Research Article

The Key Technology for the Pilot-Scale Production of Chinese Commercial Za Wine

Kaizheng Zhang, Wei Zou and Wenxue Zhang

1College of Bioengineering, Sichuan University of Science and Engineering, Zigong, 643000, 2Department of Food Engineering, College of Light Industry and Food Engineering, Sichuan University, Chengdu, 610065, China

Abstract: The study aimed to explore the key technology for the pilot-scale production of Commercial Za Wine (CZW), including processing methods of the raw materials, screening fermentation starters and yeast and other brewing technics about CZW. As an ancient low-alcohol drink, Za wine is very popular in the Qiang ethnic minority areas of China. In order to expand the production of Traditional Za Wine (TZW) to meet the increasing demands of consumers and tourists, it is necessary to industrialize the production of Qiang Za wine. Hence the key techniques for the pilot-scale production of CZW were explored and the results are as follows: hull-less barley (the main raw material) was soaked in water at 60°C for 12 h then crushed to plum petal shapes for saccharification. The koji should be rice koji made from A. flavus Q1 and Rhizopus Q303. The LI8 and YW yeasts were the best choice for brewing the wine. For the production of CZW with special requirements on flavour ingredients, an appropriate amount of aroma yeast LII 1-1 could be added. In addition, for producing the semi-sweet CZW, the concentrated saccharification liquid mix from hull-less barley and glutinous rice can be used during the postfermentation period. CZW from the pilot test was yellow and transparent, possessed the typical characteristics of TZW, had higher alcohol content, liquor yield, better wine appearance and circulation than those of TZW. Meanwhile, CZW had reached the Grade one standard for semi-sweet fermented wine of the Chinese Yellow Wine National Standard. This study initiates the research for the key producing technology of CZW.

Keywords: Commercial Za wine, hull-less barley, pilot-scale production, rice koji, Traditional Za wine, yeast

INTRODUCTION

Za wine is a unique drink made by minority ethnic groups living in southwestern China, which is similar to Japanese Sake. Qiang Za wine is a typical Za wine with a unique flavour, nutritionally rich, smooth with sweet and sour flavours and a mellow aroma, which has been very popular with the local villagers (Ying, 2007; Jie, 2007; Ying and Ming-Yu, 2009). Generally, the traditional brewing of Qiang Za Wine (TZW) is fermented from cooked whole hull-less barley in the solid-state, with xiao qu (farm koji), for 2 weeks or longer until the wine is mature. Then hollow bamboos are inserted into the jar so that people can suck up and drink the wine together while warm water is gradually added. Drinking stops when the wine becomes diluted and loses its taste (Ning, 2003; Yunyan, 2004; Song, 2007; Zhang et al., 2013). Therefore, the brewing process for TZW is to suck out the wine after solid-state fermentation typically using an ordinary starter of farm koji. However, feeding the jars with whole grain often causes poor fermentation, a low utilization rate of the raw material and low efficiency for wine production. TZW is usually brewed at home for the family to drink with occasionally a small amount left to sell, but the scale of production is small and uses out-of-date processing equipment. To meet the needs of a greater number of visiting tourists and consumers and to promote the economic development of the Qiang and Tibetan ethnic areas, it is therefore necessary to industrialize the brewing of TZW to produce Commercial Za wine (CZW). Pilot testing is an important bridge between laboratory research and industrial production and essential for the industrialization process. After piloting these scientific and technological projects, their success rate for industrialization can be up to 80%.

Therefore, based on the traditional brewing process, but using the concepts of modern fermentation technology and processes, we aim to investigate how to optimize materials handling, fermentation starters and yeast screening during the pilot-scale production of
CZW. Small-scale and pilot tests will then be used to help understand the key technology for the pilot production of CZW.

**MATERIALS AND METHODS**

**Materials:** Hull-less barley with a water content of 12-13% was purchased in Aba (Sichuan Province, China) and commercially-available glutinous rice in Chengdu, (Sichuan, China).

