The optimum technology of the mixed rice wine

Liyuan Liu¹, Lijun Nan²,³*, Xiaofang Zhang³, Ting Wang⁴, Yingxue Zhong², Ruiqun Yang², Fang Tao² and Jing Huang⁴*

¹Turpan Technology Extension Service Center for Forest and Fruit Industries, Turpan 838000, China
²School of Chemistry and Life Sciences, Chuxiong Normal University, Chuxiong 675000, China
³Engineering Technology Research Center of Grape and Wine for advanced school in Yunnan, Chuxiong 675000, China
⁴School of Mathematics and Statistics, Chuxiong Normal University, Chuxiong 675000, China

*Corresponding author: submit_paper73@126.com; jing_huang12@126.com

Abstract. In order to study the influence of raw materials, the mold culture addition and fermenting time on the quality of rice wine, the comprehensive utilization of the optimized rice wine process in practice was discussed. The aroma rice, glutinous rice, red rice and black rice in Renxing Town, Lufeng County, Chuxiong City were used as the main experimental materials, and the single-factor test was used to optimize the fermenting process of the mixed glutinous rice wine. The contents of starch, reducing sugar and soluble solids in aroma rice, black rice, red rice, and glutinous rice were measured after soaking, cooking and cooling, and the quality characteristics of the raw materials in mixed rice wine were studied. Using the black rice, red rice and aroma rice mixed with glutinous rice separately for the mixed rice wine, and using the rice wine made from single glutinous rice as blank control. The physical and chemical indexes and electronic tongue analysis of the mixed rice wines were determined, and the best rice materials were selected by combing with sensory evaluation. Using the best rice materials, different concentration gradients of the mold culture were added in for fermentation (1 g/230g, 1 g/240g, 1 g/250g and 1 g/260g), the best concentration of the mold culture were obtained. Based on the optimum materials and the optimum concentration of the mold culture, the different time gradients (5 d, 8 d, 11 d, 14 d and 17 d) were set for fermentation to study the effect of fermentation time on rice wines. It was finally determined that the best material was the aroma rice. The optimum concentration of the mold culture was 1 g/240 g, and the optimum fermentation time was 14 d. By fermenting glutinous rice wine with aroma rice can increase the rice aroma of rice wine on the one hand, and provide a useful route for the new process of rice wine on the other hand. Studying the influence of the amount of the mold culture and the fermentation time on the fermentation of rice wine not only improves the fermentation rate of rice wine, but also makes the rice wine have a unique aroma, which also provides a more perfect process route for rice wine fermenting process, thus promoting the optimization of rice wine fermentation process.

1. Introduction
Rice is a principle food in south China, the widespread cultivation of rice makes the fermenting of rice wine more popular in China. Rice wine, as a kind of nutritious and healthy fermenting wine, is suitable...
for the consumption trend of rice wine and the development direction of rice wine advocated in China, has a broad market prospect. It is of great practical significance to study the new rice wine fermenting technology for expanding rice wine industry, increasing farmers’ income and promoting rural development. In addition, rice wine as a kind of family wine widely liked by the public, has the advantages of low cost, easy fermenting, good taste, etc. It is of great significance to optimize the fermenting process of rice wine and ferment rice wine with high quality to expand the rice wine consumption market and meet the consumption demand. Through the study of the materials and fermenting technology, the optimal technology of the mixed rice wine was selected, and then through the comprehensive evaluation of basic physical and chemical indexes, and the aroma and sensory evaluation, the optimal utilization plan of rice wine was formulated. After the plan is obtained, the scientific research results can be quickly transformed into the production of enterprises, which will contribute to the development of rice wine in China, and provide reference for the further processing of cereals and grains in other regions, which will also promote the recycling of rice and promote the green and healthy development of rice wine.

At home and abroad, great attention has been paid to the improvement of traditional rice wine technology and the development of related new products [1]. The China has developed Ganoderma rice wine, corn rice wine, bitter vegetable mixed with black glutinous rice wine, black rice and glutinous rice mixed sweet wine and so on [2, 3]. Nowadays, the research on rice wine mainly focuses on the determination and production of the nutritional components of rice wine products, while the research on the improvement of rice wine processing technology is less. At present, the production of black rice wine in China has initially formed a relatively complete industrial structure, but there are still many problems to be solved in the process of rice wine fermentation, especially the control of fermentation parameters.

This experiment is closely related to the current research trends, through the determination of the four kinds of rice materials, to determine the feasibility of different rice wines’ fermentation, through the mixed fermenting of different rices and glutinous rice, and on the original basis of single glutinous rice wine, to study the influence of the amount of the mold culture and fermentation time on the mixed rice wines, and finally to optimize the process of the mixed rice wine. The traditional way of rice wine fermentation has the future of long fermentation time, slow fermentation rate and low wine yield, comparing with the traditional rice wine fermentation, this experiment has changed the mode of the traditional rice wine fermentation, by using the solid fermentation saccharification first, then adding water for liquid fermentation production, this deep fermentation method can product more juicy rice wine in a short term while maintain the unique style of rice wine, not only improve the yield of rice wine, but also reduce the production cost of rice wine.

2. Materials and methods

2.1. Materials

Raw materials: aroma rice, black rice, red rice, glutinous rice from Renxing Town, Lufeng County, Chuxiong City, China; Supplementary material: the fermenting mold culture: 400g, from Kunming Longmen Liquor Co., Ltd.

