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Original Article

Studies on the quality and flavor of ponkan (*Citrus poonensis* hort.) wines fermented by different yeasts

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ABSTRACT

Ponkan (*Citrus poonensis* hort.) juice and ponkan pulp were used as raw materials for the production of ponkan wines fermented with six different strains of *Saccharomyces cerevisiae*. We found that ponkan wines fermented with *S. cerevisiae* BCRC 22332 and commercial yeast HF-08 had higher alcohol contents (10.70–11.86%), lower contents of residual sugar (0.64–1.14%), lower degrees of browning (OD_{420} = 0.20–0.34), higher clarity (OD_{660} = 0.07–0.17), and higher sensory scores (5.15–6.25 points). Flavor analysis revealed that, in addition to the citrus flavor characters of α-pipene, limonene, and α-terpineol, ponkan wine contained the yeast-generated characteristic aromatic components of isoamyl alcohol, phenethyl alcohol, 2,3-butandiol, ethyl acetate, diethyl succinate, isoamyl acetate, ethyl 3-hydroxybutyrate, ethyl caproate, ethyl 4-hydroxybutanoate, ethyl caprylate, phenethyl acetate, ethyl caprate, and ethyl 3-methylbutyl butanedioate. The aromatic components of isobutyl alcohol, isoamyl alcohol, diethyl succinate, phenethyl alcohol, ethyl acetate, and isoamyl acetate in the ponkan wine fermented with HF-08 were higher in amounts than that fermented with BCRC 22332. In summary, *S. cerevisiae* HF-08 produced more types of aroma and higher aroma contents.

1. Introduction

The annual global orange yield is about 80 million tons, with Brazil and USA as the major producing countries, and approximately 34% of the total production is made into fruit juice. Most fruit juices are consumed in European and North American countries [1]. Based on the fact that oranges can be made into orange wines, the types of products can be diversified. It has been reported that the major yeasts used in the making of commercial fruit wines are *Saccharomyces cerevisiae* and *Saccharomyces bayanus* [2]. The ethanol productivity by yeasts is theoretically about 51%, when hexose is used as the fermentation substrate [3]. Furthermore, it has been reported that different yeast strains can produce different organic acids and alcohol contents in wine [4].

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In wine fermentation processes, the consumption of sugar is proportional to alcohol generation [5]. When fruits are used as the raw materials for wine fermentation, the shortage of nitrogen source will retard the growth of yeast [6], whereas nitrogenous nutrients can accelerate fermentation and improve the quality and yield [7,8]. Fermentation temperature also affects the quality and flavor of the wine products [9,10]. The suitable fermentation temperature for most *S. cerevisiae* strains is in the range of 22–27 °C [2,11]. In addition, the type of strain of *S. cerevisiae* used is an important factor influencing aroma contents and flavor characteristics of wine [12–18]. Because orange juice squeezed together with peel contained too much oil that consequently influenced yeast fermentation [19,20], Liou [21] has chosen deacidified peeled tangerine orange juice with the addition of 10% raw peel juice to make tangerine orange wine. It was found that the quality of wine fermented with fruit juice without the peel was much better than that of wine fermented with the peel [22]. Beyond this, Li et al [23] found that ethyl acetate, isoamyl acetate, and α-terpineol are the main aromatic constituents of the fruit wine of Glorious Oranges from Chongqing, China. Another research group also indicated that the main aromatic constituents of the fruit wine made from the Turkish oranges of Kozan are isoamyl alcohol, 2-phenethyl alcohol, ethyl hexanoate, aromatic alcohols, citronellol, terpinene, and eugenol [24]. In this study, we compared the ponkan wines made from ponkan juice and ponkan pulp by fermenting with different *S. cerevisiae* strains.

2. Methods

2.1. Sample preparation

Ponkan fruits were purchased from a local market in Taichung, Taiwan. The yeast strains of BCRC 21761, BCRC 21805, BCRC 21823, BCRC 22293, and BCRC 22332 were purchased from the Food Industry Research and Development Institute (Hsinchu, Taiwan). HF-8 (commercial yeast) was purchased from the Eherfon Biotechnology Company (Kaohsiung, Taiwan). Ponkan fruits were washed and peeled. The peeled ponkan fruits were then crushed with a stainless steel crusher (crushing aperture was 800 mm × 1,350 mm) to prepare ponkan pulp, and squeezed with a barrel basket squeezer (squeezing aperture was 270 mm × 390 mm) to prepare ponkan juice. Ponkan pulp and ponkan juice were separately adjusted to contain 24% sugar, 50 ppm of potassium metabisulfite [22,25], 500 ppm of ammonium sulfate, and 5% activated yeast culture in the 5 L conical glass flasks for wine fermentation [26]. The total volume of each flask was 3000 mL. They were then subjected to fermentation for 30 days at 25 ± 1 °C [10,11]. Samplings were carried out every 5 days to determine pH, residual sugar contents, soluble solid contents, alcohol contents, acidity, clarity, and colors. The ponkan wine made from ponkan juice was later abbreviated as PJW, and ponkan wine made from ponkan pulp was abbreviated later as PPW. Numbers 61, 05, 23, 93, 32, and 08 represented *S. cerevisiae* BCRC 21761, BCRC 21805, BCRC 21823, BCRC 22293, BCRC 22332, and commercial yeast HF-08 used for wine fermentation, respectively.

2.2. Analytical methods

Enological parameters such as oBrix, pH value, and total acidity (g citric acid/100 mL) were measured according to official Association of Office Analytic Chemists (AOAC) methods [27]. Reducing sugar contents were determined according to Zoecklein et al [28]. Browning indices were determined according to the method of Tien and Chiang [29].

2.3. Determination of ethanol contents

Ethanol contents were determined according to Zoecklein et al [28]. Approximately 1 g of acetonitrile was accurately added as the internal standard for each 10 mL of the sample wines, filtered with a Millipore filter (0.45 μm), and analyzed by gas chromatography with an FID detector (Agilent HP-6890, Wilmington, DC–>, USA). The column used was a DB-1 capillary column (60 m, 0.25 mm i.d., 1-μm thickness). Nitrogen gas was used as the carrier gas, and operated at a flow rate of 1 mL/min with a split ratio of 60:1. The gas chromatography (GC) oven temperature was initially held at 40 °C for 10 minutes, then raised from 40 °C to 240 °C at 2 °C/min, and maintained at 240 °C for 10 minutes. The injection port temperature was 200 °C, and the detector temperature was 220 °C.

