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# Optimization research of fermentation conditions of purple potato liquor

**Yaru Wan, Xiao Dou, Ying-ying Ma, Xiaohua Zhu and Jian-gang Yang***

*College of Bioengineering, Sichuan University of Science and Engineering, Zigong, China*

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**ABSTRACT**

*In this paper, we take purple potato as raw material, optimize the fermentation process of Purple Potato Wine. To obtain the optimal process parameters combination, we use orthogonal design method on the basis of single-factor experiment to optimize the conditions of liquefaction, saccharification and culturing yeast fermentation. The results showed that under the condition of the pH value being 6.5, temperature being 60℃, α-amylase addition amount being 0.15%, reaction time being 2h, the liquefaction was optimized. At this time, the hydrolysis rate could reach 90.32%; Under the condition of the pH value being 4.0, temperature being 60℃, added amount of saccharifing enzyme being 0.2%, reaction time being 2h,the saccharification was optimized. Under this condition, the reducing sugar content in the liquor was 10.87%; and under the condition of the pH value being 3.5, temperature being 20℃, ratio of material to water being 1:1, reaction time being 6d, the fermentation effect of purple potato wine was the best.*

**Keywords:** Purple potato; liquefaction; saccharification; fermentation; orthogonal design method

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## INTRODUCTION

Purple potato (*Solanum tuberosum*), also called black potato, is one kind of sweet potato, and the potato flesh is purple to dark purple. Like other kinds of sweet potatoes, purple potato also contains rich protein, carbohydrate, vitamin and starch, as well as selenium and anthocyanins, and has special physiological health function and medicinal value [1]. Anthocyanin belongs to flavonoid component, and is a kind of water-soluble pigment widely exists in plants. The potato flesh present bright purple for purple potato contains a great deal of anthocyanins [2]. Dr Musquelier, a French scientist, found that anthocyanins were natural potent scavenger of free radical [3]. During the research, Jia Zhenghua and He Haiyan also mentioned that anthocyanins were most direct; the most effective and most secure scavenger of free radical ever found for preventing and controlling of diseases and maintaining people's health, and its ability of scavenging free radical was 20 times of vitamin C and 50 times of vitamin E [4]. Selenium has anti-cancer function and the function of and protecting myocardium. Selenium can play the role in scavenging free radicals and peroxide by raising the activity of GSH-Px in the form of selenoprotein, to improve the body's immune function against tissue cancerization. In addition, it also has the healthy efficacy of softening blood vessels, lowering blood pressure and lowering blood fat [5-7]. Wang Wenjuan and Yang Jian found that selenium could improve the content of immune globulin, and had the promoting effect in lymphocyte proliferation caused by antigen stimulation [8]. Due to its special efficacy, the purple potato wine made from purple potato has great market prospect. Zhou Suguo and others researched the influence of fermentation temperature, pH value and the added amount of active dry yeast on purple potato wine. It was found from the experiment that fermentation temperature had the greatest experimental effects on alcohol content of purple potato wine, and it was concluded from the experiment that the best conditions for brewing purple potato wine were: pH value was 3.6, the added amount of active dry yeast was 3% (volume fraction) and the fermentation temperature was 20℃, as a result, the brewed purple potato wine could have bright color, strong aromas and soft sour, with the alcohol content being 9.8% (volume fraction) and the retention amount of red pigment being 75.20% [9]. Zhu Hongmei researched the influence of enzymolysis method and Chinese yeast fermentation on physical and chemical parameters of purple potato wine. The results showed that the change trends of pH of the two methods have were the same with little difference; the

method of Chinese yeast fermentation can make full use of starch and reducing sugars; the fermented mash of purple sweet potato processed by enzymolysis method had higher soluble solids than that processed by Chinese yeast fermentation; the final alcohol content of purple sweet potato wine processed by enzymolysis method was lower than the purple sweet potato wine processed by Chinese yeast fermentation; the content pigment of fermented mash and polymeric pigment processed by enzymolysis method were higher than that processed by Chinese yeast fermentation. Enzymolysis saccharification method is more suitable for the saccharification of purple potato [10]. Taking purple potato and grapes as the main raw materials, Shi Jinglue and others produced purple potato wine with healthy function fermented with wine yeast. The result showed that: the added amount of purple potato syrup was 33.57%, the temperature was 20.95℃, fermentation time was 10.84d, add 0.59g/L attapulgite to clarify the purple potato wine, and the wine quality and clarity of the purple potato obtained was the best [11]. In this paper, we take the purple potato marketed in Zigong area as the raw material, adopts the orthogonal design method to conduct optimization research of the production process of purple potato wine, aiming to provide theoretical basis for the mass production of purple potato wine.

