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APPLIED MICROBIAL AND CELL PHYSIOLOGY

Combined effects of fermentation temperature and pH on kinetic changes of chemical constituents of durian wine fermented with *Saccharomyces cerevisiae*

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Abstract This study investigated the effects of temperature (20 and 30 °C) and pH (pH 3.1, 3.9) on kinetic changes of chemical constituents of the durian wine fermented with *Saccharomyces cerevisiae*. Temperature significantly affected growth of *S. cerevisiae* EC-1118 regardless of pH with a higher temperature leading to a faster cell death. The pH had a more significant effect on ethanol production than temperature with higher production at 20 °C (5.95%, *v*/*v*) and 30 °C (5.56%, *v*/*v*) at pH 3.9, relative to that at pH 3.1 (5.25 and 5.01%, *v*/*v*). However, relatively higher levels of isobutyl alcohol and isoamyl alcohol up to 64.52 ± 6.39 and 56.27 ± 3.00 mg/L, respectively, were produced at pH 3.1 than at pH 3.9 regardless of temperature. In contrast, production of esters was more affected by temperature than pH, where levels of ethyl esters (ethyl esters of octanoate, nonanoate, and decanoate) and acetate esters (ethyl acetate and isoamyl acetate) were significantly higher up to 2.13 ± 0.23 and 4.61 ± 0.22 mg/L, respectively, at 20 °C than at 30 °C. On the other hand, higher temperature improved the reduction of volatile sulfur compounds. This study illustrated that temperature control would be a more effective tool than pH in modulating the resulting aroma compound profile of durian wine.

Keywords Durian wine · *Saccharomyces cerevisiae* · pH · Temperature · Volatiles

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Introduction

Durian, known as the "King of fruits", is a seasonal tropical fruit in Southeast Asia (Lu et al. 2016). Durian is limited to its short fruiting season (May to August) and an overproduction of durians during this period will cause wastage if they are not consumed within the short shelf time of 3–4 days at ambient temperature (Jaswir et al. 2005). Numerous preservation and processing technologies have been used to increase the commercial value and extend the shelf life of durian. Fermentation of durian to wine is an interesting option due to its unique and strong flavor as well as its popularity among Southeast Asia countries. This could create a new revenue gateway for the fruit. However, the consumption of excess durian together with alcohol might be unsafe because the rich sulfides (e.g., diethyl disulfide) in durian can inhibit the activity of aldehyde dehydrogenase (ALDH), which is the key enzyme related to ethanol metabolism (Maninang et al. 2009). Lee et al. (2012) reported that most of the sulfur-containing compounds decreased to trace levels after fermentation. This would eliminate the potential consumption risk of durian wine.

Saccharomyces is the main yeast genus that is used in alcoholic fermentation due its ability to quickly kick start

fermentation and also higher tolerance towards alcohol. This will enable almost all the sugars to be fermented completely. Ethanol and secondary metabolites (e.g., higher alcohols, ethyl and acetate esters) are formed via yeast metabolism during wine fermentation (Valero et al. 2002). These metabolites are at least partially responsible for the aroma profile of wine and then affect the organoleptic qualities, ultimately determining the vinous character of the wine (Pretorius 2000).

In grape wine fermentation, the pH ranging from 2.75 to 4.25 is crucial for the growth of yeast cells (Fleet and Heard 1993). This would significantly affect the flavor compound production (Lilly et al. 2006). Therefore, it is vital to evaluate the relationship between pH and the growth rate of *S. cerevisiae* during fermentation. Previous studies showed that there is a correlation between the pH and *S. cerevisiae* growth with yeast cells experiencing a longer lag phase when starting fermentation at pH ≤ 3 (Liu et al. 2015; Serra et al. 2005). Gao and Fleet (1988) reported that low pH might induce stress to the growth of yeast cells by increasing their sensitivity to ethanol. In addition, the starting pH could affect the production of volatiles and non-volatiles as well as the uptake of substrates (Pérez-Torrado et al. 2002). Furthermore, Morata et al. (2006) reported that the formation of proanthocyanidins and acetyl derivatives was affected by pH at the early stage of fermentation in grape wine.

Temperature is another variable that could directly affect the growth rate of yeasts and metabolic reactions (Fleet and Heard 1993; Torija et al. 2003a, b). Suitable temperatures could increase the production of secondary metabolites like succinic acid, acetic acid, and glycerol (Torija et al., 2003a, b). The wines produced at lower temperatures improved taste and aroma via increasing the production of organic volatiles like terpenes, higher alcohols, and esters (Llauradó et al. 2002; Torija et al. 2003a). As most studies are conducted on grape wine fermentation, it is not ideal to translate these findings to durian wine fermentation. The medium differences might influence the performance of yeast strains (Beltran et al. 2008). Durian has different physicochemical properties that could impact yeast strains and their physiological reactions (Beltran et al. 2008). The composition of volatile and non-volatile compounds in the final wine will eventually be different.

This research was carried out with the objective of analyzing the effects and relationships between pH and temperature on yeast growth and metabolic activities during durian wine fermentation. It was hypothesized that at different initial pH and temperatures, the physiological characteristics of *S. cerevisiae* would be affected, and thus leading to changes in metabolic activities and growth rates of the yeasts. This difference in growth and metabolic activities of the yeasts would in turn affect the biotransformation of the non-volatile components as well as volatiles, especially higher alcohols, ethyl esters, acetate esters, and volatile sulfur-containing compounds in durian wine.

