Study on Extraction of Polysaccharide by Composite Enzymatic Dilapidating Walls from Waste Wine Yeast Slurry

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Abstract—Objective: To establish a method for the extraction of polysaccharide from waste wine yeast slurry by composite enzymatic method. Methods: On the basis of single factor experiments, the effects of operating conditions, such as enzyme mixture ratio, enzyme added, composite enzymatic extraction pH, and composite enzymatic extraction temperature, were analyzed by orthogonal design methodology. Results: The optimized conditions of composite enzymatic extraction are as follows: the ratio of β-glucan enzyme to papain enzyme is 3:1, composite enzyme concentration 2.5 g/L, pH value 6.0, temperature 60°C, the polysaccharide yield is 8.97%. Conclusion: The composite enzymatic extraction method has a high extraction rate in extracting the polysaccharide of waste wine yeast slurry. The method is simple and practical.

Keywords- enzymatic extraction; cell wall disruption; polysaccharide; yeast slurry ;orthogonal design methodology

I. INTRODUCTION

Saccharomyces cerevisiae is a kind of more extensive single-celled eukaryotic organisms in the study. Yeast polysaccharide, which exists in yeast cell wall , is one of the basic components of yeast cell life [1]. With the research on the structure of the natural polymer is more and more deeply, the biological activities of natural macromolecular compound polysaccharide research has been developed rapidly [2]. Current research focuses on biological activities of polysaccharides in immunomodulatory [3], antitumor [4], antioxidation [5], antivirus[6] and lowering cholesterol. It shows good application prospect. Yeast polysaccharide, an important part of polysaccharide polymer, forits rich resources and the characteristics of natural non-toxic, is gradually becoming one of the focuses on which scholars study [7,8].

Enzymatic wall-breaking is mainly by adding exogenous enzyme on yeast cells to break the walls of outside-in. Given that the cell walls of saccharomyces cerevisiae contain a large amount of glucan, mannan, protein, a single glucanase or protease is often chosen to destroy the cell walls of yeast cells. Research of Liao Xianyan showed that only a single enzyme wall-breaking effect was not very ideal [9,10]. In practice, compound enzymatic is usually adopted. Zhao Yu used the combination of snail enzyme and protease on yeast cell wall-breaking in the preparation of amino acids [11]. Zhang Xiaoming thought that compound enzyme on wall breakingcould significantly accelerate the dissolution of insoluble substance in yeast cells after pretreatment [12]. This article is studying on enzymatic extraction of polysaccharide from grape wine yeast mud using compound enzyme, and the optimum extraction conditions were determined by orthogonal design, and it provides a new way for the comprehensive utilization of discarded yeast mud in wine.

II. MATERIALS AND METHODS

A. Materials

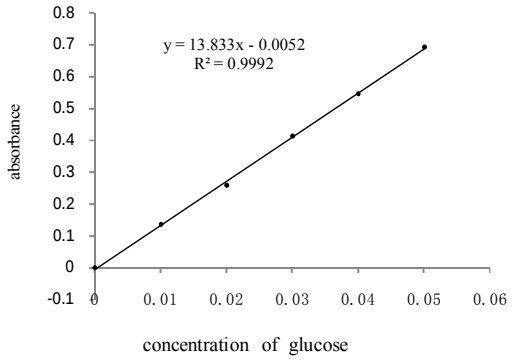
Wine yeast mud(Hebei Changli's fort wine offter)

B. Methods

1) The polysaccharide content determination (spectrophotometry)

Working liquid glucose is accurately suck up 0.0 mL , 0.2 mL, 0.4 mL , 0.6 mL, 0.8 mL , 1.0 mL, 1.2 mL , 1.4 mL and 1.6 mL, which is respectively put into 10 mL colorimetric tube. Then add the water to 2.0 mL and mix with 1.0 mL phenol sulfuric acid solution 1.0 mL . The blend is stored for 30 min at room temperature. Using 0.0mL glucose liquid group as the blank, determine absorbance values at 490 nm in 1 cm tube. A as the ordinate to the absorbance value, glucose

concentration as the abscissa draw the standard curve, and calculate the regression equation. Standard curve is shown



in Fig.1.