## MATERIALS AND METHODS

### The raw materials and main reagents

Purple potato were from Zigong Wal-Mart (water content is 65%, starch content is 20.13%);， Other experimental medicines are all AR.

### Process

Purple potato→clean and peel→slice→boil for gelatinization→ add water and pulp→adjust PH →add pectinase →adjust PH →add α-amylase for liquefaction→adjust PH→adding saccharifying enzyme for saccharification→added distiller's yeast→fermented for 6-10 days→sterilized →purple potato wine [9].

### Key points for operation

Production of distiller's yeast: Add 4mL water into 8.8g rice and boil for 45min at 110℃; distiller's yeast: 15g; Water: 20ml. Add them to the 250mL triangle bottle in proportion and saccharify for 8h at the temperature of 58-60℃. Add the yeas and lactic acid cultivated for 30h to the triangle bottle and cultivate for 24-48h at the temperature of 30℃. The yeast culture medium is 10mL liquid PDA medium, and the added lactic acid for each triangle bottle is 0.2mL. See the production method of distiller's yeast in Japanese Sake Technique [12]. The initial conditions for the liquefaction of Purple Potato: Add 100% water into the gelatinized purple potato and pulp, adjust pH to 4.0 with lactic acid and 20% NaOH solution, add 0.1% pectinase, then react for 2h, next, add 0.1% α-amylase in water bath and react for 2h at the temperature of 60℃, then measure its hydrolysis rate. The initial conditions for the saccharification of purple potato: Take the purple potato feed liquid liquefied at the best conditions as the experiment material, add 0.1% saccharifying enzyme, then react for 2h at the temperature of 60℃and measure the content of reducing sugar after the reaction. The initial conditions for the fermentation of the distiller's yeast of purple potato：Inoculate 0.1% distiller's yeast into the purple potato feed liquid which has been processed with pectinase, α-amylase and saccharifying enzyme, ferment for 8 days at the temperature of 20℃ in a ratio of material to water being 1:1, then respectively measure the alcohol content and the content of anthocyanins.

### The determination of the optimum conditions for the liquefaction of purple potato

The determination of the optimum PH for the liquefaction of purple potato：Add 100% water into the gelatinized purple potato and pulp, adjust pH to 4.0 with lactic acid and 20% NaOH solution, add 0.1% pectinase, and then react for 2h. Adjust the pH value to 5.5, 6.0, 6.5, 7.0 and 7.5, add 0.1% α-amylase in water bath and react for 2h at the temperature of 60℃, then measure their hydrolysis rate respectively. The determination of the optimum temperature for the liquefaction of purple potato：Add 100% water into the gelatinized purple potato and pulp, adjust its pH to 4.0 with lactic acid and 20% NaOH solution, add 0.1% pectinase, and then react for 2h. Adjust the pH to 6.5, then add 0.1% α-amylase and react for 2h at the temperature of 50℃, 55℃, 60℃, 65℃, 70℃, 75℃ and 80℃ respectively, then measure their hydrolysis rate respectively. The determination of the optimum reaction time for the liquefaction of purple potato：Add 100% water into the gelatinized purple potato and pulp, adjust its pH to 4.0 with lactic acid and 20% NaOH solution, add 0.1% pectinase, and then react for 2h. Adjust the pH to 6.5, then add 0.1% α-amylase into the water bath and react for 1h, 1.5h, 2h, 2.5h, 3h and 3.5h respectively at the temperature of 60℃ then measure their hydrolysis rate respectively. The determination of the optimum added amount of α-amylase for the liquefaction of purple potato：Add 100% water into the gelatinized purple potato and pulp, adjust its pH to 4.0 with lactic acid and 20% NaOH solution, add 0.1% pectinase, and then react for 2h. Adjust the pH to 6.5, then add 0.05%, 0.1%, 0.15%, 0.2% and 0.25% α-amylase into the water bath and react for 2h at the temperature of 60℃ then measure their hydrolysis rate respectively. The orthogonal experiment for the liquefying condition of purple potato：

According to the results of single-factor experiment and combining the hydrolysis rate of purple potato starch as the evaluation index, select three levels and conduct the orthogonal experiment of $L_9(3^4)$ four factors and three levels.