**Strains:**
- QJ (an *Aspergillus flavus* strain), ZZH (an *Aspergillus albicans* strain) and QZ (a *Rhizopus* strain) were bred and stored by the Food Science and Biotechnology Laboratory of Sichuan University and characterized by relevant agencies for their suitability in the fermentation industry. Q303 *Rhizopus*, originally preserved in the Southwest Center of Industrial Culture Collection (SICC) was obtained from the Guizhou Light Industry Research Institute, China.
- *Saccharomyces cerevisiae* 1300 and *Candida pelliculosa* LI8 and LIH 1-1 were bred and stored by the Food Science and Biotechnology Laboratory of Sichuan University; YW-Yeast (Yellow wine yeast) was purchased from the Hubei Angel Yeast Co. (Hubei, China).

**Main instruments and equipment:** The following equipment was used: a PHS-3C pH meter (Shanghai Kangyi Instrument Co. Ltd., Shanghai, China); a stainless steel coarse filtration press (made at our laboratory); a positive pressure bucket filter (Zhejiang Kangyi Instrument Co. Ltd., Shanghai, China); a household roller press machine (SeagullTR, China); a stainless steel coarse filtration press (made at our laboratory); a 400 L stainless steel temperature-controlled fermentation tank (made at our laboratory); a 100 L stainless steel pilot fermenters (Shandong Zhongde Equipment Co. Ltd., China); a frame filter (Xuzhou Strong Machinery Co., China); a diatomaceous earth filter and 3-5 t/h capacity fine filter (0.6 and 0.45µm) (Sichuan Kehua Filter Equipment Corp., China); and a plate heat exchanger (Jinan Xinli Corp., China).

**Methods:**

**Soaking experiments on the main raw materials:** These experiments aimed to determine the best soaking parameters for the hull-less barley used in the pilot-scale production of Za wine. Raw materials (500 g) were soaked at 15°C (about room temperature in winter and spring), 25°C (about room temperature in summer and autumn), 40 and 60°C (the minimum safe temperature for hull-less barley starch pasting) for 8, 16, 24 and 32 h. The results were judged on the presence or absence of a dry core in the hull-less barley grain, the extent of grain softness and the amount of absorbed water, as well as cracks on the grain and the exposure of starch or pasting. The grains were evaluated visually. The experiments were completely randomized with triplicate samples. **Hull-less barley fragmentation test:** Hull-less barley grain (3 kg) was soaked in water at 60°C for 12 h then treated by one of three processes: 1) pulverized to 1 mm particle size; 2) pulverized to 2 mm particle size; or 3) flattened to plum petal shapes using a roller pressing machine. The processed hull-less barley was steam-cooked for 45 min then cooled to 35°C. After mixing with 3% raw material weight of Q303 *Rhizopus koji*, the processed hull-less barley product was transferred to a slender glass specimen jar with a height-to-diameter ration of 2.3:1 for glycation for 2 d at 38°C (to mimic actual production conditions, the materials were appropriately compacted). Distilled water (2 L) was then injected into the cylinder to allow extraction for 1 h (stirring two or three times). Fifty millilitres of liquid was centrifuged for 8 min (4000 rpm) and the content of reducing sugars in the supernatant was determined. Then the saccharification rate was calculated for each treatment as follows:

Saccharification rate: The total weight of reducing sugars/raw material weight (w/w). The amount of reducing sugars was determined using the DNS (3, 5-dinitrosalicylic acid) method (Ning, 2002). The optimum degree for grinding the hull-less barley was based on the saccharification rate.

**Screening kojis for Za wine fermentation:** The starch content of hull-less barley is about 56 to 67%, but is higher in rice, so rice was used for making *koji* for the Za wine fermentation. Rice *kojis* were made from QJ (*A. flavus*), ZZH (*A. albicans*), QZ and Q303 (*Rhizopus*) using the following steps:

