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OPTIMIZATION OF EXTRACTION TECHNIQUE OF POLYSACCHARIDE FROM STAUNTON VINE (PSS) BY RESPONSE SURFACE ANALYSIS

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Abstract

For extraction of polysaccharide in staunton vine (PSS), a single factor experiment was carried out to determine the experimental factors and experimental levels. Then in combination with Box-Benhnken central composite design, a response surface analysis was carried out through three factors at three levels inorder to evaluate the influence of temperature, ratio of water to material and time on extraction rate of PSS. It is shown by the response surface figure drawn as the extraction rate that the optimal conditions for extraction of PSS include: extraction temperature of 83°C, extraction time of 2.7 hrs and water to material ratio of 30:1 ml/g, and in such case, the extraction rate reached 11.03%.

Introduction

Stauntonia chinensis is a natural specialty resource in China. The quality of *S. chinensis* is unique, with the characteristics of large fruit, thin skin, thick meat, tender meat, sweet and sour taste, gas aroma and so on. *Stauntonia chinensis* also is widely used in Traditional Chinese Medicine (TCM), it has been found that *S. chinensis* not only has the effects of relaxing muscles and activating collaterals, relieving thirst and generating fluid, calming liver and stomach, dispelling wind and dehumidification, but also can treat waist and knee pain, beriberi edema, dysentery, cholera, heartache and abdominal pain (Xu *et al*. 2018). In addition, *Stauntonia chinensis* has the functions of antibacterial and anti-inflammatory properties, protecting liver and reducing enzymes, clearing summer heat and diuresis, relieving alcohol and eliminating phlegm. According to the modern research, it has been found that polysaccharides are the main components in *Stauntonia chinensis.* In addition, the fruits are rich in organic acids, pectin, carotene, flavonoids, amino acids, vitamins, and minerals (Hu *et al.* 2019).

Polysaccharides are good adjuvants in medicine. In recent years, it has been found that the sugar chain of polysaccharide not only plays a decisive role in molecular biology, but also can control cell division and differentiation, regulate cell growth and senescence (Guzmán *et al*. 2020). Based on the epidemiological and physiological studies, it has been found that the effect of polysaccharides on human body is related to the chemical composition of cell wall polysaccharides (Oliveira *et al.* 2020). As an energy and structural substance in the life process, sugar has long been known for its taste, physiological and processing properties. In addition, according to the modern medical research, it has shown that some polysaccharides also have anti-coagulant, anti-infection, radiation control, hypoglycemia, anti-tumor and other anti-epidemic regulation functions (Wang *et al*. 2020). It can be used as a broad-spectrum immune promoter, and are widely used in functional food and clinic because of their various biological activities, so polysaccharides have become a research hotspot in natural medicine, biochemistry and life science.

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Response surface analysis (SRA) is the method used for function estimation through multiple quadratic regression, and approximate simulation of the relationship among all indexes in multi-factor experiments by polynomial (Chai *et al.* 2021). On this basis, by analyzing the response surface of function, it studies the response surface, factors and the relationship among them (Li *et al*. 2017, Liu *et al.* 2018). Hence, it has been widely adopted by the majority of personnel engaging in biology and food industry due to its reasonable design and excellent outcomes (Francini *et al*. 2019, An *et al*. 2021).

In order to further develop and utilize *S. chinensis*, a precious plant resource which can be used as food and medicine, and give full play to its hidden economic value. In the present study, the extraction process of polysaccharide in staunton vine (PSS), and use for the first time, the response surface analysis on optimization of the corresponding extraction process is reported so as to provide some reference for further development and utilization of staunton vine.

Materials and Methods

Stauntonvine bought from Zunyi Tianlou Stauntonvine Co., Ltd. Anhydrous ethanol, glucose, phenol, concentrated hydrochloric acid and concentrated sulfuric acid, all of them are analytical grade. 2K-82A vacuum drying oven, Dwf-180 electric plant crusher, UV2550 ultraviolet-visible spectrophotometer, Sbxz-1 vacuum rotary evaporator.

