The Influence of Yeast Strains on the Volatile Flavour Compounds of Chinese Rice Wine

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ABSTRACT


In order to assess the influence of yeast strains on volatile flavour profiles of Chinese rice wine, small-scale Chinese rice wine brewing was carried out with eight yeast strains from three different typical Chinese rice wine brewing regions. The volatile flavour compounds were extracted by headspace solid phase microextraction (HS-SPME) and analyzed by gas chromatography-mass spectrometry (GC-MS). The volatile flavour profiles of the different Chinese rice wines showed statistically significant differences depending on the yeast strains used. Yeast strains from the Shaoxing region showed a higher capacity to produce the esters 2-phenylethanol and 3-methylthiopropanol, while yeast strains from the Shanghai region stood out for their production of branched-chain higher alcohols. Chinese rice wine fermented with a yeast strain from the Jiangsu region had the highest levels of organic acids. Using principal component analysis of the Chinese rice wine volatile flavour compounds, the eight yeast strains could be classified into three groups according to their origins. This is the first report about the volatile flavour profiles of Chinese rice wine, small-scale Chinese rice wine brewing are the use of wheat

INTRODUCTION

Yeast has been used to produce alcoholic beverages, including wine, beer and Chinese rice wine, since ancient times. Chinese rice wine has been a traditional fermented alcoholic beverage of Chinese for more than five thousand years and now it is one of the most popular alcoholic beverages in China with an annual consumption of about 2.4 billion litres in 2008. Chinese rice wine is typically fermented from glutinous rice with wheat Qu and yeast (Saccharomyces cerevisiae). The characteristic features in Chinese rice wine brewing are the use of wheat Qu as a source of saccharification enzymes, and parallel fermentation by yeast in a semi-solid state. In a Chinese rice wine fermentation mash, glucose liberated from rice starch is fermented successively by yeast. “Parallel fermentation” refers to the combination of progressive saccharification of starch and alcoholic fermentation. This process avoids yeast cell exposure to high concentrations of sugar, and contributes to the high ethanol production, which can be over 20% (v/v) in the final fermentation mash. The quality of Chinese rice wine depends mainly on the raw materials, yeast strains, brewing conditions and maturation conditions. Because of the particular brewing process, Chinese rice wine presents peculiar sensory characteristics, and it is easy to distinguish Chinese rice wines from different regions by their sensory characteristics.

Saccharomyces cerevisiae is the dominant microorganism in Chinese rice wine fermentations. The quality of Chinese rice wine is closely related to the yeast strains used in the fermentation. Besides the main production of ethanol and CO₂, yeast produce many flavour compounds during fermentation and these greatly influence the aroma and taste characteristics of Chinese rice wine. Studies on fermented alcoholic beverages indicate that the dominant and major compounds (higher alcohols, fatty acids, acetates, ethyl esters, ketones and aldehydes) which contribute to flavour are formed during yeast fermentation. The literature on this topic shows that different yeast strains have a large influence on volatile compound production. The fermented alcoholic beverage industry is very interested in yeast strains that produce a unique flavour, and there is more and more research ongoing on yeast flavour characteristics and on the selection of yeast strains yielding excellent flavours.

In the Chinese rice wine industry, different manufacturers generally use different yeast strains, which results in significant sensory differences among the Chinese rice wines from different manufacturers, but no research on the volatile flavour characteristics of Chinese rice wine yeast has been carried out to date. In order to select the best yeast strains to brew the desired Chinese rice wine, it is important to study the influence of the different yeast strains on volatile flavour compound production in Chinese rice wine fermentation. The objective in this study was to investigate the effect of different strains of S. cerevisiae on the volatile flavour composition of Chinese rice wine.

MATERIALS AND METHODS

Chemicals

All volatile compound standards such as alcohols: 2-methylpropanol (98%), 1-butanol (99%), 2-methylbutanol (99%), 3-methylbutanol (99%), 1-pentanol (98%), 1-hexanol (99%), 1-heptanol (98%), 2-phenylethanol (98%);
Yeast strains used as solvents.

Pure Milli-Q water (Millipore Co., Bedford, USA) were supplied by China National Pharmaceutical Group Corp. (Shanghai, China). Used to adjust pH were supplied by China National Pharmaceutical Ground Corp. (Shanghai, China). Sodium hydroxide and hydrochloric acid were supplied by Sigma-Aldrich China Co. (Shanghai, China). Sodium chloride used to control the ionic strength, and lactic acid used to adjust pH were supplied by China National Pharmaceutical Ground Corp. (Shanghai, China).