An appropriate amount of rice was weighed and washed until the water was clear, soaked at room temperature for 45 min, then drained and cooked for 45 min, cooled to about 30°C, to produce steamed rice with a water content of 30% to 40%. Then a solution containing suspended spores (1×10⁵ cells/g rice) was inoculated into the steamed rice. The mixture was then incubated in an incubator at room temperature (32-34°C) at a relative humidity of 95%. After 16-20 h, the temperature increased to 44-48°C and the mixture was spread out and mixed, then cooled and piled up; after the temperature increased to 40-44°C, the mixture was spread out and mixed again, then portioned into shallow trays and the temperature was controlled at 36-40°C. After 36 h, the mixture was stirred once again. After the temperature dropped to 32-36°C (after 40 h), the moisture had been removed. The rice *koji* were then cultured at 32-34°C in ventilated dry air until their
moisture content was about 15%. The rice koji was then ready for use after a total preparation time of about 44–46 h.

The saccharifying and liquefaction powers of the four rice kojis were determined and used as two of the criteria for screening Za wine brewing koji.

Saccarifying power refers to the activity of glucoamylase in koji, which was measured using the DNS colourimetric method. Glucoamylase activity is defined as the amount of soluble starch mg converted to glucose in 1 h at 35°C, at a pH of 6.0 in 1 h (Ning, 2002); liquefaction power refers to the activity of the liquefying amylase and was determined by the iodine reaction. One unit of liquefying enzyme activity is defined as the liquefaction of 1 g soluble starch by 1 g koji at 60°C, at a pH of 6.0 in 1 h (Ning, 2002).

Meanwhile, the tests for brewing Za wine were performed using the four kinds of rice koji using the following steps. Hull-less barley (0.7 kg) was washed, soaked in warm water at 60ºC for 12 h then crushed to plum petal shapes. Glutinous rice (0.3 kg) was soaked at room temperature for 8 h. Then the processed hull-less barley and glutinous rice were mixed and steam-cooked and fermented according to the method of Deng (2013). Briefly, rice koji was added at about 8% (w/w) of raw materials, followed by solid-state saccharification for 4 d at 38ºC, then semi-solid state fermentation for 4 d at 25-30ºC after adding 1 kg of water and 100 mL of the particular seeding yeast followed by fermentation for 14 d at 14-18ºC. The mash was then filtered to obtain clear Za wine. During the experiment, the gas production after adding the particular yeast culture was recorded; the yeast activity was determined; the physical and chemical parameters of the resulting Za wine were determined; and a comprehensive sensory evaluation of the resulting Za wine was performed, to determine the best yeast for Za wine fermentation.

For seeding yeast preparation, sterilized koji juice (10º Brix) was inoculated with yeast on a fresh 2% YPD (yeast extract peptone dextrose) slant, then cultured for 2 d at 25ºC, with shaking and mixing once every day.

The physical and chemical parameters of the resulting wine samples were determined according to the methods of the Chinese Yellow Wine National Standard (GB/T 13662-2008) and the Chinese Shaoxing Wine National Standard (GB17946-2008) (Chinese rice wine (GB/T 13662-2008) and Shaoxing wine (2008) (GB/T 17946-2008)). The method is the same thereafter (Guo et al., 2008).

Small-scale testing of commercial Za wine brewing:
After optimizing the raw material processing and the koji and yeast for brewing, a small-scale test was performed using about 100 kg mash in a laboratory-made temperature-controlled 100 L stainless steel fermentation tank (Yue et al., 2007). For Za wine brewing, the combination of solid- and semi-solid fermentation methods is superior to other methods according to Deng (2013). Therefore the same method was used for the small-scale test. The specific processing steps are as follows (Fig. 1):

Raw materials processing: Twenty five kg hull-less barley and 10 kg glutinous rice were mixed and fermented.

Processing of hull-less barley: The hull-less barley was washed with water then incubated with water at 60ºC for 12 h, with the water level 15 cm above the top surface of the hull-less barley; then the water was drained away and hull-less barley crushed into plum petal shapes with a roller pressing machine.

Rice processing: the rice was washed with tap water twice then soaked in water at about 25ºC for 8 h, with the water level 15 cm above the top surface of the rice.