**The determination of the optimum conditions for the saccharification of purple potato**

The determination of the initial optimum PH for the saccharification of purple potato：Take the purple potato feed liquid which has been saccharified under the optimum conditions as the raw material, adjust its pH to 3.0, 3.5, 4.0, 4.5 and 5.0 with lactic acid and 20% NaOH solution, add 0.1% saccharifying enzyme respectively, and react for 2h at the temperature of 60℃, then measure their content of reducing sugar respectively.

The determination of the optimum temperature for the saccharification of purple potato：Take the purple potato feed liquid which has been saccharified under the optimum conditions as the raw material, adjust its pH to 4.0 with lactic acid and 20% NaOH solution, add 0.1% saccharifying enzyme, and react for 2h at the temperature of 50℃, 55℃, 60℃, 65℃ and 70℃ respectively, then measure their content of reducing sugar respectively. The determination of the optimum saccharification time for the saccharification of purple potato：Take the purple potato feed liquid which has been saccharified under the optimum conditions as the raw material, adjust its pH to 4.0 with lactic acid and 20% NaOH solution, add 0.1% saccharifying enzyme, and react for1h, 1.5h, 2h, 2.5h and 3h at the temperature of 60℃, then measure their content of reducing sugar respectively.

The determination of the optimum added amount of saccharifying enzyme for the saccharification of purple potato：Take the purple potato feed liquid which has been saccharified under the optimum conditions as the raw material, adjust its pH to 4.0 with lactic acid and 20% NaOH solution, add 0.05%, 0.1%, 0.15%, 0.2% and 0.25% saccharifying enzyme respectively, and react for 2h at the temperature of 60℃, then measure their content of reducing sugar respectively.

The orthogonal experiment for the saccharification condition of purple potato：According to the single-factor experiment results of the saccharification of purple potato and combining the content of reducing sugar after saccharification of purple potato as the evaluation index, select three levels and conduct the orthogonal experiment of $L_9(3^4)$ four factors and three levels.

**The determination of the best conditions for the fermenting conditions of purple potato**

The determination of the optimum initial PH for the fermentation of purple potato：Adjust the PH of the purple potato feed liquid which has been processed with pectinase, α-amylase and saccharifying enzyme to 3.0, 3.5, 4.0, 4.5 and 5.0 respectively with lactic acid and 20% NaOH solution, add 0.1% distiller's yeast, make the ratio of material to water be 1:1, then ferment for 8 days at the temperature of 20℃and measure their alcohol content respectively.

The determination of the optimum temperature for the fermentation of purple potato：Adjust the PH of the purple potato feed liquid which has been processed with pectinase, α-amylase and saccharifying enzyme to 4.0 with lactic acid and 20% NaOH solution, add 0.1% distiller's yeast, make the ratio of material to water be 1:1, then ferment for 8 days at the temperature of 20℃, 25℃, 30℃and 35℃respectively, and measure their alcohol content respectively. The determination of the optimum fermenting time for the fermentation of purple potato：Adjust the PH of the purple potato feed liquid which has been processed with pectinase, α-amylase and saccharifying enzyme to 4.0 with lactic acid and 20% NaOH solution, add 0.1% distiller's yeast, make the ratio of material to water be 1:1, then ferment for 6 days, 8 days, 10 days and 12 days respectively at the temperature of 20℃ and measure their alcohol content respectively. The determination of the optimum ratio of material to water for the fermentation of purple potato：

Add 100%, 75% and 50% water into the gelatinized purple potato and pulp respectively, adjust the Ph of the purple potato feed liquid which has been processed with pectinase, α-amylase and saccharifying enzyme to 4.0 with lactic acid and 20% NaOH solution, add 0.1% distiller's yeast, make the ratio of material to water be 1:1, then ferment for 8 days at the temperature of 20℃, and measure their alcohol content respectively. The orthogonal experiment for the fermenting condition of purple potato：According to the single-factor experiment results of the fermentation of purple potato and combining the alcohol content and the content of anthocyanins and total acid after fermentation of purple potato as the evaluation index, select three levels and conduct the orthogonal experiment of L9(34） four factors and three levels.