Yeast strains

Eight different yeast strains (identified as S. cerevisiae) were used in this work: strains JF and JF3 were supplied by the Jinfeng Wine Company in Shanghai city; strains SX, GS1 and GJ1 were supplied by the Guyuelongshan wine company in Shaoxing Zhejiang province; strains ZL1 and ZLJY were supplied by the Zhongliang wine company in Shaoxing Zhejiang province; the SY4 strain was supplied by Sigma-Aldrich China Co. (Shanghai, China). Sodium chloride was used as a solvent.

Small-scale Chinese rice wine brewing

Yeast strains were pre-cultured in 10 mL YPD medium at 30°C for 24 h. The pre-cultured yeast was added to 300 mL YPD medium and cultured at 30°C for 24 h. The cells were harvested by centrifugation and then mixed with 1,500 g steamed glutinous rice, 160 g wheat Qu (supplied by the Jinfeng Wine Company, Shanghai, China) and 2 L water in a 5 L flask. The pH was adjusted to 4.0 with 90% lactic acid (China National Pharmaceutical Ground Corp. Shanghai, China). The pitching rate for each yeast strain was 2 × 10^7 cells/mL. Because some of the materials used in Chinese rice wine brewing are not sterile, higher pitching rates are traditionally used to ensure that the inoculated yeast strain is the dominant strain and finishes fermentation. wheat Qu is a source of many enzymes such as amylase, glucoamylase and protease. Raw materials are degraded by these enzymes, producing nutritional components for yeast growth and fermentation. Lactic acid is used to prevent bacterial growth, since the fermentation set-up for Chinese rice wine brewing is not sterile. The mash was incubated at 30°C under stable condition for 5 days and post fermentation was carried out at 15°C for 15 days. After post fermentation, the mash was centrifuged and the supernatant (Chinese rice wine) was analysed.

Analysis of enological parameters

In order to compare the effect of different yeasts on Chinese rice wine brewing, several enological parameters were determined after fermentation. The pH was measured with a pH meter; ethanol content was measured by densitometry at 20°C after distillation; residual sugar was determined according to the Fehling method and total acid was determined by titration with 0.1N NaOH (phenolphthalein as indicator) and expressed as lactic acid (g/L); and glycerol was determined by HPLC according to the method described by Kelebek.

Analysis of volatile flavour compounds

For the rapid determination of volatile flavour compounds in Chinese rice wines, a headspace solid phase microextraction (HS-SPME) technique was used to extract the flavour compounds. Gas chromatography mass spectrometry (GC-MS) was used for separation and quantitative analysis.

SPME extraction procedure

The 50/30 μm divinylbenzene/carboxen/poly (dimethylsilyloxane) (DVB/CAR/PDMS) coated fibre (Supelco, Inc., Bellefonte, PA, USA) was used for volatile compound extraction. HS-SPME extraction was performed according to the method previously described with minor modifications. Each Chinese rice wine sample was diluted with deionised water to a final concentration of 6% (v/v) ethanol, and the total 8 mL of diluted sample, 5 μL of internal standard (2-octanol, 40.34 μg/L; octyl propionate, 25.12 μg/L) and 2.5 g sodium chloride were transferred into a 20 mL vial and hermetically sealed with a PTFE-faced silicone septum. This sample was equilibrated at 50°C for 5 min and extracted for 45 min at the same temperature under stirring in a multipurpose sampler with SPME capability (MPS 2, Gerstel, Germany). After extraction, the fibre was inserted into the injection port of a GC (250°C) for 5 min to desorb the analytes. All analyses were carried out in triplicate.