Materials and methods

Durian pulp preparation

Durian (D666), imported from Malaysia, was purchased from a supermarket of Singapore. The durian pulp and seeds were separated manually, and then durian puree was obtained by mixing the durian pulp with deionized water at a ratio of 3:7 (*w*/*w*). The pH of the durian pulp was adjusted from 6.80 to 3.15 and 3.92 respectively by using DL-malic acid (1 mol/L, Sigma-Aldrich, Singapore) and the total soluble solids (°Brix) content was adjusted from 7.82 to around 20 by adding sucrose (FairPrice, Singapore). The durian puree was subsequently pasteurized at 60 °C for 20 min, and the efficiency of pasteurization was confirmed by plating.

Preculture preparation

S. cerevisiae EC-1118 was purchased from Lallemand Inc. (Brooklyn Park, Australia). A pure culture was obtained via propagating in a sterile broth (2.5 g of yeast extract, 2.5 g of bacteriological peptone, 2.5 g of malt extract, and 20 g of glucose per liter of water, pH 5.0) at 20 °C for 48 h with the colony-forming units (CFUs) of at least ~10^7/mL and was stored in −80 °C before use. A preculture was obtained via inoculating 5% *v*/*v* pure culture into the pasteurized durian puree with subsequent incubation at 20 °C for 48 h with the yeast cell counts of at least ~10^7 CFU/mL.

Durian wine fermentation

Triplicate laboratory-scale fermentations were conducted in 500-mL sterile Erlenmeyer flasks. Each of the sterile flasks contained 300 mL of the pasteurized durian puree. Four treatments were performed to evaluate the kinetic changes of volatile and non-volatile compositions during durian wine fermentation. Treatments included the following: treatment A—fermentation incubated at pH 3.1, 20 °C; treatment B—fermentation incubated at pH 3.1, 30 °C; treatment C—fermentation incubated at pH 3.9, 20 °C; and treatment D—fermentation incubated at pH 3.9, 30 °C. The adjustment of pH was done just before pasteurization. A *S. cerevisiae* EC-1118 preculture (1%, *v*/*v*) was inoculated into each flask, and the flasks were incubated statically at 20 °C for 14 days. Samples were taken at days 0, 1, 2, 3, 4, 7, 10, and 14 to perform the following analysis: viable cell counts, pH, °Brix, sugars, organic acids, and volatile compounds. All of the samples were stored at −20 °C before analysis.

Enumeration of viable yeast cells

The yeast cell counts were monitored by spread-plating on potato dextrose agar (PDA). The PDA plates were prepared

by dissolving 39 g of PDA powder in 1 L of deionized water. This mixture was shaken to ensure the agar was completely dissolved and then autoclaved at 121 °C for 15 min before pouring. The fermentation samples were serially diluted with 0.1% (*w*/*v*) peptone water. The diluted samples (0.1 mL) were added and spread on PDA plates, which were then incubated for 48 h at 20 °C.

Determination of °Brix, pH, sugars, organic acids, and volatiles

The pH and °Brix were measured using a pH meter (Metrohm, Zofingen, Switzerland) and a refractometer (ATAGO, Tokyo, Japan), respectively. Analyses for sugars and organic acids were conducted using a high-performance liquid chromatography (HPLC, Shimadzu, Kyoto, Japan), and volatiles were analyzed by using headspace (HS) solid-phase micro-extraction (SPME), gas chromatography (GC)-mass spectrometry (MS), and flame ionization detector (FID) as reported in our previous study (Lu et al. 2015, 2016).

Statistical analysis

A one-way analysis of variance (ANOVA) and Tukey's test (SPSS Corporation, Chicago, IL, USA, version 17.0) were chosen for data analysis. The statistical significance between durian pulp and wines was evaluated at $P < 0.05$. The data is presented as mean values ± standard deviations obtained from the triplicate fermentations.

Results

Evolution of cell population during fermentation

The evolution of *S. cerevisiae* EC-1118 cell population in different treatments is shown in Fig. 1a. For treatments A and B (pH 3.1), the temperature significantly affected the growth of *S. cerevisiae* EC-1118 with the maximum cell populations of 2.12×10^8 CFU/mL (20 °C) and 1.27×10^8 CFU/mL (30 °C) at day 4, respectively (Fig. 1a). A similar trend was found in treatments C and D (pH 3.9), where the maximum cell populations were 2.79×10^8 CFU/mL (20 °C) and 1.77×10^8 CFU/mL (30 °C) at day 4, respectively (Fig. 1a). The yeast grew faster at 30 °C (treatments B and D) with slightly shorter lag phases compared to 20 °C (treatments A and C) during the first 2 days of fermentation (Fig. 1a). In addition, cell viability depended on fermentation temperature, where an increase in temperature led to a slightly earlier cell death with the cell population declining to 2.90×10^5 CFU/mL (pH 3.1) and 5.33×10^4 CFU/mL (pH 3.9) at 30 °C (Fig. 1a).

Changes in sugars

The kinetic changes of sugars are shown in Fig. 1b–d. Fructose and glucose sharply increased at day 1 in all treatments in tandem with rapid sucrose reduction, except treatment D (Fig. 1b–d). The slight increase of fructose and glucose in treatment D corresponded with the slight sucrose reduction at day 1 (Fig. 1b–d). The total residual sugar content in all treatments was less than 2 g/L (Supplementary Table S1), which indicated a completely alcoholic fermentation.

Regardless of temperature, treatments C (5.95%, *v*/*v*) and D (5.56%, *v*/*v*) fermented at pH 3.9 produced relatively higher levels of ethanol than that of treatments A (5.25%, *v*/*v*) and B (5.01%, *v*/*v*) at pH 3.1 (Supplementary Table S1). This is consistent with the growth of yeasts as shown in Fig. 1a, where *S. cerevisiae* grew better and persisted longer at pH 3.9 than at pH 3.1 and the higher cell populations could contribute to the higher ethanol production in treatments C and D, but given that there was little difference in °Brix, low pH might have adversely affected glycolysis and ethanol production by diverting more carbon (pyruvate) to energy generation (Supplementary Table S1).

