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Research Article

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

[1]Kaizheng Zhang, [1]Wei Zou and [2]Wenxue Zhang
[1]College of Bioengineering, Sichuan University of Science and Engineering, Zigong, 643000,
[2]Department 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* QJ 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

Corresponding Author: Wenxue Zhang, Department of Food Engineering, College of Light Industry and Food Engineering, Sichuan University, Chengdu, 610065, China
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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 LII 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 Haining Filtration Equipment Co., Haining, China); a household roller press machine (Seagull^{TR}, China); a 100 L stainless steel temperature-controlled fermentation tank (made at our laboratory); a 400 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 (1×10^5 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

Table 1: Organoleptic evaluation method of *Za* wine

Criterion	Colour	Aroma	Taste	Style
Maximum score	10	25	50	15

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 *koji*s were determined and used as two of the criteria for screening *Za* wine brewing *koji*.

Saccharifying 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 in mg converted to glucose in 1 h at 35°C, at a pH of 4.6 by 1 g dry *koji* (u/g) (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 rice *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 60°C 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 38°C, then semi-solid state fermentation for 4 d at 25-30°C after adding 1 kg of water. After fermentation at 15-18°C for 10 d, the mash was filtered to obtain clear *Za* wine. The rice *koji* for brewing *Za* wine was determined on the basis of the resulting wine's alcohol content and comprehensive sensory evaluation, along with rice *koji*'s saccharification and liquefaction powers.

The sensory evaluation method used a group of five trained wine tasting professionals who scored the wine aroma, taste, colour and style of each *Za* wine sample (Table 1). The final score of each sample was the average from the five assessors (Bo-Bin *et al*., 2010).

Yeast optimization: The laboratory-bred wine yeasts, 1300, LI8, LII 1-1 and the commercial YW-Yeast were studied using a single-factor test. The test procedure was as follows: 0.7 kg hull-less barley was washed, soaked in warm water at 60°C for 12 h, crushed to plum petal shapes, then mixed with 0.3 kg glutinous rice soaked at room temperature for 8 h then steam-cooked. QJ *koji* at 8% raw material weight was added, followed by solid-state saccharification for 4 d at 38°C, then semi-solid fermentation for 5 d at 25-30°C after adding 1 kg of water and 100 mL of the particular seeding yeast followed by fermentation for 14 d at 14-18°C. 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 (10° Brix) was inoculated with yeast on a fresh 2% YPD (yeast extract peptone dextrose) slant, then cultured for 2 d at 25°C, 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 60°C 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 25°C 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 40-60°C was sprayed in 2 or 3 times.

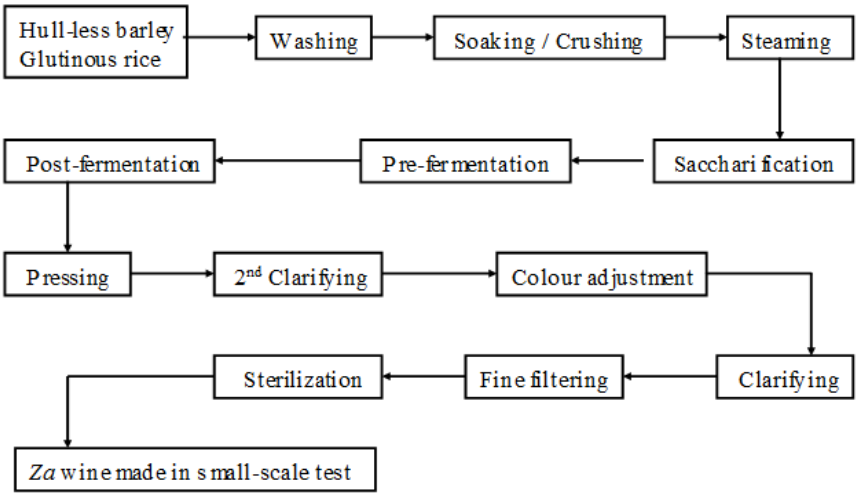

Fig. 1: Technological process for the small-scale testing of commercial *Za* wine production

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 $1\text{-}3\times10^8$ 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 m^2, 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 postfermentation, 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.

Fig. 2: Steeped hull-less barley (60°C+12h)

Theoretically, raw materials that have been crushed into smaller particles provide a better brewing efficiency, because this increases the surface area in contact with the various enzymes. However, in the actual brewing process, if the particles are too small, the gap between raw materials also becomes small, which is not conducive to aerobic microbial growth and reproduction. Almost all saccharification microbes are of oxygen consumption, so this can explain why the effects of the flattening process and the 2-mm particle treatment were better than the 1-mm particle treatment. After the hull-less barley was cooked, if the starch was significantly exposed to large amounts of raw materials, this would cause the particles to stick each other. Therefore, we can draw the following conclusions from the results of this test. In the subsequent small-scale test, pilot or industrial production of CZW, after hull-less barley has been soaked, it would be appropriate to use the flattening (plum petal shapes) treatment in the crushing process. This could prevent the agglomeration of particles to obtain a better saccharification effect.