Main regents are listed as follows: Concentrated sulfuric acid, 0.05 mol/L sodium hydroxide, 20 % NaOH, 2.5 g/L glucose, methylene blue, phenolphthalein indexe, Fehling reagent, citric acid solution, disodium hydrogen phosphate, copper tartrate solution, copper B tartrate solution, 0.1% glucose, 0.2% methyl red, ethanol, 6 mol/L hydrochloric acid.

Main instruments used in this study are listed in Table 1.
Table 1. Main instruments and equipments

| The instrument              | Specifications | The manufacturer                                      |
|-----------------------------|----------------|-------------------------------------------------------|
| electronic balance          | Sop OUINITIX224-1CN | Sartorius scientific instruments (Beijing) Co., Ltd    |
| electric blast drying oven  | DHG-9070A      | Shanghai Yiheng Scientific Instrument Co., Ltd        |
| UV spectrophotometer        | UV-5500        | Shanghai Yuan Analytical Instrument Co., Ltd          |
| acidometer                  | PB-10          | Sartorius scientific instruments (Beijing) Co., Ltd    |
| electric constant temperature water bath | HWS26 | Shanghai Yiheng Scientific Instrument Co., Ltd        |
| analytical balance          | TG328A         | Shanghai precision instrument factory                |
| magnetic stirrer             | 1kA-RTC Basic  | Bangxi Instrument Technology (Shanghai) Co., Ltd       |
| electronic tongue           | Smart Tongue   | Shanghai rifen isenso company Co., Ltd                |

2.2. Method

2.2.1. Experiment design. Experiment design 1: Based on the different components of different rices, black rice, red rice, aroma rice and glutinous rice were fermented separately to detect the changes of starch, reducing sugar and soluble solid contents of four kinds of rice in three stages after soaking, cooking and cooling.

Experiment design 2: single factor experiment was used, black rice, red rice, aroma rice were mixed and fermented with glutinous rice respectively, and rice wine fermented from single glutinous rice was used as blank control. The alcohol content, pH, reducing sugar, total acid, starch, soluble solids, liquor yield and chroma of rice wines were measured. Combined with sensory evaluation, the influence of different raw materials on rice wine was analyzed.

Experiment design 3: according to the best ratio of 1:4 and the best raw materials, adding different concentration gradients (1 g/230g, 1 g/240g, 1 g/250g, 1 g/260g) of the mold culture for fermentation, and measuring the alcohol content, pH, reducing sugar, total acid, starch, liquor yield, soluble solids and sensory evaluation of rice wines, and analyzing the effect of the addition of the mold culture on the fermenting of rice wines.

Experiment design 4: according to the best amount of the mold culture selected in the above experiment, different time gradients (5 d, 8 d, 11 d, 14 d and 17 d) were set for fermentation, and the alcohol content, pH, reducing sugar, total acid, starch, liquor yield, soluble solids of rice wine were measured, and sensory evaluation was conducted to analyze the effect of fermentation time on rice wine fermenting.

2.2.2. Technological process and key control points of rice wine fermenting. Rice wine fermenting process: Raw material → rice washing → rice soaking → rice cooking → rice pouring → saccharification with the mold culture → fermentation with water → pressing → filtration → finished product. During this period, the key control points are listed as follows:

Rice washing: wash rice with tap water to remove sundries and dust on the surface of rice. In order to improve the quality of rice products, it is necessary to wash the raw materials completely until the water is clear and free of turbidity.

Rice soaking: soak the washed raw material rice to make the starch in the rice grains expand and absorb water, and make the rice grains loose, so as to create conditions for the full gelatinization of
starch in the next step of rice steaming. The soaking time depends on the temperature of the day, which is shorter when the temperature is high, and longer when the temperature is low. Generally, it is better to ensure the integrity of rice and crush it with fingers. If the rice is not soaked thoroughly, the rice will not be fully cooked when cooking, and it will be prone to rancidity when fermenting.

Rice cooking: put glutinous rice, aroma rice, red rice and black rice into electric rice cooker separately for cooking and making starch gelatinization. The appearance of steamed rices should be soft, transparent but not rotten, cooked but not sticky and have enough oxygen supply. In the process of fermentation, over cooking, rice caking and not well-mixing are not conducive to saccharification and fermentation; insufficient cooking will lead to incomplete saccharification, increase the acidity of rice wine, and easily lead to rancidity.

Rice pouring: cool the newly cooked rices. Sprinkle the boiled water on the cooked rice to cool it quickly. It can make the hot rice cool quickly and also increase the water content of rice, make its surface greasy and moist, and make the particles smooth and non-sticky, and increase the oxygen content.

(1) adding the mold culture: after rice pouring, reduce the temperature to about 28-30 °C, so that it can reach the temperature suitable for fermentation of the mold culture. Mix well and dig a small hole in the center of the rice to increase the contact surface between the rice and the air, so as to in favor of the fermentation.

(2) saccharification: it is to use saccharifying enzyme to further decompose the starch in rices into glucose at 28 °C, which will take 48 hours.

(3) fermentation: add 1.5 times of cold boiled water to the saccharified rice at 38 °C for 14 days.

(4) filtration: use gauze to separate liquor from rice meal.

2.2.3. Determination of reducing sugar, total acid and starch content. Determination of reducing sugar, total acid and starch content was followed by references [4-6].

Sample handling, weigh 2 g rice flour (commercially available), filter and wash it with 100 mL 80% ethanol for several times, remove the soluble sugar, and then transfer the residue into a 250 mL wide mouth triangular flask with 100 mL distilled water.

