Effect of mixed fermentation (Jiuqu and Saccharomyces cerevisiae EC1118) on the quality improvement of kiwi wine

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ABSTRACT

The aim of this study was to investigate the effect of mixed fermentation of Jiuqu and Saccharomyces cerevisiae on the quality of kiwi wine. The results showed that total titratable acidity, methanol and organic acids of kiwi wine fermented by Jiuqu + Saccharomyces cerevisiae EC1118 were significantly lower (p < 0.05) than that in other groups. There was no significant difference in the content of flavor substances in kiwi wines fermented by a mixed group and Saccharomyces cerevisiae EC1118 alone, but the sensory evaluation showed a significantly large difference. On the sensory scale, kiwi wine fermented by mixed fermentation had a unique presentation and typical bouquet, better than those fermented by Jiuqu and Saccharomyces cerevisiae EC1118 alone. In conclusion, mixed fermentation of Jiuqu + Saccharomyces cerevisiae EC1118 could improve the quality of the kiwi wine, which provides a promising application prospect for kiwi wine production.

1. Introduction

Kiwifruit (Actinidia chinensis Planch), originates in China and belongs to Actinidiaceae family (Luh & Wang, 1984), is a popular fruit for its pleasant tasted and profuse nutrition, being especially rich in vitamin C, organic acids, tannins, carbohydrates, pectin, essential amino acids, trace elements as well as inositol and lutein (Nishiyama et al., 2004; Tavarini, Degl’Innocenti, Remorini, Massai, & Guidi, 2008; Wei & Guohua, 2015). There are a lot of health benefits associated with kiwi such as antioxidant and anti-cancer activity, improving immunity, reducing blood pressure and blood fat, protecting against diabetes and treatment of burns (Leontowicz et al., 2016). In recent years, the production of kiwi fruit has been remarkably increased due to the development of cultivation techniques and production management all over the world, which in turn lead to its overproduction (López-Vázquez et al., 2012). There is an urgent demand to find a solution for utilizing this overproduction for economic benefits. Kiwi wine is a popular kiwi-based product in Asia that increases its commercial value.

Saccharomyces cerevisiae is one of the most commonly used yeast for the fermentation of kiwi juice in China; however, there are a few problems associated with S. cerevisiae fermented kiwi wine such as lack of ample fruitiness, high acidity, low typicality, high methanol content. Some researchers believed that S. cerevisiae is not the ideal one for producing kiwi wine (Kang et al., 2011). Mixed microorganism fermentation may improve the quality of kiwi fruit wine as there are many successful examples on other fruit: Englezos et al. (2016) reported that compared to pure fermentation, mixed fermentation with Starmerella bacillaris and S. cerevisiae improved the quality of Barbera wine because of the synergistic effect of the two microorganisms, the glycine content can be increased to 8.8–9.8 g/L and acetic acid content can be reached up to 0.4 g/L (Gobbi et al., 2013). Garcia et al. (2002) found that mixed fermentation with Debaryomyces vanriji and S. cerevisiae could improve the quality of white wine.
significantly improved the concentration of several acids, alcohol, ester, and terpinol, and therefore better volatile profile of grape wine was detected by mixed fermentation. Minnaar, Jolly, Paulsen, Du Plessis, and Van Der Rijst (2017) reported that the titratable acid of Kei-apple juice fermented by *Schizosaccharomyces pombe* and *S. cerevisiae* yeasts decreased by 70% compared to single culture fermentations. According to these reported researches, kiwi wine produced with mixed fermentation may improve its final quality.

Jiuqu is a kind of fermentation starter for the production of Chinese sweet rice wine (CSRW), which is a traditional Chinese alcohol beverage, possessing a desirable flavor and high nutritional value, including peptides, oligosaccharides, vitamins, amino acids, and organic acids (Jiang et al., 2016; Shen et al., 2010; Shen, Ying, Li, Zheng, & Hu, 2011; Wei et al., 2017). The starters are the mixtures of yeasts, molds, and bacteria (Bora, Keot, Das, Sarma, & Barooah, 2016; Lv, Weng, Zhang, Rao, & Ni, 2012), which produce plenty of enzymes for cellular metabolisms and subsequent small molecule generation, which contribute to final quality of the products (Cai et al., 2018).

Therefore, the aim of this work was to investigate the organic acid, flavor substance, methanol and sensory quality of kiwi wines produced by the mixed fermentation of Jiuqu and *S. cerevisiae*.