2.4. Preparation of volatile extracts and gas chromatography-mass spectrometry analysis

The volatile components of the sample wines were analyzed according to Romer and Renner [30]. A wine sample (300 mL) was diluted with distilled water to a concentration of 4% alcohol (Sample A) in an Erlenmeyer flask (2000 mL), with the addition of 5 mL of internal standard (accurately weigh about 0.08 g of dodecane dissolved in 100 mL of dichloromethane). Dichloromethane (300 mL) was added to the above sample wines with vigorous stirring for 2 hours, and allowed to stand for separation of the two layers. The bottom dichloromethane layer was separated and dried with a sufficient amount of anhydrous sodium sulfate. The dichloromethane was transferred to an oval-shaped flask and distilled with a concentration tower at 45 °C. It was firstly concentrated to about 1–2 mL, followed by purging with nitrogen gas at a flow rate of 25 mL/min to a volume of about 0.5 mL (Sample B). Sample B was then analyzed with a gas chromatograph/mass selective detector (GC-MSD; Hewlett-Packard 6890 GC connected to Hewlett-Packard 5973 MSD–>). A capillary column (DB-1, J&W Scientific, Folsom, CA, i.d. 0.25 mm × 60 m, 0.25 μm film thickness) was used. The carrier gas, helium, was operated at a flow rate of 1.0 mL/min, with a split ratio of 80:1. The temperature was programmed initially at 40 °C for 5 minutes, raised to 240 °C at 2 °C/min, and maintained at 240 °C for 60 minutes. The injector temperature was 250 °C. The MS source temperature was 230 °C, the EM (electron multiplier) voltage was 2300 V, and the mass (MS) Quad temperature was 250 °C. The qualitative and quantitative determinations of the volatile components were performed according to Majlat et al [31]. Triplicate quantitative determinations were carried out by using the internal standard. The content of a specific component (C_s) was calculated by the following equation:

Table 1 – Changes in ethanol contents and residual sugar of the ponkan wines during the wine fermentation process.

Strain[c] day[d]	Ethanol content (% v/v)[a]							Residual sugar (%)[b]						
	0	5	10	15	20	25	30	0	5	10	15	20	25	30
PPW05	0	2.90 ± 0.10^{F},[e]	7.70 ± 0.26^{E}	9.80 ± 0.20^{C}	10.93 ± 0.06^{B,C}	10.90 ± 0.05^{B}	10.93 ± 0.06^{B,C,D,E}	24.00	18.23 ± 0.21^{B}	9.70 ± 0.26^{D}	5.30 ± 0.44^{D}	1.44 ± 0.39^{E}	1.27 ± 0.24^{E,F}	1.37 ± 0.31^{E}
PPW08	0	7.56 ± 0.31^{A}	10.26 ± 0.15^{A}	11.80 ± 0.30^{A}	11.63 ± 0.42^{A}	11.66 ± 0.40^{A}	11.86 ± 0.21^{A}	24.00	11.06 ± 0.15^{F}	3.56 ± 0.20^{i}	1.41 ± 0.22^{H}	0.70 ± 0.12^{G}	0.60 ± 0.04^{H}	0.68 ± 0.07^{G,H}
PPW23	0	5.23 ± 0.25^{D}	8.23 ± 0.25^{D}	8.76 ± 0.25^{E}	10.90 ± 0.10^{B,C}	11.13 ± 0.25^{B}	11.23 ± 0.15^{B}	24.00	14.20 ± 0.61^{C}	7.80 ± 0.20^{F}	6.40 ± 0.10^{C}	2.07 ± 0.31^{D}	1.87 ± 0.14^{D}	1.84 ± 0.14^{D}
PPW32	0	4.76 ± 0.23^{E}	7.56 ± 0.21^{E}	9.90 ± 0.20^{C}	10.63 ± 0.15^{C}	10.90 ± 0.10^{B}	10.70 ± 0.17^{E}	24.00	13.90 ± 0.36^{C}	9.23 ± 0.25^{D}	4.50 ± 0.30^{E}	1.63 ± 0.42^{E}	1.34 ± 0.15^{E}	1.14 ± 0.16^{E,F}
PPW61	0	2.63 ± 0.15^{F}	4.46 ± 0.35^{G}	6.96 ± 0.15^{G}	9.03 ± 0.15^{E}	9.33 ± 0.15^{D}	9.43 ± 0.12^{G}	24.00	18.13 ± 0.32^{B}	15.30 ± 0.44^{A}	10.16 ± 0.21^{A}	5.25 ± 0.25^{B}	5.45 ± 0.17^{B}	5.60 ± 0.10^{B}
PPW93	0	6.56 ± 0.21^{B}	9.01 ± 0.20^{C}	10.63 ± 0.23^{B}	10.93 ± 0.15^{B,C}	10.96 ± 0.15^{B}	10.90 ± 0.10^{C,D,E}	24.00	12.16 ± 0.21^{D}	6.86 ± 0.42^{G}	2.33 ± 0.15^{G}	1.26 ± 0.21^{E,F}	1.19 ± 0.18^{E,F,G}	1.13 ± 0.06^{E,F}
PJW05	0	5.46 ± 0.35^{C,D}	8.26 ± 0.21^{D}	9.23 ± 0.25^{D}	10.26 ± 0.21^{D}	11.06 ± 0.21^{B}	11.06 ± 0.25^{B,C,D}	24.00	14.36 ± 0.51^{C}	7.96 ± 0.21^{E,F}	5.53 ± 0.31^{D}	0.92 ± 0.03^{F,G}	0.90 ± 0.03^{G,H}	0.93 ± 0.04^{F,G}
PJW08	0	7.70 ± 0.17^{A}	9.56 ± 0.31^{B}	11.60 ± 0.46^{A}	11.80 ± 0.30^{A}	11.73 ± 0.32^{A}	11.86 ± 0.21^{A}	24.00	10.33 ± 0.20^{E}	4.53 ± 0.35^{H}	2.12 ± 0.16^{G}	0.71 ± 0.03^{G}	0.64 ± 0.04^{H}	0.64 ± 0.04^{H}
PJW23	0	2.96 ± 0.12^{F}	5.16 ± 0.35^{F}	7.50 ± 0.30^{F}	9.36 ± 0.23^{E}	10.06 ± 0.15^{C}	9.73 ± 0.21^{F}	24.00	18.76 ± 0.30^{B}	14.23 ± 0.15^{B}	5.13 ± 0.35^{D}	2.83 ± 0.16^{C}	2.69 ± 0.10^{C}	2.63 ± 0.11^{C}
PJW32	0	5.70 ± 0.26^{C}	7.70 ± 0.20^{E}	10.60 ± 0.20^{B}	10.86 ± 0.15^{B,C}	10.73 ± 0.21^{B}	10.73 ± 0.21^{E}	24.00	12.3 ± 0.43^{D}	8.36 ± 0.32^{E}	2.93 ± 0.15^{F}	1.56 ± 0.20^{E}	1.17 ± 0.20^{E,F,G}	1.21 ± 0.16^{E}
PJW61	0	2.06 ± 0.32^{G}	5.50 ± 0.30^{F}	7.46 ± 0.06^{F}	9.93 ± 0.15^{D}	10.80 ± 0.26^{B}	10.80 ± 0.10^{D,E}	24.00	20.43 ± 0.45^{A}	13.23 ± 0.32^{C}	9.10 ± 0.10^{B}	6.31 ± 0.25^{A}	6.37 ± 0.34^{A}	6.34 ± 0.27^{A}
PJW93	0	6.73 ± 0.21^{B}	8.56 ± 0.21^{C,D}	10.73 ± 0.21^{B}	11.20 ± 0.10^{B}	11.13 ± 0.15^{B}	11.16 ± 0.15^{B,C}	24.00	11.70 ± 0.26^{D}	7.50 ± 0.30^{F}	3.10 ± 0.36^{F}	0.97 ± 0.04^{F,G}	0.97 ± 0.12^{F,G}	0.89 ± 0.08^{F,G,H}

[a] The ethanol content in the fermentation processes of the ponkan wine fermented with different yeast strains.
[b] The residual sugar content in the fermentation processes of the ponkan wine fermented with different yeast strains.
[c] The *Saccharomyces cerevisiae* strain of PPW (ponkan pulp wine) and PJW (ponkan juice wine) is BCRC 21805, HF-08, BCRC 21823, BCRC 22332, BCRC 21761, and BCRC 22293, respectively.
[d] Day of fermentation.
[e] The means in column with different uppercase alphabets are significantly different at $p < 0.05$.

