

Study on Extraction Technology of Pitaya Polysaccharide

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Abstract: Using pitaya as raw material, this paper used ethanol subsiding method to study the extraction technology of pitaya polysaccharide by single factor test and orthogonal test. Through orthogonal test, we have found the optimum extraction conditions of pitaya polysaccharide as follows: extraction temperature 60°C, solid-liquid ratio 1:30, extraction time 3h. Under the optimal extraction conditions, the yield of pitaya polysaccharide was 1.13%.

1 Introduction

Hylocereus undulatus Britt, also known as Pitaya fruit, Jade Dragon Fruit, Red Dragon Fruit and Dragon Pearl Fruit, is a fruit cultivar of *Trigonocarpus* genus in Cactaceae. It is a kind of cactaceous plant that can blossom and bear fruit, and its fruit is edible. It is native to Costa Rica, Cuba, Mexico and other tropical areas in Central America. After being introduced into China, it is mainly distributed in the southern provinces, such as Guangdong, Hainan, Fujian, Taiwan and so on.

Pitaya is a kind of perennial climbing succulent. The rootstock is dark green, very stout and triangular-shaped. It has a wavy edge and clings to other plants. The flower is white and the huge ovary is inferior, which has the name of "the king flower". The fruit is olive like, the outer surface has the fleshy scale outwardly curls, and the flesh is covered with black small seeds. The texture is mild and the taste is fragrant.

The stem of pitaya is rich in plant polysaccharides, accounting for about 7% of the dried product [2]. According to the results of related scientific experiments, plant polysaccharides have high bioactivity and can maintain the biological function of organisms. This paper studied the extraction technology of pitaya polysaccharide using pitaya as raw material. The ethanol subsiding method was used to extract polysaccharide in pitaya. The optimal extraction conditions were found through single factor experiment and orthogonal test, and the content of polysaccharide was determined by phenol-sulfuric acid method [3], which provided certain theoretical basis for the further processing of pitaya fruit and the further research or development in related fields.

2 Ingredients and Methods

2.1 Experimental Materials

Ingredients: Hainan pitaya; Reagents: petroleum ether, 95%

ethanol, anhydrous ethyl ether, glucose, chloroform, anhydrous ethanol, n-butanol, phenol, acetone, sulfuric acid and other reagents are analytical pure.

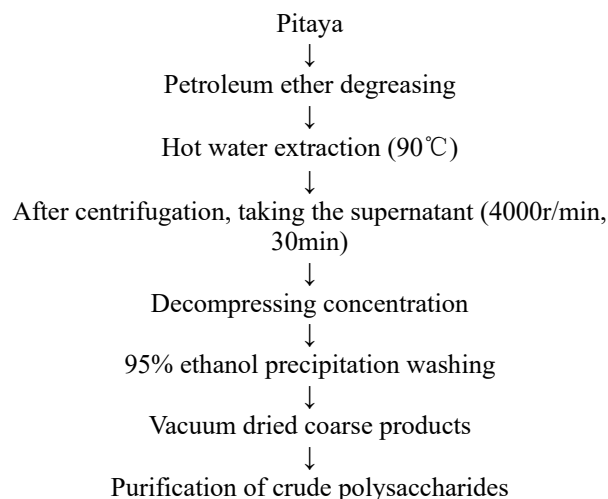
FA-244 electronic balance; digital display constant temperature water bath; rotary evaporator; UV1000 visible spectrophotometer; centrifuge; thermostatic oven.

2.2 Experimental Methods

2.2.1 Material Treatment

Wash, peel, slice, dry and grind fresh pitaya at 50°C and store it in reagent bottles for later use.

2.2.2 Extraction Process



2.2.3 Experimental Procedures

2.2.3.1 Preparation of Crude Polysaccharides

Sample treatment: peel, slice, dry and grind the pitaya, and store the sample in a dry reagent bottle;

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Degreasing: weigh 30g pitaya powder, add 100mL ether to reflux for 1h, extract and filter, and air dry^[4];

Hot water extraction: after the degreasing operation is completed, weigh 3g pitaya powder, add water in different proportions, and undertake extraction at different temperatures;

Centrifugation: centrifuge at 4000r/min for 30min, and take the supernatant;

Decompressing concentration^[5]: after centrifugation, decompress and concentrate the polysaccharide solution;

Ethanol precipitation: after the completion of the concentration step, add a certain amount of 95% ethanol to the solution, stir it while adding, place it overnight, then filter it the next day, and wash it for several times with anhydrous ethanol, acetone and anhydrous ether respectively^[6];

Vacuum drying: after filtering and washing the material, then start vacuum freeze drying, so as to get the pitaya polysaccharide crude products.

