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Optimization of Extraction Process of Lingbao Jujube Polysaccharide

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Abstract: *Lingbao jujubewhich contains polysaccharide, an important biological active material, has high nutritional and medicinal values. In this experiment, polysaccharidewas extracted fromLingbao jujube. The effects of solid-liquid ratio, extractiontemperature, extractiontime and extraction times on the extraction yield of Lingbao jujube polysaccharide were investigated by single factor test. The extraction process conditions were optimized using orthogonal test. The results of single factor experiment showed that the extraction rate of polysaccharide was higher when the solid-liquid ratio was less than 1:6 g/mL, but the difference was not significant.When the extraction temperature was between 60 and 100°C, the extraction rate of polysaccharide increased with the increase of extraction temperature.When the extraction time was 0.5~2h, the extraction rate of polysaccharide increased with the prolonging of extraction time. However, when the extraction time was more than 2 h, the extraction rate of polysaccharides decreased. When the number of extractions was less than two, the extraction rate was higher. Moreover, as the number of extractions increased, the extraction rate of polysaccharides increased slowly. The optimal extraction process parameters were obtained by orthogonal test, the extraction solid-liquid ratio was 1:10g/mL, the extraction temperature was 100°C, the extraction time was 2h, and the number of extraction was 2 times. The polysaccharide extraction rate obtained by the verification test according to the optimal extraction process parameters was 4.208%.*

Keywords: *Lingbao jujube;Polysaccharide;Extraction process*

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I. Introduction

Polysaccharides are linked by more than 10 monosaccharides through glycoside bonds. They are widely distributed in nature and exist in plants, animals and microorganisms[1-3]. Plant polysaccharides have been received increasing attention because of their obvious function in body regulation and disease prevention. Jujube is a dry mature fruit of the genus Rhamnaceae., which is rich in various nutrients. Lingbao jujube is an excellent variety of Rhamnaceae jujube genus and famous for its unique color, shape and taste. Lingbao jujube has been cultivated for more than 400 years. Polysaccharide is an important bioactive substance in jujube, which can effectively scavenge oxygen free radicals in human body and has anti-aging function[4-7]. In addition, further pharmacological studies show that jujube polysaccharides can effectively enhance the immunity of organismsbecause of their anti-complement activity and promoting effecton lymphocyte proliferation.Thus,jujube polysaccharidescan be widely used in health care products and functional foods. As a green biomedical product, jujube polysaccharide has a broad market prospect and application value[8-10].In recent years, studies have also found that jujube polysaccharide has anti-cancer, anti-AIDS and other physiological activities. Therefore, its medicinal value has attracted much attention. In this paper, the extraction technology of Lingbao jujube polysaccharides was studied, which provided theoretical basis and guidance for the extensive and mature application of Lingbao jujube polysaccharides.

II. Materials And Methods

2.1. Materials and Chemicals

Lingbao jujubeswere gathered from Lingbao city, Henan Province of China. Glucose, phenyl hydroxide, ethyl alcoholandstrong sulfuricwere purchased fromSinopharm Chemical ReagentCo., Ltd(Shanghai, China).

2.2 Determination of polysaccharide content

The polysaccharide content was determined by the sulfuric acid-phenol method using glucose as a standard.

2.2.1 Protraction of standard curve

Different concentrations of glucose standard solution (1 mL) were mixed with 1.6 ml of 5% phenol solution and 7 ml of concentrated sulfuric acid, respectively. After blended well, the mixture was placed for 10 minand

incubatedin water bath at 25 ℃ for 15 min. The absorbancevalues(A) at 490 nm was determined and the standard curve of glucose was obtained as shown in Figure 1.

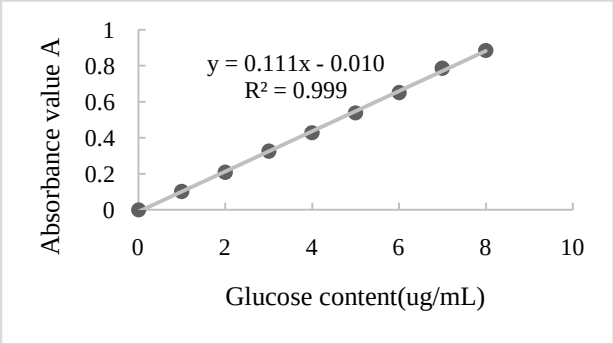

Fig. 1 Standard curve of glucose

2.2.2 Extraction rateof polysaccharide

Extraction rateof polysaccharide(%) = $M/M_0 \times 100$ (1)

Where M(g) is the weight of polysaccharide; M_0 (g) is the weight of jujube.