Technological Process of polysaccharide in staunton vine (PSS) was as follows: Firstly, taking out the seeds of staunton vine, cutting it into slices before drying, and then placing the grinded staunton vine powder into a brown bottle with ground stopper for later use. Then, 25g sample was taken and placed into the flask with a round bottom and a capacity of 500 mL. After that, 250mL petroleum ether was taken and added to the flask for reflux for 2 hours so as to remove the fat layer on the surface. And then, the samples obtained were placed in a thermostatic water bath for heating extraction for 2 hours, and centrifuged with the revolving speed of 1,000 rpm for 2 minutes. The supernatant was taken and placed in a vacuum rotary evaporator, at this time, the samples were compressed to 1/4 of the original volume through vacuum concentration. Anhydrous ethanol with 3 times the volume of that of the original samples was then added into the concentrated solution, and put at 4℃ overnight. The crude polysaccharide was obtained after filtration and vacuum drying, which was dissolved again and to which Sevag's reagent (chloroform: n-butanol =5:1) was added, to remove the protein. The operation was repeated for several times until the protein was completely removed. Anhydrous ethanol with three times the volume of that of the solution obtained was added to the solution. The mixed solution was placed at 4℃ for 12 hours. Finally, the precipitate was repeatedly washed with ethanol, acetone and ether, and then filtrated and dried to obtain refined polysaccharide.

The total sugar content was determined by classical method, the content of PSS was determined by phenol-sulfuric acid method, and the reducing sugar content was determined by DNS method. Content and extraction rate of PSS were calculated according to the following Eq.

(1) and (2): Polysaccharide content= total sugar content - reducing sugar content (1)

Extraction rate of polysaccharide % = dry polysaccharide quantity/dry matter quantity of stauntonvine ×100% (2)

Referring to the previous study (Wang *et al*. 2018, Nie *et al*. 2017), the formulation of standard curve of glucose was made. Glucose was dried at 105℃ to constant mass. 20 mg of the dry matter was accurately weighed and dissolved it in 500 and 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4 and 1.6 ml standard glucose solution were respectively adsorbed, and then placed into 10ml test tubes. And then, 1mL 6% phenol was added to each test tube after the solution was diluted to 2 ml by water. After shaking up, 5 ml concentrated sulfuric acid was quickly added to all the test tubes that were placed for 5 min at room temperature. And then, the test tubes were heated at 100℃ for 20 minutes and rapidly cooled to room temperature. The OD values were measured at 490nm. Finally, with the distilled water as blank, the standard curve was drawn with the glucose concentration as abscissa and absorbance as ordinate.

Single factor experiments were shown as follows. Effects of extraction temperature on the extraction rate of PSS. Six parts of 0.2 g stauntonvine powder were accurately weighed and added into distilled water at a water to material ratio of 25:1 ml/g for 2 hr and placed in a constant temperature water bath at 50, 60, 70, 80, 90 and 100℃, respectively.

The effect of water to material ratio on the extraction rate of PSS was as follows, six portions of 2 g wild staunton vine powder were prepared with water to material ratios of 10:1, 15:1, 20:1, 25:1, 30:1 and 35:1 ml/g, respectively. After leaching at 80℃ for 2 hrs, the supernatant was filtered to measure the PSS concentration.

The effect of extraction time on the yield of polysaccharide, 6 parts of 2 g wild staunton vine powder, water/material ratio of 25:1 ml/g, in 80℃ constant temperature water bath, treated for 1.0, 1.5, 2.0, 2.5, 3.0 and 3 hrs, respectively. Then the processed products were filtered, and the filtrate was taken to measure the PSS content, and the change of PSS extraction rate under different time was studied.