2. Materials and methods

2.1. Kiwi handling procedure and microorganisms

Fifteen kilograms kiwi fruit were purchased from the market (Ya'an, Sichuan, China) at commercial maturity and with uniform shape and size, the pH was 2.93 ± 0.06, total acidity was 14.27 ± 0.46 g/L citric acid, soluble solids content was 9.63 ± 0.55 °Brix and organic acid content was 31.28 ± 2.03 mg/g FW (Fresh Weight). The fruits were pulped with a domestic juicer. Pectinase (60 mg/L) (40 u/mg, from *Aspergillus niger*, Beijing Solarbio Science & Technology Co., Ltd., China) was added to the pulp, and incubated at 40°C for 4.5 h, and then centrifuged at 4000 rpm for 10 min (Heraeus Multifuge X3R, Thermo Scientific, USA), the supernatant was obtained with four layers of nylon, followed by addition of SO2 (80 mg/L). Twenty percent (v/v) of sucrose was added to kiwi juice and then stored at 4°C until use.

Jiuqu was purchased form Angel Yeast Co., Ltd (Yichang, Hubei, China). *S. cerevisiae* EC1118 was obtained from Xinmiao Winery (Ya'an, Sichuan, China).

2.2. Fermented kiwi juice and fermentation procedure

The Kiwi juice was divided into three groups as follows:

1. Kiwi juice fermented by *S. cerevisiae* EC1118: 600 mL of kiwi juice and 2 g dried *S. cerevisiae* EC1118 was added to a sealed jar and fermented at room temperature according to the manufacturer’s instructions.
2. Kiwi wine fermented by Jiuqu: 600 mL of kiwi juice and 6 g Jiuqu was added to a sealed jar and fermented at room temperature (22°C) according to the manufacturer’s instructions.
3. Kiwi wine fermented by Jiuqu + *S. cerevisiae* EC1118: 600 mL of kiwi juice and 6 g Jiuqu and 2 g dried *S. cerevisiae* EC1118 was added to a sealed jar and fermented at room temperature according to the manufacturer’s instructions. Transit operation for all kiwi wines was carried out after fermenting for 8 days to remove dregs and then further fermented at 22°C for 8 days. The raw wine was obtained after removing dregs. All raw wines were filled with glass jars and aged for 60 days at room temperature. All treatments were conducted in triplicate. Kiwi wines were monitored by recording the loss in mass.

2.3. Physico-chemical parameters

The analyses included the alcohol percentage, total titratable acidity (TTA), total sugar (TS) and ascorbic acid content (AA) of wine, according to the methods described by Chung, Son, Park, Kim, and Lim (2008). Clarity and chromaticity were measured by UV-1800PC visible spectrophotometer (UV-1600PC, MAPADA, China) at 680 nm, 520 nm, respectively. All treatments were conducted in triplicate.

**Determination of condition and method validation of HPLC-DAD and GC-FID of organic acids and methanol content**

Organic acids were quantified using a published HPLC-DAD method (Barreca, Bellocco, Caristi, Leuzzi, & Gattuso, 2011; Coelho et al., 2018; Mesquita & Monteiro, 2018), with slight modification. The HPLC-DAD determination of organic acids was performed using an Agilent HPLC model (Agilent Technologies, Palo Alto, CA, USA). For the test method of organic acids, an Agilent Eclipse plus C18 reserved-phase column (5 μm, 250 mm × 4.6 mm) and an Agilent Eclipse plus C18 guard column (5 μm, 20 mm × 4.6 mm) were used. The sample injection volume was 10 μL, the temperature was 30°C, the detection wavelength was 210 nm, the mobile phase consisted of deionized water-dipotassium phosphate (A: 100:0.02, v/v) and methanol (B), and the flow rate was 1 mL/min. Initial condition was A:B (98:2, v/v), linearly changed to A:B (95:5, v/v) at 8 min and then run for 10 min.

Standard solution of organic acids with different concentrations (0.0025–0.64 g/L) was prepared. The standard curves of different organic acids were plotted with the concentration of the standard solution as the abscissa and the peak area as the ordinate. For the sample determination, the sample was diluted 10 times, and passed through the 0.22 μm organic filter membrane and then injected into HPLC-DAD.

