Production of Polysaccharides from *Angelica sinensis* by Microbial Fermentation

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Angelica sinensis polysaccharides are important active ingredients and biological resources in traditional Chinese medicine. Properly changing the fermentation conditions of microorganisms may alter the yield of fermentation products. Based on single factor test results, three factors with great influence on the yield of *Angelica sinensis* polysaccharides produced by *Aspergillus niger* were optimized: initial pH value, fermentation time, and culture temperature. According to the box Behnken central combination principle, a response surface analysis scheme with three factors and three levels was designed, and the yields of active polysaccharide of *Angelica sinensis* were taken as the response value to optimize the fermentation process. The results of response surface analysis showed that under the optimized fermentation temperature of 30 °C, cultivation time of 8 days, and initial pH value of 5, the predicted yield of active polysaccharides from *Angelica sinensis* was 15.5%, while the actual value was 15.35%, which was 0.11% lower than the predicted value. This indicated that using response surface analysis to optimize the fermentation conditions of *Angelica sinensis* polysaccharides was reasonable and feasible. This method can effectively improve the biological resource utilization rate of *Angelica sinensis* polysaccharides.

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Keywords: Angelica polysaccharide; Fermentation process; Biological Resources

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INTRODUCTION

Angelica sinensis belongs to the Umbelliferae family and is mainly produced in the Gansu and Yunnan provinces of China. *Angelica sinensis*, known as the "Ten Prescription and Nine *Angelica*", means that out of ten traditional Chinese medicine prescriptions, approximately nine will use *Angelica sinensis*. *Angelica sinensis* is widely used for the treatment of various diseases and has been used as a health food for a long time, especially for female gynecological diseases with significant therapeutic effects (Wang *et al.* 2017). The main components of *Angelica sinensis* include polysaccharides (AP), ferulic acid, and volatile oil (Hu *et al.* 2004; Rong *et al.* 2011). *Angelica* polysaccharides can enhance immune function, anti-tumor effect, antioxidant, antithrombotic, promote hematopoietic function, and have resistance to radiation damage (Guan *et al.* 2013; Mao *et al.* 2015; Sun *et al.* 2009 Zhang *et al.* 2007). *Angelica* polysaccharides are an active biological resource in *Angelica sinensis* decoction. Analysis of the structure of *Angelica sinensis* polysaccharides showed that their high molecular weight and relatively complex structure make them less prone to crystallization, but at the same time making it difficult to delve

into their functional mechanisms (Zhou 2018). The scope of utilization of *Angelica sinensis* polysaccharides is becoming increasingly broad. In addition to experimental mice and human clinical applications, many reports have confirmed that *Angelica sinensis* polysaccharides can help increase immune capacity and production performance of aquatic animals. Long term (over 8 weeks) feeding of high doses (bait) of *Angelica sinensis* polysaccharides can significantly improve the non-specific immunity and disease resistance of spotted grouper (Wang 2012). There are also reports that *Angelica sinensis* polysaccharides can effectively inhibit the apoptosis of crucian carp cells caused by *Vibrio* infestation (Ren 2017). Adding a certain amount of *Angelica sinensis* polysaccharides to the feed can significantly improve the antioxidant capacity and enhance the immune system of juvenile oval pomfret and scad fish (Tan 2018). The utilization range of *Angelica* polysaccharides is becoming wider and has broader research prospects. However, according to existing reports, the extraction yield of *Angelica sinensis* polysaccharides is greatly affected by changes in extraction methods. Traditional methods such as water extraction, dilute acid, dilute alkali, ultrasonic assisted extraction, biological enzyme method, and hot water repeated extraction can be used to extract polysaccharides, and process optimization can be used to improve the extraction yield of polysaccharides (Jin *et al.* 2007; Zhang *et al.* 2012). Due to increasing demand for *Angelica sinensis* polysaccharides, it is necessary to improve the extraction yield, which can save traditional Chinese medicine resources.

Traditional Chinese medicine often undergoes changes in chemical components and metabolism of special microorganisms during microbial fermentation, especially changes in the sugar content and composition of some medicinal herbs. For example, in the study of microbial fermentation, the traditional Chinese medicine ginseng was reported to use *Lactobacillus* as the fermentation strain. The results showed that microbial fermentation significantly increased the content of effective ingredients in ginseng (Sang *et al.* 2011). There has not been much research on the microbial fermentation of *Angelica sinensis*. There are reports indicating that bidirectional fermentation can be carried out using *Ganoderma lucidum Angelica sinensis*. After optimizing the fermentation process, the data showed that the content of polysaccharides, flavonoids, and *Ganoderma* triterpenoids gradually increased with the fermentation process. The content of polysaccharides in fermented bacterial matter increased from 5.49 μg/mg on the first day of fermentation to 18.2 μg/mg on the 13th day (increasing 3.32 times) (Wei 2014). This indicates that *Angelica* polysaccharides may increase yield after fermentation.