Hydrolysis, take 30 mL 6 mol/L HCl and add it to 250 mL wide mouth triangular flask for reflux, and put it into boiling water bath for hydrolysis for 2 h. After hydrolysis, take out the triangular flask and cool it with cold water. Add 2 drops of methyl red indicator to the sample, adjust to yellow with 20% sodium hydroxide solution first. Then adjust 6 mol/L HCl to just turn red, and then use 10% sodium hydroxide solution to just turn red to fade away, so that the sample appears neutral. Transfer the sample to a 250 mL volumetric flask for dilution and constant volume, filter, collect the filtrate, and dilute the filtrate 10 times for use.

Calibration of alkaline copper tartrate solution (vs = 11.3 mL), take 5 mL of copper tartrate solution a and B respectively, put them into a 150 mL conical flask, add 10 mL of water, add several glass beads, drop 9 mL of glucose standard solution from the buret, and heat it to boiling within 2 minutes, keep it for 30 s, while it is hot Add the glucose standard solution at a low speed of D/2S until the blue color of the solution just fades away. Record the total volume of the consumed glucose standard solution. Repeat the operation three times and take the average value. Calculate the mass of glucose equivalent to 10 mL of basic tartaric acid copper B mixed solution.

Sample solution pre determination, pipette 5.0 mL of each solution of cupric tartrate A and B into a 150 mL conical flask, add 10 mL of water, add several glass beads, heat to boiling within 2 min, drop the sample solution from the buret while it is hot, keep the boiling state throughout the process, after the color of the solution turns light, drop it at the speed of 1D/s until the blue just goes away, and record the consumed volume of the sample v.

Sample solution Determination, pipette 5.0 mL of each solution of cupric tartrate A and B into a 150 mL conical flask, add 10 mL of water, add several glass beads, add 1mL of sample solution less than the predicted volume from the buret, heat it to boiling within 2min, titrate it continuously at the
speed of 1D/2S while boiling until the blue just goes away, and record the consumed volume of sample v.

Calculation were performed by:

\[
\text{Starch content} = \frac{V_c \times 0.9 \times 1}{m} \times \frac{1000}{V} \times 100\%
\]

Where: \( V_c \): volume of glucose standard solution consumed for titration of copper tartrate, 11.3 mL; \( C \): concentration of glucose standard solution, 1 g/L; \( M \): mass of sample, G; \( V \): volume of sample solution consumed during determination, mL; 0.9: conversion coefficient of glucose to starch.

2.2.4. Determination of soluble solids, Alcohol content, pH, chroma, and principal component analysis. Determination of soluble solids was using refractometer method [7], determination of Alcohol content was using density bottle method [8], determination of pH and chroma were using pH meter method [9], then preform the principal component analysis of electronic tongue [10].

First, dilute rice wine and distilled water by volume ratio of 1:4; then the data signal acquisition. Before testing the sample, clean the probe with distilled water, insert the probe into 0.01 mol/lkcl, and then set the test parameters of the electronic tongue. After setting, the electronic tongue begins to test the sample. Clean the probe with distilled water between the same products.

After that was the data processing: import data; sensor optimization and PCA analysis, a multivariate statistical analysis method, called principal component analysis, which selects a small number of important variables through a certain linear transformation for many variables. Select the data to be used; then the analysis and results, click PCA analysis after intelligent grouping; last, save model, data and drawing.

2.3. Statistical analysis

SPSS and Excel 2003 were used for data analysis and mapping.

3. Results

3.1. Indexes Changes in raw materials at different stages

3.1.1. Indexes Changes after rice soaking. It can be seen from Table 2 that after rice soaking, the content of various components in raw materials is low, and the main components in rice cannot be fully soaked[11]. From the longitudinal analysis, among red rice, black rice, aroma rice and glutinous rice, aroma rice has the highest starch content, while red rice has the lowest starch content; aroma rice has the highest reducing sugar content while red rice has the lowest; aroma rice has the highest soluble solids, while red rice and black rice have the lowest; and the differences between the physical and chemical indexes of different rice wines were different. Based on the above analysis, it can be concluded that after rice soaking, aroma rice has the highest contents of starch, reducing sugar and soluble solids, followed by glutinous rice, then black rice, and the lowest was red rice. It can be concluded that there are a lot of effective ingredients in aroma rice which can be used in rice wine fermenting.

| Rice         | Starch (%) | Reducing sugar (g/L) | Soluble solids (%) |
|--------------|------------|----------------------|-------------------|
| Red rice     | 5.80±0.10a | 24.40±0.85a          | 16±0.00a          |
| Black rice   | 6.60±0.17b | 49.08±0.10d          | 16±0.00a          |
| Aroma rice   | 11.66±0.17a| 16.00±0.00a          | 28±0.00b          |
| Glutinous rice| 6.72±0.26b | 28.30±0.26c          | 18±0.00d          |

Note: The same letter indicates that the difference is not significant, and different letters indicate that the difference is significant (P<0.05), the same below.
3.1.2. Indexes Changes after rice cooking. It can be seen from Table 3 that, compared with Table 2, all components of rices have changed after cooking, all indexes have a rising trend and there were great differences among similar indexes. As all components were gelatinized in the cooking process, cooking promoted the gelatinization of the rices [12], and part of sugar was hydrolyzed into single molecule monosaccharide due to high temperature. At the same time, the cooking stage can show the aroma components in rices. After cooking, the hardness and elasticity of rice become smaller. According to the analysis, the contents of reducing sugar, starch and soluble solids in aroma rice were the highest, followed by red rice and glutinous rice, while black rice was the lowest. The results showed that the components of aroma rice were more prominent, while other rices were less obvious.