GC-MS analysis

The analyses were performed using an Agilent 6890 GC equipped with an Agilent 5975N mass selective detector (MSD). The target analytes were separated using a DB-FFAP column (60 m length, 0.25 mm i.d., 0.25 μm film thickness, Agilent, CA, USA). The injector was in splitless mode at 250°C. The oven temperature was kept at 50°C for 2 min, and then increased to 230°C at 5°C/min and kept at 230°C for 10 min. The carrier gas was helium with a flow-rate of 2 mL/min. All mass spectra were acquired in electron impact (EI) mode at 70 eV, using full scan with a scan range of 35–550 amu. Identification of all compounds was confirmed by mass spectra and retention indices with those standards. The compounds were quantified in selected ion monitoring (SIM) mode by selecting for each compound their most characteristic ion. In order to eliminate variations in extraction efficiency caused by small differences in the sample matrix, internal standardization using 2-octanol and octyl propionate as the internal standards were applied to quantify the analytes.

Preparation of standards and SPME fibre calibration

Single standard stock solutions of the volatile flavour compounds were prepared by dissolving each compound in absolute ethanol (Sigma-Aldrich, China). Working solutions were made from the stock solutions by spiking and mixing.
mixing them with a model Chinese rice wine which was a hydro-alcohol solution (6% v/v) with the pH adjusted to 4.0 with lactic acid. The concentrations of the flavour compounds in the working solution were as follows: 2-methylpropanoic acid (77,148.59 µg/L), 1-butanol (10,536.12 µg/L), 2-methylbutanol (153,561.23 µg/L), 3-methylbutanol (229,701.54 µg/L), 1-pentanol (1,237,268 µg/L), 1-hexanol (14,568.50 µg/L), 1-heptanol (591.40 µg/L), 2-phenylethanol (253,779.95 µg/L), ethyl acetate (127,226.06 µg/L), ethyl propanoate (1,645.27 µg/L), ethyl 2-methylpropanoate (920.12 µg/L), isobutyric acid (1,134.45 µg/L), ethyl pentanoate (1,200 µg/L), ethyl hexanoate (1,134.60 µg/L), ethyl octanoate (585.08 µg/L), ethyl decanoate (1,537.23 µg/L), diethyl succinate (10,959.49 µg/L), ethyl benzoate (416.45 µg/L), ethyl 2-phenylacetate (936.98 µg/L), 2-phenylacetdehyde (482.15 µg/L), acetic acid (2,207,867.80 µg/L), propanoic acid (16,088.11 µg/L), 2-methylpropanoic acid (15,686.54 µg/L), butanoic acid (21,415.34 µg/L), 3-methylbutanoic acid (13,987.87 µg/L), pentanoic acid (930.87 µg/L), hexanoic acid (3,670.86 µg/L), octanoic acid (1,271.07 µg/L), 2-phenylacetaldheyde (906.43 µg/L), 3-methylthiopropanol (59,989.93 µg/L), γ-nonalactone (508.10 µg/L). This solution was used as the highest concentration calibration standard, and ten more calibration solutions were prepared by serially diluting the working solution by twofold with the model Chinese rice wine.

Statistical analysis

The SPSS 15.0 software (SPSS Inc., Chicago, Illinois, USA) was used for the statistical analysis of the volatile flavour compounds and the enological parameters. Statistical differences among the Chinese rice wines fermented with different yeast strains were determined using the one way ANOVA analysis. For all the statistical analyses performed, differences were considered significant at \( p \leq 0.05 \). Factor analysis was performed to reduce the dimensionality of flavour compound data and to identify underlying variables.

RESULTS AND DISCUSSION

The difference in the enological characteristics of the yeast strains

The enological parameters of the eight Chinese rice wines are shown in Table I. Results were within the normal range of values expected. During the process of Chinese rice wine brewing, rice starch was saccharified by enzymes produced by wheat Qu, and the resultant glucose was fermented to ethanol by the yeast. This process allowed for a highly condensed mash without accumulation of high concentrations of sugar, which can inhibit yeast cell growth and ethanol fermentation. The ethanol content of the Chinese rice wine samples was between 16.2 to 17.1% (v/v) and the highest ethanol yield was obtained with strains ZL1 and JF. The total acidity was relatively low in all of the Chinese rice wines (between 3.69 and 5.19 g/L). Strain ZLJY showed the lowest levels of ethanol and the highest levels of glycerol.