$C_s = (A_s/A_i) \times C_i$

C_s is the concentration (ppm) of the specific component; A_s is the area of the specific component; A_i is the area of the internal standard; C_i is the concentration (ppm) of the internal standard. The structural determinations were processed by referring to the computerized database of Heller and Miline [32,33] and Toegepast Natuurwetenschappelijk Onderzoek (TNO) [34]. Alternatively, some data were referred to the cited mass spectroscopic data.

2.5. Sensory analysis

The organoleptic evaluation [35] was performed by 47 students (from the Department of BioIndustry Technology, Da-Yeh University, Changhua, Taiwan). Data were expressed as mean scores (1 = extremely dislike; 5 = mutual; 9 = extremely like).

2.6. Statistical analysis

Samples were analyzed in triplicates. The concentration of volatile components was determined as the mean value of three independent determinations. The data were analyzed by Duncan's multiple range method with a significance of difference of $p < 0.05$ (SPSS Base 12.0).

3. Results and discussion

3.1. Analysis of general compositions of ponkan wine fermented with different S. cerevisiae strains

3.1.1. Comparison of alcohol content and residual sugar in ponkan wines during the fermentation processes with different yeast strains

As shown in Table 1, ponkan juice wines, PJW 08 and PJW 93, had better alcohol productivity.

After 15 days of fermentation, the alcohol contents were 11.60 ± 0.46% and 10.73 ± 0.21%, respectively, and the residual sugar decreased to 2.12 ± 0.16% and 3.10 ± 0.36% respectively. The alcohol analysis showed that, after 30 days of fermentation, the alcohol contents increased to 10.5%, except that of PJW 23 and PPW 61 which were <10%. As shown in Table 1, there was a significant difference in the productivities of alcohol between ponkan juice wine and ponkan pulp wine.

The fermentation rate of the ponkan juice was faster than that of the ponkan pulp, and the amount of alcohol content of the ponkan juice wine was 0.5% higher than that of the ponkan pulp wine. Furthermore, its sugar residue was 1% lower than that of the ponkan pulp wine. Table 1 also showed that the alcohol content made from the ponkan juice and ponkan pulp fermented with HF-08 yeast strain was up to 11.86 ± 0.21%. It demonstrated that HF-08 yeast strain was beneficial to the utilization of sugar and the production of alcohol. Table 1 indicates that ponkan pulp wine fermented with yeast strain BCRC 21823 had a higher alcohol content and lower sugar residue than those of the ponkan juice wine. The alcohol content of PPW 23 was 11.23 ± 0.15%, which was higher than that of PJW 23 with an alcohol content of 9.73 ± 0.25%. This study showed that BCRC 22332 and HF-08 were beneficial for the manufacturing of ponkan juice wine and ponkan pulp wine.

3.1.2. Comparison of acidity of ponkan wines during the fermentation process fermented with different yeast strains

Table 2 indicates a variation of acidity in producing PJW and PPW fermented with different yeasts. When ponkan juice and ponkan pulp were fermented with different yeasts, the acidity of mash increased during the initial 15–20 day fermentation period. In general, Table 2 indicates that the acidities of ponkan wines were mainly dependent on the yeasts used for fermentation. The acidities of PPW 08, PPW 32, PJW 08, and PJW -32 were 0.64%, 0.81%, 0.75%, and 0.5%, respectively.

Table 2 – Changes in the acidities of the ponkan wines during the wine fermentation process.

Strain[b] day[c]	Acidity (%)[a]						
	0	5	10	15	20	25	30
PPW05	$0.42 \pm 0.02^{B,d}$	0.82 ± 0.03^{B}	0.89 ± 0.03^{A}	1.11 ± 0.10^{A}	1.11 ± 0.10^{A}	0.99 ± 0.05^{A}	1.00 ± 0.06^{A}
PPW08	0.41 ± 0.03^{B}	0.64 ± 0.04^{E}	0.55 ± 0.04^{E}	$0.70 \pm 0.04^{D,E}$	0.64 ± 0.03^{F}	$0.64 \pm 0.03^{E,F}$	0.64 ± 0.04^{F}
PPW23	0.43 ± 0.02^{B}	0.90 ± 0.02^{A}	0.81 ± 0.03^{B}	0.90 ± 0.03^{B}	0.94 ± 0.02^{B}	0.91 ± 0.01^{B}	$0.94 \pm 0.02^{B,C}$
PPW32	0.41 ± 0.02^{B}	$0.72 \pm 0.02^{C,D}$	0.73 ± 0.04^{C}	0.82 ± 0.03^{C}	$0.84 \pm 0.03^{C,D}$	0.82 ± 0.03^{C}	0.81 ± 0.01^{D}
PPW61	0.41 ± 0.03^{B}	0.47 ± 0.02^{G}	$0.77 \pm 0.02^{B,C}$	0.82 ± 0.02^{C}	$0.91 \pm 0.04^{B,C}$	0.92 ± 0.05^{B}	0.91 ± 0.03^{C}
PPW93	$0.44 \pm 0.02^{A,B}$	0.55 ± 0.03^{F}	0.62 ± 0.02^{D}	0.68 ± 0.04^{E}	0.62 ± 0.02^{F}	0.60 ± 0.02^{F}	0.62 ± 0.01^{F}
PJW05	$0.44 \pm 0.02^{A,B}$	$0.71 \pm 0.03^{C,D}$	0.61 ± 0.02^{D}	$0.72 \pm 0.04^{D,E}$	$0.90 \pm 0.04^{B,C}$	0.89 ± 0.03^{B}	0.89 ± 0.04^{C}
PJW08	0.49 ± 0.02^{A}	$0.73 \pm 0.04^{C,D}$	0.75 ± 0.03^{C}	$0.75 \pm 0.02^{C,D,E}$	0.75 ± 0.04^{E}	$0.76 \pm 0.02^{C,D}$	0.75 ± 0.01^{E}
PJW23	0.43 ± 0.03^{B}	0.65 ± 0.04^{E}	0.87 ± 0.02^{A}	0.91 ± 0.03^{B}	0.97 ± 0.02^{B}	1.02 ± 0.06^{A}	$0.98 \pm 0.03^{A,B}$
PJW32	0.42 ± 0.04^{B}	0.42 ± 0.02^{H}	0.47 ± 0.04^{F}	0.44 ± 0.04^{F}	0.51 ± 0.03^{G}	0.45 ± 0.05^{G}	0.50 ± 0.03^{G}
PJW61	$0.44 \pm 0.04^{A,B}$	$0.68 \pm 0.04^{D,E}$	0.90 ± 0.05^{A}	$0.77 \pm 0.02^{C,D}$	$0.77 \pm 0.03^{D,E}$	0.73 ± 0.04^{D}	0.74 ± 0.04^{E}
PJW93	0.42 ± 0.04^{B}	0.75 ± 0.02^{C}	0.73 ± 0.03^{C}	$0.75 \pm 0.04^{C,D,E}$	0.72 ± 0.02^{E}	$0.70 \pm 0.03^{D,E}$	$0.75 \pm 0.02^{D,E}$