**Measured indicators and methods**

The hydrolysis rate of starch: see the measuring method in the part of measuring method for starch from the *Pandect of Wine Production Technology* written by Shen Yifang [13]. The content of reducing sugar: see the measuring method in the part of measuring method for reducing sugar from the *Pandect of Wine Production Technology* written

by Shen Yifang [13]. The alcohol content: measure the alcohol content according to the method of specific gravity in GB/T13662-2008. The content of total acid: see the measuring method in the part of measuring method for reducing total acid from the *Pandect of Wine Production Technology* written by Shen Yifang [13]. The content of Anthocyanins: adopt the method described in the article of Optimization of the Measuring Conditions for Anthocyanins of the Blueberry Wine with pH Differential Method written by Sun Jingchao, Liu Yutian, Zhao Yuping, etc. [14]

## RESULTS AND DISCUSSION

### 2.1 The influence of PH, temperature, liquefaction time and α-amylase on the liquefaction of purple potato starch

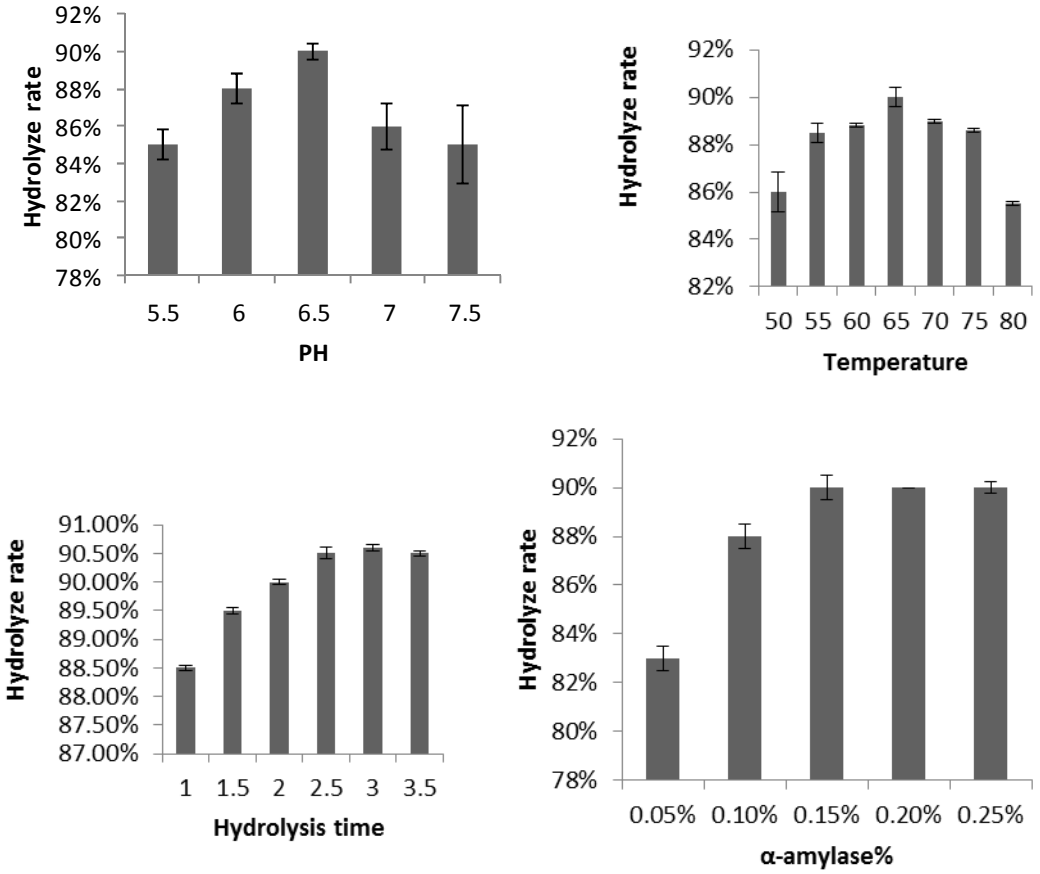


**Fig. 1 Effects of temperature，time，α-amylase ( %) on the hydrolysis rate**

According to the single-factor experiment results above (Fig.1), we can find that the optimum PH for the liquefaction of purple potato starch is 6.5 or so, the optimum temperature is at 65℃or so, the optimum liquefaction time is 2.5h and the optimum added amount of enzyme is 0.15%.