Changes in pH and organic acids

The pH of all fermented samples decreased initially (Fig. 2). For the samples treated at pH 3.1, the pH declined from the initial 3.14 to 3.02 (treatment A, 20 °C) and 3.07 (treatment B, 30 °C) at days 2 and 1, respectively, and then gradually increased to 3.28 and 3.36 (Supplementary Table S1 and Fig. 2). The pH of the treatments C and D decreased from the initial 3.92 to 3.61 (20 °C) and 3.71 (30 °C) at days 3 and 2, respectively, and then gradually increased to 4.00 and 4.07 by day 14 (Supplementary Table S1 and Fig. 2). The samples fermented at 20 °C (treatments A and C) showed a greater initial decrease but a lower subsequent incease in pH than those fermented at 30 °C (treatments B and D).

The kinetic changes of organic acids differed with treatments, except for citric acid and tartaric acid (Fig. 2). The dynamics of these two acids followed similar trends regardless of different pH and temperatures (Fig. 2). Citric acid and tartaric acid originally present in the durian pulp decreased gradually to low levels throughout the fermentation, except treatment C (pH 3.9, 20 °C), which showed a slight increase in citric acid at day 1 (Fig. 2). Tartaric acid reduction was not attributed to yeast metabolism and could be related to precipitation of tartrate salts such as potassium bitartrate and calcium tartrate.

α-Ketoglutaric acid and succinic acid are intermediate compounds of the citric acid (TCA) cycle. It was observed that the production of α-ketoglutaric acid correlated with the reduction of citric acid due to the rapid conversion of citric acid into α-ketoglutaric acid via the TCA cycle (Fig. 2). The treatment C produced the highest amount of succinic acid

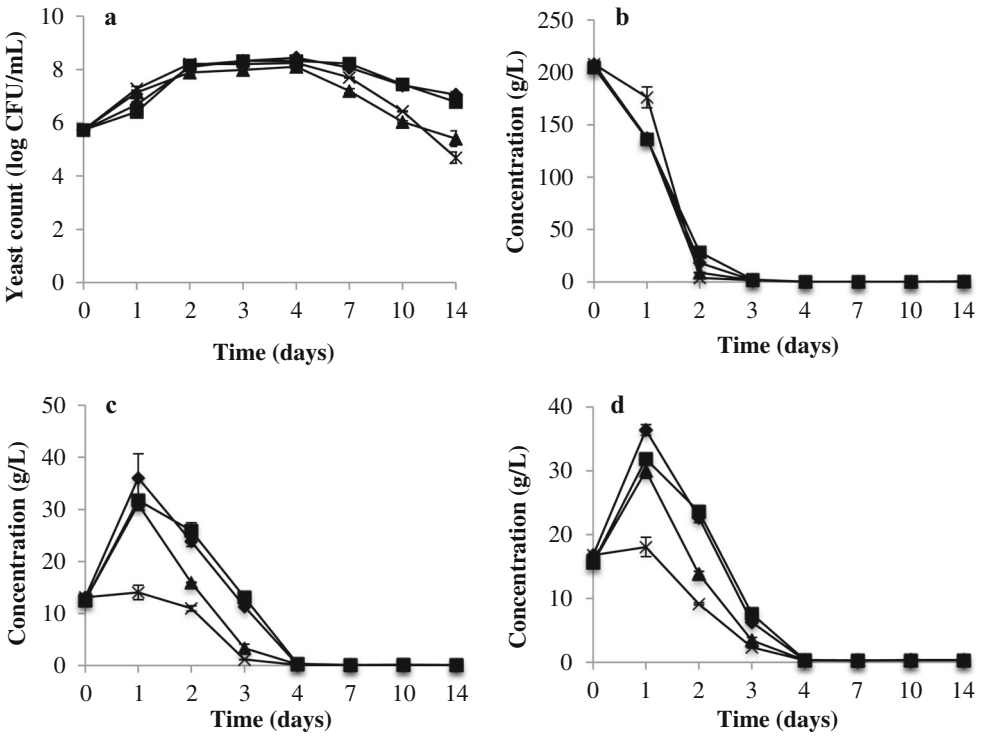

Fig. 1 Evolution of *S. cerevisiae* EC-1118 cell population (**a**), sucrose (**b**), fructose (**c**), and glucose (**d**) during durian wine fermentation. Treatment A (*squares*): pH 3.1, 20 °C; treatment B (*triangles*): pH 3.1, 30 °C; treatment C (*diamonds*): pH 3.9, 20 °C; treatment D (*crosses*): pH 3.9, 30 °C. The data is presented as mean values ± standard deviations obtained from the triplicate fermentations

(2.63 g/L), followed by treatment D (1.96 g/L), treatment A (1.73 g/L), and treatment B (1.68 g/L) (Supplementary Table S1 and Fig. 2).

Malic acid was used for pH adjustment, and the kinetic changes of malic acid in all samples were similar (Fig. 2). It was noted that the decrease rate of malic acid was relatively higher at 30 °C than at 20 °C (Fig. 2). In addition, a significant decline of malic acid was found in lower pH treatments (pH 3.1), which decreased by 5.64 g/L (treatment A) and 6.78 g/L (treatment B), respectively. However, malic acid was only decreased by around 2.5 g/L in treatments C and D (Supplementary Table S1 and Fig. 2).

Temperature and pH did not have significant effects on the production of lactic acid (except treatment B) (Supplementary Table S1 and Fig. 2). The production of acetic acid was significantly affected by temperature, where the samples fermented at 30 °C (treatments B and D) had higher levels of acetic acid (1.02 and 1.09 g/L) than the respective samples (treatments A and C) fermented at 20 °C (0.88 and 0.98 g/L).

