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Optimization conditions for extracting polysaccharide from *Angelica sinensis* and its antioxidant activities

Suyang Tian [a,b], *Changchun Hao* [a,*], *Guangkuan Xu* [a], *Juanjuan Yang* [a], *Runguang Sun* [a,*]

[a] *Laboratory of Biophysics and Biomedical Engineering, School of Physics and Information Technology, Shaanxi Normal University, Xi'an 710062, China*

[b] *Lintong Middle School, Xi'an 710600, China*

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ABSTRACT

In this study, polysaccharides from *Angelica sinensis* were extracted using the ultrasound-assisted extraction method. Based on the results of single factor experiments and orthogonal tests, three independent variables—water/raw material ratio, ultrasound time, and ultrasound power—were selected for investigation. Then, we used response surface methodology to optimize the extraction conditions. The experimental data were fitted to a quadratic equation using multiple regression analysis, and the optimal conditions were as follows: water/raw material ratio, 43.31 mL/g; ultrasonic time, 28.06 minutes; power, 396.83 W. Under such conditions, the polysaccharide yield was 21.89 ± 0.21%, which was well matched with the predicted yield. *In vitro* assays, scavenging activity of superoxide anion radicals, hydroxyl radicals, and 2,2-diphenyl-1-picry-hydrazyl radical showed that polysaccharides had certain antioxidant activities and that hydroxyl radicals have a remarkable scavenging capability. Therefore, these studies provide reference for further research and rational development of *A. sinensis* polysaccharide.

1. Introduction

The rhizome of *Angelica sinensis* Diels, which belongs to the Umbelliferae family [1,2], is a well-known Chinese traditional natural herbal medicine. It has many biological functions, for example, promoting blood circulation, regulating the menstruation, and lubricating the intestines [3].

Polysaccharides, a type of macromolecule carbohydrate polymer, are found in a host of herbal plants and fungus [4–6]. Researchers found that polysaccharides extracted from numerous plants possess effective bioactivities [7–9]. Furthermore, several studies have reported that *A. sinensis* polysaccharides (ASPs) have antiproliferative effects [10] and the function of healing gastric ulcer [11]. Meanwhile, research

* *Corresponding authors*. Laboratory of Biophysics and Biomedical Engineering, School of Physics and Information Technology, Shaanxi Normal University, Chang'an South 199, Xi'an 710062, China.
E-mail addresses: haochangchun@snnu.edu.cn (C. Hao), Biophymed@snnu.edu.cn (R. Sun).

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results have shown that ASP showed a strong antioxidant activity [12]. It could improve aging and age-related diseases by reducing intracellular free radicals [13].

Hot water extraction, enzymolysis collection, and alkaline extraction are the traditional extraction methods for polysaccharides. They have many obvious shortcomings, such as they are time-consuming and expensive, and produce low yield. Therefore, the traditional extraction methods and conditions for polysaccharides need to be improved. In recent years, numerous studies have shown that ultrasound-assisted extraction has many obvious superior characteristics [14], such as reduced operating times, more economic solvent consumption, and enhanced efficiency. Acoustic cavitation could increase the motion frequency and speed of material molecules by the mechanical effect and thermal effect of ultrasonic waves [15,16]. Thus, ultrasonic-assisted extraction is an appropriate technique for the extraction of polysaccharides.

Response surface methodology (RSM) is an effective multivariate statistical method for optimizing complex experimental processes. It generates a polynomial equation and a cambered model by regression fitting of response surface analysis to evaluate the polytomy variables and its interaction, and then determine the best level. The most important advantage of RSM is the reduced number of experiments on process optimization [17,18]. Box–Behnken design (BBD), a type of RSM, is an independent, spherical, rotatable quadratic method that consists of three interlocking 2^2 factorial designs with points locating on the surface of a sphere surrounding the center of the design. It has been successfully used to optimize various biochemical and biotechnological processes [19,20].

In this study, we used the ultrasonic-assisted method to extract ASP and then optimized the extraction condition of using BBD of RSM. Through the results of single factor experiments and orthogonal test design, the applicable variables (water/raw material ratio, ultrasound extraction time, and ultrasound extraction power) and levels were screened for BBD. The measurement index of selecting extraction conditions was the extraction yield of *A. sinensis* crude polysaccharide (ASP) (%). The antioxidant activities on superoxide anion radicals, hydroxyl radicals, and 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) radicals of ASP were investigated using *in vitro* assays.