Table 3. Determination of indexes of rice after cooking

| Rice            | Starch (%)  | Reducing sugar (g/L) | Soluble solids (%) |
|-----------------|-------------|----------------------|--------------------|
| Red rice        | 13.70±0.17a | 57.50±0.10b          | 25±0.00c           |
| Black rice      | 8.36±0.17c  | 25.20±0.11c          | 24±0.00b           |
| Aroma rice      | 14.44±0.36b | 60.79±0.26e          | 28±0.00e           |
| Glutinous rice  | 9.85±0.10d  | 41.47±0.15d          | 20±0.00e           |

3.1.3. Indexes Changes after rice cooling. According to Table 4, when rice was cooled down after cooking, there was no significant change in all components. According to the comprehensive analysis of horizontal and vertical axes, the contents of starch, soluble solids and reducing sugar were the highest in aroma rice, while the lowest in black rice. Comparing the changes of all indexes after cooking and after cooling, it was found that cooling has no significant effect on rice components[13].

Table 4. Determination of indexes of rice after cooling

| Rice            | Starch (%)  | Reducing sugar (g/L) | Soluble solids (%) |
|-----------------|-------------|----------------------|--------------------|
| Red rice        | 12.97±0.10b | 54.59±0.62b          | 27±0.00c           |
| Black rice      | 8.53±0.51c  | 35.90±0.10c          | 26±0.00a           |
| Aroma rice      | 14.12±0.17a | 59.44±0.10b          | 36±0.00b           |
| Glutinous rice  | 9.93±0.20d  | 41.79±0.26d          | 26±0.00a           |

In summary, according to Table 2, 3 and 4, among the four rice materials, aroma rice has the highest contents of starch, soluble solids and reducing sugar, followed by glutinous rice, red rice and black rice. Through the determination of the components of rice materials in three stages of soaking, cooking and cooling, it also showed that during the soaking process, some components in rice can be extracted but the amount was less, cooking had a greater impact on the components of raw materials, while cooling has no significant effect on the components.

3.2. Effect of different kinds of raw materials on rice wine

3.2.1. Changes of physical and chemical indexes of different mixed rice wines. It can be seen from Table 5 that the mixing ratio of glutinous rice and other rice was 1:4 for mixed fermentation, the temperature was controlled at 38 °C, and rice wines were fermented after 14 days of fermentation [14]. Due to the different fermentation materials, the indexes of the finished wines were also changed. After fermentation, the glutinous rice and red rice mixed wine had the highest contents of starch, reducing sugar and soluble solids, with the color was bright red, and the alcohol content was the lowest, which indicated that the red rice and glutinous rice mixed wine had not been completely fermented; however, the indexes of single glutinous rice wine had no significant effect, its alcohol content was the highest with the color of golden yellow, the color was light yellow; glutinous rice and aroma rice mixed wine had the highest alcohol yield, and the chroma, soluble solids, reducing sugar, total acid and starch were all in the second place; glutinous rice and black rice mixed wine had the lowest alcohol yield, with the purple red color. As the higher of pH value, the lower the starch, soluble solids,
acidity of rice wines were, which showed that the content of soluble solids of rice wines was in direct
ratio with starch.

### Table 5. Determination of indexes of different mixed rice wines

| Rice             | Soluble solids (%) | Reducing sugar (g/L) | Total acid (g/L) | pH    | Alcoholic content (%vol) | Alcoholic yield (%) | Chroma | Starch (%) |
|------------------|-------------------|---------------------|------------------|-------|--------------------------|---------------------|--------|------------|
| Red rice         | 26 ± 0.10c        | 196.26 ± 0.10a      | 3.99 ± 0.00b     | 3.96 ± 0.00b | 15.10 ± 0.00a            | 158 ± 0.00a         | 75.32 ± 0.01d | 10.33 ± 0.00a |
| Black rice       | 23 ± 0.20a        | 110.41 ± 0.20d      | 3.87 ± 0.10b     | 3.89 ± 0.00a | 18.30 ± 0.00b            | 132 ± 0.00c         | 47.30 ± 0.36b | 6.90 ± 0.00b  |
| Aroma rice       | 24 ± 0.26b        | 170.96 ± 0.17b      | 4.59 ± 0.20c     | 4.37 ± 0.00a | 24.70 ± 0.57c            | 164 ± 0.00b         | 69.73 ± 0.00c | 9.93 ± 0.20a  |
| Glutinous rice   | 23 ± 0.20a        | 147.22 ± 0.17c      | 4.47 ± 0.34a     | 3.89 ± 0.00a | 44.50 ± 0.00d            | 160 ± 0.00d         | 26.71 ± 0.01a | 6.11 ± 0.87b  |

#### 3.2.2. Sensory analysis of different rice mixed wine.

It can be seen from Table 6 that the characteristics of rice wine fermented by glutinous rice and
aroma rice were outstanding[15], the sugar and acid were balanced, with the unique aroma of rice
wine. The integration of the aroma of aroma rice into rice wine increased the unique aroma of the
mixed rice wine, and also made the body of the wine harmonious; secondly, the wine with good taste
was the glutinous rice and red rice mixed wine, with outstanding sweetness, fruit candy aroma, and
long aftertaste, but the sweetness concealed the wine aroma, making the wine aroma less prominent;
the wine was made from glutinous rice alone, had harmonious, clear and yellowish body, but the
aroma was dull; the mixed wine of glutinous rice and black rice had unpleasant flavor of rice bran, and
the aftertaste was bitter. Six out of ten people who were in tasting group preferred the mixed rice wine
of glutinous rice and aroma rice. It can also be clearly seen from Fig. 1 that the score area of the mixed
rice wine of glutinous rice and aroma rice was the largest, indicating the best tasting result; the score
area of the mixed rice wine of glutinous rice and black rice was the smallest, indicating that the quality
characteristics of all aspects of the rice wine were not very good.