Volatile profile of durian wine

Alcohols contributed to over 90% relative peak area (RPA) of the total volatiles with ethanol as the highest contributing alcohol (Supplementary Table S2). Relatively higher levels of ethanol and 2-phenylethyl alcohol were produced at pH 3.9 in treatments C and D than that in treatments A and B (pH 3.1) regardless of temperature (Supplementary Tables S2 and S3). However, treatments A and B (pH 3.1) produced relatively higher levels of isobutyl alcohol and isoamyl alcohol than treatments C and D, regardless 20 and 30 °C (Fig. 3, Supplementary Tables S2 and S3).

The concentration of isobutyl alcohol in treatment B (pH 3.1, 30 °C) is 64.52 mg/L, which is higher than its odor detection threshold (40 mg/L in 10% ethanol, *v*/*v*) and may impart an ethereal flavor note to durian wines, while the concentration of isobutyl alcohol in other treatments was lower than 40 mg/L and may not significantly affect the aroma note of the wines. The concentrations of isoamyl alcohol (ranging from 44.15 to 56.27 mg/L) and 2-phenylethyl alcohol (ranging from 28.33 to 38.04 mg/L) in all treatments were higher than their corresponding odor detection thresholds (30 and 10 mg/L, respectively, *v*/*v*) and could impart whiskey, winey, and floral notes to the wines (Supplementary Tables S3).

Esters are the second major group of volatiles produced. Most of the endogenous esters in the durian pulp were reduced to trace or undetectable levels after fermentation (Supplementary Tables S2 and S3, Fig. 4) and may weaken the fruity odor. However, the production of ethyl esters (e.g., ethyl octanoate and ethyl decanoate) and acetate esters (ethyl acetate and isoamyl acetate) could make up the loss of fruity notes (Supplementary Tables S2 and S3). In contrast with alcohols, the formation of esters was more affected by temperature than pH (Supplementary Tables S2 and S3). The production of ethyl esters of octanoate, nonanoate, and decanoate was significantly higher at 20 °C (treatments A and C) than at 30 °C (treatments B and D) as shown in Supplementary Tables S2 and S3.

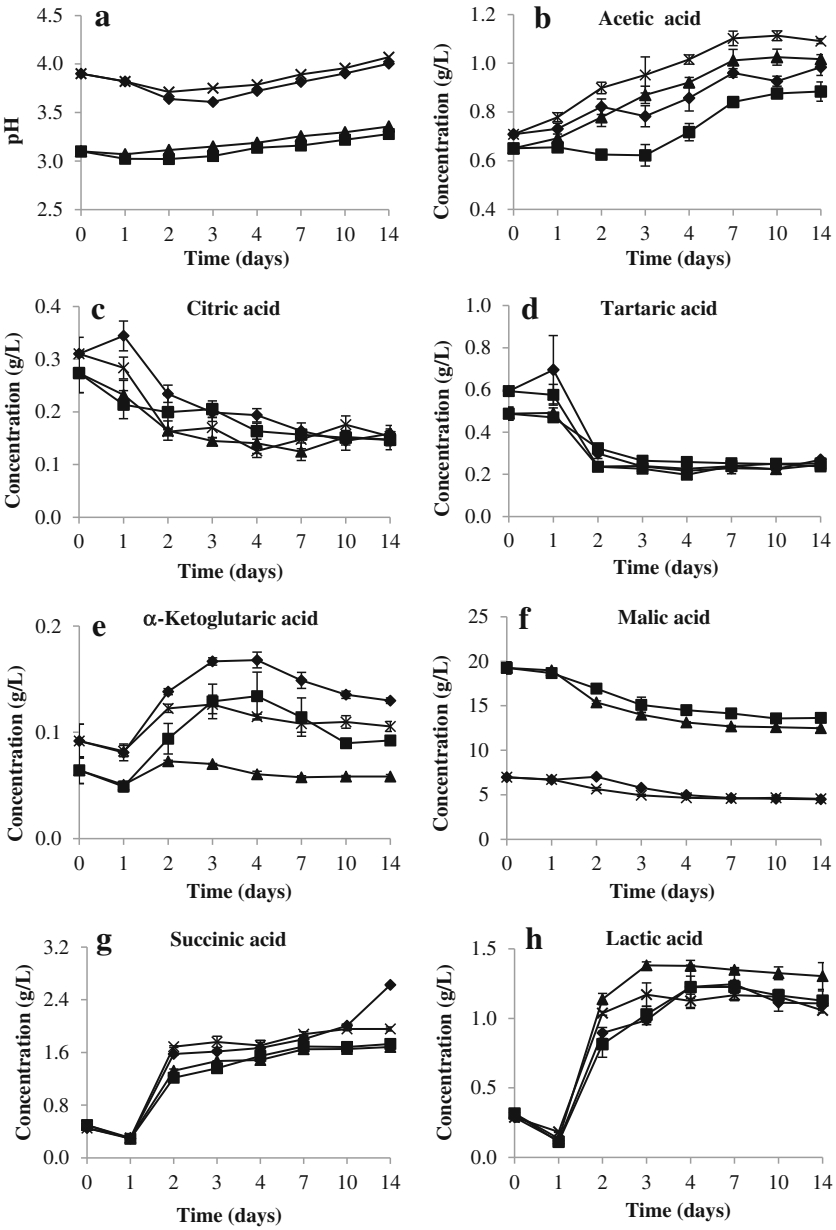

Fig. 2 Changes in pH and organic acids during durian wine fermentation. Treatment A (*squares*): pH 3.1, 20 °C; treatment B (*triangles*): pH 3.1, 30 °C; treatment C (*diamonds*): pH 3.9, 20 °C; treatment D (*crosses*): pH 3.9, 30 °C. The data is presented as mean values ± standard deviations obtained from the triplicate fermentations

Similar trends were also found for the production of ethyl acetate and isoamyl acetate with treatment C producing the highest levels (4.59 and 0.017 mg/L, respectively), followed by treatments A (3.31 and 0.016 mg/L, respectively), B (2.88 and 0.012 mg/L, respectively), and D (2.59 and 0.006 mg/L, respectively) as shown in Supplementary Tables S2. However, their concentrations were below their corresponding odor detection thresholds (7.50 and 0.03 mg/L, respectively, in 10% ethanol, *v*/*v*).