Figure 1. Standard curve of glucose

Determination of the sample: Dilute the 0.2 mL sample with water to the appropriate multiple. Accurately learn the diluent 2.0 mL, then measure absorbance value as above, calculate its concentration ccording to the regression equation. And then calculate the total sugar content in the sample.

2) The determination of polysaccharide yield

The polysaccharide yield

$$the\ polysaccharide\ yield(\%) = \frac{C * V * F}{M * (1 - W)} * 100 \quad (1)$$

C——the polysaccharide concentrations calculated according to glucose standard curve , g/L;

V——The volume of a solution of yeast , L;

F——Diluted multiples;

M——Yeast weight, g;

W——The moisture content of yeast, %.

3) Single factor experiment

The wall breaking process: yeast mud centrifugal (4000 r/min, 15min) → sediment → dry(37°C, 24 h) → dry yeast → disperse → the enzymatic wall-breaking → centrifugal (3000 r/min, 15 min) → supernatant → concentration (rotary evaporators) → alcohol sink → dry → crude polysaccharide

Wall of wine yeast were broken according to the wall breaking process, heated at 95°C for 15 min after enzymolysis. The enzymes were β-glucan enzyme and papain. With ratio of 0:1,0.5:1,1:1,2:1,3:1,4:1,1:0,the adding amount of 0.5 g/L, 1.0 g/L, 1.5 g/L, 2.0 g/L, 2.5 g/L, 3.0 g/L; pH of 5.0, 5.5, 6.0, 5.5, 7.0, 7.5; temperature of 50°C, 55 °C, 60 °C, 65 °C, 70 °C, 75 °C; duration of 2 h, 4 h, 6 h, 8 h, 10 h, 12 h, 14 h, 16 h, single factor experiments were carried out. Determined the content of polysaccharide of supernatant and calculated polysaccharide yield. Each test was done 3 times in parallel, taking the average value.

4) Process optimization test

The optimum parameters were obtained by the orthogonal analysis method, which was used to study the ratio of compound enzyme, enzyme dosage, pH value and temperature of four factors. With composite enzyme ratio, enzyme amount, pH, temperature, four factors as investigation object, using the method of orthogonal experiment arrangement to obtain the optimal process parameters.

III. RESULTS AND DISCUSSION:

A. The influence of enzyme mixture ratio of polysaccharide

Took seven 50mL of 10% yeast cell sap, add β-glucan enzyme and papain compound enzyme 100 mg, mixed as the proportion of 0:1,0.5:1,1:1,2:1,3:1,4:1,1:0. Sampled and determined polysaccharide content after adjusting pH to 5.0 and water bath heating at 50°C for 2 h . Bar charts of the percentage of the dry weight of the polysaccharide content of yeast were drawn, shown in figure 2.

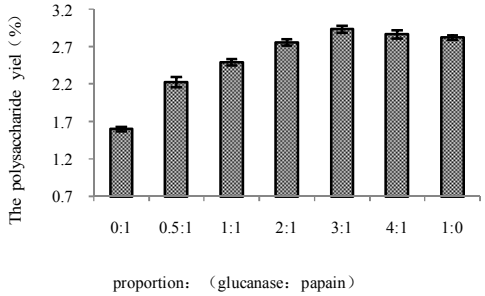


Figure 2. The effect of mixing ratio on the content of polysaeeharide

As shown in the figure 2, papain effect of pure yeast polysaccharide yield is smaller. With the increase of proportion of β-glucan, polysaccharide content increased. When the mixing ratio was 3:1 polysaccharide yielded maximum. Therefore at the condition of 50°C, the optimum mixture ratio was 3:1.

B. The influence of enzyme addition on the content of polysaccharide

Took six 50mL of 10% yeast cell sap, add β-glucan enzyme and 3:1 of compound enzyme papain 25 mg, 50 mg, 75 mg, 100 mg, 125 mg, 150 mg. Sampled and determined polysaccharide content after adjusting pH to 5.0 and water bath heating at 50°C for 2 h . Bar charts of the percentage of the dry weight of the polysaccharide content of yeast were drawn, shown in figure 3.