### Table 6. The average scores of ten person tasting group

| Sensory indexes (20 ponits) | Single glutinous rice wine | Glutinous rice + aroma rice mixed wine | Glutinous rice + black rice mixed wine | Glutinous rice + red rice mixed wine |
|-----------------------------|----------------------------|---------------------------------------|--------------------------------------|-------------------------------------|
| Appearance and color         | 15±0.24b                   | 16±0.18c                              | 14±0.74b                            | 16±0.47a                           |
| Aroma (20 ponits)            | 15±0.73b                   | 16±0.18c                              | 14±0.14b                            | 16±0.68a                           |
| Taste (30 ponits)            | 24±0.18d                   | 26±0.28d                              | 22±0.24a                            | 25±1.22c                           |
| Characteristics (30 ponits)  | 16±0.89a                   | 20±0.22a                              | 14±0.89b                            | 19±0.55b                           |
| Total scores (100 ponits)    | 70±0.40d                   | 78±0.96b                              | 64±1.22c                            | 76±0.87d                           |
According to the analysis of Table 5, 6 and Fig. 1, the best materials of the mixed rice wine were the glutinous rice and aroma rice, which had the clear, bright and glossy appearance, typical golden yellow, with the unique mellow and strong fragrance, harmonious and elegant body, no obvious bitterness, and unique style of fermented rice wine.

3.2.3. PCA analysis of electronic tongue of different mixed rice wines. According to Fig. 2, the relative distances between the four different mixed rice wines were large, which can be clearly distinguished, and the sample tests of each wine sample have certain repeatability. It can be seen from Fig. 2 that except for the single wine making of glutinous rice, the differences between the duplicate samples of other mixed rice wine were small. The DI value of the principal component analysis of the four wines was 99.09, and the total contribution rate of the principal component was 99.16%, which can completely represent the overall information of the samples. The results showed that the electronic tongue can be used to identify and distinguish different glutinous rice wine, which reflects the agility and practicability of the electronic tongue to identify and analyze different rice wine [16].
3.3. Effects of the mold culture addition on mixed rice wines’ fermentation

3.3.1. Changes of physicochemical indexes of mixed rice wine with different amount of the mold culture addition. It can be seen from Table 7 that with the change of the amount of the mold culture added, each index will change differently after the rice wine were fermented [17].

From the longitudinal analysis and comparison, it can be concluded that the amount of mold culture was 1 g/230g got the highest soluble solids, the amount of mold culture was 1 g/250g got the lowest reducing sugar, the amount of mold culture was 1 g/230g got the lowest total acid, the amount of mold culture was 1 g/250g got the lowest pH value, the amount of mold culture was 1 g/240g got the highest alcohol degree, the amount of mold culture was 1 g/240g got the highest alcohol content, and the amount of mold culture was 1 g/240g got the lowest starch content. It can be concluded from the above that it was not conducive to complete fermentation when the amount of mold culture was low, which will make rice wine sour, reducing sugar and acidity would rise. As the mold culture can not use all the sugar, it will cause bad smell. However, it will promote fermentation quickly when the amount of the mold culture was high, but it will reduce the yield of rice wine, reduce the conversion of starch, and it was not conducive to normal fermentation. Therefore, the optimal addition of the mold culture was 1 g/240g. A reasonable addition of the mold culture was helpful to the fermentation.

### Table 7. Determination of indexes of mixed rice wines with different amounts of the mold culture addition

| Amount of the mold culture | Soluble solids (%) | Reducing sugar (g/L) | Total acid (g/L) | pH | Alcohol degrees (%vol) | Alcohol content (%) | Starch (%) |
|---------------------------|-------------------|---------------------|-----------------|----|------------------------|-------------------|------------|
| 1 g/230g                  | 16±0.10<sup>a</sup> | 76.42±0.10<sup>a</sup> | 4.77±0.00<sup>d</sup> | 3.81±0.00<sup>a</sup> | 9.19±0.01<sup>b</sup> | 224±0.00<sup>a</sup> | 7.56±0.10<sup>b</sup> |
| 1 g/240g                  | 13±0.17<sup>c</sup> | 76.42±0.10<sup>b</sup> | 4.23±0.05<sup>c</sup> | 3.80±0.00<sup>a</sup> | 11.46±0.03<sup>a</sup> | 272±0.00<sup>c</sup> | 6.62±0.20<sup>c</sup> |
| 1 g/250g                  | 11±0.10<sup>d</sup> | 23.99±0.10<sup>d</sup> | 3.75±0.05<sup>a</sup> | 3.72±0.00<sup>b</sup> | 8.13±0.00<sup>c</sup> | 260±0.00<sup>b</sup> | 8.70±0.20<sup>b</sup> |
| 1 g/260g                  | 12±0.26<sup>b</sup> | 76.42±0.10<sup>c</sup> | 3.87±0.00<sup>b</sup> | 3.75±0.00<sup>b</sup> | 8.61±0.00<sup>c</sup> | 232±0.00<sup>d</sup> | 6.76±0.10<sup>c</sup> |