Temperature and pH did not significantly affect the metabolism of volatile fatty acids (VFAs) in all treatments with most of VFAs being metabolized to trace or undetectable levels after fermentation (Supplementary Tables S2 and S3).

Aldehydes are intermediate compounds that can be metabolized by yeast cells during fermentation. Temperature and pH did not significantly affect the metabolism of aldehydes (Supplementary Tables S2 and S3). Aldehydes like acetaldehyde, hexanal, nonanal, benzaldehyde, and 4-tolualdehyde that were detected in the durian pulp were metabolized to trace or undetectable levels after fermentation (Supplementary Tables S2 and S3).

Most of the volatile sulfur compounds (VSCs) were metabolized to trace or undetectable levels after fermentation (Fig. 5). It was important to note that volatile thiols such as ethanethiol and 1-propanethiol were metabolized to undetectable levels after fermentation (Supplementary Table S2, Fig. 5). Volatile sulfides such as methyl ethyl disulfide, diethyl

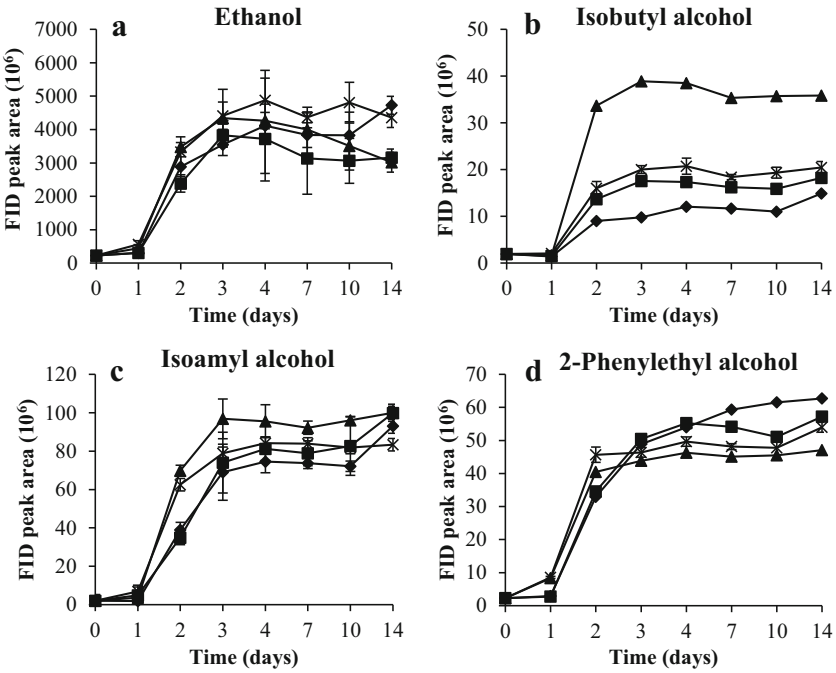

Fig. 3 Changes in higher alcohols during durian wine fermentation. Treatment A (*squares*): pH 3.1, 20 °C; treatment B (*triangles*): pH 3.1, 30 °C; treatment C (*diamonds*): pH 3.9, 20 °C; treatment D (*crosses*): pH 3.9, 30 °C. The data is presented as mean values ± standard deviations obtained from the triplicate fermentations

disulfide, ethylisopropyl disulfide, dipropyl disulfide, and diethyl trisulfide were reduced to trace levels (Supplementary Table S2, Fig. 5).

Principal component analysis of different treatments of durian wines

The selected key volatile compounds of durian wines treated with different pH and temperatures were quantified (Supplementary Table S3). These quantified compounds as well as ethanol from Supplementary Table S1 were subjected to principal component analysis (PCA). The first two principal components accounted for 88.68% of total variation with PC 1 and PC 2 explaining 61.48 and 27.20%, respectively (Fig. 6). Treatments A and C are located on the positive semi-axis of PC 1 while treatments B and D are located on the negative semi-axis due to relatively higher levels of ethyl esters (ethyl decanoate and ethyl octanoate) and acetate esters (ethyl acetate and isoamyl acetate) of the former two treatments (Fig. 6). PC