Based on the single factor experimental results and Box-Benhnken central composition experimental design principle (Li *et al.* 2017, Raza *et al*. 2017), extraction time, water to material ratio and temperature were taken as the factors that significantly affected the extraction of polysaccharide in stauntonvine (PSS). Moreover, RSA method with three factors at three levels was adopted.

Results and Discussion

The regression equation of the standard curve was y = 0.0108x+0.0098, and the correlation coefficient R^2 = 0.9995, which showed high reliability.

Many factors such as PH, temperature and the ratio of water to material exert certain influence on extraction rate for polysaccharide in staunton vine (PSS). As a result, single factor experiments shall be conducted at first to determine the experimental factors and experimental levels. And then, the response surface analysis was used to select the optimal conditions for extraction of PSS.

The influence of extraction temperature is as follows. Firstly, 0.2g staunton vine powder samples were accurately weighed and added into distilled water with a water to material ratio of 25:1 ml/g and fixed duration of 2 hrs. And then, the solutions were respectively placed in thermostat water bath at 50, 60, 70, 80, 90 and 100℃. The specific extent of changein extraction rate of polysaccharide under all the temperatures are presented in Fig. 1.

It can be seen from Fig. 1 that, the extraction rate of polysaccharide goes up with the rise of temperature between 50-90℃, and the change is particularly obvious before 80℃, which is with a steep increase. When the temperature is higher than 80℃, the rising tendency is relatively slowand shows a downward trend in the range of 90-100℃. It is found that the color of dry products deepens and the viscosity increases as the temperature rises. The reason for this might be that as the rise of temperature, the structure of polysaccharides was damaged and the activity was deprived therefrom. Based on the above reasons and results, 80-90℃ was selected as the optimum temperature range for extraction of polysaccharide.

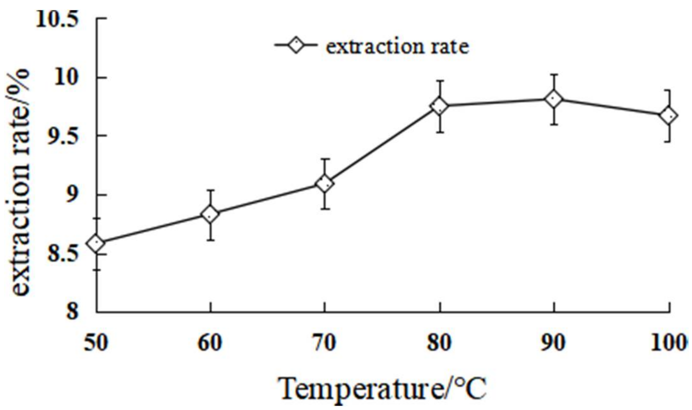

Fig. 1. Extraction temperature on the extraction rate of PSS.

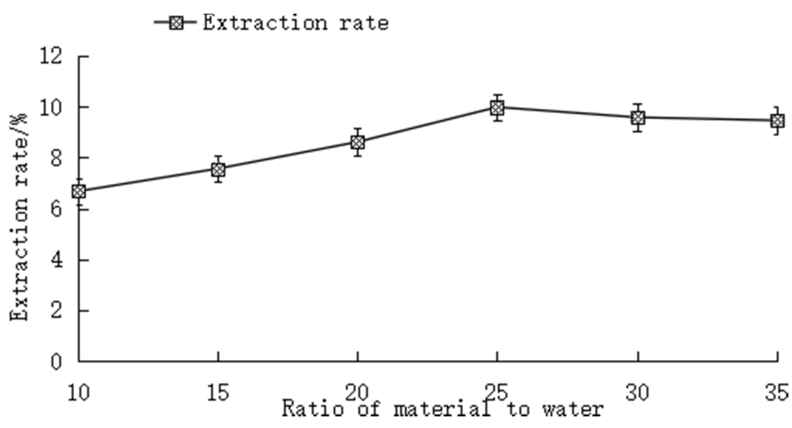

Fig. 2. The ratio of water to material on the extraction rate of PSS.