2.3Single-factor experimental design

2.3.1Effect of solid-liquid ratio on extraction rate

Under the fixed conditions of extraction temperature 80 °C and extraction time 1 h, the solid-liquid ratios of 1:4 g/mL, 1:6 g/mL, 1:8 g/mL, 1:10 g/mL and 1:12 g/mL were selected to extract Lingbao jujube once, respectively.The mixture was filtered, and then 8 g/L ofactivated carbon was added to the filtrate, which was decolorized in 60°C water bath for 30 min, followed by secondary filtration and centrifugation of 3000 r/min for 15 min. Finally, the supernatant was taken and diluted appropriately. The absorbance was measured according to the determination method of polysaccharide, and the extraction rate of polysaccharide was calculated.

2.3.2Effect of temperature on extraction rate

The Lingbao jujube was extracted once at 60 °C, 70 °C, 80 °C, 90 °C and 100 °Cwiththesolid-liquid ratio of 1:8 g/mL and extraction time of 1 h. The following operations were the same as above.

2.3.2Effect of time on extraction rate

The solid-liquid ratio and extraction temperature were fixed at 1:8g /mL and 80, and the extraction time was set at 0.5h, 1h, 1.5h, 2h and 2.5h, respectively. Lingbao jujube was extracted once, and the following operations were the same as above.

2.3.2Effect of times on extraction rate

Under the conditions of solid-liquid ratio of 1:8 g/mL, extraction temperature of 80 °C and extraction time of 1 h, the extracts of Lingbao jujube were extracted once, twice, three times, four times and five times respectively. The following operations were the same as above.

2.4 Orthogonal experimental design

On the basis of single factor experiment, orthogonal experiment was carried out to determine the optimum extraction conditions of jujube polysaccharide.

III. Results and Discussion

3.1 Single-factor experiment

3.1.1Effect of solid-liquid ratio on extraction rate of polysaccharide

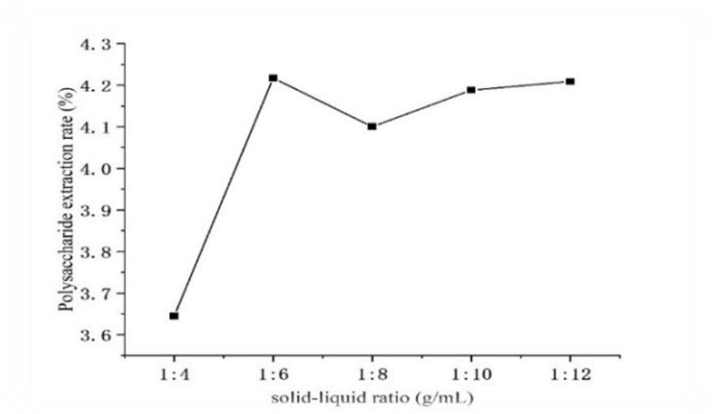

Fig. 2 Effect of solid-liquid ratio on extraction rateofpolysaccharide

The effect of solid-liquid ratio on the extraction rate of polysaccharides was shown in Figure2. From Figure2, it can be seen that when the solid-liquid ratio was less than 1:6 g/mL, the extraction rate of polysaccharides was relatively high, but the difference is not significant. When the solid-liquid ratio was greater than 1:6 g/mL, the extraction rate of polysaccharides decreased slightly, and the solid content decreased, making subsequent operations more difficult. In order to reduce the energy consumption of concentration and other operations in the polysaccharide extraction process and to make the process simpler, the solid-liquid ratio was initially selected to be 1:6g/mL.

3.1.2Effect oftemperature on extraction rateof polysaccharide

The effect of extraction temperature on the extraction rate of polysaccharides was shown in Figure 3. As can be seen from Figure3, when the temperature of the extract was between 60°C to 100°C, the polysaccharide extraction yield increased with the rising of temperature, and the polysaccharide extraction rate was the highest when the temperature was 100 °C. Due to the limitation of conditions, the temperature cannot reach a high level, so the extraction temperature should be selected at 100 °C.

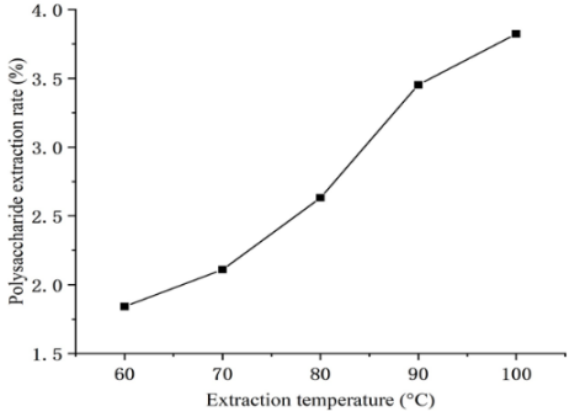

Fig. 3 Effect of extraction temperature on extraction rateofpolysaccharide

3.1.3Effect ofextractiontime on extraction rateof polysaccharide

The effect of extraction time on the extraction rate of polysaccharides is shown in Figure4. According totheFigure 4, when the extraction time was between 0.5-2 h, the extraction rate of polysaccharides increased with the increase of extraction time, but when the extraction temperature was higher than 2 h, the extraction rate of polysaccharides decreased. In order to shorten the working hours and reduce energy consumption, the extraction time should be selected for 2 h.