Response surface methodology (RSM) is a method of optimizing the rationality of experimental conditions, including experimental design, model establishment, and suitability for model verification. Combining the single factor test results with the response surface test scheme can make up for the shortcomings of the single factor test. The single factor test can provide a reference for the response surface test method, provide conditions for determining the factors and levels of the test design, and obtain the best combination of training conditions (Wang and Wang 2005; Diao *et al.* 2019). There has been more and more research on response surface methodology in optimizing the fermentation process of traditional Chinese medicine ingredients. Rong *et al.* (2016) indicated that based on the principle of response surface optimization experiments, the optimal optimization conditions were obtained as follows: fermentation time of 5.5 days, rotational speed of 240 r/min, and initial pH value of 6.6. The final yield of pullulan polysaccharides was 26.3 mg/mL, which was 63.1% higher than the initial fermentation yield of 16.1 mg/mL. Chen *et al.* (2014) showed that the best fermentation process conditions were obtained by

combining single factor and response surface methodology: the strain used for fermentation was SZ-2, the temperature was controlled at 33 ℃, the number of seed added was 4%, and the fermentation reaction time was 3.5 days. The improvement of the total peak area was 31.2%. Liu *et al.* (2017), used yeast as the fermentation strain and response surface analysis to optimize the fermentation process conditions of *Astragalus membranaceus*. The fermentation time was 9.46 days, the temperature was 29.1 ℃, the percentage of seeds inserted was 10.4%, the initial pH value was 6.52, and the final flavonoid content obtained was 0.866 mg/mL. Many fermentation experiments on the changes in the effective ingredients of medicinal herbs have confirmed that the effective ingredients of Chinese medicinal herbs may change to varying degrees after microbial fermentation, which can save resources and fully utilize the medicinal value of each medicinal herb. Response surface methodology has created a new method for the optimal utilization of Chinese medicinal materials. This article aimed to apply this method to the fermentation experiment of *Angelica sinensis* polysaccharides, with the goal of improving their polysaccharide yield.

EXPERIMENTAL

Materials

Test materials

Angelica sinensis was purchased from Minxian Hetai Traditional Chinese Medicine Co., Ltd.). *Aspergillus niger* is a strain preserved in the 613 laboratory of Anhui Agricultural University Science and Technology Building.

Culture medium

PDA medium was made of potato 30%, glucose 3%, and agar 1.5%. Fermentation medium was made of *Angelica sinensis* 16%, peptone 1%, bran 81%, $(NH_4)\ 2SO_4$ 0.5%, KH_2PO_4 0.5%, $MgSO_4$ 0.7%, $CaCl_2$ 0.3%, and water content 65%.

Methods

Preparation of bacterial fermentation broth

Activated strain: *Aspergillus niger* strain was added to the PDA culture medium plate and incubated at 28 ℃ for 3 days before activation for backup.

To prepare seed culture medium (PDA liquid medium), 5 mL of sterile water was poured into an activated bacterial culture dish. A pipetter was used to suck 1 mL and transfer it to 100 mL of seed culture medium, which was fermented and cultivated in a shaking bed at 28 ℃ and 150 r/min for 72 h.

Fermentation medium: Under the same conditions, the seed culture solution was poured into a conical flask containing 50 g of fermentation medium according to the amount of inoculation required for the experiment.

Standard curve

50 mg of glucose standard substance was accurately weighed which dried to constant weight at 105 °C. The standard substance was placed in a 100 mL volumetric flask with distilled water to volume. It was used as a standard solution. Standard solution was measured at 0, 0.2, 0.4, 0.6, 0.8, 1.0, and 1.2 mL, and placed in 25 mL volumetric flasks to constant volume. 2 mL standard solutions of various concentrations were transferred into

a test tube. 1 mL of 5% phenol solution was added in sequence and shook well. 5 mL concentrated sulfuric acid was added to the test tube, mixed well, and placed in a boiling water bath for 30 min before removing and cooling to room temperature. The absorbance value was measured at 490 nm (Zhang *et al.* 2012). Linear regression analysis was performed using absorbance values as the vertical axis and the concentration of glucose solution as the horizontal axis. The polysaccharide content was calculated using Eq. 1,

$$W = CV/M \times 100\%. \quad (1)$$

where W is the yield of polysaccharides, C is the concentration of polysaccharide extract (standard glucose solution) (mg/mL), V is the volume (mL) of polysaccharide extract sample solution, and M is the mass of *Angelica* powder sample (g).

Extraction method of Angelica sinensis polysaccharides

Five grams of *Angelica sinensis* were weighed through 40 mesh sieve and put into a round bottom flask. 100 mL of 80% ethanol solution was added and refluxed for 1.5 h in 80 ℃ water bath. Afterward, a vacuum pump was used for suction filtration, and a filter residue was taken for standby and placed into a round bottom flask with 100 mL of distilled water. Finally, it was placed into an 80 ℃ water bath for heating and reflux, and the filtrate was collected into a volumetric flask. The determination method of *Angelica sinensis* polysaccharides was the same as described in the previous section (Standard curve). According to the standard curve regression equation, the extraction percentage of *Angelica sinensis* polysaccharides was calculated.

Single factor experiments

Temperature: Five groups of identical fermentation media were prepared and placed at 24, 26, 28, 30, and 32 ℃ for fermentation under the condition of keeping *Ceteris paribus* unchanged (fermentation time 8 d, pH 5.0, liquid volume 100 mL, rotating speed 100 r/min, inoculation volume 10 mL). *Angelica sinensis* polysaccharide content was sampled and measured in fermentation samples using the phenol-sulfuric acid method.