The influence of water to material Ratio is as follows. Firstly, 0.2 g stauntonvine powder samples were accurately weighed and provided with water to material ratios of 10 : 1, 15 : 1, 20 : 1, 25 : 1, 30 : 1 and 35 : 1 ml/g, respectively; after being placed in a thermostatic water bath at 80℃ for extraction for 2 hrs, the supernatant was filtered to measure the polysaccharide concentration. It can be seen from Fig. 2 that the increase of water to material ratio exerts significant impact on extraction rate of polysaccharide in stauntonvine (PSS). When the water to material ratio is less than 25:1 ml/g, significant difference in the extraction rate would be brought by each adjustment of water to material ratio; when the water to material ratio is greater than 25 : 1 ml/g, there would be slight change in the extraction rate with adjustment of the water to material ratio. Given that the extract needs to be further concentrated in the subsequent process, there would be large energy consumption in the subsequent process if too much water was added in the initial process. On this account, the ratio of water to material was set at about 25 :1 ml/g.

The influence of extraction time is as follows. Firstly, 0.2 g stauntonvine powder samples were accurately weighed and mixed with water at the proportion of 25 : 1 ml/g respectively, and placed in a thermostatic water bath at 80℃ for extraction for 1, 1.5, 2, 2.5, 3 and 3.5 hrs, respectively. And then, the products obtained were filtrated to measure the polysaccharide content.

The changes in extraction rate of polysaccharide in stauntonvine (PSS) at different durations are as shown in Fig. 3.

It can be seen from Fig. 3 that, when the water to material ratio is set at 25:1 ml/g and the temperature is at 80°C, the extraction rate of polysaccharide increases as the duration expands, which is especially obvious when the treatment duration is less than 2 hrs. And extraction rate tends to change gently as the duration increases when the treatment duration is longer than 2 hrs. In order to shorten the working time and reduce energy consumption, optimal extraction time is set at 2-3 hrs.

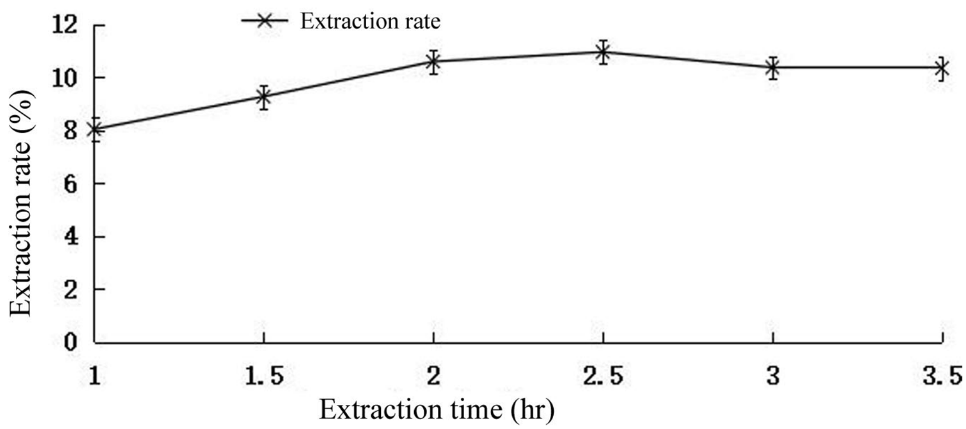

Fig. 3. Extraction time on the extraction rate of PSS.

Based on the single factor experimental results and Box-Benhnken central composition experimental design principle (Wu *et al.* 2017, Tian *et al*. 2021), extraction duration, water-material ratio and temperature were taken as the factors that significantly affected the extraction of polysaccharide in staunton vine (PSS). Moreover, RSA method with three factors at three levels was adopted. The design of experimental factors and levels are presented in Table 1.

Table 1. Factors and the levels of experiment of response surface analysis.