2.2.3.2 Purification of crude polysaccharides -- Sevage method

Take the advantage of the denaturation of proteins in organic solvents such as trichloroethane, and mix the crude polysaccharide extract with Sevage reagent (5:1). Then shake, stir it for 20min, and put it into a centrifuge at 4000r/min for 20min. The denatured protein is located at the junction of the extract and Sevage reagent. Remove the

denatured protein and repeat the steps several times until there is no turbidity precipitation in the middle^[7].

2.2.4 Determination of Polysaccharide Content

2.2.4.1 Preparation of Solution

Preparation of glucose standard solution: accurately weigh 60mg of dried glucose, put it in a 100mL volumetric flask, dilute it with water to the scale, shake it well, and then get the glucose standard solution.

Preparation of 4% phenol: take a precise measure of 4mL phenol, put it into a 100mL volumetric flask, dilute it with water to the scale, shake it evenly, and get it.

2.2.4.2 Production of Standard Curve

Take a precise measure of glucose standard solution, respectively 0.0 mL, 1.0 mL, 1.5 mL, 2.0 mL, 2.5 mL and 3.0 mL in the 50 mL volumetric flasks, add water dilute to scale, and shake them well. Take 2 mL of each diluted solution and place them in tubes with plug, label group No. 1-6, add 1 mL of 4% phenol solution respectively, and shake them well. Then add 7.0 mL concentrated H₂SO₄ immediately, shake them while adding, heat them in water bath at 40°C for 30 minutes, then place them in ice water bath for 5 minutes. Measure the absorbance of each group at 490nm, and the experimental data are shown in Table 1.

Table 1 Measured values of standard curve

	1	2	3	4	5	6
C(ug·mL ⁻¹)	0	2.4	3.6	4.8	6.0	7.2
Absorbance/(g·cm)	0	0.136	0.202	0.257	0.323	0.384

The solution concentration is taken as the abaxial axis, the unit is ug·mL⁻¹, the absorbance is taken as the vertical axis, and the unit is L/(g·cm). Draw the standard curve, then obtain the linear regression equation as follows: $Y = 0.053x + 0.0049$, $R^2 = 0.999$. The results are shown in Figure 1.

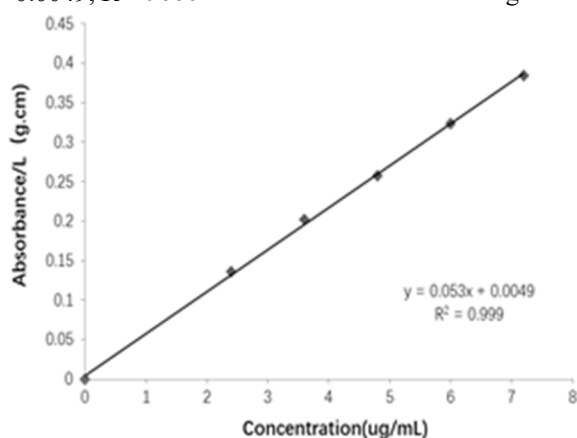


Fig. 1 Standard curve of glucose

2.2.4.3 Determination of Polysaccharide Content of the Sample

Dissolve and dilute the extracted polysaccharide into a 100 mL volumetric flask. Precisely take 2 mL diluted and constant volume into a 50 mL volumetric flask as sample solution. Take a precise measure of sample solutions of 0.0 mL and 2.0 mL, place them in 10 mL tubes with plug respectively. Then add 1 mL of 4% phenol solution respectively, shake them well, and add 7.0 mL concentrated H₂SO₄ immediately. Shake them while adding, heat them in water bath at 40 °C for 30 minutes, and then place them in ice water bath for 5 minutes. Take the first group as the blank control group, measure the absorbance of each group at 490 nm, and calculate the polysaccharide content in sample solution by standard curve^[8].

2.2.4.4 Polysaccharide Yield

According to the polysaccharide content of pitaya fruit, calculate the total amount of polysaccharide in the treated

pitaya, and then divide the polysaccharide mass obtained by the total amount of polysaccharide, so as to obtain polysaccharide yield.