Steaming and cooking the raw materials: The processed hull-less barley and rice were mixed, put into a stainless steel cooker then cooked for 50 min when the steam had started to appear. During the steam cooking period, warm water at 40-60ºC was sprayed in 2 or 3 times.
Solid saccharification: The steam-cooked hull-less barley and rice were cooled to a temperature of 30-35°C by spraying with cold water. Then Q303 *Rhizopus koji* was added at a proportion by weight of 2‰ raw materials and then put into a 100 L laboratory-made temperature-controlled fermentation tank for solid-state saccharification. The temperature was controlled at 28-30°C on the first day and then at 35-40°C on the 2-3 d.

Pre-fermentation treatment: After saccharification, QJ *koji* at 8 % raw material weight and seeding yeast (yeast 1300 at a concentration of 1-3×10^8 cfu/mL) at 5-7 % of total mash, were added, along with water at the same weight as that of the raw material. All components were mixed well then pre-fermented with the temperature controlled at 24-32°C, for 4-5 d with stirring three times each day.

Post-fermentation treatment: When the pre-fermented mash temperature was reduced to 14-18°C, the post-fermentation period started with stirring once a day for the first 5 d, then stirring once in 3 days. After fermentation for about 20 d, the wine mash should be mature.

Pressing and clarifying operations: The mature mash was preliminarily filtered using a laboratory-made stainless steel filter, then the filtrate was clarified at 10-15°C for 16-24 h. The precipitate was removed and the wine saved.

Colour adjustment and fine filtering processes: The colour was adjusted using edible caramel and then the mash was clarified at 10-15°C for 4-6 h. The preliminarily filtered wine was filtered using a 0.45-µm bucket filter. The final small scale test Za wine was obtained after sterilization. The alcohol content, acidity, sugar content, pH, amino nitrogen, non-sugar solids and volatile esters were also determined.

Pilot production of commercial Za wine: The main equipment in the pilot production was the 400 L stainless steel fermentation tank and its auxiliary equipment: the kitchen steaming machine, the brewing roller crusher, the frame pressing filter, the diatomaceous earth filter and the 0.60-µm and 0.45-µm fine filters. The design of 400 L stainless steel fermentation tank is basically the same as that of a 10 t fermentation tank that would be used for the full-scale production of Za wine. The total volume of the pilot-scale tank was 550 L with an effective volume of 400 L, a heat exchange area of 2.5 m², a ratio of diameter to height of 1:2.3, a mechanical stirrer and automatic temperature control of the jacket. Its bottom was designed to allow sterile compressed air to enter and help optimize and regulate the fermentation (Liang, 2004; Jin, 2003).

For the pilot testing, small-scale test processes were used. The raw materials were 80 kg of hull-less barley, 33 kg of rice for mixed fermentation (loading factor, 85%). Because the target product was the semi-sweet Za wine, after 5 d of post-fermentation, 10 kg concentrated saccharification liquid was added (from hull-less barley and glutinous rice (1:1) mixed with solid saccharification -55° Brix). After filtration through the frame filter press, colour-adjustment with edible caramel-colour, fine filtration, sterilization with the plate heat exchanger and filling, the pilot Za wine was finally obtained.

Data analysis: All tests were performed in triplicate and the data were analysed using DPS software version 12.05 (Hangzhou Rui Feng Information Technology Co. Ltd., Zhejiang, China). A probability level of *p*<0.05 was considered as statistically significant between mean values and *p*<0.01 was considered as very significant.
RESULTS AND DISCUSSION

Main soaking parameters of raw materials: From the hull-less barley soaking tests and the comprehensive evaluation of sensory attributes and water uptake, it was found that the combinations of 40°C for 32 h and 60°C for 12 h were both the best with enough water soaking (approximately 36-39%), softened material, the cracking of only a small amount of grain but without any gelatinized starch (Fig. 2), which favoured the later breaking or crushing of hull-less barley. The other treatment groups showed a low water uptake, dry and hard grain hearts, or over-soaking, too much cracking, losses of exposed starch and stickiness from gelatinization.