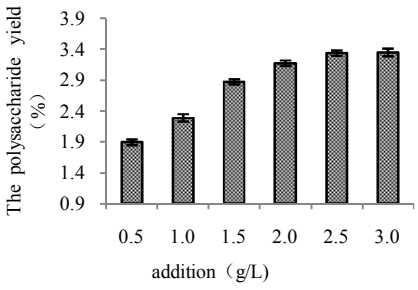


Fig. 3 The effect of enzyme addition on the content of polysaeeharide

As shown in figure 3, with the increase of adding amount of the compound enzyme, polysaccharide content gradually increased. After the mixing amount of 2.5 g/L, polysaccharide content changes tended to be gentle. Taking cost into consideration, best adding compound enzyme quantity is 2.5 g/L.

C. The influence of the pH of polysaccharide

Took six 50mL of 10% yeast cell sap. Respectively pH 5.0, 5.5, 6.0, 5.5, 7.0, 7.5, with 125 mg of β-glucan enzyme and composite enzyme papain 3:1.Sampled and determined polysaccharide content after adjusting pH to 5.0 and water bath heating at 50°C for 2 h . Bar charts of the percentage of the dry weight of the polysaccharide content of yeast were drawn, shown in figure 4.

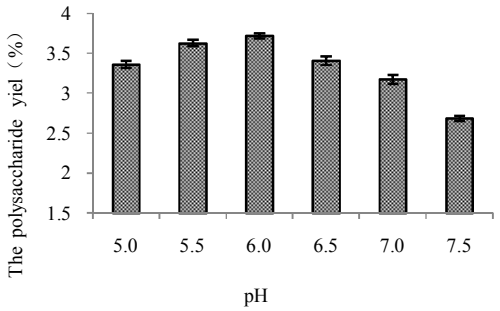


Fig. 4 The effect of pH on the content of polysaccharide

The figure 4 showed that polysaccharide yield reached maximum when pH was 6.0, pH increase or decrease polysaccharide yield were significantly decreased. pH 6.0 is the optimum. When the pH was greater than 6.0, polysaccharide yield droped rapidly, because of a higher percentage of β-glucan incompound enzymes , and β-glucan enzyme's optimum pH was5.5. enzyme activity began to decline when pH was greater than 5.5, after pH6.0 fell more rapidly, and as the optimum pH was between 6.0 - 7.0, with the increase of pH, the activity of papain was enhanced. when compound enzyme pH was 6.0, polysaccharide yield was maximum. When pH was greater than 6.0, falling fast the polysaccharide yield fell rapidly with β-glucan enzyme activity fell fast. Optimum pH for compound enzyme is 6.0.

D. The influence of temperature on polysaccharide yield

Took six 50mL of 10% yeast cell sap. Adjusted pH value of 6.0, with 125 mg of β-glucan enzyme and 3:1 composite enzyme papain, respectively in 50 °C and 55 °C, 60 °C, 65 °C, 70 °C, 75 °C water bath, 2 h after sampling determination of polysaccharide. Bar charts of the percentage of the dry weight of the polysaccharide content of yeast were drawn, shown in figure 5.