Polysaccharide yield (%) = polysaccharide mass /pitaya powder mass ×100%

3 Results and Analysis

3.1 Single factor test

The yield of polysaccharides is affected by a variety of factors. This experiment focuses on the impact of three factors on the yield of polysaccharides, namely the extraction temperature, solid-liquid ratio and extraction time.

3.1.1 Influence of Extraction Temperature on the Yield of Polysaccharide

Under the same conditions of one-hour extraction time, two extraction times and a solid-liquid ratio of 1:20, change extraction temperature, and study its impact on the yield of polysaccharide. The experimental results are shown in Figure 2.

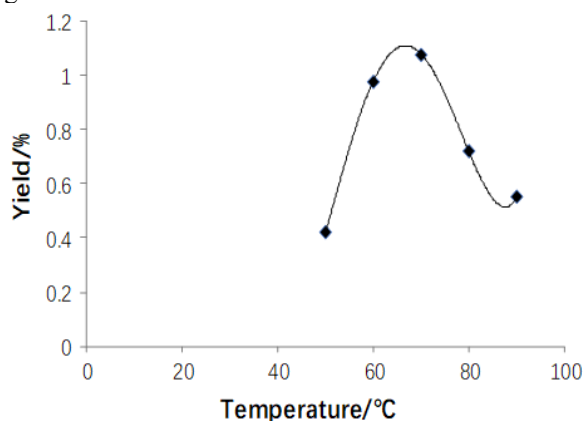


Fig. 2 Effect of extraction temperature on the yield of polysaccharide

As can be seen from Figure 2, under 70°C, the yield of polysaccharides gradually increased with the increase of extraction temperature. Above 70 °C , the yield of polysaccharides decreased with the increase of extraction temperature.

The analytical reason is that the yield of polysaccharides decreases when the temperature is too high. It is likely that the temperature is too high, so the polysaccharides are destroyed, the glycosidized bond is broken, and the polysaccharide degrades continuously, which finally leads to the decrease of the yield of polysaccharides [9]. Therefore, the appropriate temperature is 70°C.

3.1.2 Effect of Solid-liquid Ratio on the Yield of Polysaccharide

Under the same conditions of extraction temperature 70°C, one-hour extraction time and two extraction times, change solid-liquid ratio, study its impact on the yield of

polysaccharide. The experimental results are shown in Figure 3.

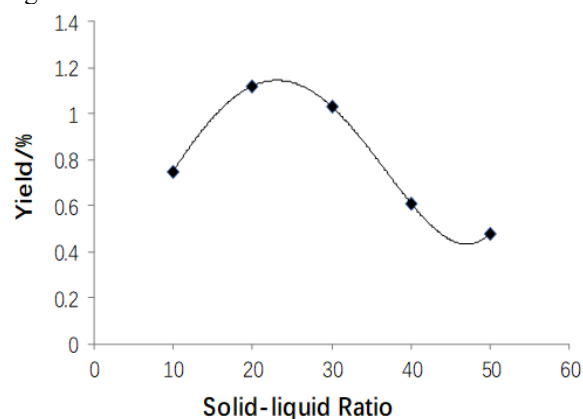


Fig. 3 Effect of solid-liquid on the yield of polysaccharide

As can be seen from Figure 3, the yield of polysaccharide increased with the increase of the solid-liquid ratio when it was under 1:20, and after the solid-liquid ratio increased above 1:20, the yield of polysaccharide was inversely proportional to the solid-liquid ratio. The analytical reason is as follows. When the solid-liquid ratio is above 1:20, with the increase of solid-liquid ratio, more and more distilled water is used for extraction, which increases the consumption of subsequent processes, and the loss of polysaccharides is also increasing [10]. Therefore, in order to ensure a good polysaccharide yield, the solid-liquid ratio of 1:20 is the most appropriate choice in the experiment.

3.1.3 Influence of Extraction Time on the Yield of Polysaccharides

Under the same conditions of extraction temperature 70°C, a solid-liquid ratio of 1:20 and two extraction times, change extraction time, study its effects on the yield of polysaccharide. The experimental results are shown in Figure 4.