Because the coat of the hull-less barley grain is thick and the grain is rich in protein and β-glucan, it is harder for hull-less barley to absorb water and swell compared with other grains. Therefore, after 32 h of soaking at room temperature (25°C), it still had a hard core. Therefore, during the following tests or a factory production process, a heat preservation soaking process would be needed to achieve the desired soaking effect and shorten the soaking time and so improve the efficiency of the test or factory production. Therefore, the combination of 60°C with 12 h soaking was the best choice.

Tests on the degree of crushing of hull-less barley:
The rates of saccharification using the three crushing treatments are shown in Fig. 3. For hull-less barley crushed into 1-mm particles, the saccharification effect was poor with a saccharification rate of only about 26%, which was significantly lower than that of the other two experimental treatments (p<0.01). Both the flattening (plum petals) processing and breaking into 2-mm particles provided better results with saccharification rates of more than 35%, with no significant difference (p>0.05) between these two treatments.

Screening rice koji for commercial Za wine: In wine fermentation and brewing, rice koji generally accounts for about 6-12% of the weight of the raw materials. Rice koji is a solid culture of Aspergillus on rice or steamed rice and can produce large amounts of biological enzymes and flavours. Rice koji is also the raw material for brewing, playing an important role in the production process. Enzymes in koji and their activities will have a significant impact on the fermentation process and wine quality (Kang, 2004; Gu, 2005). Therefore, for the process of Za wine brewing, selecting an excellent Aspergillus to make koji

Fig. 4: Aerogenesis of analyzed yeasts

Table 2: Results of rice koji tests

<table>
<thead>
<tr>
<th>Rice koji</th>
<th>Saccharification power (u/g)</th>
<th>Liquefaction power (u/g)</th>
<th>Alcohol content % (v/v)</th>
<th>Sensory evaluation score</th>
</tr>
</thead>
<tbody>
<tr>
<td>QJ (A. flavus)</td>
<td>686±14&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.32±0.45&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.45±0.43&lt;sup&gt;b&lt;/sup&gt;</td>
<td>65±4&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>ZZH (A. albicans)</td>
<td>563±12&lt;sup&gt;c&lt;/sup&gt;</td>
<td>7.13±0.34&lt;sup&gt;c&lt;/sup&gt;</td>
<td>5.33±0.32&lt;sup&gt;b&lt;/sup&gt;</td>
<td>58±4&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>QZ (Rhizopus)</td>
<td>571±12&lt;sup&gt;c&lt;/sup&gt;</td>
<td>7.11±0.54&lt;sup&gt;c&lt;/sup&gt;</td>
<td>5.24±0.41&lt;sup&gt;b&lt;/sup&gt;</td>
<td>55±3&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Q303 (Rhizopus)</td>
<td>671±11&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.87±0.32&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.61±0.53&lt;sup&gt;a&lt;/sup&gt;</td>
<td>67±3&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

*: Different letters in the same column indicate a significant difference (p<0.05)

and using the koji for wine production, has important implications for improving the quality of Za wine.

From the koji production process, A. flavus QJ rice koji, A. albicans ZZH rice koji, Rhizopus QZ and Q303 rice koji were obtained. First, the saccharification power and liquefaction power were measured, followed by the determination of alcohol content and overall sensory evaluation of the Za wine produced (Table 2). The experimental results showed that saccharification power was the highest in QJ rice koji, followed by that of Q303 rice koji, while those of ZZH rice koji and QZ rice koji had no significant difference (p>0.05). On liquefaction power, Q303 rice koji had the best performance with up to 7.87 u/g, QJ rice koji liquefaction power was 7.32 u/g and ranked second and there was no significant difference (p>0.05) in liquefaction power between ZZH koji and QZ koji. This was because the screening test of these rice koji used no added yeast for fermentation, so the alcohol content of the Za wine produced in this test was relatively low. Specifically, the alcohol content of Za wine produced from Q303 rice koji was the highest with the alcohol contents of Za wine from the other three rice koji being relatively low. Based on the overall sensory scores, Q303 rice koji and QJ rice koji were similar, having 67 points and 65 points, respectively, which were higher than the score of ZZH rice koji of 58 points with QZ rice koji having the lowest score of 55 (p<0.01).