3.3.2. Sensory analysis of mixed rice wine with different amount of the mold culture addition. Fig. 3 Radar chart of different amount of the mold culture addition in mixed rice wine.
It can be seen from Table 8 that when the added amount of the mold culture was 1g/240g, the sensory evaluation score of rice wine was the highest, while when the added amount of the mold culture was 1g/260g, the sensory evaluation score of rice wine was the lowest; combined with the analysis of Fig. 3, when the added amount of the mold culture was 1g/240g, the area was the largest, indicating that when the added amount of the mold culture was 1g/240g, the rice wine had a unique style, good taste and fresh fragrance; however, whether the amount of the mold culture was high or low, it will have a certain impact on the aroma and taste of rice wines.

Table 8. The average scores of ten person tasting group

| Sensory indexes                  | Amount of the mold culture addition |
|---------------------------------|-------------------------------------|
|                                 | 1g/230g | 1g/240g | 1g/250g | 1g/260g |
| Appearance and color (20 points) |         |         |         |         |
| Aroma (20 points)               | 16±0.87b | 17±0.13a | 17±0.46d | 15±0.16a |
| Taste (30 points)               | 26±0.29c | 27±0.34c | 25±0.94c | 23±0.46c |
| Characteristics (30 points)     | 17±0.11b | 24±0.74d | 19±0.13a | 16±0.83a |
| Total scores (100 points)       | 73±0.93d | 84±1.36b | 76±0.73e | 70±0.42b |

Figure 3. Radar chart of mixed wine with different quantities of wine stater

From the comprehensive analysis of Table 7, 8 and Fig. 3, it can be concluded that the best addition amount of mold culture of the rice wines was 1g/240g. After fermentation, the rice wine had a typical aroma of rice wine, with a long aftertaste, while the rice wine added with the amount of 1g/230g had an obvious smell of rotten eggs. The reason was that in fermentation process, when the temperature was too high, it would result in secondary fermentation, so that the rice wine had an oxidation and vulcanization taste [18].

3.3.3. PCA analysis of electronic tongue of mixed rice wines with different amount of the mold culture addition. According to Fig. 4, the relative distances between the four kinds of the mixed rice wines fermented with different added amount of the mold culture were relatively large, which can be clearly distinguished.

The DI value of the four kinds of wine was 98.1, which indicated that the electronic tongue could be used for the identification and differentiation of the different amount of rice wine. The total contribution rate of the principal component was 87.38%, which can fully represent the overall
information of the sample. When the addition of the mold culture was 1 g/260g, the difference was large and it was not representative.

3.4. Effect of fermentation time on mixed rice wine fermentation

3.4.1. Indexes changes of rice wine with different fermentation time. It can be seen from Table 9 that different fermentation time of rice wine had certain influence on rice wines.

From the horizontal analysis, when rice wine was fermented for 5 days, the reducing sugar content of rice wine was high, most of the sugar was not fermented, the alcohol degree was low, the alcohol content was the lowest, and the degree of saccharification was low, only a small amount of starch was converted; after 8 days of fermentation, most of the sugar was converted, the alcohol degree was increased, but the acidity was also increased; After 11 days of fermentation, the pH value of rice wine was the lowest, while the acidity and alcohol degree had increased, resulting in the imbalance between sugar, alcohol and acidity; after 14 days of fermentation, reducing sugar, alcohol degree and alcohol content were all high; after 17 days of fermentation, the pH value of rice wine was the lowest, resulting in the increase of acidity, the consumption of reducing sugar was quickly completed, and the alcohol degree was very low, resulting in the incongruity of the three. In conclusion, the best fermentation time of rice wine was 14 days.

Table 9. Determination of indexes of mixed rice wine with different fermentation time

| Fermentation time (day) | Soluble solids (%) | Reducing sugar (g/L) | Total acid (g/L) | pH  | Alcohol degree (%vol) | Alcohol content (%) | Starch (%) |
|-------------------------|-------------------|---------------------|-----------------|-----|-----------------------|---------------------|------------|
| 5                       | 17±0.20<sup>d</sup> | 112.77±0.10<sup>b</sup> | 4.11±0.10<sup>b</sup> | 3.67±0.00<sup>d</sup> | 6.74±0.00<sup>d</sup> | 212±0.00<sup>b</sup> | 10.50±0.10<sup>b</sup> |
| 8                       | 13±0.00<sup>b</sup> | 25.00±0.10<sup>d</sup> | 6.27±0.05<sup>d</sup> | 3.31±0.00<sup>b</sup> | 10.42±0.00<sup>c</sup> | 240±0.00<sup>c</sup> | 9.14±0.10<sup>b</sup> |
| 11                      | 12±0.17<sup>a</sup> | 14.30±0.20<sup>c</sup> | 5.07±0.10<sup>d</sup> | 3.35±0.00<sup>b</sup> | 11.67±0.04<sup>a</sup> | 256±0.00<sup>a</sup> | 8.14±0.10<sup>c</sup> |
| 14                      | 14±0.10<sup>c</sup> | 79.16±0.10<sup>b</sup> | 3.57±0.10<sup>a</sup> | 3.78±0.00<sup>b</sup> | 11.95±0.00<sup>c</sup> | 260±0.00<sup>d</sup> | 7.56±0.10<sup>c</sup> |
| 17                      | 12±0.20<sup>a</sup> | 11.00±0.20<sup>c</sup> | 7.71±0.10<sup>c</sup> | 3.13±0.00<sup>d</sup> | 7.84±0.00<sup>d</sup> | 256±0.00<sup>d</sup> | 7.99±0.10<sup>d</sup> |