[a] The acidity in the fermentation processes of the ponkan wine fermented with different yeast strains.
[b] The *Saccharomyces cerevisiae* strain of PPW (ponkan pulp wine) and PJW (ponkan juice wine) is BCRC 21805, HF-08, BCRC 21823, BCRC 22332, BCRC 21761, and BCRC 22293, respectively.
[c] Day of fermentation.
[d] The means in column with different uppercase alphabets are significantly different at $p < 0.05$.

Table 3 – Changes in browning clarity of the ponkan wines during wine fermentation process.

Strain[c]	Browning index (A 420 nm)[a]							Clarity (660 nm)[b]						
day[d]	0	5	10	15	20	25	30	0	5	10	15	20	25	30
PPW05	0.48 ± 0.94 ± 0.04^{A,e}	0.89 ± 0.05^{A}	0.74 ± 0.04^{C}	0.65 ± 0.01^{B}	0.40 ± 0.02^{C}	0.40 ± 0.02^{C}	0.23 ± 0.04^{A,B}	0.51 ± 0.02^{C}	0.47 ± 0.03^{C}	0.33 ± 0.03^{C}	0.24 ± 0.02^{C}	0.12 ± 0.03^{D,E}	0.13 ± 0.02^{D}
PPW08	0.48 0.84 ± 0.02^{B}	0.77 ± 0.03^{B}	0.65 ± 0.05^{D}	0.41 ± 0.03^{D}	0.37 ± 0.02^{C,D}	0.34 ± 0.04^{D}	0.29 ± 0.02^{A}	0.85 ± 0.06^{B}	0.74 ± 0.05^{B}	0.65 ± 0.05^{B}	0.59 ± 0.01^{B}	0.22 ± 0.07^{B}	0.17 ± 0.03^{B,C}	
PPW23	0.48 0.85 ± 0.06^{B}	0.83 ± 0.01^{A,B}	0.81 ± 0.03^{B}	0.63 ± 0.03^{B}	0.54 ± 0.04^{B}	0.54 ± 0.02^{B}	0.23 ± 0.03^{A,B}	0.54 ± 0.04^{C}	0.40 ± 0.03^{D}	0.38 ± 0.01^{C}	0.22 ± 0.02^{C}	0.20 ± 0.02^{b,C}	0.21 ± 0.01^{B}	
PPW32	0.48 0.88 ± 0.03^{A,B}	0.82 ± 0.03^{A,B}	0.58 ± 0.03^{E}	0.37 ± 0.04^{D,E}	0.27 ± 0.03^{E}	0.22 ± 0.03^{E}	0.22 ± 0.04^{B}	0.53 ± 0.03^{C}	0.39 ± 0.04^{D}	0.24 ± 0.04^{D}	0.09 ± 0.02^{E}	0.07 ± 0.02^{E}	0.07 ± 0.02^{E,F}	
PPW61	0.48 0.85 ± 0.05^{B}	0.81 ± 0.03^{B}	0.69 ± 0.04^{C,D}	0.51 ± 0.02^{C}	0.49 ± 0.01^{B}	0.49 ± 0.01^{B}	0.23 ± 0.04^{A,B}	0.51 ± 0.03^{C}	0.41 ± 0.04^{D}	0.32 ± 0.03^{C}	0.20 ± 0.01^{C}	0.20 ± 0.02^{B,C}	0.19 ± 0.02^{B}	
PPW93	0.48 0.94 ± 0.05^{A}	0.89 ± 0.04^{A}	0.88 ± 0.04^{A}	0.89 ± 0.05^{A}	0.84 ± 0.04^{A}	0.84 ± 0.05^{A}	0.84 ± 0.05^{A}	0.22 ± 0.04^{B}	1.12 ± 0.01^{A}	1.25 ± 0.05^{A}	1.22 ± 0.10^{A}	1.10 ± 0.03^{A}	1.05 ± 0.04^{A}	1.04 ± 0.03^{A}
PJW05	0.48 0.32 ± 0.02^{C}	0.27 ± 0.02^{F}	0.23 ± 0.01^{i}	0.19 ± 0.02^{G}	0.18 ± 0.04^{F}	0.18 ± 0.04^{F}	0.20 ± 0.02^{E}	0.22 ± 0.04^{B}	0.19 ± 0.01^{D}	0.13 ± 0.01^{F}	0.09 ± 0.02^{F}	0.09 ± 0.02^{E}	0.07 ± 0.02^{E}	0.06 ± 0.01^{F}
PJW08	0.48 0.31 ± 0.02^{C}	0.34 ± 0.04^{E}	0.30 ± 0.02^{H}	0.26 ± 0.08^{F}	0.19 ± 0.02^{F}	0.20 ± 0.01^{E}	0.22 ± 0.03^{B}	0.16 ± 0.03^{D,E}	0.17 ± 0.03^{E,F}	0.14 ± 0.01^{E,F}	0.09 ± 0.02^{E}	0.08 ± 0.03^{E}	0.07 ± 0.02^{E,F}	
PJW23	0.48 0.33 ± 0.01^{C}	0.54 ± 0.03^{C}	0.45 ± 0.04^{F,G}	0.41 ± 0.01^{D}	0.40 ± 0.03^{C}	0.40 ± 0.03^{C}	0.21 ± 0.02^{B}	0.12 ± 0.02^{E}	0.20 ± 0.01^{E}	0.16 ± 0.02^{D,E,F}	0.11 ± 0.01^{D,E}	0.11 ± 0.01^{D,E}	0.10 ± 0.02^{D,E}	
PJW32	0.48 0.37 ± 0.03^{C}	0.35 ± 0.05^{E}	0.42 ± 0.03^{G}	0.33 ± 0.03^{E,F}	0.32 ± 0.02^{E}	0.31 ± 0.02^{D}	0.21 ± 0.04^{B}	0.17 ± 0.02^{D,E}	0.19 ± 0.04^{E}	0.22 ± 0.03^{D}	0.12 ± 0.03^{D,E}	0.09 ± 0.01^{E}	0.09 ± 0.01^{E,F}	
PJW61	0.48 0.34 ± 0.01^{C}	0.43 ± 0.04^{D}	0.39 ± 0.03^{G}	0.31 ± 0.02^{E,F}	0.3 ± 0.02^{D,E}	0.31 ± 0.01^{D}	0.20 ± 0.01^{B}	0.19 ± 0.01^{D}	0.22 ± 0.02^{E}	0.22 ± 0.02^{D}	0.11 ± 0.01^{D,}	0.11 ± 0.03^{D,e}	0.11 ± 0.02^{D,e}	
PJW93	0.48 0.38 ± 0.03^{C}	0.49 ± 0.05^{C,D}	0.50 ± 0.02^{F}	0.44 ± 0.04^{D}	0.41 ± 0.04^{C}	0.40 ± 0.04^{C}	0.22 ± 0.01^{B}	0.16 ± 0.01^{D,e}	0.22 ± 0.02^{e}	0.20 ± 0.03^{D,e}	0.14 ± 0.02^{D}	0.16 ± 0.02^{C,D}	0.14 ± 0.02^{C,D}	