Gas chromatograph (7890A/59750) (Agilent Technologies, Palo Alto, CA, USA) was used for the determination of methanol. Chromatographic separation was performed on an Agilent HP-INNOWAX capillary column (0.25 mm × 30 m). Helium was used as the carrier gas at a flow rate of 0.5 mL/min, the split ratio was 50:1, the flow rate of hydrogen was 40 mL/min, with a corresponding airflow rate of 40 mL/min, and makeup gas flow rate was 25 mL/min, accompanied with hydrogen flame ionization detector. Detector and injector temperatures were set at 220°C. The following GC oven temperature program was applied: 40°C for 4 min, 3.5°C/min to 96°C, 96°C hold for 2 min, 20°C/min to 200°C, 200°C hold for 10 min; the sample injection volume was 1 μL. Detection and quantitation limits, precision, recovery and linearity were assessed in terms of analytical methods established in previous studies (Coelho et al., 2018; Gupta, 2015; Mesquita & Monteiro, 2018).

Standard solution of methanol of different concentrations (0.01–0.64 g/L) was prepared. The internal standard of 4-methyl-2-pentanol was added to the standard solution and
the final concentration was made to 0.2 g/L. Methanol was tested according to the method mentioned previously. The standard curve of methanol was plotted with the concentration of the standard solution as the abscissa and the peak area ratio of methanol to 4-methyl-2-pentyl alcohol as the ordinate.

2.4. Validation of the applied method

In this study, the optimal chromatographic condition was determined for the isolation of organic acids, methanol and 4-methyl-2-pentanol. As shown in Figure 1(a,b), the standard samples of organic acids and methanol were isolated in the optimal condition. The standards of different concentrations of organic acids and methanol had a good linear relationship with the peak area ($R^2 > 0.9997$), which is in accordance with the report by Coelho et al. (2018) that the $R^2$ must over 0.99. According to the results, the established method could be used to assay organic acids and methanol.

Table 1 shows that the LOD of organic acids was in the range of 0.0004–0.0032 g/L, and LOQ was in the range of 0.0016–0.0063 g/L. The LOD and LOQ of methanol were less than 0.01 g/L and 0.04 g/L, respectively. The results were in agreement with previous other reports (Chinnici, Spinabelli, Riponi, & Amati, 2005; Coelho et al., 2018; Eyéghé-Bickong, Alexandersson, Gouws, Young, & Vivier, 2012). The standard samples of organic acids and methanol were added to measure their recovery rate, which was in the range of 93.90%–103.2%, and all RSD of repeatability was less than 2.77%. Therefore, the method could be used to detect the organic acids and methanol in this study.

2.5. Analysis of aroma compounds

The wine aroma compounds were isolated and pre-concentrated following a solid-phase extraction (SPE)
procedure (García-Carpintero, Sánchez-Palomo, Gallego, & González-Viñas, 2011) and then assayed by gas chromatography-mass spectrometry (GC-MS) as previously described (Velázquez, Zamora, Álvarez, Álvarez, & Ramírez, 2016).

2.6. Sensory evaluation

Sensory evaluation of kiwi wine was followed by Friedman test as described by Sherwood (1993) to compare the differences in different kiwi wines. A panel of 15 experts who had extensive experience in tasting kiwi wine were employed for the sensory test. The kiwi wines were presented in clear flute-shaped wine glasses. Synthetic ranking test of different kiwi wines was carried out by experts and graded according to the following criteria: 1-common, 2-good and 3-perfect. According to the evaluation results, order and rank sum were attained.

2.7. Statistical analysis

The determinations were conducted in triplicates. One-way analysis of variance (ANOVA) by Tukey test \((p < 0.05)\) was applied to check the statistical significance of differences among different treatments with Statistics 22.0 (SPSS Inc., Chicago, IL, USA). Origin Lab original 8.5 pro software was used for plotting.

3. Results and discussion

3.1. Physico-chemical parameters

Figure 2 shows the fermentation curve of kiwi wine by different treatments at 22°C. It can be seen that kiwi wine produced with Jiuqu + \(S. \text{cerevisiae}\) EC1118 had the fastest rate of fermentation than that produced by \(S. \text{cerevisiae}\) EC1118 and Jiuqu alone. The release of \(\text{CO}_2\) reached to the highest level on the third day in mixed fermentation group, while in \(S. \text{cerevisiae}\) and Jiuqu fermented group, the peak value was at fourth and fifth day, respectively. Moreover, the peak \(\text{CO}_2\) value in the mixed group was much higher than that in other groups.