3.4.2. Sensory analysis of rice wine at different fermentation time. It can be seen from Table 10 that different fermentation time has certain influence on the taste of rice wine [19].
The average score of rice wine fermented for 14 days was the highest, and the average sensory evaluation value of rice wine fermented for 17 days was the lowest. According to Fig. 5, the area of rice wine fermented for 14 days was the largest, and the area of rice wine fermented for 5 days was the smallest. Because the rice wine fermented for five days was not fresh and dull, the fermentation was not fully conducted, resulting in a certain sweetness in the rice wine. However, for the rice wine fermented for 17 days, almost all the sugars in the the mold culture were fermented, which resulted in rice wine rancidity and no obvious style of rice wine.

According to Table 8, 9 and Fig 5, through physical and chemical analysis and sensory evaluation analysis, the best fermenting time of rice wine was 14 days. After 14 days of fermenting, the glutinous rice and aroma rice mixed for fermentation, highlighting the aroma of rice wine, and the wine quality was appropriate.

Table 10. The average scores of ten person tasting group

| Sensory indexes                  | Fermentation for five days | Fermentation for eight days | Fermentation for eleven days | Fermentation for fourteen days | Fermentation for seventeen days |
|----------------------------------|----------------------------|----------------------------|------------------------------|--------------------------------|---------------------------------|
| Appearance and color (20 points) | 17±0.20d                   | 16±0.09c                   | 16±1.36d                     | 17±0.23c                       | 14±0.15d                        |
| Aroma (20 points)                | 15±0.13c                   | 16±0.23c                   | 17±0.23d                     | 17±0.21c                       | 15±0.83d                        |
| Taste (30 points)                | 22±0.14b                   | 24±0.36b                   | 23±0.42c                     | 27±0.42a                       | 21±1.23a                        |
| Characteristics (30 points)      | 18±0.71d                   | 20±0.49d                   | 22±0.61d                     | 25±0.62d                       | 19±0.39b                        |
| Total scores (100 points)        | 72±0.36c                   | 76±0.11d                   | 78±1.23b                     | 86±0.94b                       | 69±1.23c                        |

Figure 5. Radar chart of mixed rice wines at different fermentation time

3.4.3. PCA analysis of rice wine with electronic tongue at different fermentation time. According to Fig. 6, the relative distance between the five kinds of the mixed rice wines fermented at different fermenting times was relatively large, which can be clearly distinguished.
The results of principal component analysis showed that the DI value of the five wines was 97.52, which indicated that the electronic tongue could be used to distinguish the fermentation time of different mixed rice wines. The contribution rate of principal components was 96.59%, which can completely represent all the information of fermented rice wine at different times. When the fermentation time was 14 days, the differences among the three parallel samples were small, indicating that the wine had certain representativeness.

**Figure 6.** The results of PCA analysis at different fermentation times

4. Discussion
Electronic tongue has certain advantages, accuracy and reliability in distinguishing different kinds of rice wines [20]. It is a quick and convenient identification method of rice wine with comprehensive information and low cost. Different rice wine fermenting conditions have a certain impact on the fermenting of rice wine. Different rice materials are suitable for different fermenting conditions. In order to optimize the fermenting process of rice wine, it is necessary to carry out multi-level experimental design. Through the experiment, the advantage of this experiment was that it can clearly select the best raw materials for the mixed fermentation with glutinous rice, and optimize the fermenting process of rice wine through parameter control; while the disadvantage was that the fermentation process was directly added with the mold culture, so that the rice wine fermentation was slow and the saccharification was not sufficient. After the fermentation, the reducing sugar content was still very high. And in the process of tasting, because some rice wine had bubbles, resulting in the bottom turbidity and clear rice wine mixed, so that the taste team can not accurately judge the quality of rice wine from the appearance, aroma and taste.

This experiment changed the traditional mode of rice wine fermentation, using solid-state fermentation and saccharification first, then adding water to liquid fermentation. Compared with traditional rice wine fermentation, this method made multi-juice rice wine by deep fermentation in a short period of time, keeping the unique style of rice wine as much as possible, not only improving the yield of rice wine, but also reducing the production cost of rice wine. At the same time, taking the raw rice material, the amount of the mold culture added and the fermentation time as variables, through the final index of sensory evaluation and electronic tongue analysis, the fermenting process of rice wine was optimized, which not only had great value in the fermenting of the mixed glutinous rice wine, but also had certain research significance in the application of the mold culture in rice wine. Through the comprehensive evaluation of the basic physical and chemical indexes, electronic tongue and sensory evaluation of rice wine, the optimal utilization plan of the glutinous rice wine was finally worked out, fermenting rice wine according to the best plan was conducive to the promotion of rice wine industry.
Through the research of this experiment, it was found that the fermenting cost of rice wine was low, the technology was simple, easy to operate, and had a good development prospect. In the future, we can further carry out orthogonal test, put more high-quality technology into production, and better drive the development of grain wine such as rural rice wine, corn wine and sorghum wine. In the future, the technology of rice wine optimization will have great application prospects.