Volatile flavour compounds in Chinese rice wines

Flavour compounds play an important role in the typical odour and taste of Chinese rice wine. These compounds are mainly the result of yeast metabolism during fermentation, although some are derived from the raw materials and maturation. Higher alcohols, esters and volatile acids are the major flavour compounds in Chinese rice wine, whereas some carbonyl compounds, sulphur compounds, phenols, lactones, furans and nitrogen-containing compounds are present at lower concentrations. The concentrations of the volatile flavour compounds of Chinese rice wine produced during fermentation are shown in Table II. The odour activity values (OAVs) of the volatile flavour compounds in different Chinese rice wines are shown in Table III. The differences observed in the volatile composition of the Chinese rice wines obtained from the different yeast strains appeared to be quantitative, rather than qualitative. The concentrations of most volatile compounds were significantly influenced by the identity of the inoculated yeast strain, due to the metabolic differences between strains.

The concentration of higher alcohols in the Chinese rice wine samples represented important variables for the differentiation of yeast strains. The amount of total higher alcohols varied from 376,787 µg/L (GJ1 strain) to 423,921 µg/L (GS1 strain). The major aroma compounds in Chinese rice wine are 2-methylpropanol, 3-methylbutanol and 2-phenylethyl alcohol. These showed odour activity in all Chinese rice wine samples analysed (Table III) suggesting that these three higher alcohols contribute to the overall sensory complexity of the Chinese rice wines. Branched-chain higher alcohols, including 2-methylpropanol, 2-methylbutanol and 3-methylbutanol, were the major aliphatic alcohols and these can be produced directly from sugar fermentation or through the catabolism of amino acids. The concentrations of amino acids (the precursors for higher alcohols), yeast strain identity, fermentation temperature and pH have a large effect on the concentration of higher alcohols in the final product. The concentrations of 2-methylbutanol and 3-

Table I. Enological parameters of Chinese rice wines produced by different yeast strains.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>JF</th>
<th>JF3</th>
<th>SX</th>
<th>GS1</th>
<th>GJ1</th>
<th>SY4</th>
<th>ZL1</th>
<th>ZLJY</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>4.26</td>
<td>4.16</td>
<td>4.54</td>
<td>4.34</td>
<td>4.53</td>
<td>4.32</td>
<td>4.45</td>
<td>4.15</td>
</tr>
<tr>
<td>Ethanol (% Vol)</td>
<td>17.1</td>
<td>16.3</td>
<td>16.4</td>
<td>16.8</td>
<td>16.9</td>
<td>16.7</td>
<td>17.1</td>
<td>16.2</td>
</tr>
<tr>
<td>Residual sugar (g/L)</td>
<td>4.28</td>
<td>5.07</td>
<td>4.80</td>
<td>4.43</td>
<td>4.56</td>
<td>5.02</td>
<td>3.92</td>
<td>6.28</td>
</tr>
<tr>
<td>Total acid (g/L)a</td>
<td>4.80</td>
<td>4.58</td>
<td>3.80</td>
<td>4.21</td>
<td>3.97</td>
<td>5.19</td>
<td>4.10</td>
<td>4.98</td>
</tr>
<tr>
<td>Glycerol (g/L)</td>
<td>4.52</td>
<td>5.42</td>
<td>5.35</td>
<td>5.33</td>
<td>5.62</td>
<td>5.98</td>
<td>4.72</td>
<td>5.96</td>
</tr>
</tbody>
</table>

a g/L as lactic acid.
## Table II. Volatile flavour compound concentrations (µg/L) found in Chinese rice wine fermented by different yeast strains\(^a\).

