardless of the inoculation regimes, aldehydes that were initially present in durian pulp, including acetaldehyde, hexanal and nonanal, were catabolised to undetectable levels in all treatments (data not shown). These aldehydes might be reduced to the respective alcohols or oxidised to acids, and even be used to form esters (Sumby *et al*., 2010). Benzaldehyde was metabolised to low levels in all treatments (Table 2), and may be transformed to benzylalcohol (Delfini *et al*., 2015), L-phenylacetyl carbinol (Agarwal *et al*., 1987) or benzoic acid by yeast employing benzaldehyde as the precursor. MLF wines contained relatively higher residual levels of benzaldehyde (Table 2), which could be ascribed to the metabolism of L-phenylalanine by LAB, such as *O. oeni*.

Volatile sulphur compounds (VSCs) are significant compounds in durian pulp and can also contribute to the wine aroma. Previous studies showed that some VSCs could even determine wine quality, with a positive bouquet aroma contribution based on their concentrations (Landaud *et al*., 2008). Most of the endogenous VSCs in durian pulp were catabolised to undetectable (e.g. ethanethiol, 1-propanethiol) or trace levels (diethyl disulphide) (Table 2). This agrees with previous studies (Lee *et al*., 2012; Lu *et al*., 2016a, 2016b). The reactive thiols, such as ethanethiol and 1-propanethiol, might be oxidised to their respective disulphides by trace levels of metal ions in the fermented medium, or form non-volatile thiols by reacting with polymeric phenols (Nikolantonaki *et al*., 2010). In addition, thiols could also be consumed by cell wall mannoproteins of yeast lees by forming disulphide bridges (Nikolantonaki *et al*., 2010). Furthermore, sulphides could be reduced to the corresponding thiols by yeast under anaerobic conditions (Gómez-Plaza & Cano-López, 2011), and the thiols that form could be degraded further, as mentioned above.

The production of new VSCs such as 3-(ethylthio)-1-propanol (Fig. 3) could make up for the weak onion-like odour caused by the loss of initial VSCs. SIM wines produced comparable levels of 3-(ethylthio)-1-propanol to the *S. cerevisiae* EC-1118 control, which were significantly higher than that in the *T. delbrueckii* Biodiva control and SEQ (Fig. 3). The presence of 3-(ethylthio)-1-propanol has been noted in grape wines (Moreira *et al*., 2011), which might be from the metabolism of methionine (Noguerol-Pato *et al*., 2011). The production of 3-(ethylthio)-1-propanol could be affected by the nitrogen content of the fermented medium (Moreira *et al*., 2011). Amino acids are the most important nitrogen source for LAB metabolism during wine fermentation, and *O. oeni* could convert methionine into various VSCs, such as methional and 2-oxo-4-(methylthio)-butyric acid (Vallet *et al*., 2008). However, Izquierdo *et al*. (2014) reported that *O. oeni* only played a minor role in the production of VSCs during MLF. Above all, the metabolic pathway for the formation of 3-(ethylthio)-1-propanol in *O. oeni* has not yet been elucidated.

Multivariate data analysis of durian wine

Acetic acid and ethanol from Table 1 and all volatile compounds from Table 2 were analysed by applying principal component analysis (PCA) to distinguish the particular characteristics of durian wines produced by different treatments. The first two principle components (PCs) accounted for 84.78% of the total variation, with PC1 and PC2 accounting for 51.05% and 33.73% respectively (Fig. 4). Wines were separated on the basis of their different aroma compositions, with the *S. cerevisiae* EC-1118 control and SIM on the right half of the plot, while the *T. delbrueckii* Biodiva control, SEQ 4th and SEQ 7th are located on the left half (Fig. 4). PC2 separates SIM from other treatments due to its relatively higher levels of higher alcohols (2-phenylethyl alcohol, isoamyl alcohol, isobutyl alcohol and 1-octanol), ethyl hexanoate, ethyl nonanoate and ethyl decanoate (Fig. 4). SEQ 4th and SEQ 7th, positioned in the negative part of PC1 with the *T. delbrueckii* Biodiva control, indicate their similar volatile compositions, which are higher in acetic acid, diethyl disulphide and ethyl acetate.

CONCLUSIONS

This work reported for the first time the impact of MLF inoculation regimes on the fermentation kinetics and chemical components of durian wines fermented with *T. delbrueckii* Biodiva and *O. oeni* PN4. The inoculation time of *O. oeni* significantly adversely affected the population persistence of *T. delbrueckii*, especially in SIM and SEQ 4th. However, the metabolism of yeasts in the early stage of AF may stimulate the growth of the malolactic bacteria in SEQ 4th and SEQ 7th. The final concentrations of organic acids and the production of esters in MLF wines varied with the timing of inoculation. SIM produced the highest amount of ethanol (6.93%), with sugars almost depleted. In addition, the relatively higher levels of ethyl esters, acetate esters and succinic acid produced in SIM could contribute fruity properties and modify mouthfeel. Therefore, SIM treatment would be an effective tool to produce durian wines with more fruity notes while retaining unique durian odour.

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