Table 2 shows the physico-chemical parameters of the produced kiwi wines. For ethanol content, Jiuqu fermented

### Table 1. The retention time, regression equation, linear range, \(R^2\), LOD, LOQ, recovery and repeatability of organic acids and methanol determination.

| Organic acids | Retention time (min) | Calibration curve \((N = 3)\) | Range \((g \text{ L}^{-1})(N = 6)\) | \(R^2\) | LOD \((g \text{ L}^{-1})\) | LOQ \((g \text{ L}^{-1})\) | Recovery \((100\%)\) \((N = 6)\) | Repeatability \((\% \text{ RSD})\) \((N = 6)\) |
|---------------|---------------------|------------------------------|---------------------------------|--------|----------------|----------------|-------------------------------|-----------------------------|
| Oxalic        | 2.075               | \(Y = 7.069.20X + 8.2963\)  | 0.0025–0.64                     | 0.9997 | 0.0004         | 0.0016         | 98.9€                          | 0.4€                        |
| Tartaric      | 2.187               | \(Y = 66.62X – 2.4895\)    | 0.0025–0.64                     | 0.9988 | 0.0032         | 0.005          | 98.2€                          | 1.03€                       |
| Malic         | 2.443               | \(Y = 451.72X – 0.7297\)  | 0.0025–0.64                     | 0.9998 | 0.0032         | 0.005          | 95.9€                          | 2.65€                       |
| Lactic        | 2.819               | \(Y = 186.63X + 0.4949\)  | 0.0025–0.64                     | 0.9999 | 0.0032         | 0.005          | 97.2€                          | 2.76€                       |
| Acetic        | 3.089               | \(Y = 316.36X + 0.7903\)  | 0.0025–0.64                     | 0.9997 | 0.0008         | 0.0025         | 93.7€                          | 2.12€                       |
| Citric        | 3.448               | \(Y = 659.30X + 0.4944\)  | 0.0025–0.64                     | 0.9999 | 0.0008         | 0.0032         | 95.1€                          | 0.64€                       |
| Methanol FID  | 6.388               | \(Y = 1.645X + 0.0138\)   | 0.01–0.64                       | 1     | 0.01           | 0.04           | 103.2€                         | 1.94€                       |

DAD, photodiode array detector; \(R^2\), linear correlation coefficient; LOD, limit of detection limit; LOQ, limit of quantification.

DAD, detector de matriz de fotodiodos; \(R^2\), coeficiente de correlación lineal; LOD, límite de detección; LOQ, límite de cuantificación.

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**Table 1.** Tiempo de retención, ecuación de regresión, rango lineal, \(R^2\), LOD, LOQ, recuperación y repetibilidad de ácidos orgánicos y determinación de metanol.

**Tabla 1.** Tiempo de retención, ecuación de regresión, rango lineal, \(R^2\), LOD, LOQ, recuperación y repetibilidad de ácidos orgánicos y determinación de metanol.
kiwi wine recorded the highest ethanol content, that was 13.47 ± 0.02%. The ethanol in Jiuqu + *S. cerevisiae* EC1118 group was 11.94 ± 0.02%, and it was 11.14 ± 0.02% in *S. cerevisiae* EC1118 fermented group. But Jiuqu treated group showed relatively higher residual sucrose content. The high ethanol and residual sucrose content might be caused by ethanol production ability of different microorganisms (Hong et al., 2016), the results indicated that the micro-flora in Jiuqu showed higher ethanol production ability than *S. cerevisiae* EC1118.

For TTA content, lower TTA content was observed in mixed fermentation group than other groups, which may indicate a low sour taste in the final products.

Clarity serves as one of the key factors in judging overall wine quality (Valentín, Parr, Peyron, Grose, & Ballester, 2016). For the clarity parameters, that were transmittance and absorbency, mixed fermentation showed higher transmittance (98.7 ± 0.21) but lower absorbency (0.029 ± 0.00), suggesting higher clarity of kiwi wine produced by Jiuqu + *S. cerevisiae* EC1118 fermentation.

### 3.2. Organic acids and methanol

The contents of organic acids from all wines were analyzed by HPLC-DAD, and their values are presented in **Table 3**. Total organic acids were in the range from 16336.69 to 22952.05 mg/L for all the wines. Organic acid significantly affected the pH of kiwi wine, which contributed the rough and harsh taste to the final products (Colangelo, Torchio, De Faveri, & Lambri, 2018). The kiwi wine produced with mixed fermentation showed significantly lower (p < 0.05) total organic acids than the kiwi wines produced by pure fermented groups, which were accord with the titratable acid content (Ribéreau-Gayon, Glories, Maujean, & Dubourdieu, 2006, Table 2).