<table>
<thead>
<tr>
<th>Yeast strains</th>
<th>(2\text{-Methylpropanol})</th>
<th>(1\text{-Butanol})</th>
<th>(2\text{-Methylbutanol})</th>
<th>(3\text{-Methylbutanol})</th>
<th>(1\text{-Pentanol})</th>
<th>(1\text{-Hexanol})</th>
<th>(1\text{-Heptanol})</th>
<th>(2\text{-Phenylethanol})</th>
<th>(\sum\text{esters})</th>
<th>(\sum\text{organic acids})</th>
</tr>
</thead>
<tbody>
<tr>
<td>JF</td>
<td>95331</td>
<td>89074</td>
<td>85756</td>
<td>70468</td>
<td>81501</td>
<td>89074</td>
<td>85756</td>
<td>70468</td>
<td>81501</td>
<td>89074</td>
</tr>
<tr>
<td>JF3</td>
<td>8743</td>
<td>8917</td>
<td>5275</td>
<td>5880</td>
<td>6051</td>
<td>6665</td>
<td>6051</td>
<td>5880</td>
<td>6051</td>
<td>6665</td>
</tr>
<tr>
<td>SX</td>
<td>28400</td>
<td>26014</td>
<td>24600</td>
<td>23882</td>
<td>21520</td>
<td>23374</td>
<td>21520</td>
<td>23882</td>
<td>21520</td>
<td>23374</td>
</tr>
<tr>
<td>GS1</td>
<td>181924</td>
<td>195319</td>
<td>151486</td>
<td>170001</td>
<td>143258</td>
<td>164377</td>
<td>143258</td>
<td>170001</td>
<td>143258</td>
<td>164377</td>
</tr>
<tr>
<td>GJ1</td>
<td>261</td>
<td>248</td>
<td>348</td>
<td>429</td>
<td>365</td>
<td>311</td>
<td>365</td>
<td>429</td>
<td>365</td>
<td>311</td>
</tr>
<tr>
<td>SY4</td>
<td>769</td>
<td>724</td>
<td>1087</td>
<td>1078</td>
<td>929</td>
<td>928</td>
<td>929</td>
<td>1078</td>
<td>928</td>
<td>928</td>
</tr>
<tr>
<td>ZL1</td>
<td>82</td>
<td>91</td>
<td>61</td>
<td>62</td>
<td>77</td>
<td>65</td>
<td>62</td>
<td>77</td>
<td>65</td>
<td>62</td>
</tr>
<tr>
<td>ZLJY</td>
<td>83403</td>
<td>97427</td>
<td>139271</td>
<td>152120</td>
<td>123086</td>
<td>96991</td>
<td>132301</td>
<td>121965</td>
<td>123086</td>
<td>96991</td>
</tr>
</tbody>
</table>

*ns*: Not significantly different; *, **, *** denotes significance at \(p < 0.05, p < 0.01, p < 0.001\), respectively.

\(a\) The RSD values in the quantification of flavour compounds did not exceed ±10%.

\(b\) \(\sum\text{higher alcohols}\) refers to the sum of all higher alcohols.

\(\sum\text{esters}\) refers to the sum of all esters.

\(\sum\text{organic acids}\) refers to the sum of all organic acids.