2. Materials and methods

2.1. Materials

The rhizomes of *A. sinensis* were collected from a local market (Xi'an, China). Pyrogallic acid, phenanthroline, and DPPH, L-(+)-ascorbic acid (Vc) were purchased from Sigma-Aldrich (St. Louis, MO, USA). All solvents and chemicals used in this study were of analytical grade.

2.2. Extraction of crude polysaccharide from A. sinensis and determination of the yield

The dried *A. sinensis* were powdered by a disintegrator and sieved through a 60-mesh screen. The powders were recirculated three times with 95% (v/v) alcohol by an ultrasonic crasher (JY92-II, 25 kHz; Ningbo Scientz Biotechnology Co., Ltd., Zhejiang, China), and then lipids, polyphenols, monosaccharides, oligosaccharides, and some small molecule materials were removed, followed by filtration, and drying at 45°C for the following procedures.

The polysaccharide was extracted from a suitable amount of pretreated dry powders in a designated water/raw material ratio, ultrasound extraction time, and ultrasound extraction power. The supernatants were separated by centrifugation at 4000 rev/min for 10 minutes and concentrated under reducing pressure. Four volumes of 95% ethanol were added to the condensed solution to precipitate the polysaccharide. The precipitates were collected by centrifugation and dissolved by distilled water, then dialyzed and lyophilized to obtain water-soluble crude polysaccharide defined as ASP. The content of ASP was measured using the phenol–sulfuric acid method, which used D-glucose as a standard. The percentage of extraction ASP yield (%) was calculated as follows:

$$\text{Yield of ASP}(\%) = \frac{M_{ASP}(g)}{M_{AS}(g)} \times 100\%, \quad (1)$$

where M_{ASP} is the dried ASP mass and M_{AS} is the dried powder of *A. sinensis* mass.

2.3. Experimental design

2.3.1. Single factor experiments

Single factor experiments mainly studied the effects of water/raw material ratio, ultrasound extraction time, ultrasound extraction power, and ultrasonic radiation method on the extraction yield of ASP. Among these factors, the water/raw material ratio ranged from 10 mL/g to 50 mL/g, the ultrasound extraction time ranged from 20 to 40 minutes, the ultrasound extraction power ranged from 200 W to 600 W, and the ultrasonic radiation method ranged from 5:10 to 25:30 (ultrasonic treatment time/intermission). One variable was studied at each experiment while the other factors were kept constant.

2.3.2. Orthogonal test design and analysis

The orthogonal experimental design referenced the results of single factor experiments, an L_9 (3^4) orthogonal test design with three levels was constructed, and four main factors were used (water/raw material ratio, 30–50 mL/g; ultrasound extraction time, 20–30 minutes; ultrasound extraction power, 300–500 W; ultrasonic radiation method range, 5:10–15:20). The subscript 9 indicated the number of experiments, which was much lower than the possible number of experiments. Each experiment was repeated three times [21,22].

2.3.3. Optimization of extraction conditions by BBD and statistical analysis

The extraction parameters of BBD with three factors at three levels based on orthogonal experimental design were optimized using RSM. The three main factors (water/raw material ratio, ultrasound extraction time, and ultrasound extraction power) were investigated, and the optimal conditions were determined to the maximal yield of ASP via a statistical analysis of RSM. Each independent variable was coded at

three levels between −1 (low), 0 (center), and +1 (high). The variables were coded according to the following equation [23]:

$$x_i = \frac{X_i - X_0}{\Delta X_i} \quad i = 1, 2, 3, \tag{2}$$

where x_i is the dimensionless value of an independent variable; X_i is the actual value of an independent variable; X_0 is the actual value of an independent variable at the center point; and ΔX_i is the step change of the actual value [24].

In the Box–Behnken method, the whole design matrix comprised 17 experiments in random order. The design of experiments is given in Table 1. For the three variables, the experimental data of the system was fitted to the following quadratic equation [25]:

$$Y = \sum \beta_0 + \sum_{i=1}^{3} \beta_i X_i + \sum_{i=3}^{3} \beta_{ii} X_i^2 + \sum_{i=1}^{2} \sum_{j=i+1}^{3} \beta_{ij} X_i X_j, \tag{3}$$

where Y is the dependent variable and X_i, X_j are the independent variables; β_0, β_i, β_{ii}, and β_{ij} are the regression coefficients of the independent variables that were estimated by the model for intercept, linear, quadratic, and interaction terms, respectively. Design-Expert 8.0.5.0 was used for statistical analysis of variance for each response and predicting the optimal conditions for the extraction of ASP [26,27].