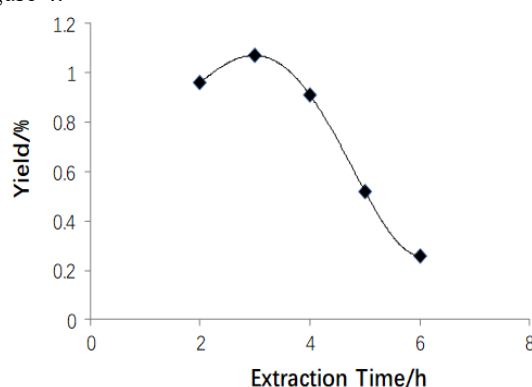


Fig. 4 Influence of extraction time on the yield of polysaccharide

According to the change rule in Figure 4, the yield of polysaccharides increased with the extension of extraction time. However, after 3 hours, the yield of polysaccharides decreased significantly with the increase of extraction time. The reason is as follows. As the extraction is done in hot water, the effect of too long extraction time is similar to that of too high temperature, which will lead to the breaking of the glycosidized bond

and the continuous degradation of polysaccharides. Therefore, too long extraction time will lead to a decrease in the yield of polysaccharides [11]. Therefore, 3 hours is the most appropriate extraction time.

3.2 Orthogonal Test

Orthogonal test is a design method to study multi-factors and multi-levels. On the basis of single factor experiment,

orthogonal test is carried out by selecting some representative points, and the horizontal combination selected by orthogonal test is listed into a table, which is called orthogonal table [12].

According to the conditions obtained from the single-factor test and considering various factors, this experiment took the extraction temperature, liquid-solid ratio and extraction time as factors, and $L_9(3^4)$ orthogonal method was adopted to design the experiment, as shown in Table 2.

Table 2 Factor level table

Levels	Factors		
	Extraction Temperature /°C A	Extraction Time /h B	Solid-liquid Ratio C
1	60	2	1:10
2	70	3	1:20
3	80	4	1:30

Table 3 Orthogonal test

NO.	A	B	C	Yield /%
1	60	2	1:10	0.84
2	60	3	1:30	1.13
3	60	4	1:20	0.43
4	70	2	1:20	0.88
5	70	3	1:10	0.97
6	70	4	1:30	0.45
7	80	2	1:30	0.79
8	80	3	1:20	0.68
9	80	4	1:10	0.52
K ₁	2.40	2.51	2.33	
K ₂	2.30	2.78	1.99	
K ₃	1.99	1.40	2.37	
R	0.41	1.38	0.38	

According to the range analysis in the table above, it can be seen that the degree of influence of various factors on the experimental results is B>A>C, namely extraction time>extraction temperature>solid-liquid ratio. According to the experimental results, the optimal extraction conditions are as follows. The extraction time is maintained at 3h, the extraction temperature is 60°C, and the solid-liquid ratio is 1:30g/mL, under which the yield of polysaccharide is 1.13%.

Table 4 Analysis of Variance Table

Factors	SS	Freedom	MS	F
Extraction Temperature(A)	0.0305	2	0.01525	0.6531
Extraction Time(B)	0.3556	2	0.17830	7.6360
Solid-liquid Ratio (C)	0.0291	2	0.01455	0.6231
Error	0.0467	2	0.02335	

Look up the F distribution threshold table, we can get $F_{0.10(2,2)} = 9.00$, $F_{0.05(2,2)} = 19.00$. According to the table above, the value of F is small, which can be concluded that the three factors have no significant

influence on the yield of polysaccharides. The reason may be that the experimental error in this experiment is large, and the error degree of freedom is small, which is only 2, leading to low sensitivity of the experiment [13]. Since the influence of each factor is not significant, there is no need to carry out multiple comparisons between each factor level. Therefore, the result obtained from range analysis in Table 3 is the optimal level combination [14].

4 Conclusion

This paper used the ethanol subsiding method to extract polysaccharide from pitaya and investigated the effects of extraction temperature, solid-liquid ratio, extraction time and other factors on the extraction yield of polysaccharides. The optimum extraction technology of pitaya was determined by orthogonal test. The optimized conditions are as follows. The sample is added into water according to the solid-liquid ratio of 1:30, the extraction temperature is kept at 60°C, and the extraction time is 3h. Under the optimized conditions, the yield of pitaya polysaccharide was 1.13%. This experiment provides a

theoretical basis for industrial large-scale extraction of pitaya polysaccharide.

Fund program

Natural Science Research Foundation of the Department of Education of Anhui Province (No. KJ2020A0789); Anhui provincial-level quality engineering project: (2020jyxm0790, 2020zyrc077, 2020zyrc081, 2019jxjj45); school-level grassroots teaching and research office demonstration project of Anhui Xinhua University (2019jyssfx02); Research project of Anhui Xinhua University (kytd201908).

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