[a] The Browning index (OD_{420} nm) in the fermentation processes of ponkan wine fermented with different yeast strains.
[b] The Clarity at OD_{660} nm in the fermentation processes of ponkan wine fermented with different yeast strains.
[c] The *Saccharomyces cerevisiae* strain of PPW (ponkan pulp wine) and PJW (ponkan juice wine) is BCRC 21805, HF-08, BCRC 21823, BCRC 22332, BCRC 21761, and BCRC 22293, respectively.
[d] Day of fermentation.
[e] The means in column with different uppercase alphabets are significantly different at $p < 0.05$.

3.1.3. *Comparison of browning and clarity of ponkan wines during the fermentation processes fermented with different yeast strains*

Table 3 values determined with spectrum photometer at 420 nm show the variation in browning indices and clarity of PJW and PPW fermented with different yeasts. Table 3 reveals that during the 30-day fermentation period, the browning index of that made from pulp was higher than that made from juice, except that the browning index of PJW 32 was higher than that of PPW 32. The browning index of PPW 32 was 0.22 ± 0.03 (OD 420). The main reason for the browning of PPW might be due to the polyphenols presented in ponkan pulp. Table 3 shows that both browning indices and clarity parameters decreased throughout the entire fermentation process. This might be due to the sedimentation of the colloids presented in the ponkan juice and ponkan pulp, especially in the ponkan pulp.

3.2. *Comparison of the results of sensory evaluation of PJW and PPW fermented with different yeast strains*

Table 4 shows the results of sensory evaluation of the ponkan wines after fermenting for 30 days. Concerning the color of four of the six ponkan wines, PJW had higher preference scores than PPW. In general, the ponkan juice wines had better sensory evaluation scores, and the bitterness of ponkan pulp wines might lower the sensory evaluation scores. The most preferred ponkan wines were PJW 08 and PJW 32, with the overall preference scores of 6.25 and 6.15, respectively. They also had better colors, flavors, and taste preferences.

3.3. *Differences between aromatic constituents in the ponkan wine fermented with BCRC 22332 and HF-08 yeast strains*

Based on the data in Table 4, PJW 08 and PJW 32 were the most preferred ponkan wines. Therefore, they were selected for further studies of their flavor components.

Table 4 – Sensory evaluation scores of ponkan wines.[a,b]

Wine product	Color	Flavor	Taste	Overall preference
PPW05	6.75 ± 0.56^{B}	6.02 ± 0.57^{A}	3.34 ± 0.52^{B,C}	4.58 ± 0.68^{B,C}
PPW08	6.36 ± 0.79^{B}	6.16 ± 0.72^{A}	4.12 ± 0.45^{A,B}	5.15 ± 0.88^{B}
PPW23	4.18 ± 0.64^{C}	5.75 ± 0.74^{B}	3.24 ± 0.43^{B,C}	3.42 ± 0.50^{D}
PPW32	6.50 ± 0.59^{B}	5.82 ± 0.61^{A,B}	3.95 ± 0.70^{A,B}	5.55 ± 0.72^{A,B}
PPW61	6.53 ± 0.65^{B}	4.25 ± 0.57^{C}	2.45 ± 0.77^{C}	4.12 ± 0.80^{C,D}
PPW93	6.15 ± 0.72^{B}	5.52 ± 0.66^{B}	2.72 ± 0.62^{C}	4.98 ± 0.53^{B,C}
PJW05	7.25 ± 0.44^{A}	6.47 ± 0.55^{A}	3.95 ± 0.60^{A,B}	5.49 ± 0.59^{A,B}
PJW08	7.88 ± 0.40^{A}	6.58 ± 0.74^{A}	4.55 ± 0.62^{A}	6.25 ± 0.74^{A}
PJW23	3.63 ± 0.68^{C}	5.43 ± 0.71^{B}	4.64 ± 0.49^{A}	4.98 ± 0.49^{B,C}
PJW32	7.72 ± 0.62^{A}	6.79 ± 0.66^{A}	4.61 ± 0.85^{A}	6.15 ± 0.75^{A}
PJW61	6.93 ± 0.53^{A,B}	4.86 ± 0.65^{B,C}	3.12 ± 0.58^{B,C}	4.36 ± 0.79^{C}
PJW93	7.28 ± 0.45^{A}	5.53 ± 0.58^{B}	3.45 ± 0.65^{B}	5.25 ± 0.67^{B}

[a] The PPW (ponkan pulp wine) and PJW (ponkan juice wine) of strain is *Saccharomyces cerevisiae* BCRC 21761, 21805, 21823, 22293, 22332 and HF-08 is 61, 05, 23, 93, 32, and 08, respectively.
[b] Symbols with different uppercase alphabets in the same column are significantly different ($p < 0.05$, $n = 47$).

Table 5 – Contents of volatile compounds in the ponkan wines fermented with BCRC 22332 and HF-08 strains