Level	Factor		
	A Temperature/°C	B Time/h	C Ration of water to material/ mL/g
-1	70	20	20:1
0	80	25	25:1
1	90	30	30:1

With temperature A, extraction time B and water to material ratio C as independent variables, and extraction rate of polysaccharide in staunton vine (PSS) as the response value, RSA experiments were carried out. The experimental scheme and results are given in Table 2.

Table 2. Program and experimental results of response surface analysis.

Test number	A	B	C	Extraction%
1	70	2	25	9.09
2	90	2	25	9.81
3	70	3	25	9.92
4	90	3	25	9.61
5	70	2.5	20	7.99
6	90	2.5	20	9.04
7	70	2.5	30	10.48
8	90	2.5	30	11.01
9	80	2	20	8.61
10	80	3	20	8.87
11	80	2	30	8.99
12	80	3	30	10.51
13	80	2.5	25	10.94
14	80	2.5	25	10.78
15	80	2.5	25	10.88

RSA software was used for the quadratic regression response surface analysis of the test results in Table 2, and based on which, a multiple quadratic regression response surface model was established; Y=10.87+0.25A+0.30B+0.81C-0.26AB-0.13AC+0.31BC-0.44A2-0.82B2-0.80C2

The variance analysis results of each factor are as shown in Table 3.

Table 3. Analyze of regression analysis.

Source	Sum of square	Degree of freedom	Mean square	F value	Prob > F
Model	12.09	9	1.34	6.14	0.0298
A-A	0.50	1	0.50	2.26	0.1928
B-B	0.73	1	0.73	3.32	0.1281
C-C	5.25	1	5.25	24	0.0045
AB	0.27	1	0.27	1.21	0.3210
AC	0.068	1	0.068	0.31	0.6022
BC	0.40	1	0.40	1.81	0.2358
A^2	0.71	1	0.71	3.22	0.1325
B^2	2.50	1	2.50	11.41	0.0197
C^2	2.36	1	2.36	10.79	0.0218
Residual error	1.09	5	0.22		
Lack of fit	1.08	3	0.36	55.13	0.0179
Pure error	0.013	2	6.533E-003		
Total deviation	13.19	14			

It can be seen from Table 3 that, the "Prob > F" value in the model is 0.0298, clearly less than 0.05, indicating that the model is significant. The determination coefficients of the regression model include A (temperature), B (extraction time), C (water to material ratio), AB (interaction between temperature and extraction time), AC (interaction between temperature and water to material ratio) as well as BC (interaction between extraction time and water to material ratio). The corresponding "Prob > F" values of the above coefficients are 0.1928, 0.1281, 0.0045, 0.3210, 0.6022 and 0.2358, respectively.

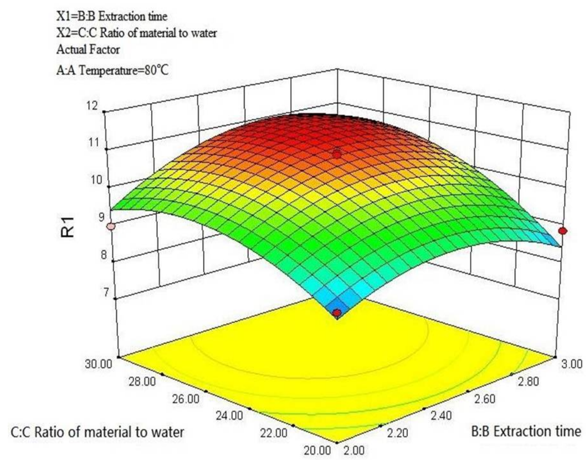

Fig. 4. Response Surface of interrelated influence of ratio of water to material and time to PSS extraction.