Fermentation time: Six sets of identical fermentation media were prepared, and samples were taken at different fermentation times under the same conditions (temperature 30 ℃, pH 5.0, liquid volume 100 mL, rotational speed 100 r/min, inoculation volume 10 mL). The content of *Angelica sinensis* polysaccharides in the samples was determined using the phenol-sulfuric acid method. The fermentation time was set to 0, 5, 6, 7, 8, or 9 days.

pH: Five sets of fermentation media were prepared with different pH values, and fermented under the same conditions (temperature 30 ℃, fermentation time 8 d, liquid volume 100 mL, rotational speed 100 r/min, inoculation volume 10 mL). The pH of the fermentation broth was 3.0, 4.0, 5.0, 6.0, and 7.0, respectively. The content of *Angelica sinensis* polysaccharides were sampled and measured in the fermentation broth using the phenol-sulfuric acid method.

Liquid loading capacity: Five fermentation mediums with different amounts of liquid were placed into 250 mL triangular bottles. The liquid contents in the bottles were 50, 75, 100, 125, and 150 mL respectively, with *ceteris paribus* unchanged (temperature 30 ℃, fermentation time 8 d, pH 5.0, rotational speed 100 r/min, and inoculation amount

10 mL). After a period of fermentation, samples were taken separately and the content of *Angelica sinensis* polysaccharides was determined using the phenol-sulfuric acid method.

Speed: The five groups of fermentation broth with identical preparation were fermented and cultured at different rotational speeds. The oscillating culture speed was set as 0, 50, 100, 150, or 200 r/min, and *ceteris paribus* remained unchanged (temperature 30 ℃, fermentation time 8 d, pH 6.0, liquid loading 100 mL, inoculation 10 mL). After a certain period of fermentation, the content of *Angelica sinensis* polysaccharides in the fermentation broth was determined using the phenol sulfuric acid method.

Inoculation volume: Under the same fermentation conditions (temperature 30 ℃, fermentation time 8 days, pH 5.0, liquid volume 100 mL, rotational speed 100 r/min), different inoculums of seed culture medium were added to six sets of fermentation medium, with inoculums of 2, 4, 6, 8, 10, or 12 mL. After the same fermentation time, the content of *Angelica sinensis* polysaccharides in the fermentation broth was determined using the phenol-sulfuric acid method.

Response surface optimization test

According to the principle of Box Behnken central combination experimental design, integrating the results of previous single factor experiments, three factors that have a significant impact on the yield of *Angelica sinensis* polysaccharides were selected, namely cultivation time, cultivation temperature, and initial pH value. Three levels for each factor were taken, and the high-level values were encoded as 1, while the low level values were encoded as -1. Based on the previous single factor experiment, a response surface optimization test plan with three factors and three levels was designed. The factors and levels are shown in Table 1.

Table 1. Box-Behnken Experimental Factors and Levels

Levels	Factors		
	A: Culture temperature (°C)	B: Culture time (d)	C: Initial pH value
-1	28	7	4.5
0	30	8	5
1	32	9	5.5

RESULTS AND DISCUSSION

Glucose Standard Curve

According to the standard curve in the experimental method, the standard curve for measuring glucose content was obtained through the experiment. The glucose standard curve was $Y = 0.0047x + 0.0315$, and $R^2 = 0.9996$.

Single Factor Experiment

Temperature

According to the single factor experimental plan of temperature, 5 different cultivation temperatures were used. After 8 days of fermentation, samples were taken at the same time point (three replicates for each sample), and the content of *Angelica sinensis* polysaccharides in the fermentation samples was measured using the phenol sulfuric acid

method. As shown in Fig. 1, the polysaccharide yield of *Angelica sinensis* fermented at 5 different temperatures reached its maximum at approximately 30 ℃. The yield of *Angelica sinensis* polysaccharides gradually increased with the increase of cultivation temperature, until it reached nearly 30 ℃ (the set temperature of machine is slightly lower than the actual temperature).

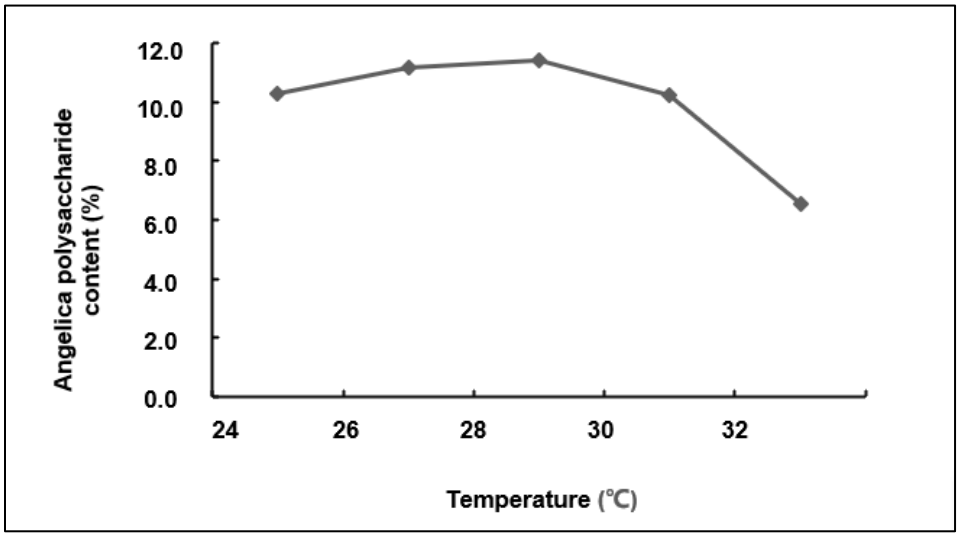

Fig. 1. Effect of different fermentation temperatures on the yield of *Angelica sinensis* polysaccharides

Fermentation time

As shown in Fig. 2, with the change of time, the polysaccharide content of *Angelica sinensis* gradually increased, and after 8 days of fermentation, the polysaccharide content gradually stabilized. Within 0 to 8 days, the experimental results showed that the longer the fermentation time, the higher the yield. After 8 days, the yield tended to stabilize and basically reached its maximum.