Compound	RI[a]	Concentration (ppm)				
	(DB-1)	Ponkan[b]	PPW32[c]	PJW32[d]	PPW08[e]	PJW08[f]
Alcohols						
Isobutyl alcohol	632	n.d.^{B,g}	n.d.^{B}	n.d.^{B}	11.32 ± 4.81^{A}	17.61 ± 6.09^{A}
Butyl alcohol	669	n.d.^{B}	n.d.^{B}	1.53 ± 0.90^{A}	n.d.^{B}	n.d.^{B}
2-Methyl-3-buten-2-ol	685	n.d.^{B}	n.d.^{B}	n.d.^{B}	n.d.^{B}	0.37 ± 0.14^{A}
3-Penten-2-ol	686	n.d.^{C}	0.09 ± 0.05^{B}	0.24 ± 0.08^{A,B}	0.42 ± 0.16^{A}	0.36 ± 0.12^{A}
Isoamyl alcohol	747	0.67 ± 0.12^{C}	9.19 ± 2.02^{B}	6.61 ± 3.50^{B}	57.17 ± 3.67^{A}	79.95 ± 35.37^{A}
Amyl alcohol	755	n.d.^{C}	4.77 ± 1.97^{A}	2.59 ± 0.44^{B}	2.55 ± 0.25^{B}	3.06 ± 0.67^{A,B}
2,3-Butanediol	808	35.63 ± 19.58^{A}	37.78 ± 14.02^{A}	49.04 ± 12.47^{A}	32.35 ± 9.97^{A}	61.39 ± 19.20^{A}
3-Methyl-1-pentanol	882	n.d.^{C}	n.d.^{C}	n.d.^{C}	3.03 ± 0.50^{A}	0.51 ± 0.14^{B}
Hexanol	895	n.d.^{C}	n.d.^{C}	n.d.^{C}	0.39 ± 0.04^{A}	0.25 ± 0.11^{B}
Methionol	998	n.d.^{C}	n.d.^{C}	n.d.^{C}	1.96 ± 0.54^{A}	0.01 ± 0.004^{B}
Phenethyl alcohol	1139	n.d.^{D}	8.40 ± 1.04^{C,D}	10.94 ± 1.78^{C}	60.87 ± 9.52^{A}	36.40 ± 0.70^{B}
4-Hydroxyphenethyl alcohol	1480	n.d.^{D}	0.08 ± 0.03^{C}	0.31 ± 0.04^{C}	21.76 ± 2.80^{A}	16.82 ± 3.98^{B}
Elemol	1596	n.d.^{C}	0.20 ± 0.09^{B}	0.33 ± 0.06^{B}	0.29 ± 0.15^{B}	0.40 ± 0.01^{A}
Farnesol	1611	n.d.^{C}	n.d.^{C}	n.d.^{C}	0.04 ± 0.01^{B}	0.12 ± 0.06^{A}
2,6-Dimethoxy-4-allylphenol	1634	n.d.^{D}	0.54 ± 0.07^{B}	0.50 ± 0.20^{B}	2.25 ± 0.27^{A}	0.11 ± 0.03^{C}
4-Methyl-2,6-di-tert-butylphenol	1681	n.d.^{B}	n.d.^{B}	0.05 ± 0.02^{A}	n.d.^{B}	n.d.^{B}
2,4-Di-tert-butylphenol	1690	n.d.^{B}	n.d.^{B}	0.28 ± 0.05^{A}	n.d.^{B}	n.d.^{B}
t-Muurolol	1693	n.d.^{B}	1.29 ± 0.26^{A}	1.16 ± 0.23^{A}	1.00 ± 0.61^{A}	1.20 ± 0.35^{A}
β-Eudesmol	1700	n.d.^{C}	1.42 ± 0.14^{A}	0.20 ± 0.05^{B}	n.d.^{C}	n.d.^{C}
α-Cadinol	1720	n.d.^{C}	n.d.^{C}	n.d.^{C}	0.43 ± 0.23^{B}	1.07 ± 0.44^{A}
Subtotal		36.30 ± 19.70	63.76 ± 19.69	73.78 ± 19.82	195.83 ± 33.53	219.63 ± 67.41
Ketones						
3-Hydroxy-2-butanone	696	n.d.^{B}	0.48 ± 0.06^{A}	n.d.^{B}	n.d.^{B}	n.d.^{B}
Butyrolactone	898	n.d.^{C}	n.d.^{C}	n.d.^{C}	0.13 ± 0.05^{B}	0.31 ± 0.12^{A}
Geranyl acetone	1408	0.16 ± 0.02^{A}	n.d.^{B}	n.d.^{B}	n.d.^{B}	n.d.^{B}
α-Ionone	1441	0.11 ± 0.02^{A}	n.d.^{B}	n.d.^{B}	n.d.^{B}	n.d.^{B}
Farnesyl acetone	1861	0.12 ± 0.04^{A}	n.d.^{B}	n.d.^{B}	n.d.^{B}	n.d.^{B}
Subtotal		0.39 ± 0.08	0.48 ± 0.06	n.d.	0.13 ± 0.05	0.31 ± 0.12
Esters						
Ethyl acetate	609	24.37 ± 9.10^{A}	10.94 ± 0.81^{B}	9.08 ± 1.15^{B}	7.03 ± 2.79^{B}	11.48 ± 4.19^{B}
Methyl tiglate	638	n.d.^{B}	n.d.^{B}	1.11 ± 0.32^{A}	n.d.^{B}	n.d.^{B}
Methyl lactate	741	n.d.^{C}	0.20 ± 0.02^{B}	n.d.^{C}	0.28 ± 0.04^{A}	n.d.^{C}
Isobutyl acetate	751	n.d.^{C}	n.d.^{C}	11.53 ± 2.36^{A}	n.d.^{C}	0.96 ± 0.44^{B}
Ethyl isobutyrate	771	n.d.^{C}	n.d.^{C}	0.09 ± 0.02^{A}	n.d.^{C}	0.02 ± 0.01^{B}
Ethyl lactate	831	n.d.^{D}	21.83 ± 6.53^{A}	13.70 ± 4.09^{B}	4.52 ± 0.41^{C}	6.82 ± 1.55^{C}
Isoamyl acetate	896	n.d.^{C}	0.16 ± 0.02^{B}	0.21 ± 0.07^{B}	0.35 ± 0.12^{AB}	0.58 ± 0.28^{A}
Ethyl 3-hydroxybutyrate	962	n.d.^{C}	n.d.^{C}	n.d.^{C}	0.94 ± 0.07^{A}	0.33 ± 0.09^{B}
Ethyl caproate	1029	n.d.^{C}	n.d.^{C}	n.d.^{C}	0.25 ± 0.05^{A}	0.03 ± 0.02^{B}
Methyl 3-methoxypropionate	1068	n.d.^{C}	n.d.^{C}	0.35 ± 0.11^{B}	n.d.^{C}	2.53 ± 0.78^{A}
Ethyl 4-hydroxybutanoate	1088	n.d.^{C}	n.d.^{C}	n.d.^{C}	2.31 ± 0.33^{B}	33.33 ± 2.29^{A}
Ethyl 2-hydroxycaproate	1093	n.d.^{B}	0.09 ± 0.06^{A}	n.d.^{B}	0.15 ± 0.04^{A}	n.d.^{B}
Ethyl caprylate	1234	n.d.^{C}	n.d.^{C}	n.d.^{C}	0.23 ± 0.10^{B}	1.55 ± 1.09^{A}
Phenethyl acetate	1280	n.d.^{C}	n.d.^{C}	n.d.^{C}	0.39 ± 0.14^{A}	0.03 ± 0.01^{B}
Trans-2-hexenyl butyrate	1431	n.d.^{B}	n.d.^{B}	n.d.^{B}	n.d.^{B}	1.46 ± 0.53^{A}
Ethyl caprate	1439	n.d.^{C}	n.d.^{C}	n.d.^{C}	0.06 ± 0.01^{B}	1.45 ± 1.01^{A}
Ethyl 3-methylbutyl butanedioate	1460	n.d.^{C}	n.d.^{C}	n.d.^{C}	0.33 ± 0.12^{B}	11.90 ± 7.23^{A}
Diethyl succinate	1471	n.d.^{D}	2.58 ± 0.98^{B}	0.15 ± 0.08^{C}	64.95 ± 10.84^{A}	58.73 ± 3.16^{A}
Ethyl 2-hydroxy-3-phenylpropanoate	1478	n.d.^{C}	0.16 ± 0.03^{B}	0.18 ± 0.09^{B}	0.43 ± 0.18^{A}	n.d.^{C}
Ethyl 2-hydroxypentanedioate	1656	n.d.^{C}	n.d.^{C}	n.d.^{C}	0.04 ± 0.01^{B}	0.41 ± 0.18^{A}
Methyl linoleate	2030	0.74 ± 0.42^{A}	n.d.^{B}	n.d.^{B}	n.d.^{B}	n.d.^{B}
Methyl linolenate	2036	2.59 ± 1.18^{A}	n.d.^{B}	n.d.^{B}	n.d.^{B}	n.d.^{B}
Methyl oleate	2042	1.11 ± 0.45^{A}	n.d.^{B}	n.d.^{B}	n.d.^{B}	n.d.^{B}
Methyl trans-8-octadecenoate	2047	3.24 ± 1.43^{A}	n.d.^{B}	n.d.^{B}	n.d.^{B}	n.d.^{B}
Ethyl linolenate	2103	4.25 ± 2.35^{A}	n.d.^{B}	n.d.^{B}	n.d.^{B}	n.d.^{B}
Ethyl oleate	2112	17.97 ± 1.19^{A}	n.d.^{B}	n.d.^{B}	n.d.^{B}	n.d.^{B}
Methyl linolelaidate	2167	0.29 ± 0.14^{A}	n.d.^{B}	n.d.^{B}	n.d.^{B}	n.d.^{B}
Subtotal		54.56 ± 16.26	33.38 ± 8.45	36.4 ± 8.29	82.26 ± 15.25	131.61 ± 22.86
Acid						
Acetic acid	644	13.27 ± 2.66^{BC}	20.17 ± 0.65^{C}	22.34 ± 5.22^{B,C}	35.60 ± 12.40^{B}	97.10 ± 34.20^{A}
Hexanoic acid	1045	n.d.^{C}	n.d.^{C}	n.d.^{C}	0.50 ± 0.06^{B}	0.89 ± 0.34^{A}
Benzoic acid	1270	n.d.^{C}	n.d.^{C}	n.d.^{C}	0.13 ± 0.04^{B}	0.58 ± 0.30^{A}
Caprylic acid	1271	n.d.^{B}	n.d.^{B}	n.d.^{B}	0.16 ± 0.08^{A}	0.26 ± 0.05^{A}