2.4. Antioxidant activity of polysaccharide from A. sinensis

2.4.1. Superoxide anion radicals scavenging assay

The assay was carried out on the basis of the methods described by Tahmouzi [28] with several modifications. Briefly, 0.4 mL of the polysaccharide sample solution was mixed with 4.5 mL of 0.05M Tris–HCl buffer (pH 8.2) that was kept in a water bath at 25°C for 20 minutes. Then, 0.1 mL of 2.5mM pyrogallic acid was added and incubated at 25°C for 5 minutes after obtaining uniformity. Next, 0.1 mL of 10M HCl solution was added to terminate the reaction. The absorbance of the reaction mixture was detected at 325 nm. The superoxide radical scavenging activity was calculated using the following equation:

$$\text{Superoxide scavenging activity}(\%) = \frac{A_c - A_s}{A_c} \times 100(\%), \tag{4}$$

where A_c is the A_{325} absorbance of the superoxide radical solution without sample and A_s is the absorbance of the ASP sample.

2.4.2. Hydroxyl radicals scavenging assay

Briefly, 1 mL of the polysaccharide sample was mixed with 1.5 mL of 0.2M sodium phosphate buffer (pH = 7.4) and then mixed with 1 mL of 7.5mM $FeSO_4$, 1 mL of 0.1% H_2O_2, and 1.5 mL of 5mM phenanthroline. The reaction mixture was incubated at 37°C for 1 hour, and the absorbance was determined at 510 nm. The scavenging activity of the hydroxyl radical was calculated as follows:

$$\text{Hydroxyl scavenging activity}(\%) = 1 - \frac{A_s - A_{sb}}{A_c} \times 100(\%), \tag{5}$$

where A_c is the absorbance of the hydroxyl radical solution without sample, A_s is the absorbance of ASP mixed with the hydroxyl radical solution, and A_{sb} is the absorbance of the sample only.

2.4.3. DPPH scavenging assay

The assay was carried out based on methods described by Do et al [29] and Sharma et al [30] with several modifications. The DPPH free radical scavenging activity of ASP was determined using the procedures. In brief, 1 mL of polysaccharide solution was mixed with 2 mL of 0.1M DPPH solution. The mixture was shaken and left in the dark at room temperature for 60 minutes. The absorbance of the reaction mixture was measured at 517 nm. The DPPH radical scavenging activity was calculated using the following equation:

$$\text{DPPH scavenging activity}(\%) = \frac{A_c - A_s}{A_c} \times 100(\%), \tag{6}$$

where A_c and A_s are the A_{517} absorbance of the DPPH radical solution and the ASP sample mixed with the DPPH solution, respectively.

Table 1 – Box–Behnken experimental design and the results for extraction yield of ASP.

Run number	Coded levels			Yield of ASP (%)
	X_1	X_2	X_3	
1	−1	0	−1	18.40
2	−1	0	1	17.87
3	−1	1	0	18.93
4	0	0	0	21.29
5	0	1	−1	19.43
6	1	0	1	17.85
7	0	−1	1	17.72
8	1	0	−1	20.29
9	0	0	0	21.65
10	0	−1	−1	19.00
11	1	−1	0	19.04
12	0	1	1	20.83
13	−1	−1	0	18.43
14	0	0	0	21.37
15	0	0	0	21.98
16	1	1	0	21.44
17	0	0	0	21.90

ASP = *Angelica sinensis* polysaccharide.

3. Results and discussion

3.1. Single factor experiments of ASP

The water/raw material ratio, ultrasonic power, ultrasonic time, and ultrasound radiation method for the polysaccharide extraction of *A. sinensis* are described in Figure 1.