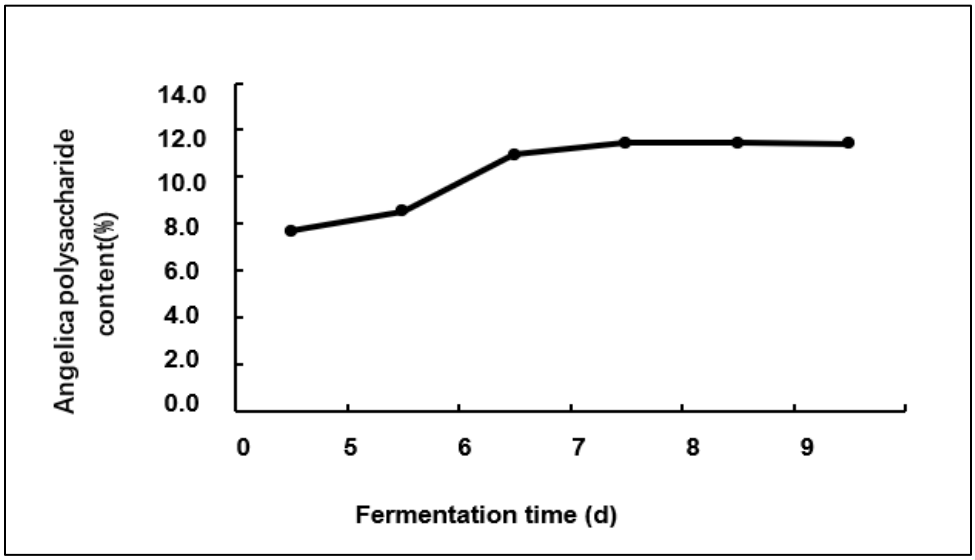

Fig. 2. Effect of different fermentation times on the yield of *Angelica sinensis* polysaccharides

pH

As shown in Fig. 3, the yield of *Angelica sinensis* polysaccharides varied greatly under different pH conditions, with a higher yield at pH 5.0. The production of polysaccharides at pH 3 to 5 gradually increased, then followed by a significant decrease, indicating that 5 was a suitable setting value.

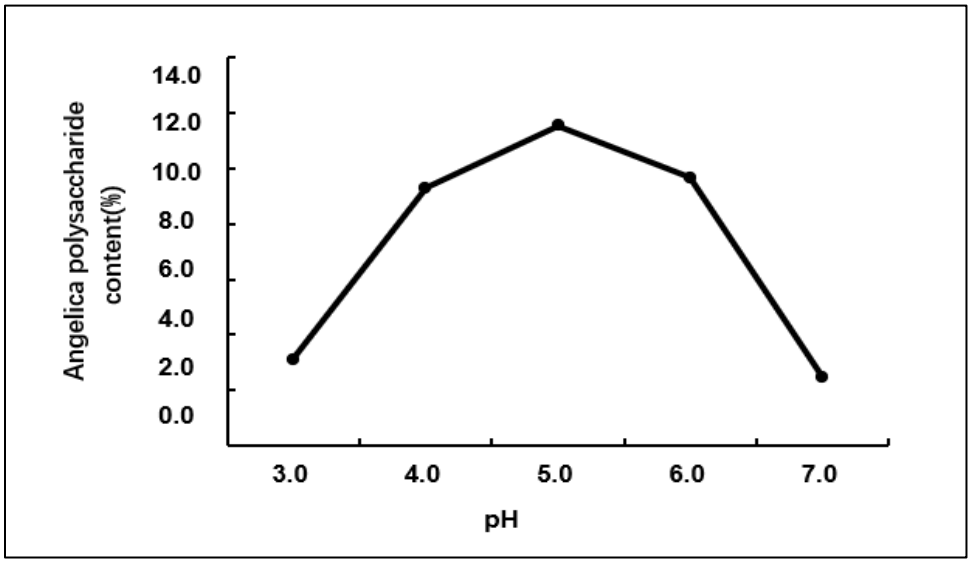

Fig. 3. Effect of different pH on the yield of *Angelica sinensis* polysaccharides

Liquid loading capacity

In Fig. 4, the yield of *Angelica sinensis* polysaccharides varied greatly depending on the amount of liquid in the triangular flask. When the amount of liquid in the 250 mL triangular flask reached 100 mL, the yield of *Angelica sinensis* polysaccharides reached its maximum value.