Table 5 – (*continued*)

Compound	RI[a]	Concentration (ppm)				
	(DB-1)	Ponkan[b]	PPW32[c]	PJW32[d]	PPW08[e]	PJW08[f]
Nonoic acid	1331	n.d.^{B}	n.d.^{B}	n.d.^{B}	0.07 ± 0.01^{A}	n.d.^{B}
Capric acid	1424	n.d.^{B}	n.d.^{B}	n.d.^{B}	0.13 ± 0.07^{A}	0.21 ± 0.07^{A}
Lauric acid	1576	0.40 ± 0.22^{B}	n.d.^{C}	n.d.^{C}	0.48 ± 0.17^{B}	3.89 ± 1.19^{A}
Myristic acid	1726	1.54 ± 0.86^{A}	n.d.^{C}	0.25 ± 0.05^{B}	n.d.^{C}	n.d.^{C}
9-Hexadecenoic acid	1899	2.55 ± 1.48^{A}	n.d.^{B}	n.d.^{B}	n.d.^{B}	n.d.^{B}
Palmitic acid	2015	61.38 ± 34.65^{A}	0.40 ± 0.26^{B}	0.25 ± 0.04^{B}	0.28 ± 0.15^{B}	0.11 ± 0.03^{B}
Octadecanoic acid	2108	8.9 ± 1.91^{A}	n.d.^{B}	n.d.^{B}	n.d.^{B}	n.d.^{B}
Linoleic acid	2112	94.39 ± 62.08^{A}	n.d.^{B}	n.d.^{B}	n.d.^{B}	n.d.^{B}
Oleic acid	2145	80.19 ± 45.88^{A}	n.d.^{B}	n.d.^{B}	n.d.^{B}	n.d.^{B}
Subtotal		262.62 ± 149.74	20.57 ± 0.91	22.84 ± 5.31	37.35 ± 12.98	103.04 ± 36.18
Terpenes and terpene alcohol						
α-Pipene	955	0.05 ± 0.02^{B}	n.d.^{C}	0.11 ± 0.04^{B}	n.d.^{C}	1.46 ± 0.29^{A}
γ-Terpinene	1037	0.15 ± 0.03^{A}	n.d.^{B}	n.d.^{B}	n.d.^{B}	n.d.^{B}
Limonene	1073	2.14 ± 0.28^{A}	0.19 ± 0.06^{B}	3.42 ± 0.36^{A}	0.38 ± 0.08^{B}	2.06 ± 0.70^{A}
α-Terpineol	1220	2.54 ± 0.35^{B}	0.28 ± 0.11^{D}	1.34 ± 0.59^{C}	0.48 ± 0.19^{C,D}	3.42 ± 0.39^{A}
δ-Cadinene	1574	n.d.^{C}	0.02 ± 0.01^{B}	0.20 ± 0.09^{A}	n.d.^{C}	n.d.^{C}
α-Calacorene	1594	n.d.^{C}	0.05 ± 0.04^{B}	0.06 ± 0.03^{B}	0.07 ± 0.04^{B}	0.51 ± 0.17^{A}
Cadina-1,4-diene	1685	n.d.^{C}	0.17 ± 0.04^{B}	n.d.^{C}	n.d.^{C}	0.72 ± 0.33^{A}
α-Cubebene	1695	n.d.^{B}	0.27 ± 0.06^{A}	n.d.^{B}	n.d.^{B}	n.d.^{B}
γ-Elemene	1705	n.d.^{C}	n.d.^{C}	n.d.^{C}	1.52 ± 1.03^{A}	0.31 ± 0.13^{B}
α-Cedrene	1904	n.d.^{B}	n.d.^{B}	0.03 ± 0.01^{A}	n.d.^{B}	n.d.^{B}
α-Copaene	2024	n.d.^{B}	n.d.^{B}	0.31 ± 0.03^{A}	0.40 ± 0.29^{A}	n.d.^{B}
Subtotal		4.88 ± 0.68	0.98 ± 0.32	5.47 ± 1.11	2.85 ± 1.63	8.48 ± 2.01
Total		358.75 ± 186.46	119.17 ± 29.43	138.49 ± 34.53	318.42 ± 63.44	463.07 ± 128.58

n.d. = not detected.
[a] RI = GC retention index calculated on DB-1 column by using c5-c25 paraffins as standards.
[b] Ponkan = ponkan (*Citrus poonensis* hort.) peeled and pressed into juice.
[c] PPW32 = fermented ponkan pulp wine using BCRC 22332 as starter.
[d] PJW32 = fermented ponkan juice wine using BCRC 22332 as starter.
[e] PPW08 = fermented ponkan pulp wine using HF-08 as starter.
[f] PJW08 = fermented ponkan juice wine using HF-08 as starter.
[g] The means in row with different uppercase alphabets are significantly different at $p < 0.05$.