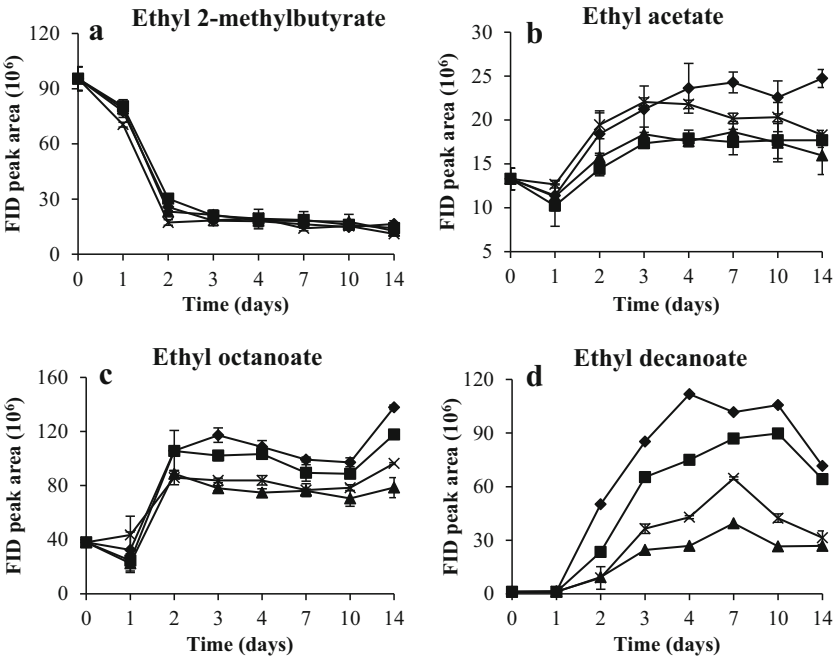

Fig. 4 Changes in ethyl and acetate esters during durian wine fermentation. Treatment A (*squares*): pH 3.1, 20 °C; treatment B (*triangles*): pH 3.1, 30 °C; treatment C (*diamonds*): pH 3.9, 20 °C; treatment D (*crosses*): pH 3.9, 30 °C. The data is presented as mean values ± standard deviations obtained from the triplicate fermentations

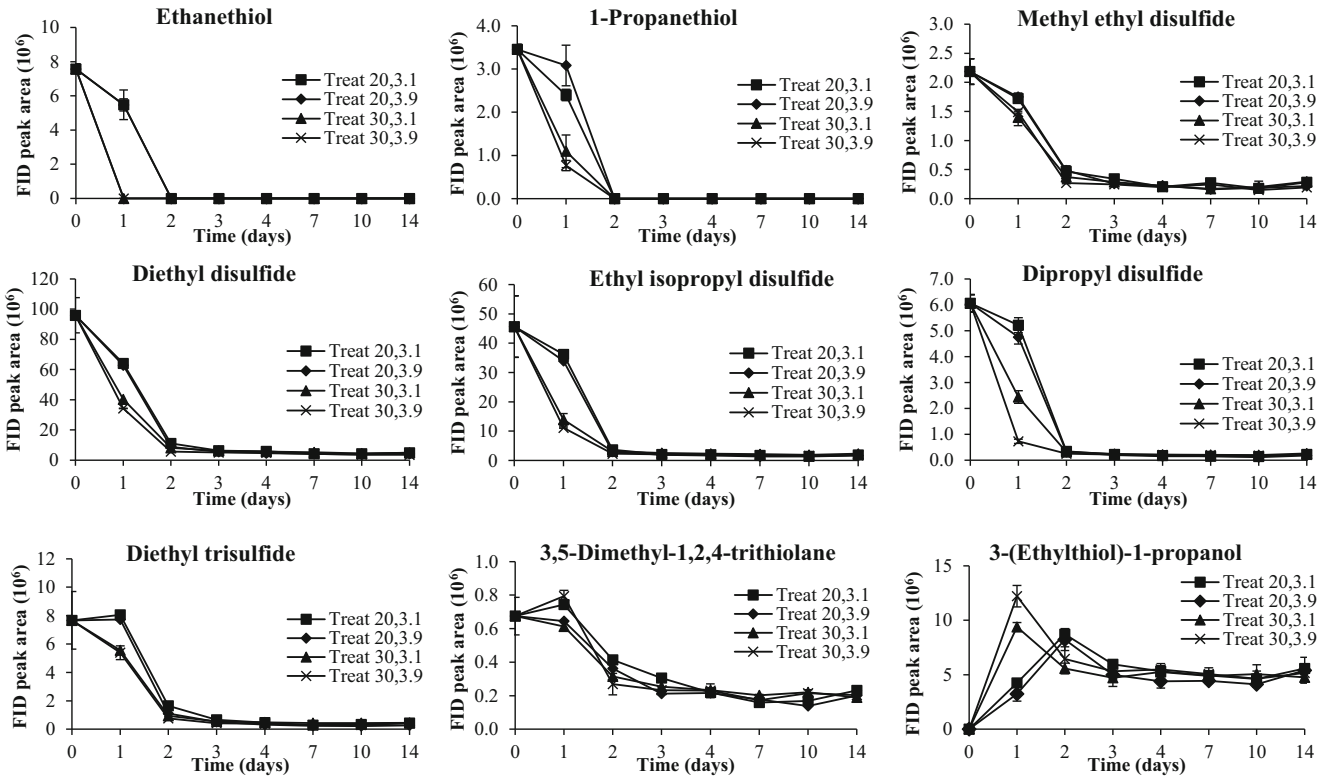

Fig. 5 Changes in volatile sulfur compounds during durian wine fermentation. Treatment A (*squares*): pH 3.1, 20 °C; treatment B (*triangles*): pH 3.1, 30 °C; treatment C (*diamonds*): pH 3.9, 20 °C; treatment D (*crosses*): pH 3.9, 30 °C. The data is presented as mean values ± standard deviations obtained from the triplicate fermentations

2 is separated treatments A and B from treatments C and D due to their relatively higher levels of higher alcohols (isobutyl alcohol, isoamyl alcohol, and 2-phenylethyl alcohol) as shown in Fig. 6.