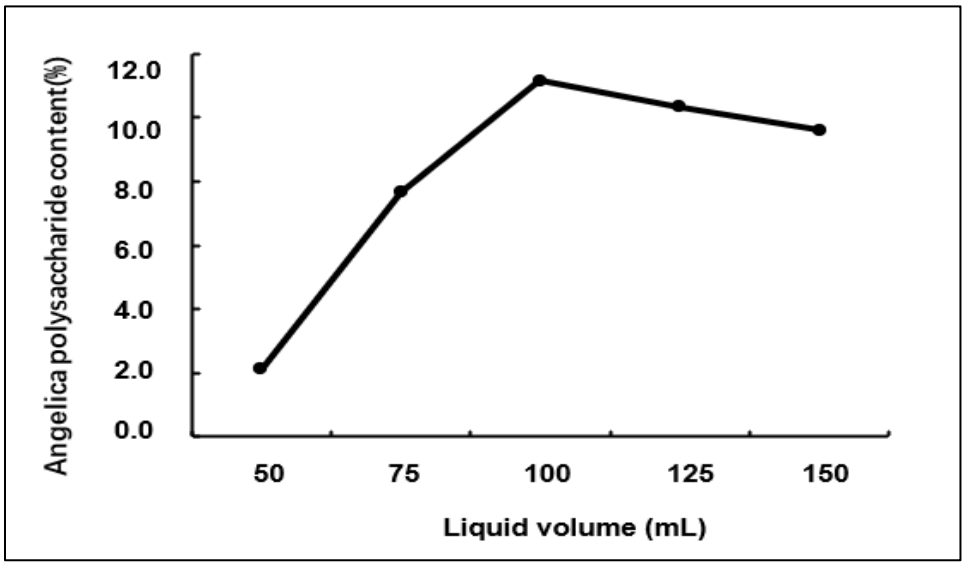

Fig. 4. Effect of different liquid volume on the yield of *Angelica sinensis* polysaccharides

Speed

In Fig. 5, under oscillation culture, high-speed oscillation had an adverse effect on the fermentation of *Angelica sinensis* polysaccharides, and the polysaccharide yield reached its maximum at lower speeds.

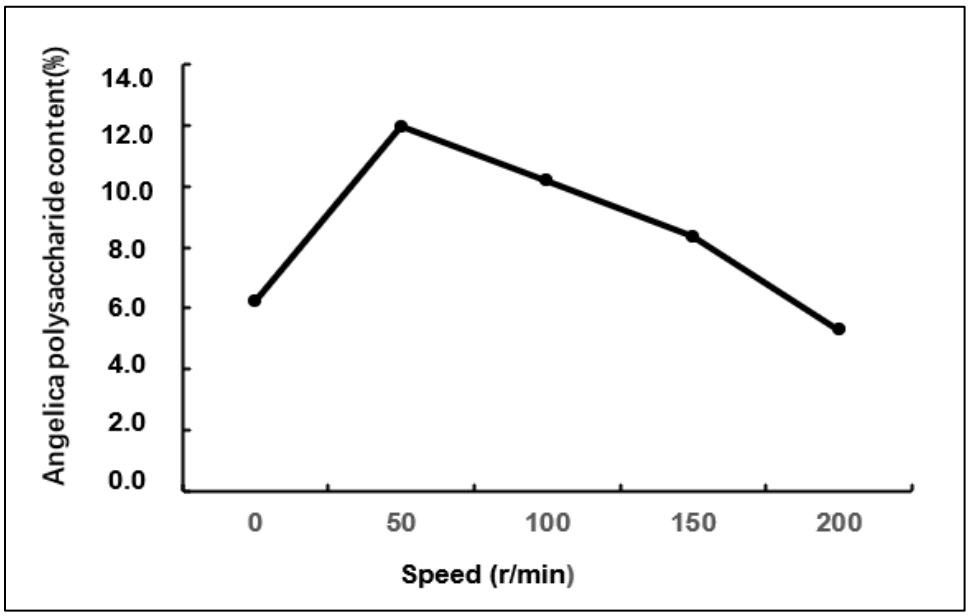

Fig. 5. Effect of different rotational speeds on the yield of *Angelica sinensis* polysaccharides

Inoculation volume

In Fig. 6, the yield of *Angelica sinensis* polysaccharides increased linearly and finally stabilized after the inoculation amount reached 10 mL, indicating that 10 mL inoculation amount was the optimal fermentation condition.

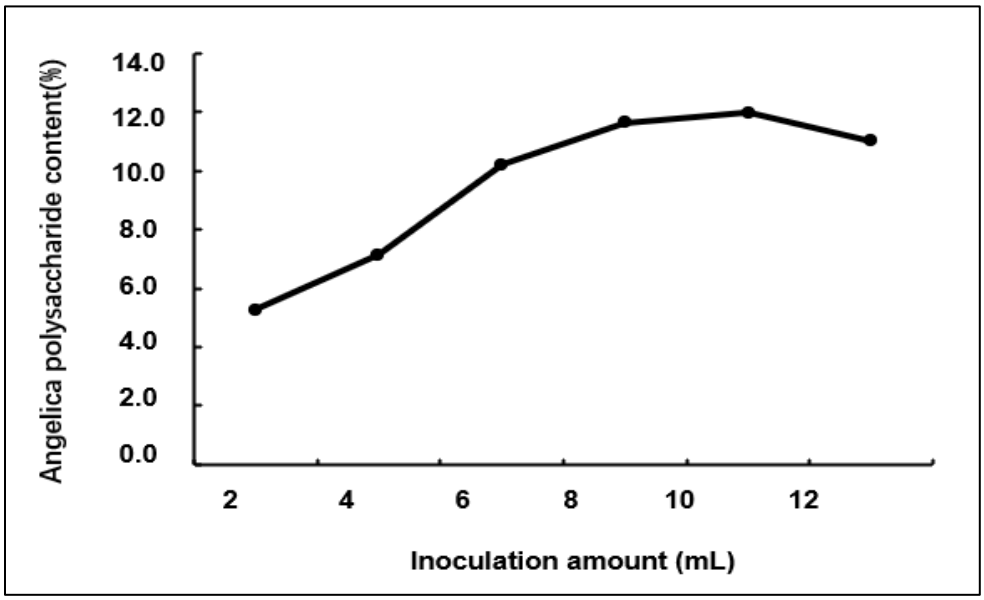

Fig. 6. Effect of different inoculation levels on the production of *Angelica sinensis* polysaccharides