In this experiment, the best mixed rice materials were studied firstly. Through physical and chemical analysis, sensory evaluation and electronic tongue analysis, the indexes of red rice, black rice and aroma rice blending were comprehensively analyzed. The best materials were aroma rice and glutinous rice mixed fermentation, and the average scores of 10 tasting groups was 78 points, which was the highest among the four samples. Through the selected aroma rice wine, the different amount of the mold culture addition were studied. According to the experimental data and the final average score of the tasting group, 84 points were obtained, and the best adding amount of the mold culture was 1g/240g. According to the determined raw materials and the mold culture adding amount, the different fermentation time was conducted. According to the physical and chemical indexes and 86 points of tasting score, the best fermentation time of the mixed glutinous rice wine was 14 days. In the fermentation process of rice wine, the mixed glutinous rice and aroma rice were selected for fermentation. The mixing ratio was 1:4, the fermentation temperature was controlled at 38 °C, the added amount of the mold culture was 1 g/240g, and the fermentation time was 14 days. The appearance and color of the fermented rice wine were clear, transparent, glossy, typical golden yellow, with the unique mellow and rich aroma of glutinous rice wine, and no bad smell, the wine was full-bodied, mellow and harmonious with exquisite taste, and had the unique style of fermented glutinous rice wine.

5. Conclusion
In order to develop the best technology of the mixed glutinous rice wines, the rice wines fermenting technology was optimized by designing different rice materials, different amount of the mold culture addition and different fermentation time of rice wine. The physical and chemical index analysis, sensory evaluation analysis and electronic tongue analysis were used to analyze the rice wines, so as to determine the best raw material as rice, the best amount of the mold culture was 1 g/240g, and the optimal fermentation time was 14 days.

Acknowledgments
Thanks for Special fund for 2018 High level talent project of Xinjiang Uygur autonomous region, Surface Project of Joint Special Fund Project on Basic Research for Local Undergraduate Colleges and Universities (part) in Yunnan province (2018FH001-043), Engineering Technology Research Center of Grape and Wine for advanced school in Yunnan, 12th Five - Year Degree Authorized Construction Discipline in Biology of Yunnan Province, University - level Academic Backbone Training Project for Chuxiong Normal College (XJGG1603), Youth Project of Yunnan Applied Basic Research Project s(2016FD088), key major construction projects on ‘biotechnology’ for Chuxiong Normal College, scientific research fund major special projects for education department of Yunnan province (ZD2015016), support plan of scientific and technological innovation team for research and development of characteristic plant resources in colleges and universities in Yunnan province (IRTSTYN).

Reference
[1] Chen S and Xu Y 2010 J. Ins. Brewing 116 190.
[2] Ling Y, Hou W G, Dong Y R, Lian B, Yuan S and Dai C 2005 Food Sci. 26 648
[3] Xu B B, Mu Y C, Chen H W, Lei X C and Su W 2012 China brewing 31 62
[4] Alves T M, Farias F C, Alcarde A R, De O F and John H 2018 Braz. J. Food Tech. 18 151
[5] China National Standardization Administration. General Administration of quality supervision, inspection and Quarantine of the people's Republic of China 2006 Determination of total
acid in GB/T 15038-2006 (Beijing: China Standards Press) chapter 7 pp 53-64

[6] Rahul T, Penta P, John B G, Costas E S, Christophe S, Michael B, Sukhvinder P S and Quan V V 2018 Starch Starke 70 1700099

[7] Ma W, Shi Y Z, Duan Y, Wang C L 2018 Chin. Melon Vege. 31 1.

[8] Wang J, Chen R Y and M G G 2016 Chem. Design Commu. 6 98.

[9] Zhang Y L, Shi L and Wei D M 2005 Northwest A & F uni. Sci. Tech. 2005 171

[10] Yunhee J, Namhyeok C, Su W P, Bong S N, Jeong Y J and Kwon J H 2016 Food Sci. Biotech. 25 1313.

[11] Kazuya K, Yoshio N, Masanari A, Nobuko Y, Iwao M, Makoto H and Hajime T 2005 J. Appli. Glycosci. 52 393.

[12] Cao Z, Jia C, Niu M, Zhang B, Zhao S, Xiong S and Fang Z. J 2019 Chin. Cereals Oils Associ. 34 1.

[13] Pan Z L, Khir R and Thompson J F 2013 Cereal Chem. 90 107.

[14] Lai Q; Li Y; Wu Y; Ouyang J 2019 J. Food Sci. Technol. 56 1988.

[15] Lin X, He Z, Liang Z, Shen P, Lin X and Li W 2019 J. Chin. Cereals Oils Associ. 34 43

[16] Fan X P, Li D, Liu H, Liu Y M, Liu J B and Ma L Z 2020. Earth Envir. Sci. 440 022011

[17] Dung N T P, Rombouts F M and Nout M J R 2007 Food Microbio. 23 331

[18] Leal-Guerra C S, Pérez-Ortega E, Damas-Buenrostro L, Cabada J C, Galán-Wong L and Pereyra-Alfárez B 2010 Microorg. From Sci. Industr. Res. Consu. Pro. 2010 575-8

[19] Park J, Seo J S, Kim S A, Shin S Y, Park J H and Han N S 2017 J. Microbio. Biotech. 27 1736

[20] Fredrik W and Peter W I L 1997 Analy. Chimi. Acta. 357 21