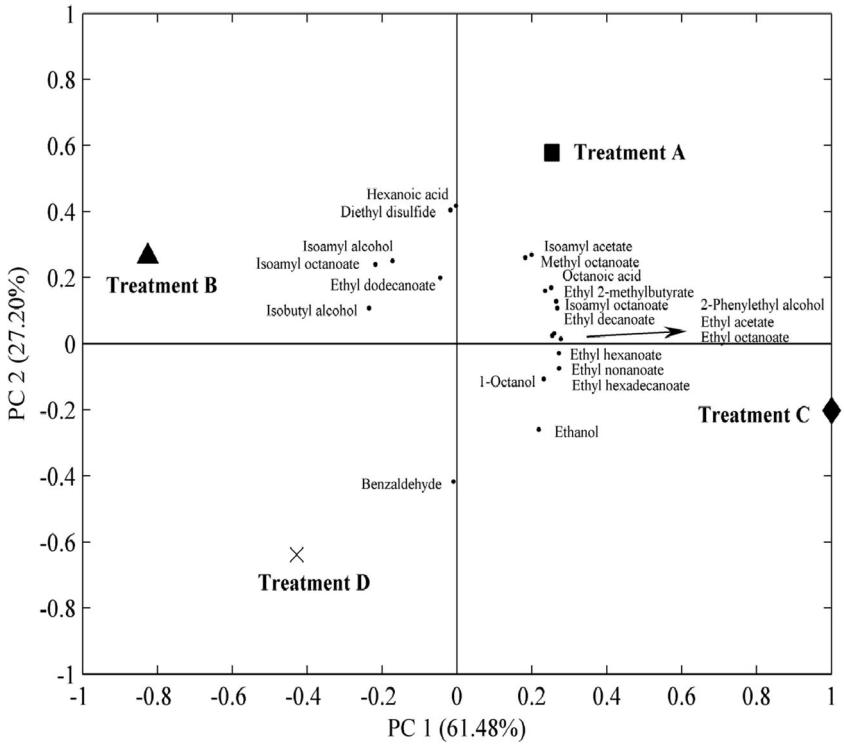

Fig. 6 Principal component analysis of durian wines treated with different conditions. Treatment A (*squares*): pH 3.1, 20 °C; treatment B (*triangles*): pH 3.1, 30 °C; treatment C (*diamonds*): pH 3.9, 20 °C; treatment D (*crosses*): pH 3.9, 30 °C

Discussion

The faster cell death at higher temperature (30 °C, treatments B and D) might be due to accumulation of intracellular ethanol in higher amounts at higher temperatures in a short time. This is toxic to yeast cells and may also alter the structure of the membrane, leading to its functionality decline (Lucero et al. 2000; Torija et al. 2003b). Our results agree with previous studies that temperature could directly affect the growth rate of yeasts and moderately low temperature could enhance the persistence of yeasts during alcoholic fermentation (Fleet and Heard 1993; Lucero et al. 2000; Torija et al. 2003a, b; Sun et al. 2016). Bisson (1999) reported that too low temperature (e.g., 13 °C) would be a restriction that may increase the risk of stuck or sluggish fermentations for *S. cerevisiae*. In addition, temperature has been reported to be the main influencing factor for the growth of *S. cerevisiae* VL3c and *S. bayanus* var. *uvarum* P3 (Serra et al. 2005) as well as other yeasts including *Pichia anomala*, *Debaryomyces hansenii*, *Saccharomyces kudriavzevii*, and *Torulaspora delbrueckii* (Arroyo-López et al. 2006, 2009; Sørensen and Jakobsen 1997; Sun et al. 2016).

For pH, the yeast cell viability was better in treatments at pH 3.9 relative to pH 3.1 but the difference was negligible compared to the effects of temperature on the dynamics of the *S. cerevisiae* population (Fig. 1a). This is in line with the results of Arroyo-López et al. (2009), who reproted that the proper initial pH did not significantly affect the growth of *S. cerevisiae* and *S. kudriavzevii*. However, several studies showed that the selection of pH value (2.75–4.25) is important and sometimes may determine the initiation of growth of *S. cerevisiae* (Fleet and Heard 1993; Serra et al. 2005).

The sharp increase of fructose and glucose at day 1 except treament D (pH 3.9, 30 °C) was likely due to rapid sucrose hydrolysis (Fig. 1b–d) by yeast invertase during fermentation (Lu et al. 2015, 2016), with the rates of sucrose hydrolysis (e.g., release of fructose and glucose) being greater than that of fructose and glucose utilization. In contrast, the slight increase of fructose and glucose at day 1 in treament D (pH 3.9, 30 °C) corresponded with the more moderate rate of sucrose hydrolysis as shown in Fig. 1b, which indicated the rates of release and utilization of fructose and glucose were broadly in tandem with each other.

It was observed that pH had a more significant effect on the production of ethanol than temperature (Supplementary Table S1). Serra et al. (2005) reported that a suitable pH value is a determinant for the initiation of growth of *S. cerevisiae*. On the other hand, the production of ethanol was relatively higher at 20 °C than that of 30 °C when comparing at pH 3.1 or 3.9, respectively (Supplementary Table S1). The results agree with the finding of Bozoglu et al. (2015), who reported that low temperature (18 °C) produced 0.7% (*v*/*v*) higher levels of ethanol than samples fermented at 25 °C. In addition, similar results were also reported by Torija et al. (2003b) with the findings that fermentations at low temperatures (15, 20, and 25 °C) produced more ethanol than that at high temperatures (30 and 35 °C). Although higher temperatures could improve enzyme reactions to increase fermentation rates (Roza et al. 2003), too high temperatures could resulte in a faster decline of yeast cell populations (Fig. 1a). In addition, too higher temperatures may also lead to more evaporative losses of volatiles during fermentation.

Succinic acid is one of the main organic acids produced by *S. cerevisiae* during wine fermentation, and they are important flavor precursors (Song and Lee 2006; Taniasuri et al. 2016). Akram (2014) reported that α-ketoglutarate could be converted into succinyl-CoA and its conversion rate depended on the activity of α-ketoglutarate dehydrogenase. It seemed that α-ketoglutaric acid was converted more efficiently into succinic acid at higher pH (treatments C and D) as shown in Fig. 2.