As shown in Figure 1A, the influence of the different ratio of water to raw material on the yield of ASP, and the ratio of water to raw material of the extraction process was carried out at 10 mL/g, 20 mL/g, 30 mL/g, 40 mL/g, and 50 mL/g, whereas the other three variables were set as follows: ultrasound power, 400 W; ultrasound extraction time, 25 minutes;

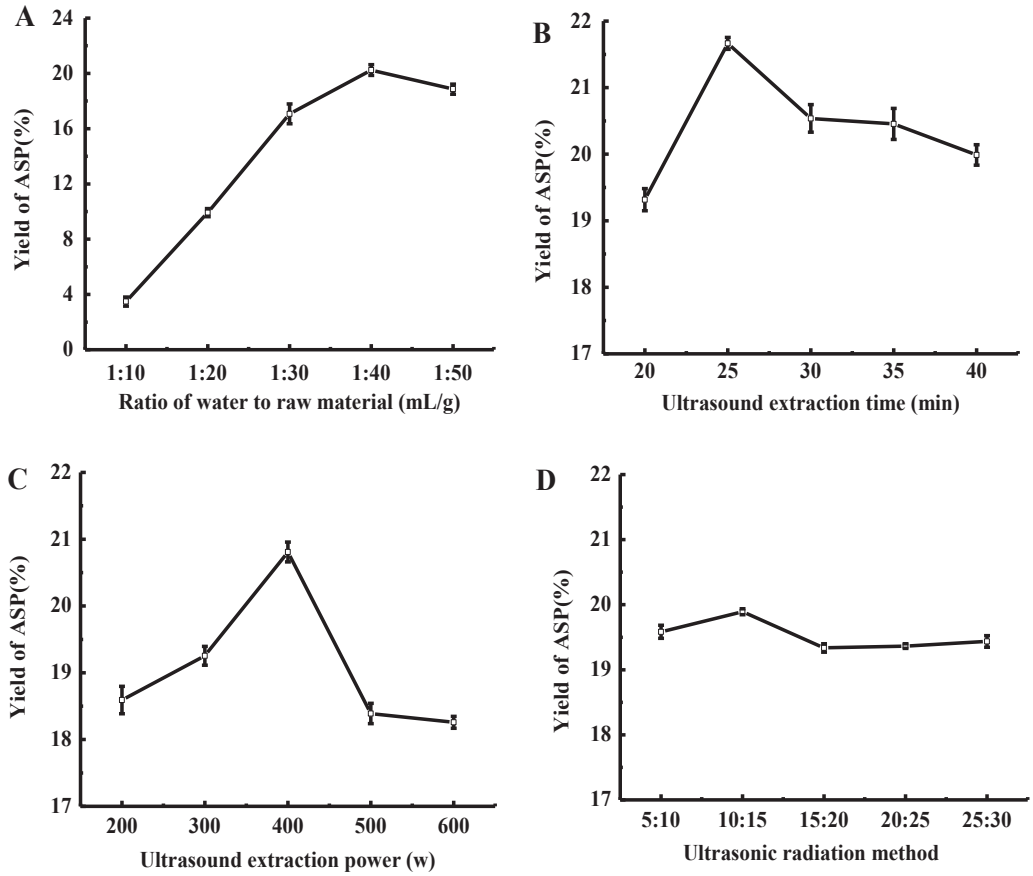

Figure 1 – Effects of (A) water/raw material ratio, (B) ultrasound extraction time, (C) ultrasound extraction power, and (D) ultrasound radiation method on the extraction yield of ASP (%). Values are means ± SD ($n = 3$). ASP = *Angelica sinensis* polysaccharide; SD = standard deviation.

ultrasound radiation method, 10:15. It showed that the yield of ASP increased clearly as the water/raw material ratio was increased from 10 mL/g to 40 mL/g, and then declined from 40 mL/g to 50 mL/g. A previous study showed that a higher water/raw material ratio leads to lower density and viscosity of the extraction solvent, which facilitates the dissolution of more polysaccharide molecules in the water [31]. The maximum yield of ASP was 20.24 ± 0.39% when it was 40 mL/g. So, the water/raw material ratio of 40 mL/g was selected, and a similar result was obtained in the extraction of polysaccharides from *Zagros oak* leaf [28].

Ultrasonic time is also an important factor to affect the extraction yield of ASP. Briefly, 20 minutes, 25 minutes, 30 minutes, 35 minutes, and 40 minutes of ultrasound time were used in the extraction of ASP when the water/raw material ratio, ultrasound power, and ultrasound radiation method were held at 40 mL/g, 400 W, and 10:15, respectively. Figure 1B indicates that the tendency of ultrasound time was to increase first and then decline. The yield of ASP increased linearly when the ultrasonic time ranged from 20 minutes to 25 minutes, and the maximum was 21.66 ± 0.09%. When ultrasound time exceeded 25 minutes, the yield of ASP no longer increased. This might be attributable to the long extraction time, which leads to the thermal instability and degradation of polysaccharides [32]. The ultrasound time of 25 minutes was deemed appropriate for the experiment.