Malic acid was the most abundant organic acids detected in produced kiwi wines, which amounted to more than 55% of total organic acids, followed by citric, tartaric and acetic content. The content of malic and citric acids in kiwi wine fermented by Jiuqu + *S. cerevisiae* EC1118 was lower, while the lactic acid content was higher when compared with that in kiwi wine fermented by *S. cerevisiae* EC1118 alone, which may be caused by the convert of malic acid and citric acid to lactic acid and CO₂ (Suárez-Lepe, Palomero, Benito, Calderón, & Morata, 2012). Besides, lactic acid has lower acidity and higher stability than that of malic acid (Zhao, Fan, Hang, & Yang, 2006), the high content of lactic acid in mixed fermentation group increased the stability and sensory profile of produced kiwi wine.

Acetic acid is an important microbial growth inhibitor, that is produced during normal yeast metabolism in biotechnological processes (Palma, Guerreiro, & Sá-Correia, 2018). The content of acetic in Jiuqu group was the highest one, which was about 4 times than that by mixed fermentation and about 10 times higher than that fermented by *S. cerevisiae* EC1118 alone. This can be attributed to the high metabolism of *Rhizopus* in Jiuqu (Londoño-Hernández et al., 2017).

The methanol content in kiwi wines measured by GC-FID was listed in **Table 3**. For all kiwi wines the methanol content ranged from 162.64 to 212.05 mg/L, and the order of methanol content in different fermentations was *S. cerevisiae* EC1118 > Jiuqu > mixed fermentation (p < 0.05). The decline of methanol content in mixed fermentation group was decreased by 49.41mg/L in comparison with *S. cerevisiae*

### Table 2. The effect of different processes on the physico-chemical parameters of kiwi wine.

| Parameters measured | Sample (n = 3) | 
|---------------------|---------------|
|                      | Jiuqu         | Yeast EC1118 | Jiuqu + Yeast EC1118 |
| Alcohol (% v/v)     | 13.47 ± 0.02a | 11.14 ± 0.02c | 11.94 ± 0.02b |
| TTA (g/L)           | 10.51 ± 0.01a | 10.15 ± 0.01b | 9.14 ± 0.03c |
| Content of Vc (g/L) | 0.50 ± 0.01b  | 0.61 ± 0.01a  | 0.49 ± 0.03b  |
| Residual sucrose (g/L) | 6.21 ± 0.17a  | 6.06 ± 0.05ab | 5.93 ± 0.07b  |
| Transmittance (%)   | 98.4 ± 0.06b  | 83.2 ± 0.1c   | 98.7 ± 0.21a  |
| Absorbency ($)      | 0.040 ± 0.00c | 0.169 ± 0.00a | 0.029 ± 0.00c |

Super indexes a, b, and c in the same row indicate significant differences in different treatments according to the least significant difference test (p < 0.05).

### Table 3. Organic acids and methanol content of kiwi wines.

| Organic acids (DAD 210 nm) | Treatment (n = 3) | 
|---------------------------|-----------------|
|                          | Jiuqu           | Yeast EC1118 | Jiuqu + Yeast EC1118 |
| Oxalic (mg/L)            | 250.11 (±1.76)a | 166.59 (±3.99)c | 195.59 (±0.49)b |
| Tartaric (mg/L)          | 1,839.55 (±2.41)b | 2,305.74 (±1.03)a | 1,709.38 (±1.89)c |
| Malic (mg/L)             | 10,009.46 (±4.32)b | 13,196.84 (±3.59)a | 9,023.11 (±2.64)c |
| Lactic (mg/L)            | 1,182.60 (±2.63)a | 439.16 (±2.32)c | 517.84 (±0.32)b |
| Acetic (mg/L)            | 4,674.50 (±0.77)a | 474.52 (±0.52)c | 1,267.42 (±2.71)b |
| Citric (mg/L)            | 3,936.36 (±0.06)c | 6,369.20 (±5.49)a | 3,623.32 (±1.32)c |
| Total organic acids      | 21,912.59 (±6.17)b | 22,952.05 (±6.70)a | 16,336.69 (±6.92)c |
| Methanol FID (mg/L)      | 183.91 (±5.30)b | 212.05 (±3.06)a | 162.64 (±2.43)c |

Super indexes a, b, and c in the same row indicate significant differences in different treatments according to the least significant difference test (p < 0.05).

Los superíndices a, b y c en la misma fila indican diferencias significativas en diferentes tratamientos de acuerdo con la prueba de diferencia menos significativa (p < 0.05).
EC1118 group. Methanol is a kind of toxin substance to humans (Zhang, Lin, Chai, & Barnes, 2015), its formation depends on many factors such as raw materials, pectinase, fermentation temperature (Cabaroglu, 2005). Microbial metabolism and the degradation of pectin by pectinase could generate methanol (Amerine & Ough, 1980; Cordonnier, 1987, Ribéreau-Gayon et al., 2006). At the same time, Rogerson, Vale, Grande, and Silva (2000) pointed out that S. cerevisiae can replace glycine to produce methanol in raw materials. In this study, the methanol content of wine

![Figure 3](image3.png)

**Figure 3.** Content of volatile flavor substances in different kiwi wines. (mean ± SD, n = 3). Kiwi juice fermented by Jiuqu (■), EC1118 (●) and Jiuqu + EC1118 (■).