Generally, A. flavus and A. albicans, with their higher α-amylase contents, could quickly liquefy the starch, so their liquefaction power should be relatively strong while Rhizopus should both have good saccharification and liquefaction powers (Gu, 2005). All Aspergillus have both the ability to break down starch and contain some alcohol producing capacity. Therefore, in the rice koji screening test, yeast was not added to the mash in order to avoid its effects so testing the effects of the rice koji only. During the experiment, A. flavus QJ was found to have excellent saccharification power and the saccharification and liquefaction powers of Rhizopus Q303 power were also very good. Regarding the alcohol contents of the Za wines produced and in particular their overall sensory score, it could be concluded that Q303 rice koji and QJ rice koji, or a mixture of them would be the best rice koji for brewing CZW.

Yeast preference test: For the laboratory-bred wine yeasts 1300, L18, L11 1-1 and the YW yeast under study, using the fermentation tests, their Za wine fermentation capacities and effects were investigated with the results shown in Fig. 4 and Table 3.

As shown in Figure 4, on the first and second days, the amount of gas produced from yeast 1300 was relatively high (about 40 and 48 g, respectively) then it fell quickly later with almost no gas being produced on the 8<sup>th</sup> and 9<sup>th</sup> days. Fermentations using YW and L18 yeasts had similar levels of gas production, which remained relatively stable during the first 4 days with an average gas yield of about 35 g. This decreased slightly, but fermentative activity continued between 6 and 12 d with 4-5 g of gas produced on the 12<sup>th</sup> day. This indicated that the two yeasts had relatively strong alcohol-resistant abilities and were not prone to premature aging during the later fermentation period or to cause rancid wine later. Yeast L11 1-1 exhibited a similar behaviour to yeast 1300, but its gas production was significantly lower than those of the other three
The acidity of Za wines from the four yeasts tested was slightly high, because these were the original Za wines without the appropriate acid-decreasing treatment. Except for Za wine brewed from yeast LII 1-1, the total sugar contents of the other three Za wines were less than 15 g/L, classified as dry-type fermented wines. However, the total sugar contents (i.e., residual sugar) of wines brewed from the LI8 and YW yeasts were lower than those brewed using yeast 1300 and LII 1-1 (p<0.01). Their alcohol contents were higher than those of Za wines from both the 1300 and LII 1-1 yeasts, indicating that YW and LI8 yeasts were better than 1300 and LII 1-1 regarding wine producing capacity and the utilization rate of starch, but there was no significant difference (p>0.05) between LI8 and YW yeasts. Because Za wine has neither an industry nor a national standard, the indicators and requirements from the national standard for Chinese yellow wine, GB/T13662-2008 were analysed and compared. For the amino acid N and non-sugar solid indicators, Za wines from LI8 and YW yeasts matched the standards for the national Grade one wine and Excellent wine. Amino nitrogen indicators of Za wines from yeast 1300 and LII 1-1 only met the standards for a national Grade two wine. The non-sugar solids of Za wine brewed from yeast 1300 satisfied the national standard for Excellent wine, but LII 1-1 did not meet the standard. At the same time, using the overall sensory scores, Za wines brewed from YW and LI8 yeasts were better than those from 1300 and LII 1-1 yeasts regarding colour, aroma, taste and flavour with scores of 78, 75 and 67 and 57, respectively, while sensory scores of wines brewed with LII 1-1 yeasts were not significantly different (p>0.05).

From examining the fermentation conditions for the four yeasts, the physical and chemical indicators of the Za wines and their overall sensory scores, the LI8 and YW yeasts were chosen for CZW fermentation yeast. For the production of wines with special requirements on flavour ingredients, LII 1-1 could be a suitable yeast.

Small-scale testing of CZW brewing: A total of 35 kg raw materials were used for the small-scale test, producing about 105 L mash and 73 kg clarified Za wine after saccharification with added koji, pre-fermentation and post-fermentation and press filter, with an alcohol content of 11.57% (v/v). The liquor yield of 2.086 (ratio of wine to raw materials, w/w) was slightly lower than that of Chinese yellow wine (2.1-2.2). This is mainly because the homemade initial filter press had a limited power, lower than that of the large frame gas membrane filter press. The lees had more than 60% volatile content, higher than that from Chinese yellow wine (40–50%). Sixteen kilograms of lees were obtained a yield of 45.71%, which is slightly higher than that from Chinese yellow wine. The Za wine from the small pilot-scale test was transparent, with an amber colour, elegant and mellow, with no bad odours.