Previous studies showed that the reduction of malic acid was caused by the passive diffusion into yeast cells and not be metabolized due to the lack of proper malic acid metabolic system in *S. cerevisiae* (Lu et al. 2016; Redzepovic et al. 2003; Sauer et al. 2008). Benito et al. (2016) reported that *S. cerevisiae* strains IFI87/CECT12512 and IFI88/CECT12513 could degrade 11–24% of the initial malic acid content in the grape juice. In our study, around 35% of the initial malic acid content was declined after fermentation (Supplementary Table S1). This might be due to the different *S. cerevisiae* strain EC-1118 used. It is important to note that higher temperature hightened the decline of malic acid at pH 3.1 (treatment B), but there was no significant difference from pH 3.9 regardless of temperatures (Supplementary Table S1 and Fig. 2). The reason might be due to that the interaction of the high temperature (30 °C) and low pH (3.1) altered the membrane structure of *S. cerevisiae* (Lucero et al. 2000; Torija et al. 2003b), leading to its functionality decline, and therefore, more malic acid diffused into yeast cells. Woo et al. (2014) also reported that high temperature may affect the capacities of *S. cerevisiae* cellular membrane, which can be structurally destabilized and permeabilized with various adverse effects on membrane-associated processes. This needs further investigation.

Comparing the treatments at 30 °C (treatments B and D), the low pH (3.1) raised the production of lactic acid, while it is not the case at 20 °C (Fig. 2), where treatments A and C produced comparable levels of lactic acid but all were lower than that in treatment B (pH 3.1, 30 °C). Relatively higher levels of lactic acid may provide more precursors for the production of ethyl lactate (Taniasuri et al. 2016). However, Valli et al. (2006) reported that high amounts of lactic acid could exert a high level of stress on *S. cerevisiae* cells at low pH. In addition, Benito et al. (2015) reported that the relatively higher production of lactic acid could affect the growth of yeasts, which might be one of the reasons for the relatively sharp

decline of viable yeast cell counts (treatment B) as shown in Fig. 1a.

Significantly higher levels of acetic acids were produced at high temperature (30 °C, treatments B and D) regardless of pH; however, the pH did not significantly affect its production when being compared at the respective temperature (Supplementary Table S1 and Fig. 2). Our results are consistent with the findings of Woo et al. (2014), who reported that high temperature could stimulate the accumulation of acetic acid in a modified medium fermented by *S. cerevisiae* (BY4741). On the other hand, the higher amount of acetic acid would result in an adverse effect with more reactive oxygen species being produced during mitochondrial respiration via leakage of electrons from the electron transport chain and reduction of molecular oxygen (Woo et al. 2014). The acetic acid stress might synergize with other factors such as temperature and ethanol, leading to decreased growth and cell instability of *S. cerevisiae* and subsequent earlier death (Fig. 1a). This reasoning concurs with the findings of Ždralević et al. (2012), who reported that acetic acid could be an inducer of programmed cell death of *S. cerevisiae*.

Alcohols are the most abundantly produced volatile compounds. The overall alcohol production was more affected by pH than temperature (Supplementary Tables S2 and S3). The production of higher alcohols was related to respective enzymes (e.g., amino acid aminotransferase and pyruvate decarboxylase) and the corresponding amino acid precursors like L-valine, L-leucine and L-phenylalanine (Buijs et al. 2013; Hazelwood et al. 2008).

The higher production of ethyl esters at 20 °C (treatments A and C) could be attributed to the relatively larger cell populations relative to that at 30 °C (treatments B and D) regardless of pH (Figs. 1a and 4). Our results agree with the findings of Torija et al. (2003b), who reported that temperature is the key variable that determined the secondary metabolits development and fermentation capacity of *Saccharomyces* yeasts.

The metabolism of VSCs is more affected by temperature than pH within 1–2 days of fermentation, where VSCs reduced faster at 30 °C than that at 20 °C (Fig. 5). When comparing samples fermented at the same temperature, there was no significant difference (Fig. 5). The volatile thiols might be oxidized by metal ions to form non-volatiles or absorbed by yeast cell wall mannoproteins via forming new sulfide bridges (Nikolantonaki et al. 2010; Lu et al. 2015, 2016). Previous studies indicated that sulfides could be reduced to their corresponding thiols (Bobet et al. 1990; Gómez-Plaza and Cano-López 2011) and the thiols could then be metabolized as discussed above, but this needs to be further studied. In contrast, Masneuf-Pomarède et al. (2006) reported that the temperature is a key factor that affected the production of volatile thiols (4-mercapto-4-methylpentan-2-one, 3-mercaptohexan-1-ol, and 3-mercaptohexyl acetate) irrespective of the yeast strain used with higher production at 20 °C than at 13 °C, mainly via releasing from the transformation of the corresponding *S*-cysteine conjugate during fermentation.

In conclusion, this study investigated the effect of temperature and pH on transformation of volatile and non-volatile compounds of durian wines. The results demonstrated that temperature had a greater effect than pH on the growth and survival of *S. cerevisiae*. Temperature and pH impacted the production of different volatile compounds, and the treatment at a lower temperature and a higher pH (treatment C, 20 °C, 3.9) might be considered to be the best condition to apply in durian wine fermentation. Treatments at low temperature (20 °C) produced relatively higher levels of ethyl esters and acetate esters; however, treatments at high pH (pH 3.9) produced higher levels of ethanol but relatively lower levels of higher alcohols than treatments at low pH (pH 3.1). In addition, the metabolism of VSCs was more affected by temperature than pH with faster reduction at high temperature. This study illustrated that temperature would be a more effective control variable than pH in modulating the aroma profile of durian wine.

Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

Research involving human participants and/or animals This article does not contain any studies with human participants or animals performed by any of the authors.

Informed consent Informed consent was obtained from all individual participants included in the study.

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