### 2.2 The orthogonal experiment results of the liquefaction of purple potato with hydrolysis rate as the evaluation index

It can be seen from table 1 that the order of all the factors can be obtained according to the degree to which they affect the hydrolysis effect of α-amylase hydrolysis: A> D> B> C, temperature has the great experiment effect on the hydrolysis of α-amylase, pH value has the minimal effect on the hydrolysis of α-amylase. The optimum condition for the hydrolysis of α-amylase is A1B1C2D3, but this combination is not within the nine groups of orthogonal experiment, verify the hydrolysis rate of α-amylase separately under the conditions, it's 90.32%, higher than that of the nine groups of the orthogonal experiment. So the optimum condition for the hydrolysis of α-amylase is that: temperature is 60℃, reaction time is 2 h, pH value is 6.5 and the content of α-amylase is 0.15%.

**Table 1 The design scheme of orthogonal experiment and results of liquefaction of purple potato starch**

| Experiment number | Factors | | | | |
|---|---|---|---|---|---|
| | Temperature(℃) | Time （h） | pH | Added amount of enzyme （%） | Hydrolysis rate（ %） |
| 1 | 1（60） | 1（2 ） | 1（6.0） | 1（0.05） | 88.75 |
| 2 | 1 | 2（2.5 ） | 2（6.5） | 2（0.10） | 90.19 |
| 3 | 1 | 3（3 ） | 3（7.0） | 3（0.15） | 89.64 |
| 4 | 2（65） | 1 | 2 | 2 | 89.16 |
| 5 | 2 | 2 | 3 | 3 | 84.98 |
| 6 | 2 | 3 | 1 | 1 | 84.57 |
| 7 | 3（70） | 1 | 3 | 3 | 83.86 |
| 8 | 3 | 2 | 2 | 1 | 83.79 |
| 9 | 3 | 3 | 1 | 2 | 82.35 |
| $k_1$ | 89.527 | 87.257 | 85.703 | 85.360 | Sequence |
| $k_2$ | 86.237 | 86.230 | 87.233 | 86.207 | A＞D＞B＞C |
| $k_3$ | 83.333 | 85.520 | 86.160 | 87.530 | The best combination |
| R | 6.194 | 1.737 | 1.530 | 2.170 | $A_1B_1C_2D_3$ |

**2.3 The influence of PH, temperature, saccharification time and saccharifying enzyme on the saccharification of purple potato**