![Figure 4](image4.png)

**Figure 4.** GC-MS chromatogram of different samples fermented by Jiuqu, EC1118 and Jiuqu + EC1118.

**Figura 3.** Contenido de sustancias de sabor volátiles en diferentes vinos de kiwi (media ± DE, n = 3). Jugo de kiwi fermentado por Jiuqu (■), EC1118 (●) y Jiuqu + EC1118 (■).

**Figura 4.** Cromatograma GC-MS de diferentes muestras fermentadas por Jiuqu, EC1118 y Jiuqu + EC1118.
fermented by Jiuqu + *S. cerevisiae* EC1118 was significantly decreased, indicated a higher safety of the kiwi wine by the mixed fermentation.

The different metabolites in different samples were mainly caused by the different microorganism flora. Jiuqu consisted a mixture of yeasts, molds, and bacteria (Bora et al., 2016; Lv et al., 2012). Since each kind of microorganisms had their specific metabolic pathways, they could generate abundant substances from the same source. For example, obligately homofermentative lactic acid bacteria can only convert hexoses to lactic acid bacteria through Embden–Meyerhof–Parnas pathway, but obligately heterofermentative lactic acid bacteria degrade hexoses by the phosphogluconate pathway, producing not only lactic acid as the end product but also ethanol or acetic acid and carbon dioxide (Buron-Moles, Chailyan, Dolejs, Forster, & Mikš, 2019). Therefore, with the help of a rich microbial community, the produced wine had higher quality but lower methanol content.

### 3.3. Volatile profile

The volatile flavor substances in different kiwi wines were shown in Figure 3. The detail of identified components for