Analysis

According to the results of each single factor experiment and the comparison with the content of *Angelica* polysaccharide extracted by chemical method, *Aspergillus niger* was used as the fermentation strain to ferment *Angelica* polysaccharide. Different factors had certain effects on the fermentation. The best conditions of each factor can improve the yield of *Angelica* polysaccharide to a certain extent, which indicated that *Aspergillus niger* fermentation had a certain role in promoting the yield of *Angelica* polysaccharide.

Table 2. Box Behnken Experimental Design and Results

Factors	Factor 1 A: Culture temperature (°C)	Factor 2 B: Culture time (d)	Factor 3 C: Initial pH value	Yield of *Angelica sinensis* polysaccharides (%)
1	1	-1	0	13.49
2	-1	-1	0	11.03
3	-1	0	-1	9.76
4	-1	1	0	10.19
5	0	1	1	12.23
6	0	-1	-1	7.12
7	0	-1	1	13.96
8	0	0	0	16.78
9	1	1	0	9.67
10	0	0	0	15.36
11	0	0	0	15.54
12	1	0	1	14.78
13	0	0	0	15.01
14	1	0	-1	8.85
15	0	0	0	14.65
16	0	1	-1	6.79
17	-1	0	1	11.34

Response Surface Optimization Test

Based on the previous single factor analysis, the factors affecting the yield of *Angelica sinensis* polysaccharides included culture temperature, initial pH value, and fermentation time. The Box Behnken experimental design method was used to optimize the experimental design of three factors and three levels, with the yield of active polysaccharides from *Angelica sinensis* as the response value to determine the optimal fermentation process. The results of the Box Behnken experiment are shown in Table 2.

Model Establishment and Adherence Testing

After using Design Expert 8.0.6 software to perform quadratic multiple regression fitting on the data in Table 2, the quadratic multiple regression equation between the yield of active polysaccharides from *Angelica sinensis* and the cultivation temperature (A), fermentation time (B), and initial pH value (C) was obtained: the yield of polysaccharides from *Angelica sinensis*, Y =15.47 + 0.56A - 0.84B + 2.47C -0.74AB + 1.09A C - 0.35BC - 1.61A2 - 2.77B2 - 2.68C2.

Afterward, variance analysis of the regression model was performed. The results are shown in Table 3. The established regression model was significant, while the p-value of the mismatched term was 0.2705, which was not significant. This indicates that the experimental data and the model fit well. Regression diagnosis shows that the model's

complex correlation coefficient was $R^2 = 0.9583$, and adjusted was $R^2 = 0.9046$, indicating that the equation had good fitting and reliability. By comparing the values of P, the cultivation time (fermentation time), pH value, and their quadratic terms had a significant impact on the yield of active polysaccharides from *Angelica sinensis*. Therefore, the experiment can predict the response value well based on the obtained multiple regression equation. The degree of influence of each factor on the response value based on the P-value is shown in Table 3. The results show that the order of the degree of influence of temperature (A), cultivation time (B), and initial pH (C) on the response value was A < B < C.

Table 3. Regression Equation Analysis Results

Source	Sum of squares	Degrees of Freedom	Mean square	F-value	P-value	Significance
Model	145.84	9	16.20	17.86	0.0005	significant
A- temperature	2.50	1	2.50	2.75	0.1410	
B- Culture time	5.64	1	5.64	6.22	0.0413	*
C-pH	48.96	1	48.96	53.97	0.0002	**
AB	2.22	1	2.22	2.45	0.1617	
AC	4.73	1	4.73	5.22	0.0563	
BC	0.49	1	0.49	0.54	0.4862	
A^2	10.88	1	10.88	12.00	0.0105	*
B^2	32.20	1	32.20	35.49	0.0006	**
C^2	30.19	1	30.19	33.28	0.0007	**
Residual	6.35	7	0.91			
Lack of Fit	3.73	3	1.24	1.90	0.2707	non-significant
Pure Error	2.62	4	0.65			
Cor Total	152.19	16				
$R^2 = 0.9583$ $R^2adj = 0.9046$ Signal to Noise Ratio: 17.86						
Note: * * indicates a significant difference (p<0.01); * The difference is significant (p<0.05)						

Response Surface Plots and Contour Lines

Using the Design Expert 8.0.6 software, response surface plots (Figs. 7 to 9) were obtained based on the data in Table 2, showing the interaction relationship between the three key factors of culture temperature, culture time, and pH value.