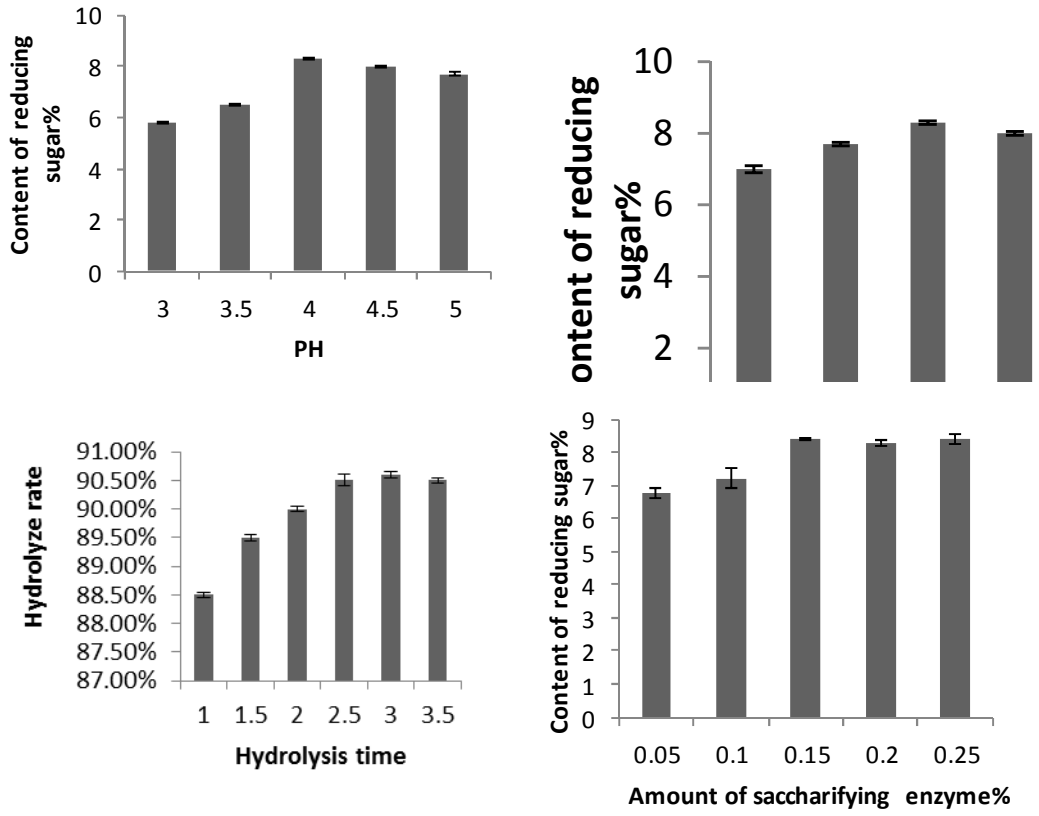


**Fig. 2 Effects of temperature，reaction time，added amount of saccharifying enzyme ( %) on the content of reducing sugar( %)**

According to the single-factor experiment results of saccharification above(Fig.2), we can conclude that the optimum PH for the saccharification of purple potato is 4.0 or so, the optimum temperature is at 55℃or so, the optimum saccharification time is 2.5h and the optimum added amount of saccharifying enzyme is 0.15% or so.

**2.4 The orthogonal experiment results of the saccharification of purple potato with the content of reducing sugar as the evaluation index**

It can be seen from table 2 that the order of all the factors can be obtained according to the degree to which they affect the saccharifying enzyme: the added amount of enzyme> pH> temperature> reaction time. The best combination for the reaction of saccharifying enzyme is $A_2B_3C_1D_3$, but this combination is not within the nine groups of orthogonal experiment, verify the content of reducing sugar separately under the conditions, it's 10.87%, higher than that of the nine groups of the orthogonal experiment. So the optimum condition for the reaction of saccharifying enzyme is that: pH value is 4.0, added amount of enzyme is 0.2%, enzymolysis time is 2h and the temperature is 60℃.

**Table 2 The design scheme of orthogonal experiment and results of saccharification of purple potato**

| Experiment number | Factors | | | | |
|---|---|---|---|---|---|
| | pH | Added amount of enzyme % | Reaction time | Temperature℃ | Content of reducing sugar% |
| 1 | 1(3.5) | 1(0.1) | 1(2) | 1(50) | 10.27 |
| 2 | 1 | 2(0.15) | 2(2.5) | 2(55) | 10.34 |
| 3 | 1 | 3(0.2) | 3(3) | 3(60) | 10.51 |
| 4 | 2(4.0) | 1 | 2 | 2 | 10.37 |
| 5 | 2 | 2 | 3 | 3 | 10.34 |
| 6 | 2 | 3 | 1 | 1 | 10.66 |
| 7 | 3(4.5) | 1 | 3 | 3 | 10.12 |
| 8 | 3 | 2 | 2 | 1 | 10.44 |
| 9 | 3 | 3 | 1 | 2 | 10.23 |
| $k_1$ | 10.374% | 10.257% | 10.456% | 10.281% | Sequence |
| $k_2$ | 10.456% | 10.373% | 10.316% | 10.374% | B＞A＞D＞C |
| $k_3$ | 10.265% | 10.465% | 10.323% | 10.439% | The best combination |
| R | 0.191 | 0.208 | 0.140 | 0.158 | $A_2B_3C_1D_3$ |

**2.5 The influence of initial PH, temperature, ratio of added water and fermentation time on the fermentation of purple potato**