3.3.1. *Comparisons of aroma components between PJW and PPW*

Table 5 shows the aroma components of the ponkan wines in this study. The ponkan wines contained high amounts of α-pipene, γ-terpinene, limonene, and α-terpineol, similar to those of other studies [33,34]. The main volatile components of the ponkan juice were fatty acids such as palmitic acid (61.38 ± 34.65 ppm), linoleic acid (94.39 ± 62.08 ppm), oleic acid (80.19 ± 45.88 ppm), and esters such as ethyl acetate (24.37 ± 9.10 ppm) and ethyl oleate (17.97 ± 1.19 ppm). The main difference of volatile composition between ponkan juice and ponkan wine was that ponkan juice contained more fatty acids, fatty acid esters, and fruity aroma of ethyl acetate, whereas ponkan wine contained high amounts of alcohols such as isoamyl alcohol, amyl alcohol, phenethyl alcohol, elemol, 2,6-dimethoxy-4-allylphenol, and esters such as ethyl lactate, isoamyl acetate, and diethyl succinate.

Ethyl acetate, acetic acid, isoamyl alcohol, α-terpineol, 2,3-butanediol, limonene, and palmitic acid in ponkan wines were also found in ponkan juice. The result was similar to that of a previous report [35]. The data revealed that, after the yeast fermentation, the ponkan wine retained the original aroma components of citrus fruits. Selli et al [27] found that the characteristic aroma of citrus wine was ethyl acetate and phenethyl alcohol. This study also showed that ethyl acetate and phenethyl alcohol, respectively, had fruity [36], sweet, and honey [37,38] flavor characteristics. The volatile components of PJW were mainly isobutyl acetate (fruity), ethyl isobutyrate, methyl 3-methoxypropionate, and α-pipene (fruity, piney) [39]. These aroma components were not present in PPW. The main volatile components of the PPW were methyl lactate and ethyl 2-hydroxycaproate. Compared with Table 4, the sensory evaluation results showed that PJW had better preference. This might be due to PJW having higher main aroma components than PPW, such as 2,3-butanediol (PJW 49.04 ± 12.47 > PPW 37.78 ± 14.02), phenethyl alcohol (PJW 10.94 ± 1.78 > PPW 8.40 ± 1.04), limonene (PJW 3.42 ± 0.36 > PPW 0.19 ± 0.06), α-terpineol (PJW 1.34 ± 0.59 > PPW 0.28 ± 0.11), and higher contents and varieties of esters. PJW contained phenethyl alcohol (sweet, honey), ethyl acetate (fruity) and isoamyl acetate (fruity). In addition to the aforementioned aroma components, PPW also contained a large amount of isoamyl alcohol, amyl alcohol, and α-pipene.

3.3.2. *Comparisons of the aroma components of ponkan wines fermented with different yeasts*

The aroma components of PJW and PPW fermented with BCRC 22332 yeast were mainly 3-penten-2-ol, isoamyl alcohol, amyl alcohol, 2,3-butanediol, phenethyl alcohol, 4-hydroxyphenethyl

alcohol, elemol, 2,6-dimethoxy-4-allylphenol, t-muurolol, β-eudesmol, ferruginol, ethyl acetate, ethyl lactate, isoamyl acetate, ethyl 2-hydroxy-3-phenylpropanoate, acetic acid, palmitic acid, limonene, and δ-cadinene, with no significant differences in the content of most of the aroma components. The aroma components of PJW and PPW fermented with HF-08 yeast were isobutyl alcohol, 3-ethoxy-1-propanol, 3-methyl-1-pentanol, hexanol, dimethyl carbitol, methionol, farnesol, α-cadinol, butyrolactone, 2-methyl-3-thiolannone, ethyl 3-hydroxybutyrate, ethyl caproate, ethyl 4-hydroxybutanoate, ethyl caprylate, phenethyl acetate, ethyl caprate, ethyl 3-methylbutyl butanedioate, diethyl 2-hydroxypentanedioate, hexanoic acid, benzoic acid, caprylic acid, capric acid, lauric acid, and γ-elemene. Most of the aroma components in PJW were higher than those in PPW. Table 4 shows that PJW fermented with HF-08 had better preferences.

In terms of aroma property, the ponkan wine fermented with HF-08 contained more volatile components, such as isobutyl alcohol (wine aroma), isoamyl alcohol, diethyl succinate (grape), phenethyl alcohol (sweet, honey), ethyl acetate (fruity), and isoamyl acetate (fruity), than that fermented with BCRC 22332. Table 5 shows that PJW fermented with HF-08 produced more alcohols, esters, acids, and terpenes. The main volatile components of ponkan wine were ethyl acetate, phenethyl alcohol, isoamyl alcohol, lethyl hexanoate, citronellol, terpinene, α-pipene, α-terpineol, limonene, γ-elemene, and α-octadecene. Compared with the previous studies [22–25,40,41], the ponkan wine fermented with HF-08 bacterial strain from ponkan juice had more and higher amounts of components in ponkan wine. The study results showed that the amount of aroma components such as isoamyl alcohol, phenethyl alcohol, and α-terpineol of PJW 08 was higher than that fermented with BCRC 22332. The wine fermented with HF-08 contained higher amounts of aroma components such as ethyl acetate, phenethyl alcohol [24,25,42], α-pipene, α-terpineol, and limonene [39,40,41], which are the important aromas found in citrus fruit and ponkan wine. The aforementioned results showed that PJW and PPW fermented with HF-08 contained more alcohols, acids, esters, terpenes, and other aroma compounds than that fermented with BCRC 22332. Lin [10] found that alcohols, esters, and acids had a significantly positive effect on wine flavor. Therefore, it was speculated that more types of volatile components fermented with HF-08 yeast produced high-quality taste and aroma of ponkan wines.

4. Conclusions

Our study indicated that the ponkan juice was preferable for ponkan wine fermentation. Ponkan wines fermented with BCRC 22332 and HF-08 yeasts had more aromatic constituents and better acceptability than other tested yeasts. Both BCRC 22332 and HF-08 strains had characteristics of higher alcohol productivity, lower residual sugar, less browning, and better sensory evaluation. However, the ponkan wine fermented with HF-08 strain contained more isobutyl alcohol, isoamyl alcohol, diethyl succinate, phenethyl alcohol, ethyl acetate, and isoamyl acetate, as compared with that fermented with BCRC 22332. Apparently, for ponkan wine fermentation, yeast HF-08 was superior to BCRC 22332.

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