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*

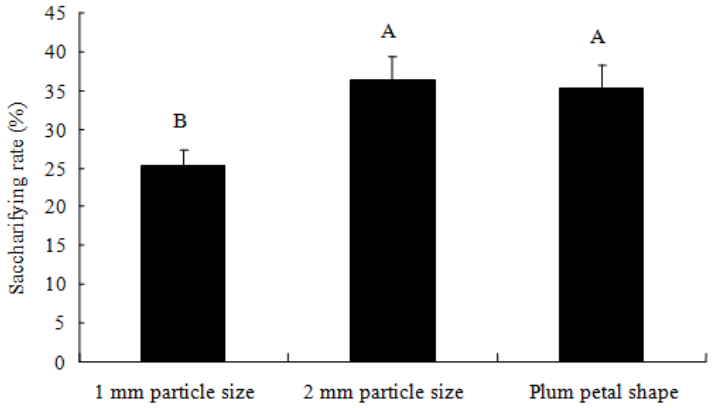

Fig. 3: Saccharification tests of crushed hull-less barley

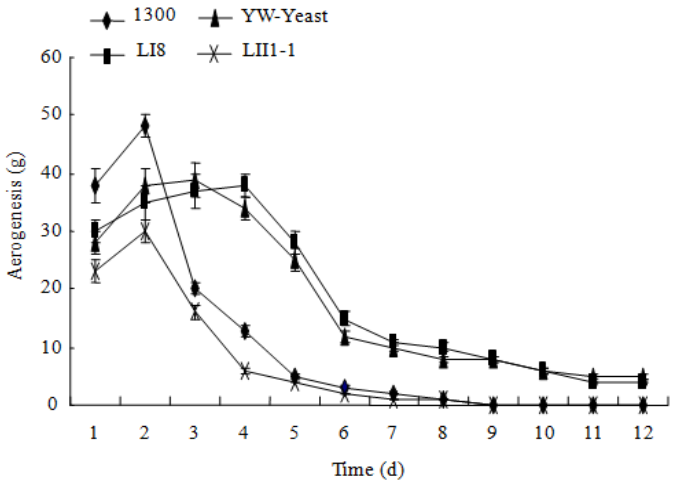

Fig. 4: Aerogenesis of analyzed yeasts

Table 2: Results of rice *koji* tests

Rice *koji*	Saccharification power (u/g)	Liquefaction power (u/g)	Alcohol content % (v/v)	Sensory evaluation score
QJ (*A. flavus*)	686±14[a]	7.32±0.45[b]	5.45±0.43[b]	65±4[a]
ZZH (*A. albicans*)	563±12[c]	7.13±0.34[c]	5.33±0.32[b]	58±4[b]
QZ (*Rhizopus*)	571±12[c]	7.11±0.54[c]	5.24±0.41[b]	55±3[c]
Q303 (*Rhizopus*)	671±11[b]	7.87±0.32[a]	6.61±0.53[a]	67±3[a]

*: 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 *kojis* 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 *kojis* 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, LI8, LII 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 8th and 9th days. Fermentations using YW and LI8 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 12th 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 LII 1-1 exhibited a similar behaviour to yeast 1300, but its gas production was significantly lower than those of the other three

Table 3: Properties of *Za* wines from tested yeasts

Yeast	Total acid (g/L)	pH	Total sugar (g/L)	Amino acid nitrogen (g/L)	Non-sugar solids (g/L)	Alcohol content (%, v/v)	Sensory evaluation score
1300	8.61±0.62	3.9±0.2	13.03±0.79^{B}	0.32±0.02	21.68±1.13	8.23±0.62^{b}	67±4^{b}
LI8	8.72±0.54	3.8±0.3	9.73±0.57^{C}	0.43±0.02	23.12±1.32	10.97±0.58^{a}	75±3^{a}
YW-Yeast	8.66±0.47	3.8±0.2	9.57±0.66^{C}	0.41±0.03	24.35±1.45	10.89±0.69^{a}	78±4^{a}
LII 1-1	6.61±0.42	3.6±0.2	24.03±1.26^{A}	0.22±0.01	11.68±0.93	4.67±0.23^{c}	57±4^{c}

*: Different letters in the same column indicate a significant difference (lowercase letters, $p<0.05$; uppercase letters, $p<0.01$)

yeasts ($p<0.01$). This indicated a lower ability to ferment wine, which might be related to yeast LII 1-1 belonging to the *C. pelliculosa* species.

In Table 3, 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, classed 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 LI8 and YW 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 4.50-7.50% (v/v) and a liquor yield of 1.50-1.80, meanwhile, TZW is installed in heavy terrine and with turbid appearance, which make it cannot be taken along conveniently and be inferior impressing on the consumer, so CZW was better than TZW in many aspects (Ning, 2003; Jie, 2007; Song, 2007). The main

Table 4: Properties of *Za* wine from the pre-pilot trial

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

*: Calculation based on ethyl acetate

Table 5: Properties of conmmercial *Za* wine from the pilot test

Total acid (g/L)	pH	Total sugar (g/La)	Amino acid nitrogen (g/L)	Non-sugar solid (g/L)	Volatile ester* (g/L)	Alcohol content (%, v/v)	Sensory evaluation score
7.23±0.42	4.0±0.23	41.03±2.4	0.45±0.03	24.43±2.11	0.23±0.01	13.55±1.21	82±6

*: 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; and 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* QJ and *Rhizopus* Q303. The LI8 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.

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