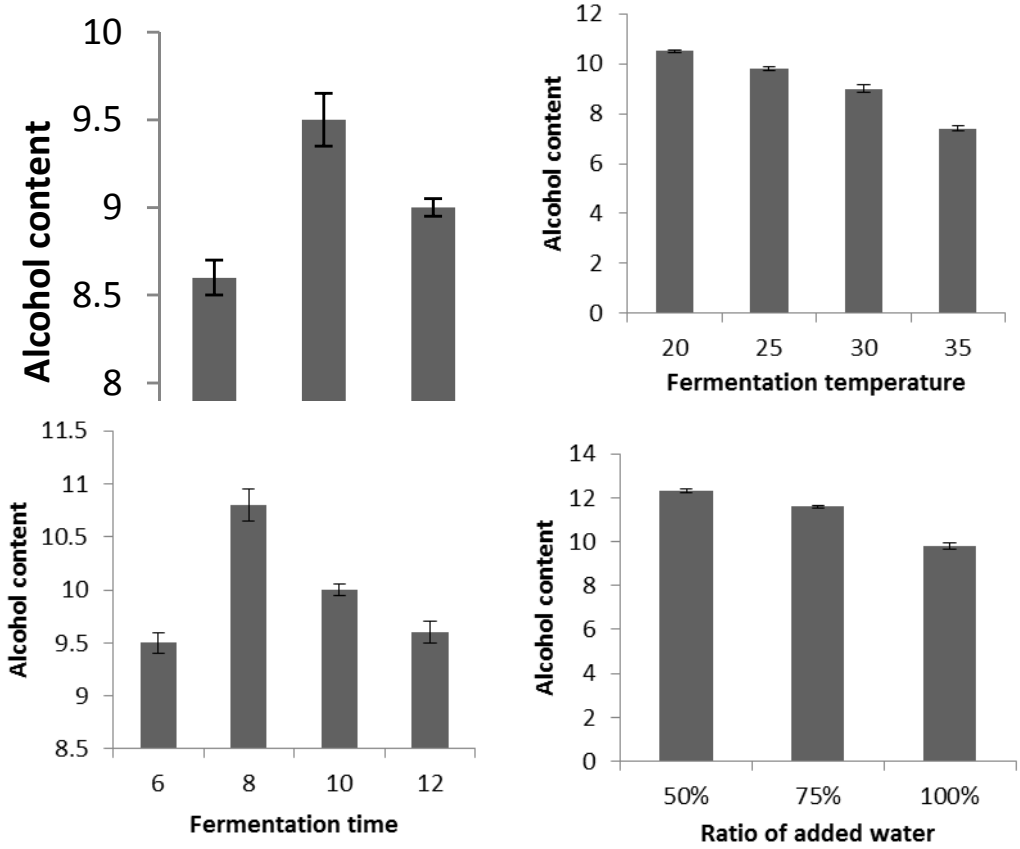


**Fig.3 Effects of initial pH，fermentation temperature，fermentation time，ratio of added water on the alcohol content**

According to the single-factor experiment results (Fig.3) we can find that, the optimum PH for the production of alcohol through fermentation is 3.5 or so, the optimum temperature is within 20℃,the optimum fermentation time is within about 8 days and the optimum ratio of added water is about 50%.

**2.6 The orthogonal experiment results taking the alcohol content and the content of total acid content and anthocyanins as the evaluation index**

It can be seen from following table that the order of all the factors can be obtained according to the degree to which they affect the alcohol content: ratio of added water> PH> fermentation time> temperature, and the best combination is A2B1C1D2; The order of all the factors can be obtained according to the degree to which they affect the content of total acid: PH > ratio of added water > fermentation time > temperature, and the best combination is A1B1C1D1; The order of all the factors can be obtained according to the degree to which they affect the content of anthocyanins: PH > ratio of added water > temperature > fermentation time, and the best combination is A2B3C1D1; Therefore, by comprehensive comparison and analysis, all the factors can be obtained according to the degree to

which they affect the purple potato wine: PH > ratio of added water > fermentation time > temperature, and the best combination is A2B1C1D1. When the initial PH is 3.5, fermentation temperature is 20 ℃, the ratio of added water is 50%, the fermentation time is 6 days, the optimal value can be obtained, and the alcohol content through the fermentation experiment after optimization can reach 11.3°, the total acid content can reach 2.77g/L, the content of anthocyanins can reach 225.37mg/L, the content of all the materials having a significant improvement compared with the value before optimization.