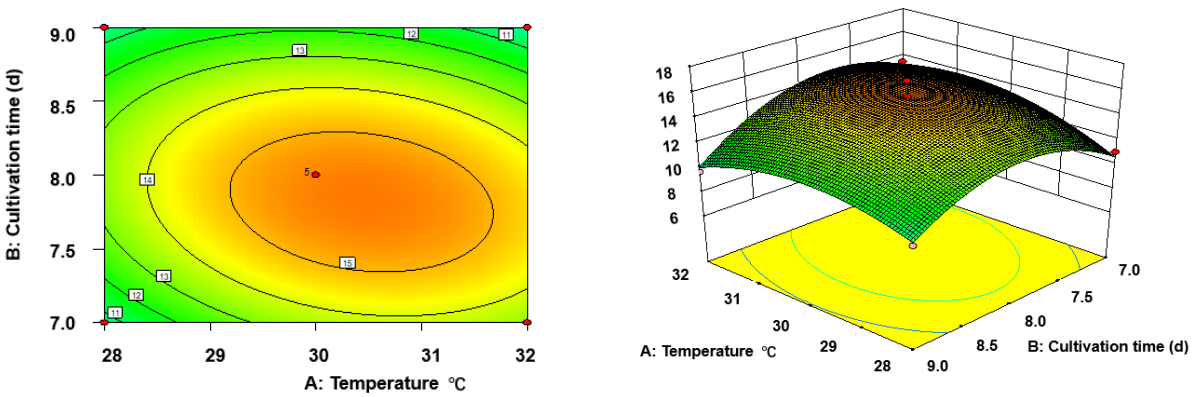

Fig. 7. Effect of the interaction between culture temperature and culture time on the yield of *Angelica sinensis* polysaccharides

This is the interaction between cultivation temperature and cultivation time. In Fig. 7, the interaction between cultivation temperature (A) and cultivation time (B) reached a significant level. The initial pH value (C) was fixed at 0 level, and the equation for the effect of temperature and cultivation time on the yield of *Angelica sinensis* polysaccharides was obtained: *Angelica sinensis* polysaccharide yield Y = 15.47 + 0.56A - 0.84B - 0.74AB - $1.61A^2$ - $2.77B^2$. With the increase of cultivation temperature and time, the yield of *Angelica sinensis* polysaccharides significantly increased. When the temperature and cultivation time levels were between -0.5 and 0.5, the response surface had the highest point, which was 15.47%.

In Fig. 8, the interaction between temperature (A) and pH (C) reached a significant level. The cultivation time (B) was fixed at 0 level, and the equation for the effect of temperature and pH on the yield of *Angelica sinensis* polysaccharides was obtained: *Angelica sinensis* polysaccharide yield Y = 15.47 + 0.56A + 2.47C + 1.09AC - $1.61A^2$ - $2.68C^2$. As the temperature and pH values increased, the yield of *Angelica sinensis* polysaccharides also increased. When both values were between -0.5 and 0.5, the response surface had the highest point, which was 16.04%.

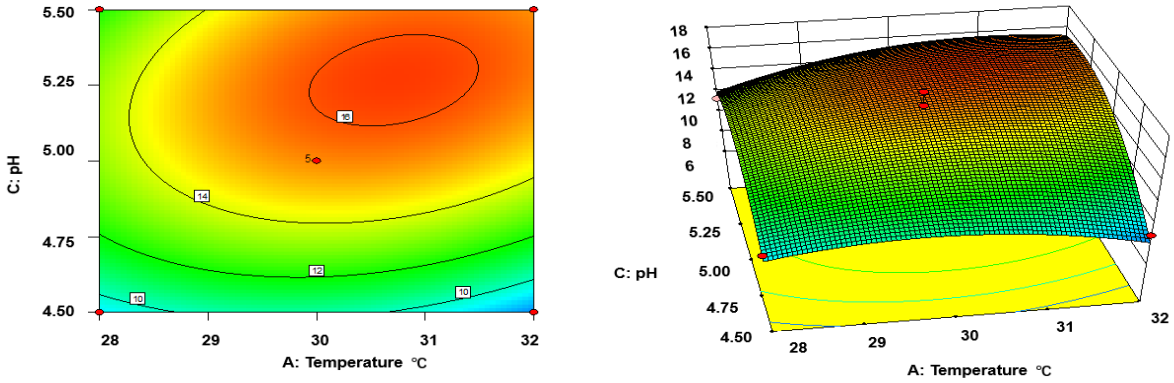

Fig. 8. Effect of temperature and initial pH interaction on the yield of *Angelica sinensis* polysaccharides

Figure 9 shows that the interaction between cultivation time (B) and pH value (C) reached a significant level. By fixing the cultivation temperature (A) at 0 level, the equation for the effect of cultivation time and pH value on the yield of *Angelica sinensis* polysaccharides was obtained:

The yield of *Angelica sinensis* polysaccharides Y = 15.47 - 0.84B + 2.47C -0.35BC - $2.77B^2$ - $2.68C^2$.

With the increase of cultivation time and pH value, the yield of *Angelica sinensis* polysaccharides increased. When both values were between -0.5 and 0.5, the response surface reached the highest point, which was 15.50%. Based on the above, when the values of temperature, cultivation time, and pH were all between -0.5 and 0.5, there was a high point in the response surface obtained from the interaction between each two factors. This indicates that when the values of the three factors were between -0.5 and 0.5, the yield of *Angelica sinensis* polysaccharides can obtain a larger response value. The maximum predicted value of the fitting equation model for the yield of *Angelica sinensis* polysaccharides was 15.5%. At this point, the values of A, B, and C were all between -0.5 and 0.5, which is consistent with the results displayed on the response surface and contour plot. After converting A, B, and C codes, it was obtained that A = 30 °C, B = 8 days, and

C = pH 5. Therefore, the optimized fermentation process conditions for *Angelica sinensis* polysaccharides were as follows: temperature 30 ℃, cultivation conditions for 8 days, and pH value 5.