The physical and chemical parameters were determined and listed in Table 4. Whereas the non-sugar solids content was higher than that allowed for the Chinese national Superior grade yellow wine standard, the ammonia nitrogen content reached the national Grade one yellow wine standard. The volatile ester content reached the 3-year-old Shaoxing wine standard and the alcohol content conformed to the national yellow wine standard. Overall, Za wine brewed from small-scale test was a dry-type of fermented wine. Because the acid was not adjusted, the total acid was higher than the national standard by about 1.76 g/L, but was within the normal range permitted.

Pilot production test of CZW: Pilot tests are essential for converting scientific and technological concepts into a practical industrial production process. Using pilot studies, the success rate for industrialization can be up to 80% but falls to 30% with no pilot study.

After pilot testing Za wine production for about a month, at the end of post-fermentation, the lees had completely sunk to the bottom of the tank so that the wine in the upper layer was clear and transparent, yellow, with a full wine taste, all of which satisfied the standards for a mature wine mash. The wine mash was pumped into the frame filter press to extract the wine then the wine was filtered through the diatomaceous earth filter with a secondary filtration using a cartridge-type fine filter. Eventually, 267 kg of clarified Za wine (i.e. commercial Za wine (CZW)) was obtained, with an alcohol content of 13.55% (v/v) and a liquor yield of 2.36, higher than that for TZW and higher than that from the small-scale test. Furthermore, CZW was yellow and transparent, with an elegant ester aroma, tasted mellow and sweet, with no bad odour thus providing the typical characteristics of a Qiang Za wine. In fact, TZW is generally with an alcohol content of 13.55% (v/v) and a liquor yield of 2.36, higher than that for TZW and higher than that from the small-scale test. Furthermore, CZW is installed in heavy terrine and with turbid appearance, which make it cannot be taken along conveniently and be superior impressing on the consumer, so CZW was better than TZW in many aspects (Ning, 2003; Jie, 2007; Song, 2007). The main

Table 3: Properties of Za wines from tested yeasts

<table>
<thead>
<tr>
<th>Yeast</th>
<th>Total acid (g/L)</th>
<th>pH</th>
<th>Total sugar (g/L)</th>
<th>Amino acid nitrogen (g/L)</th>
<th>Non-sugar solids (g/L)</th>
<th>Alcohol content (% v/v)</th>
<th>Sensory evaluation score</th>
</tr>
</thead>
<tbody>
<tr>
<td>1300</td>
<td>8.61±0.62</td>
<td>3.9±0.2</td>
<td>13.03±0.79</td>
<td>0.32±0.02</td>
<td>6.8±0.25</td>
<td>10.8±0.69</td>
<td>12±4.5</td>
</tr>
<tr>
<td>LI8</td>
<td>8.72±0.54</td>
<td>3.8±0.3</td>
<td>9.73±0.57</td>
<td>0.43±0.02</td>
<td>8.1±0.63</td>
<td>9.0±0.62</td>
<td>11±3.5</td>
</tr>
<tr>
<td>YW-Yeast</td>
<td>8.66±0.47</td>
<td>3.8±0.2</td>
<td>9.57±0.66</td>
<td>0.41±0.03</td>
<td>23.35±1.45</td>
<td>10.89±0.69</td>
<td>14±4.5</td>
</tr>
<tr>
<td>LII 1-1</td>
<td>6.61±0.42</td>
<td>3.6±0.2</td>
<td>24.03±1.26</td>
<td>0.22±0.01</td>
<td>11.62±0.93</td>
<td>4.67±0.23</td>
<td>18±4.5</td>
</tr>
</tbody>
</table>