**Table 3 The design scheme of orthogonal experiment and results of fermentation of purple potato**

| Experiment number | | Factors | | | | Subject of evaluation | | |
|---|---|---|---|---|---|---|---|---|
| | | A pH | B Temperature ℃ | C Ratio of added water | D Fermentation time d | Alcohol content ° | Content of total acid g/L | Content of Anthocyanins mg/L |
| 1 | | 1 | 1 | 1 | 1 | 11 | 4.83 | 210.06 |
| 2 | | 1 | 2 | 2 | 2 | 9 | 3.91 | 204.28 |
| 3 | | 1 | 3 | 3 | 3 | 9.5 | 3.56 | 195.3 |
| 4 | | 2 | 1 | 2 | 3 | 9 | 2.67 | 217.56 |
| 5 | | 2 | 2 | 3 | 1 | 10.2 | 2.78 | 207.57 |
| 6 | | 2 | 3 | 1 | 2 | 11 | 3.47 | 223.03 |
| 7 | | 3 | 1 | 3 | 2 | 9.3 | 2.8 | 205.06 |
| 8 | | 3 | 2 | 2 | 3 | 8.9 | 3.17 | 217.07 |
| 9 | | 3 | 3 | 1 | 1 | 8 | 3.14 | 221.79 |
| Alcohol content ° | k1 | 9.833 | 9.767 | 10.3 | 9.733 | | | |
| | k2 | 10.067 | 9.367 | 9.667 | 9.767 | Sequence C>A>D>B | | |
| | k3 | 8.733 | 9.5 | 8.667 | 9.133 | The best combination A2B1C1D2 | | |
| | R | 1.334 | 0.4 | 1.633 | 0.634 | | | |
| Content of total acid g/L | k1 | 4.1 | 3.43 | 3.81 | 3.58 | | | |
| | k2 | 2.97 | 3.29 | 3.25 | 3.39 | Sequence A>C>D>B | | |
| | k3 | 3.04 | 3.37 | 3.05 | 3.13 | The best combination is A1B1C1D1 | | |
| | R | 1.13 | 0.14 | 0.76 | 0.45 | | | |
| Content of anthocyanins mg/L | k1 | 203.21 | 210.89 | 218.29 | 213.14 | | | |
| | k2 | 216.05 | 209.64 | 209.64 | 210.79 | Sequence A>C>B>D | | |
| | k3 | 214.64 | 213.37 | 202.62 | 209.98 | The best combination is A2B3C1D1 | | |
| | R | 12.84 | 3.73 | 15.67 | 3.16 | | | |

## CONCLUSION

In this paper, we optimize the three main processes of liquefaction, saccharification and fermentation of distiller's yeast during the production adopting orthogonal experiment design on the basis of single-factor experiment, which provides theory basis for the condition control during industrial production of purple potato wine. Through the process optimization, we can obtain that the optimal conditions for the liquefaction of purple potato starch are that, the temperature is 60℃, reaction time is 2h, pH is 6.5, the added amount of α-amylase is 0.15%, then the hydrolysis rate of α-amylase after optimization can reach 90.32%; The optimal conditions for saccharification are that, pH value is 4.0, the added amount of enzyme is 0.2%, enzymolysis time is 2h, the temperature is 60℃, then the content of reducing sugar of the feed liquid after optimization can reach 10.87%; The optimal conditions for the fermentation of distiller's yeast are that, the initial PH is 3.5, fermentation temperature is 20℃, the ratio of added water is 50%, fermentation time is 6 days, then the alcohol content after optimization can reach 11.3 °, the content of total acid can reach 2.77g/L, the content of anthocyanins can reach 225.37mg/L, the content of all the materials having a significant improvement compared with the value before optimization. By optimizing the initial conditions of the three processes of liquefaction, saccharification and fermentation of distiller's yeast, this experiment basically determines the optimum conditions of each process, which provides necessary data parameters for the industrial production of purple potato wine. But the scope of all the conditions is still a little large, and we need to conduct experiment for exploration if we want to get more accurate optimization parameters.

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