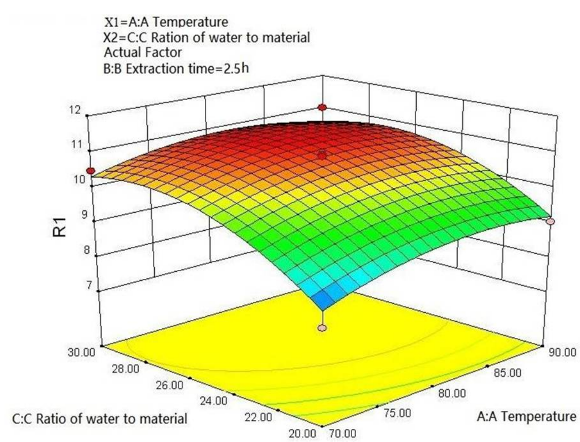

Fig 5. Response Surface of interrelated influence of ratio of water to material and temperature to PSS extraction.

Figures 4 to 6 intuitively reflect the various factors and mutual influence on the extraction rate of polysaccharide in staunton vine (PSS). And it is shown that, water to material ratio (C) has the most significant influence on the response value, which is manifested by the steep curve, while, the extraction time (B) and temperature (A) have less influence on the extraction rate of polysaccharide in staunton vine (PSS), which is manifested by the relatively flat curve. In addition, there are slight changes in the response value as A and B values vary.

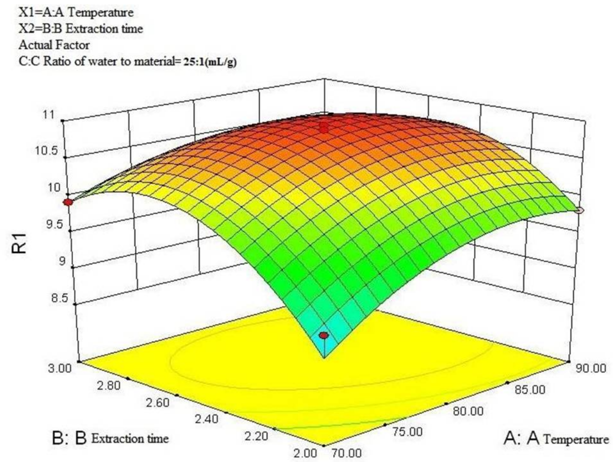

Fig. 6. Response Surface of interrelated influence of extraction time and temperature to PSS extraction.

Through solutions of the inverse matrix to the quadratic multinomial mathematical models on extraction rate of PSS, we come to the conclusion that the extraction rate of PSS is estimated to be 11.04% at the conditions with a temperature of 83.32℃, extraction time for 2.7 hrs, and a water to material ratio of 29.21 : 1 g/ml. To verify the reliability, the above optimal conditions were provided for extraction, and out of easy operation, those conditions were adjusted to: a temperature of 83℃, extraction time for 2.7 hrs, and a water to material ratio of 30:1 g/ml, the actual extraction rate measured was 11.03%, which was very close to the predicated value. On this ground, the parameters of extraction conditions optimized by response surface analysis are reliable and with practical application value. Researcher extracted polysaccharides by hydrothermal method, purified the water-soluble components of polysaccharides by alcohol precipitation method, studied the effects of extraction factors on the yield of products and the effects of different extraction temperatures on the composition of products, and analyzed the degradation law of sugars in papaya papaya. The results showed that hydrothermal extraction temperature had a great influence on the extraction effect. Appropriately increasing extraction temperature, prolongating extraction time and reducing extraction liquid were better than polysaccharide extraction. Higher extraction temperature could promote hemicellulose dissolution, but could not degrade lignin and xylan. The extraction temperature had little effect on the structure of polysaccharides from *Chaenomeles sinensis* (Ge, 2021).

Orthogonal design can only deal with discrete horizontal values, but fails to find the optimal combination of each factor and the optimum response value, which is overcome by response surface analysis method.

In conclusion, the optimal technological conditions of PSS extraction are: extraction temperature of 83℃, extraction time for 2.7 hrs, and water to material ratio of 30:1 g/ml and under which, the extraction rate of PSS is 11.03%.

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