| Volatile flavor       | Content (mg/L) | Jiuqu + EC1118 | Jiuqu | EC1118 |
|-----------------------|----------------|----------------|-------|--------|
| Ethyl caproate        | 1.83 ± 0.81    | 0.94 ± 0.25    | 1.88 ± 0.53 |
| Ethyl 2-furoate       | 0.16 ± 0.01    | 0.24 ± 0.07    | 0.49 ± 0.07 |
| Ethyl benzoate        | 0.43 ± 0.20    | 0.32 ± 0.02    | 0.33 ± 0.02 |
| Diethyl succinate     | 2.65 ± 0.28    | 2.44 ± 0.40    | 3.56 ± 1.07 |
| Ethyl caprylate       | 14.69 ± 2.92   | 5.08 ± 2.53    | 15.02 ± 1.39 |
| Ethyl phenylacetate   | 0.18 ± 0.03    | 0.20 ± 0.01    | 0.17 ± 0.03 |
| Phenethyl acetate     | 4.91 ± 1.09    | 8.26 ± 0.49    | 3.00 ± 0.60 |
| Ethyl nonanoate       | 0.17 ± 0.04    | 0.00 ± 0.00    | 0.16 ± 0.05 |
| Ethyl 3-phenylpropionate | 0.07 ± 0.04 | 0.00 ± 0.00    | 0.00 ± 0.00 |
| 9-Hexadecenoic acid, ethyl ester | 1.25 ± 0.52 | 0.91 ± 0.14 | 0.00 ± 0.00 |
| Ethyl caprate         | 3.25 ± 0.36    | 1.97 ± 0.38    | 4.70 ± 0.24 |
| Ethyl myristate       | 0.39 ± 0.06    | 0.36 ± 0.13    | 0.41 ± 0.23 |
| Ethyl palmitate       | 1.35 ± 0.55    | 2.19 ± 1.13    | 1.09 ± 0.12 |
| **Subtotal**          | 31.35          | 22.89          | 30.80 |
| Other esters          |                |                |       |
| Butanoic acid, 4-hydroxy- | 0.00 ± 0.00 | 0.07 ± 0.04 | 0.00 ± 0.00 |
| Carbonochloridic acid, octyl ester | 1.74 ± 0.45 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| Methyl benzoate       | 0.54 ± 0.11    | 0.75 ± 0.22    | 0.95 ± 0.12 |
| Ethyl isopentyl succinate | 0.28 ± 0.08 | 0.00 ± 0.00 | 0.21 ± 0.09 |
| Isopropyl palmitate   | 0.00 ± 0.00    | 0.00 ± 0.00    | 0.61 ± 0.32 |
| **Subtotal**          | 2.56           | 0.82           | 1.78 |
| Alcohols              |                |                |       |
| 3-(Methy1thio)propanol | 0.11 ± 0.05 | 0.11 ± 0.04 | 0.00 ± 0.00 |
| Benzyl alcohol        | 0.15 ± 0.04    | 0.11 ± 0.03    | 0.14 ± 0.05 |
| 1-Octanol             | 1.86 ± 0.25    | 0.00 ± 0.00    | 0.00 ± 0.00 |
| Phenethyl alcohol     | 28.84 ± 2.20   | 47.16 ± 11.42  | 25.95 ± 7.16 |
| 6-Octen-1-ol, 1,7-dimethyl- | 0.22 ± 0.06 | 0.17 ± 0.03 | 0.55 ± 0.03 |
| Decyl alcohol         | 0.13 ± 0.02    | 0.00 ± 0.00    | 0.06 ± 0.02 |
| 1-Pentadecanol        | 0.16 ± 0.06    | 0.07 ± 0.04    | 0.00 ± 0.00 |
| (±)-Dihydrafarnesol   | 0.15 ± 0.04    | 0.18 ± 0.05    | 0.21 ± 0.03 |
| **Subtotal**          | 31.62          | 47.79          | 26.60 |
| Acids                 |                |                |       |
| Octanoic acid         | 3.87 ± 0.63    | 0.00 ± 0.00    | 3.31 ± 0.82 |
| Nonanoic acid         | 0.37 ± 0.12    | 0.31 ± 0.30    | 1.51 ± 1.15 |
| Decanoic acid         | 1.60 ± 0.09    | 0.91 ± 0.10    | 4.79 ± 0.63 |
| Lauric acid           | 0.11 ± 0.05    | 0.18 ± 0.05    | 0.18 ± 0.07 |
| Tetradecanoic acid-1-13c | 0.09 ± 0.06 | 0.14 ± 0.08 | 0.00 ± 0.00 |
| Palmitic acid         | 2.15 ± 1.07    | 2.69 ± 0.60    | 0.00 ± 0.00 |
| Oleic acid            | 0.47 ± 0.10    | 1.50 ± 1.06    | 1.44 ± 1.24 |
| **Subtotal**          | 8.66           | 5.73           | 11.22 |
| Volatile furans and phenols |       |                |       |
| 3-Methyl-4-isopropylphenol | 0.17 ± 0.07 | 0.07 ± 0.05 | 0.26 ± 0.09 |
| 4-Hydroxy-3-methoxystyrene | 1.00 ± 0.70 | 0.46 ± 0.11 | 0.33 ± 0.06 |
| 2,6-Di-tert-butyl-4-methylphenol | 0.13 ± 0.02 | 0.14 ± 0.03 | 0.12 ± 0.04 |
| 2,4-Di-tert-butylphenol | 0.17 ± 0.05 | 0.18 ± 0.06 | 0.14 ± 0.08 |
| 3,4-Epoxytetrahydrofuran | 0.15 ± 0.08 | 0.24 ± 0.13 | 0.15 ± 0.00 |
| **Subtotal**          | 1.63           | 1.09           | 0.85 |
| Other compounds       |                |                |       |
| Furfural              | 1.66 ± 0.44    | 1.86 ± 0.69    | 2.55 ± 0.29 |
| Benzaldehyde          | 0.00 ± 0.00    | 0.27 ± 0.06    | 0.00 ± 0.00 |
| Phenylacetaldehyde    | 0.00 ± 0.00    | 0.09 ± 0.06    | 0.10 ± 0.03 |
| 1-Nonanal             | 0.23 ± 0.07    | 0.18 ± 0.09    | 0.00 ± 0.00 |
| 2-Methyltetrahydrothiophen-3-one | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.33 ± 0.07 |
| Acetophenone          | 0.24 ± 0.02    | 0.00 ± 0.00    | 0.00 ± 0.00 |
| 2-Undecanone          | 0.00 ± 0.00    | 0.00 ± 0.00    | 0.10 ± 0.06 |
| Damascenone           | 0.00 ± 0.00    | 0.14 ± 0.02    | 0.00 ± 0.00 |
| Bicyclo[2.2.1]hept-2-ene-1,7,7-trimethyl- | 0.05 ± 0.03 | 0.00 ± 0.00 | 0.15 ± 0.12 |
| Cyclodecane           | 0.00 ± 0.00    | 0.00 ± 0.00    | 0.12 ± 0.03 |
| **Subtotal**          | 2.19           | 2.54           | 3.35 |
each sample was reported in Figure 4 and Table 4. A total of 44 volatiles were detected for the three samples. Jiuqu fermented wine had higher alcohol content than other groups, which was constant with the results shown in Table 2, that Jiuqu fermented wine showed higher ethanol content. But for ethyl esters and acids content, Jiuqu fermented wine was significantly lower than S. cerevisiae EC1118 and Jiuqu + S. cerevisiae EC1118 groups. The results indicated that Jiuqu could help increase the ethanol production, but cannot increase the volatile profile of the final products. These results also confirmed in the mixed fermentation group, there were no significant differences between the wines produced by S. cerevisiae EC1118 alone and Jiuqu + S. cerevisiae EC1118.