## Table III. Odour activity values (OAVs) of some volatile flavour compounds (OAV\(>1\)) in different Chinese rice wines.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Aroma</th>
<th>Threshold(^a) (µg/L)</th>
<th>JF</th>
<th>JF3</th>
<th>SX</th>
<th>GS1</th>
<th>GJ1</th>
<th>SY4</th>
<th>ZL1</th>
<th>ZLJY</th>
</tr>
</thead>
<tbody>
<tr>
<td>Higher alcohols</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2-Methylpropanol</td>
<td>fusel, spirituous</td>
<td>40,000(^{[9]})</td>
<td>2.4</td>
<td>2.2</td>
<td>2.1</td>
<td>1.8</td>
<td>2.0</td>
<td>2.1</td>
<td>1.9</td>
<td>2.0</td>
</tr>
<tr>
<td>3-Methylbutanol</td>
<td>harsh, nail polish</td>
<td>30,000(^{[9]})</td>
<td>6.1</td>
<td>6.5</td>
<td>5.0</td>
<td>5.7</td>
<td>4.8</td>
<td>5.5</td>
<td>4.8</td>
<td>5.1</td>
</tr>
<tr>
<td>2-Phenylalcohol</td>
<td>floral, rose</td>
<td>14,000(^{[4]})</td>
<td>6.0</td>
<td>7.0</td>
<td>9.9</td>
<td>10.9</td>
<td>8.8</td>
<td>6.9</td>
<td>9.5</td>
<td>8.7</td>
</tr>
<tr>
<td>Ester</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>fruity, solvent-like</td>
<td>7,500(^{[9]})</td>
<td>1.4</td>
<td>1.2</td>
<td>2.3</td>
<td>2.8</td>
<td>1.6</td>
<td>1.7</td>
<td>1.9</td>
<td>2.4</td>
</tr>
<tr>
<td>3-Methylbutyl acetate</td>
<td>pear, banana</td>
<td>30(^{[9]})</td>
<td>3.4</td>
<td>2.9</td>
<td>2.0</td>
<td>2.9</td>
<td>1.9</td>
<td>2.2</td>
<td>1.8</td>
<td>2.2</td>
</tr>
<tr>
<td>Ethyl butanoate</td>
<td>floral, fruity</td>
<td>20(^{[4]})</td>
<td>4.5</td>
<td>5.1</td>
<td>9.2</td>
<td>15.7</td>
<td>8.3</td>
<td>8.4</td>
<td>8.4</td>
<td>12.3</td>
</tr>
<tr>
<td>Ethyl hexanoate</td>
<td>apple</td>
<td>14(^{[4]})</td>
<td>6.8</td>
<td>8.2</td>
<td>9.6</td>
<td>11.4</td>
<td>10.3</td>
<td>10.5</td>
<td>10.0</td>
<td>10.0</td>
</tr>
<tr>
<td>Ethyl octanoate</td>
<td>sweet, soap</td>
<td>5(^{[4]})</td>
<td>12.5</td>
<td>10.0</td>
<td>13.0</td>
<td>9.2</td>
<td>11.0</td>
<td>11.9</td>
<td>7.4</td>
<td>8.5</td>
</tr>
<tr>
<td>Ethyl decanoate</td>
<td>floral, rose</td>
<td>200(^{[4]})</td>
<td>4.0</td>
<td>4.2</td>
<td>7.5</td>
<td>5.4</td>
<td>7.1</td>
<td>6.0</td>
<td>6.3</td>
<td>6.8</td>
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<tr>
<td>Organic acids</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acetic acid</td>
<td>vinegar</td>
<td>200,000(^{[9]})</td>
<td>1.6</td>
<td>1.6</td>
<td>1.4</td>
<td>1.3</td>
<td>1.3</td>
<td>1.5</td>
<td>2.0</td>
<td>1.2</td>
</tr>
<tr>
<td>2-Methylpropanoic acid</td>
<td>acidic, rancid</td>
<td>2,300(^{[4]})</td>
<td>2.1</td>
<td>2.0</td>
<td>1.6</td>
<td>1.5</td>
<td>1.7</td>
<td>1.7</td>
<td>1.4</td>
<td>1.6</td>
</tr>
<tr>
<td>Butanoic acid</td>
<td>rancid, cheesy</td>
<td>173(^{[4]})</td>
<td>5.7</td>
<td>6.8</td>
<td>9.4</td>
<td>15.9</td>
<td>8.3</td>
<td>9.6</td>
<td>11.5</td>
<td>13.2</td>
</tr>
<tr>
<td>3-Methylbutyric acid</td>
<td>rancid, acidic</td>
<td>33.4(^{[4]})</td>
<td>30.7</td>
<td>28.6</td>
<td>27.2</td>
<td>29.2</td>
<td>24.3</td>
<td>25.3</td>
<td>27.5</td>
<td>34.0</td>
</tr>
<tr>
<td>Hexanoic acid</td>
<td>sour, cheese</td>
<td>420(^{[4]})</td>
<td>2.0</td>
<td>1.9</td>
<td>2.4</td>
<td>2.0</td>
<td>2.6</td>
<td>3.4</td>
<td>2.2</td>
<td>2.1</td>
</tr>
<tr>
<td>Others</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phenylacetaldehyde</td>
<td>floral, rose</td>
<td>1(^{[2]})</td>
<td>35.7</td>
<td>23.7</td>
<td>47.1</td>
<td>49.8</td>
<td>42.8</td>
<td>56.3</td>
<td>34.8</td>
<td>44.8</td>
</tr>
<tr>
<td>3-Methylthiopropionate</td>
<td>cabbage</td>
<td>1,000(^{[9]})</td>
<td>6.5</td>
<td>7.1</td>
<td>12.7</td>
<td>23.4</td>
<td>16.8</td>
<td>10.7</td>
<td>18.5</td>
<td>15.5</td>
</tr>
<tr>
<td>γ-Nonalactone</td>
<td>sweet, coconut</td>
<td>30(^{[4]})</td>
<td>2.9</td>
<td>2.7</td>
<td>4.3</td>
<td>6.4</td>
<td>4.6</td>
<td>5.8</td>
<td>5.4</td>
<td>5.4</td>
</tr>
</tbody>
</table>

\(a\) References from which the threshold values have been taken are given in brackets. In [2] the matrix was a 10% water/ethanol solution containing 5 g/L of tartaric acid at pH 3.2. In [4] the matrix was an 11% water/ethanol solution containing 7 g/L glycerol and 5 g/L tartaric acid at pH 3.2. In [9] the matrix was a 10% water/ethanol solution. In [18] the matrix was 10% water/ethanol solution containing a condensate of Chenin Blanc wine.