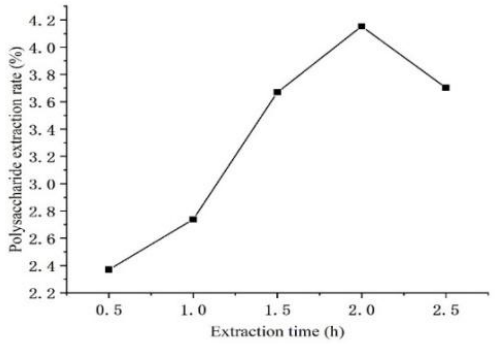

Fig. 4 Effect of extraction time on extraction rateofpolysaccharide

3.1.4 Effect of extractiontimes on extraction rateof polysaccharide

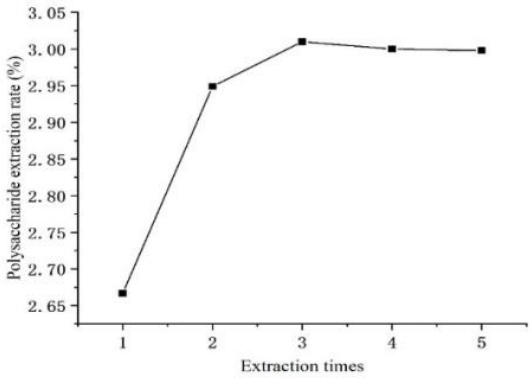

Fig. 5 Effect of extraction times on extraction rateofpolysaccharide

The effect of extraction times on the extraction rate of polysaccharide was shown in Figure 5.According to Figure 5, when the number of extraction times was no less than 2, the extraction rate of polysaccharide was relatively high, but the difference was not significant.When the number of times of extraction exceeds 2, the rate of polysaccharide extraction slowly increased.If the polysaccharide was extracted only once, the maximum amount of polysaccharide cannot be obtained, which simplifies the extraction process and saves time.Therefore, it was more appropriate to choose two extractions.

3.2Analysis of Orthogonal Test and Verification Test

Base on single factor experiment, four factors and three levels orthogonal experiment was used to determine the optimum extraction conditions. The orthogonal experiment design was shown in Table 1. The range analysis method was used to analyze the test results. As can be seen from Table 1, the order of influence of each factor on the results was B > D > C > A. The polysaccharide extraction rate of the sample showed that the extraction rate of $A_3B_3C_2D_3$ was the highest. However, there was no significant difference between extraction times of 2 and 3. Thus,the best combination was $A_3B_3C_2D_2$, i.e. solid-liquid ratio of 1:10g/mL, extraction temperature of 100°C, extraction time of2 handextraction timesoftwice. The extraction rate of polysaccharides was 4.208% in the optimal combination validation test, and the optimal combination was $A_3B_3C_2D_2$ according to the validation test data.

Table 1 Orthogonal test and results

Runs	Solid-liquid radio(g/mL) (A)	Extraction temperature (°C) (B)	Extraction time (min) (C)	Extraction Times (°C) (D)	Extractionrate of polysaccharide (%)
1	1:6	80	1.5	1	1.907
2	1:6	90	2.0	2	3.743
3	1:6	100	2.5	3	4.119
4	1:8	80	2.0	3	3.317
5	1:8	90	2.5	1	3.078
6	1:8	100	1.5	2	3.539

7	1:10	80	2.5	2	3.495
8	1:10	90	1.5	3	3.415
9	1:10	100	2.0	1	3.700
K1	9.768	8.718	8.862	8.685	
K2	9.933	10.236	10.761	10.776	
K3	10.611	11.358	10.692	10.851	
k1	3.256	2.906	2.954	2.895	
k2	3.311	3.412	3.587	3.592	
k3	3.537	3.786	3.564	3.617	
R	0.281	0.880	0.633	0.722	

III. Conclusion

In this experiment, Single factor and orthogonal experiments were used to optimize the extraction rate of polysaccharide from Lingbao jujube.Using glucose as a standard, a method for determining the polysaccharide content by phenol-sulfuric acid method was established.The measurement results of this method are accurate and reproducible. The single factor test results showed that when the solid-liquid ratio was less than 1:6 g/ml and the extraction times were more than twice, the extraction rate of polysaccharide was higher but the difference was not significant. When the extraction temperature was 100 ℃ and the extraction time was 2 h, the extraction rate of polysaccharide of jujube was significantly increased.The optimal extraction process parameters were obtained by orthogonal test, i. e. solid-liquid ratio was 1:10 g/ml, extraction temperature was 100 °C, extraction time was 2 h, and extraction times were twice.According to the process parameters, the extraction rate of Lingbao jujube polysaccharide was 4.208%.

Acknowledgements

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