3.4. Sensory evaluation

Friedman Test was carried out and the results of ranking are mentioned in Table 5. The results of the Friedman Test were F = 20.033, and \( F' = 20.372 \). According to the order of rank and rank sum of Friedman test approximate threshold table, the threshold of J, P, \( \alpha \), \( \alpha \) (15, 3, 0.05) was 6.400. The results showed significant difference in the three kinds of wines (\( F' > 6.400 \)). At the significance level of 5%, the least significant difference (LSD) was 10.37; however, the absolute value of each other could be drawn (\( p < 0.05 \)). Particularly, the sum rank of kiwi wine fermented by Jiuqu + S. cerevisiae EC1118 was 42.5, which was better than that of kiwi wine fermented by yeast S. cerevisiae EC1118 or Jiuqu alone (\( p < 0.05 \)). The contents of ethyl ester, organic acids, alcohol, volatile furan and phenol of kiwi wine fermented by S. cerevisiae EC1118 alone and that fermented by Jiuqu + S. cerevisiae EC1118 had no significant difference (Table 3). The results are in accordance with the previous study which stated that there was no significant difference in the total volatile flavors, but the sensory evaluation of kiwi wine produced by different fermentations had a significantly large difference (Velázquez et al., 2016). This phenomenon indicates that the quality of kiwi wines depends on multiple factors.

| Treatment (n = 3) | Expert | Jiuqu | Yeast EC1118 | Jiuqu + Yeast EC1118 | Rank sum |
|------------------|--------|-------|--------------|----------------------|----------|
| 1                | 2      | 1     | 3            | Jiuqu                | 6        |
| 2                | 3      | 1     | 2            | Yeast EC1118         | 6        |
| 3                | 2.5    | 1     | 2.5          | Jiuqu + Yeast EC1118 | 6        |
| 4                | 2      | 1     | 3            |                      | 6        |
| 5                | 2      | 1     | 3            |                      | 6        |
| 6                | 1      | 2     | 3            |                      | 6        |
| 7                | 2      | 1     | 3            |                      | 6        |
| 8                | 1      | 2     | 3            |                      | 6        |
| 9                | 3      | 1     | 2            |                      | 6        |
| 10               | 2      | 1     | 3            |                      | 6        |
| 11               | 2      | 1     | 3            |                      | 6        |
| 12               | 1      | 2     | 3            |                      | 6        |
| 13               | 2      | 1     | 3            |                      | 6        |
| 14               | 2      | 1     | 3            |                      | 6        |
| 15               | 2      | 1     | 3            |                      | 6        |
| Rank sum         | 29.5   | 18    | 42.5         | Jiuqu                | 90       |

1 – common, 2 – better and 3 – perfect.
1 – común, 2 – mejor y 3 – perfecto.

4. Conclusion

In this study, kiwi wine was produced in three different culture conditions S. cerevisiae EC1118, Jiuqu and Jiuqu + S. cerevisiae EC1118. Mixed fermentation showed high quality of the final products: it showed lower total organic acids but higher lactic acid content, lower methanol content and higher sensory quality. As S. cerevisiae fermented kiwi wine was not satisfying enough for consumers, the present study provided a promising strategy to improve the quality of kiwi wine by mixed fermentation of Jiuqu + S. cerevisiae EC1118. Even though Jiuqu is designed to ferment rice in China, and the microbial composition of Jiuqu is not fully understood yet, the results indicated that it is useful to utilize combinations of microorganisms to optimize the kiwi wine production. Therefore, it is of great value to explore the microbial combination which is suitable for kiwi wine in further studies.

Disclosure statement

No potential conflict of interest was reported by the authors.

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