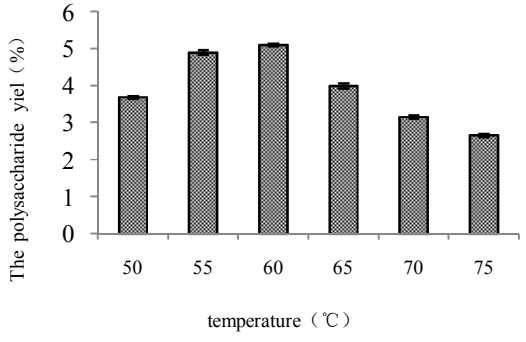


Fig.5 The effect of temperature on the content of polysaccharide

Figure 5 showed that, the polysaccharide content increased gradually ,with the increase of temperature. at 60°C content polysaccharide reached to maximum, and polysaccharide content reduced with the temperature after 60°C, the optimum temperature was 60 °C. This may be because of the optimal effect of β-glucan enzyme temperature of 55 °C, with the increase of temperature, polysaccharide content gradually decreased, but the decrease is gentle. And papain's optimal temperature may be higher than 55 °C, so the two enzymes of composite enzyme action temperature of 60 °C is to maximum. Then with the increase of temperature, activity of β-glucan decreased, and papain activity also began to decline after reaching a certain temperature. So when the temperature was above 60°C, the polysaccharide content changing with temperature was larger. The optimum temperature was 60 °C.

E. Orthogonal test

Through the above four other factors on the yeast cell enzyme wall-breaking experiment, determined the basic parameters of various factors. Were respectively selected β-glucan enzyme and enzyme papain compound ratio of 2:1, 3:1 ,4:1,and the added amount of 2.0 g/L, 2.5 g/L, 3.0 g/L, pH value of 5.5, 5.5, 6.0, temperature of 50 °C, 60 °C,65 °C. According to the $L_9(3^4)$ orthogonal experiment was carried out, the effect of the optimum conditions. The orthogonal experiment results such as table 1 and 2.

TABLE 1 THE FACTORS OF ENZYMATIC HYDROLYSIS OF YEAST

standard	A ratio	B amount(g/L)	C pH	D temperature(°C)
1	2:1	2.0	5.5	55
2	3:1	2.5	6.0	60
3	4:1	3.0	6.5	65

TABLE 2 THE ORTHOGONAL EXPERIMENT OF ENZYMATIC HYDROLYSIS OF YEAST

test	A	B	C	D	polysaccharide yield(%)
1	1	1	1	1	6.36
2	1	2	2	2	8.71
3	1	3	3	3	6.05
4	2	1	2	3	8.06
5	2	2	3	1	6.57
6	2	3	1	2	8.28
7	3	1	3	2	6.80
8	3	2	1	3	7.72
9	3	3	2	1	8.16
K1	7.040	7.073	7.453	7.030	
K2	7.637	7.667	8.310	7.930	
K3	7.560	7.497	6.473	7.277	
R	0.597	0.594	1.837	0.900	
optimal decision	A_2	B_2	C_2	D_2	

What we can see in table 2 is that $A_2B_2C_2D_2$ is the optimal scheme, according to the range C > D > A > B, so the pH > the temperature > the proportion> the adding quantity. PH is the biggest influence factor, the composite enzyme content is minimum. $A_2B_2C_2D_2$ in nine test had not been done, but only in the test2 factor A (ratio) was not in the best level, the effect of proportion on the yield of polysaccharide is the smallest. No2 obtained the highest polysaccharide yield, which meant that the optimal scheme we find out was practical.

In order to determine the optimal solution, under the condition of $A_2B_2C_2D_2$ verification experiment was done again. Results showed that under the condition of the β-glucan enzyme and enzyme papain compound ratio of 3:1, the adding amount of 2.5 g/L, pH value of 6.0, the effect of temperature of 60 °C the polysaccharide yield was 8.97%. And the experimental results were close to No2, but higher than No2 experimental results. So the optimal solution was β-glucan enzyme and enzyme papain compound ratio of 3:1, the adding amount of 2.5 g/L, pH value of 6.0, temperature of 60 °C.

IV. CONCLUSION

According to single factor experiment and orthogonal experiment method, composite enzymatic extraction of grape sugar and wine yeast polysaccharide in the best process conditions were as follows: under the condition of the effect of temperature of 60 °C, β-glucan enzyme and enzyme papain compound ratio of 3:1, the adding amount of 2.5 g/L, pH value of 6.0. Verification results showed that under the optimum technological conditions: the yeast polysaccharide yield was 8.97%. Compound polysaccharide of enzymatic extraction of grape wine yeast mud. The process is simple, and polysaccharide yield is high, and it has practical application value.

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