\(b\) OAV calculated as concentration/threshold.
methylbutanol were significantly higher in the Chinese rice wines fermented by strains JF and JF3 from the Shanghai region (Table II) and this result was in accordance with the previous studies carried out on Chinese rice wines from different regions13.

It is worth mentioning that 2-phenylethyl alcohol, a compound with a rose-honey-like flavour, is an important compound in Chinese rice wine24. The national standard
demand for sugar by
in the fermentation medium, by yeast during amino acid
production from the conversion of 2-phenylethyl alcohol in Chinese rice wine24, and
gave high values for ethyl de-
pounds. Chinese rice wine samples fermented with strains JF showed the lowest concentrations of these two com-
pounds.

Ethyl esters are the largest group of flavour compounds and affect the quality of fermented beverages due to their low threshold concentrations and desirable fruity aromas. Most esters found in alcoholic beverages are secondary metabolites produced by yeast during fermentation. A total of 16 esters were identified in the Chinese rice wine samples. Among them, only four acetates (ethyl acetate, isobutyl acetate, isoamyl acetate and 2-phenylethyl acetate) were detected. Ethyl acetate was found at the highest level, ranging from 8,820 µg/L (JF3 strain) to 20,818 µg/L (GS1 strain). The Chinese rice wines fermented with strains GS1, SX and ZLJY had high concentrations of ethyl acetate. Conversely, strains JF and JF3 from Shanghai region produced the lowest levels of ethyl acetate. The concentration of 3-methylbutyl acetate, typically described as a ‘banana, pear’ flavour, was higher in the samples fermented by strain JF than those fer-
mented by other strains, with a maximum value of 101 µg/L. Because of its low concentration, 3-
methylbutyl acetate showed odour activity in all of the Chinese rice wine samples and contributed to the fruity aroma of the Chinese rice wine.

Ethyl esters are the largest group of flavour compounds found in Chinese rice wine, and they produce a fruity and flower aroma in fresh Chinese rice wine. The medium chain fatty acids esters (C4–C6) are the most important group in this respect and their concentrations were found to be above the threshold level in all of the Chinese rice samples. Chinese rice wine samples fermented with strain GS1 showed the highest amounts of ethyl butanoate and ethyl hexanoate, whereas samples fermented with strain JF showed the lowest concentrations of these two compounds. Chinese rice wine samples fermented with strains GJ1 and ZLJY1 gave the highest values for ethyl decanoate. These values were twice as high as the minimum concentration obtained with strain JF. The high odour activity values (OAV) of ethyl butanoate, ethyl hexanoate, ethyl octanoate and ethyl decanoate, in all the Chinese rice wine samples, confirmed the importance of these compounds in Chinese rice wine.

The yeast strains SX, GS1 and ZLJY (all from the Shaoxing region) showed the greatest capacity for the synthesis of esters, with a mean concentration of about 21,000 µg/L. Chinese rice wines obtained using yeast strains JF and JF3 from the Shanghai region, gave the lowest values for the esters, mostly due to the low concentration of ethyl acetate.

Volatile acids are important to the flavour and taste characteristics of Chinese rice wine. The results showed that the concentration of volatile acids in Chinese rice wines analyzed in this study was dependant on the inoculated yeast strain (Table II). Acetic acid was the most important volatile acid and constituted over 90% of the total volatile acids. Samples fermented by strain SY4 showed the largest amount of acetic acid, with a maximum concentration of 391,018 µg/L. The strains GS1 and ZLJY were noted for their high level of butanoic acid, while strain SY4 produced the highest concentrations of 3-methylbutanoic acid and hexanoic acid.

It is worth mentioning that the concentration of 3-methylthiopropanol (methionol) is much higher in Chinese rice wines (13,906 µg/L in average) than in other alcoholic beverages such as wine and beer. Although 3-methylthiopropanol is usually described as an off-flavour in wines16, it is an important flavour compound in soy sauce1. Since Chinese rice wine has some of the flavour characteristics of soy sauce, 3-methylthiopropanol may contribute to the special flavour characteristics of Chinese rice wine. Methionine can be metabolized by yeast to form 3-methylthiopropanol through the Ehrlich pathway16. Yeast strains differed in their ability to produce 3-methylthiopropanol and the strains from the Shaoxing region showed a higher capacity to produce 3-methylthiopropanol than the strains from other regions (Table II).