* Different letters in the same column indicate a significant difference (lowercase letters, p<0.05; uppercase letters, p<0.01)
Table 4: Properties of Zai wine from the pre-pilot trial

<table>
<thead>
<tr>
<th>Total acid (g/L)</th>
<th>pH</th>
<th>Total sugar (g/L)</th>
<th>Amino acid nitrogen (g/L)</th>
<th>Non-sugar solid (g/L)</th>
<th>Volatile ester (g/L)</th>
<th>Alcohol content (% v/v)</th>
<th>Sensory evaluation score</th>
</tr>
</thead>
<tbody>
<tr>
<td>8.76±0.47</td>
<td>3.9±0.2</td>
<td>7.65±0.58</td>
<td>0.42±0.03</td>
<td>24.35±2.12</td>
<td>0.24±0.01</td>
<td>11.57±0.71</td>
<td>76±4</td>
</tr>
</tbody>
</table>

*: Calculation based on ethyl acetate

Table 5: Properties of commercial Zai wine from the pilot test

<table>
<thead>
<tr>
<th>Total acid (g/L)</th>
<th>pH</th>
<th>Total sugar (g/L)</th>
<th>Amino acid nitrogen (g/L)</th>
<th>Non-sugar solid (g/L)</th>
<th>Volatile ester (g/L)</th>
<th>Alcohol content (% v/v)</th>
<th>Sensory evaluation score</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.23±0.42</td>
<td>4.0±0.23</td>
<td>41.03±2.4</td>
<td>0.45±0.03</td>
<td>24.43±2.11</td>
<td>0.23±0.01</td>
<td>13.55±1.21</td>
<td>82±6</td>
</tr>
</tbody>
</table>

*: Calculation based on ethyl acetate

reason for this is that the mechanized frame filter press was strong (maximum pressure of about 9-10 kg), so that filter cake (lees) of CZW was dry and compact, with a volatile content of only 42%, which corresponded with the increased liquor yield and clear wine appearance. Moreover, the temperature was more precisely controlled in the pilot fermenter, with aeration and agitation also creating more favourable conditions for saccharification and fermentation.

The physical and chemical parameters determined of CZW are listed in Table 5. It can be observed that CZW had a higher overall sensory score of 82 points. This is mainly because the increased sugar level in CZW made it more refreshing and sweet, softer, mellower and more in agreement with human drinking preferences. In terms of the specific physical and chemical parameters, first, the alcohol content met the Chinese national yellow wine standard; the total acid and total sugar levels were in line with the national semi-sweet yellow wine standards; the non-sugar solids and amino acid nitrogen contents reached the national Grade one semi-sweet yellow wine standards; the volatile esters content was above the Shaoxing wine 3-year-old standards. Overall CZW has reached the Grade one standard for semi-sweet fermented wine.

CONCLUSION

Using a series of experiments, the optimal methods for soaking and crushing the main raw material, hull-less barley, for the pilot-scale production of CZW were successfully determined and the key techniques, including producing koji and brewing yeast, were also optimized. In the current method, hull-less barley was soaked in water at 60°C for 12 h then crushed to plum petal shapes for saccharification. The Koji should be rice koji made from A. flavus Q4 and Rhizopus Q303. The L18 and YW yeasts were the best choice for brewing CZW. If necessary, an appropriate amount of aroma yeast LII 1-1 could be added. In addition, a novel technique has been developed, where a concentrated saccharification liquid mix from hull-less barley and glutinous rice can be used to produce a semi-sweet CZW during the fermentation period and, along with the aforementioned techniques, constitute the key technology for the pilot production of CZW. CZW from the pilot test was yellow and transparent, possessed the typical characteristics of TZW and had higher alcohol content, liquor yield, better wine appearance and circulation than those of TZW. This study initiate the research for the key producing technology of CZW.

ACKNOWLEDGMENT

This study was financially supported by the opening fund of Sichuan Province Key Laboratory of Liquor Making Biotechnology and Application (no. NJ2015-07) and the authors gratefully acknowledge financial support from Sichuan University of Science & Engineering (no. 2014RC26, no. 13ljzk08 and no. 20140622026).

REFERENCES