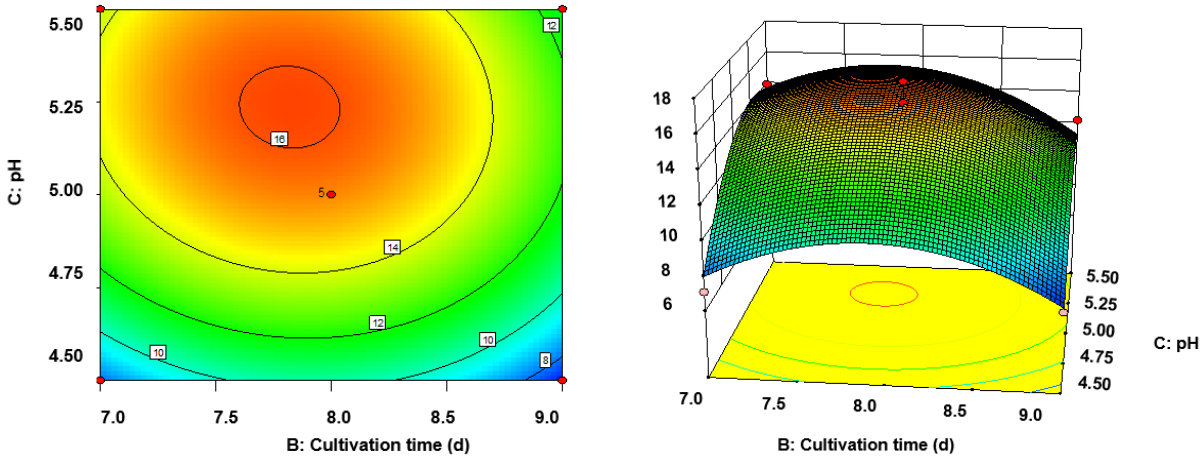

Fig. 9. Effect of pH value and culture time interaction on the yield of *Angelica sinensis* polysaccharides

Verification of Optimal Fermentation Process Conditions

By optimizing the response surface conditions and using a regression model, the optimal fermentation process parameters for *Angelica sinensis* active polysaccharides were determined: temperature of 30 ℃, cultivation time of 8 days, and initial pH value of 5. Under these conditions, the predicted value of *Angelica sinensis* active polysaccharides was 15.5%. To verify the reliability of response surface methodology in optimizing the fermentation process of *Angelica sinensis* active polysaccharides, three extraction validation experiments were conducted using the fermentation process parameters obtained from the above optimization. The average value obtained was 15.4%, which was close to the predicted value obtained from response surface optimization. It indicated that the process conditions obtained from response surface optimization had a certain level of reliability.

DISCUSSION

Using microorganisms to ferment traditional Chinese medicine can theoretically degrade a large amount of cellulose in traditional Chinese medicine plants, reduce the encapsulation effect of high polymers such as starch on traditional Chinese medicine components, and facilitate the dissolution of some effective ingredients. This can increase the content of some effective ingredients, and it may also produce new active substances, which is becoming increasingly important in the utilization of traditional Chinese medicine resources (Wang *et al.* 2010). During the fermentation experiment, microorganisms may produce some enzymes that may alter the original spatial structure of certain components in traditional Chinese medicine and produce new substances (Lei 2001). Dong *et al.* (2001) showed that there are 49 types of microorganisms that have biotransformation effect on ginsenoside Rg1, among which the small filamentous fungus *Aspergillus niger* 3.1858 and *Absidia coerulea* 3.3538 have better effects. Ning *et al.* (2003) showed that *Aspergillus*

niger had a clear biotransformation effect on lactone, an effective component of *Tripterygium wilfordii*, and obtained three new lactone compounds.

The present study has not yet analyzed the composition and structure of *Angelica sinensis* polysaccharides. This is about to be the team's next research focus. Also there is a need to understand whether microbial fermentation has caused changes in the composition and structure of *Angelica sinensis* polysaccharides.

CONCLUSIONS

1. *Angelica* polysaccharide is an important active biological resource that is present within *Angelica sinensis*. Using *Aspergillus niger* to ferment *Angelica sinensis* can effectively produce the polysaccharide. The Box Behnken central combination design of response surface methodology was used as a means to optimize the yield of active polysaccharides from *Angelica sinensis* as the evaluation index. The results of fermentation condition that based on the single factor test results, three factors that had a greater impact on the yield of *Angelica* polysaccharide produced by *Aspergillus niger* fermentation were selected: initial pH value, fermentation time, and culture temperature.
2. The results of response surface analysis showed that under the conditions of optimal fermentation temperature of 30 ℃, incubation time of 8 days, and initial pH value of 5, the predicted yield of *Angelica* polysaccharide was 15.46%, but the actual value was 15.35%, 0.11% lower than the predicted value, but the error was within a reasonable prediction range.
3. It is reasonable and feasible to optimize the fermentation conditions of *Angelica* polysaccharide by response surface analysis. This method effectively improves the utilization rate of biological resources, *Angelica sinensis* polysaccharides. Therefore, using response surface analysis to optimize the fermentation process parameters of *Angelica sinensis* active polysaccharides can obtain better process parameters and have certain reference value. However, further research is needed to optimize the yield of *Angelica sinensis* active polysaccharides and compare it with traditional methods for extracting polysaccharides.

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