Statistical analysis of volatile flavour compounds

Factor analysis using the principal component method is shown in Fig. 1. Yeast volatile fermentation compounds enabled good discrimination between Chinese rice wines inoculated with different yeast strains (Fig. 1). When all the flavour compounds were included, the first principal component (PC1) explained 53% of the total variation, and PC2 explained 38% of the total variation. Of these, the first two principal components were statistically significant in explaining the difference among the yeast strains.

Positive loading of PC1 was related to 1-hexanol, 1-
-pentanol, 2-phenylethyl alcohol, ethyl decanoate and 3-
methylthiopropanol, whereas 1-butanol, 1-heptanol, 3-
methylbutanol and 2-methylpropionic acid showed the negative loadings. For PC2, loadings were characterized by hexanoic acid, acetic acid, 3-methylbutanoic acid and pentanoic acid with positive values, whereas isobutyl acetate and 3-methylbutyl acetate showed negative values. The PCA showed a good separation of the different Chinese rice wines and three groups were clearly defined.

Chinese rice wines fermented with yeast strains from the Shaoxing region formed a clear group, which was associated with most of the esters, 2-phenylethyl alcohol and 3-
methylthiopropanol. Chinese rice wines fermented with yeast strains from Shanghai city formed another group, associated with 1-butanol, 1-pentanol, 3-methylbutanol and 2-methylpropionic acid. Chinese rice wine samples inoculated with yeast strains from the Jiangsu region were...
Fig. 1. (a) Principal component analysis (PCA) of volatile flavour compounds in Chinese rice wines fermented by different yeast strains. Score plot for the first two principal components. Group 1: yeast strains from Zhejiang province, Group 2: yeast strains from Shanghai city, Group 3: yeast strain from Jiangsu province. (b) PCA loadings plot. C1, 2-methylpropanol; C2, 1-butanol; C3, 2-methylbutanol; C4, 3-methylbutanol; C5, 1-pentanol; C6, 1-hexanol; C7, 1-heptanol; C8, 2-phenylethanol; C9, ethyl acetate; C10, isobutyrl acetate; C11, 3-methylbutyl acetate; C12, ethyl propanoate; C13, ethyl 2-methylpropanoate; C14, ethyl butanoate; C15, ethyl pentanoate; C16, ethyl hexanoate; C17, ethyl octanoate; C18, ethyl decanoate; C19, diethyl succinate; C20, ethyl benzoate; C21, 2-phenyl-ethyl acetate; C22, ethyl 2-phenylacetate; C23, acetic acid; C24, propanic acid; C25, 2-methylpropanoic acid; C26, butanoic acid; C27, 3-methylbutanoic acid; C28, pentanoic acid; C29, hexanoic acid; C30, octanoic acid; C31, phenylacetaldehyde; C32, 3-methylthiopropanol; C33, γ-nonalactone.
located in a different quadrant of the PCA plot and were largely associated with most of the acids.

CONCLUSIONS

In conclusion, the data obtained in this study showed how the yeast strains affected the final chemical and volatile flavour composition of Chinese rice wine. The differences obtained were quantitative, rather than qualitative, and the inoculated yeast strain played an important role in the volatile aroma composition of the final Chinese rice wine.

Strains SX, GS1, and ZLY produced the largest amounts of esters. These esters are favourable for the fruity aroma of Chinese rice wine. Strain GS1 strain was also noted for high levels of 2-phenylethanol and γ-nonalactone, both of which are considered to contribute to Chinese rice wine flavour. Strains JF and JF3 from the Shanghai region showed the highest capacity for producing higher alcohols and this result was in accordance with the highest levels of higher alcohols detected in the Chinese rice wine from the Shanghai region. Strain SY4 showed the highest level of organic acids, especially acetic acid, which can exert a negative effect on Chinese rice wine at high concentrations. The PCA analysis clearly showed a differentiation in flavour characteristics due to different yeast strains from different regions. This research has provided a chemical basis for the regional character of Chinese rice wine.

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