The effect of ultrasonic power (200 W, 300 W, 400 W, 500 W, and 600 W) on the extraction yield of ASP is displayed in Figure 1C. The water/raw material ratio, ultrasound time, and ultrasound radiation method were maintained at 40 mL/g, 25 minutes, and 10:15, respectively. In Figure 1C, the yield of ASP shows an escalating trend when ultrasonic power ranged from 200 W to 400 W. The ASP yield was highest when the ultrasonic power was 400 W and the quantitative value was 20.81 ± 0.15%. This might be because the high ultrasonic power could increase the motion frequency and speed of material molecules. But the ASP yield decreased after the ultrasonic power of 400 W. This result indicated that a too high ultrasonic power would lead to the hydrolyzation and decomposition of polysaccharides [31–33].

On the basis of the test and equipment, the ultrasound radiation method in extraction of ASP was defined as 5:10, 10:15, 15:20, 20:25, and 25:30, and other parameters were as follows: water/raw material ratio, 40 mL/g; ultrasound power, 400 W; ultrasound extraction time, 25 minutes. As shown in Figure 1D, the maximum yield of ASP was 19.89 ± 0.04% when the ultrasound radiation method was 10:15, and the minimum was 19.34 ± 0.06% when the ultrasound radiation method was

15:20. The difference between the maximum and minimum levels was small. We inferred that this factor was not the important one for extracting ASP. Moreover, the other three factors (water/raw material ratio, ultrasonic power, and ultrasonic time) were found to have a more significant impact on the yield of ASP.

3.2. Orthogonal analysis of ultrasound-assisted extraction

The results of ASP extraction are listed in Table 2. Table 2 shows that the yield of ASP ranged from 19.60% to 21.48%. K_{ij} ($j = 1, 2, 3$) is the mean value of the test results with the same level of k in the i column of the table; R_i is the range of K_{ij} ($R_i = \max(K_{ij}) - \min(K_{ij})$). For each factor, the impact is different. And a bigger mean value (K_{ij}) indicated the greater impact of the factor on the yield of ASP, and the best level for each factor can be determined based on the largest mean value (K_{ij}) of the extraction condition [34]. Thus, the optimized conditions for ASP extraction were obtained as follows: water/raw material ratio, 40 mL/g (21.24%); ultrasound time, 25 minutes (20.85%); ultrasound power, 300 W (20.78%); ultrasound radiation method, 10:15 (20.69%). The best optimal portfolio was $A_2B_1C_2D_2$ of the extraction of ASP. Because the R_i of X_4 (ultrasound radiation method) was the smallest (0.096) factor, the other three factors were selected for the following experiments.

Table 2 – Orthogonal test design and the results for extraction yield of ASP.

Run number	Coded levels				Yield of ASP (%)
	X_1	X_2	X_3	X_4	
1	30	20	300	5:10	19.60
2	30	25	400	10:15	19.70
3	30	30	500	15:20	19.71
4	40	25	300	15:20	21.48
5	40	30	400	5:10	21.11
6	40	20	500	10:15	21.13
7	50	30	300	10:15	21.25
8	50	20	400	15:20	20.60
9	50	25	500	5:10	21.36
K_{i1}	19.670	20.443	20.777	20.690	
K_{i2}	21.240	20.847	20.470	20.693	
K_{i3}	21.070	20.690	20.733	20.597	
R_i	1.570	0.404	0.307	0.096	

R_i refers to the result of extreme analysis.
ASP = *Angelica sinensis* polysaccharide.

3.3. BBD analysis

A group of 17 experiments were performed using RSM of BBD to observe the combined effects of water/raw material ratio (mL/g), ultrasound extraction time (minutes), and ultrasound extraction power (W) on ASP extraction. The ASP yield ranged from 17.72% to 21.98%, whereas the extraction parameters are different as shown in Table 1.

3.3.1. Fitting of second-order polynomial equation and statistical analysis

A quadratic equation was used to establish a statistical model to confirm the optimum conditions and the response of the combined factors. By using multiple regression analysis on the experimental data, the yield of ASP was obtained using the following quadratic equation:

$$Y = 21.64 + 0.62X_1 + 0.81X_2 - 0.74X_3 + 0.47X_1X_2 - 0.48X_1X_3 + 0.67X_2X_3 - 1.41X_1^2 - 0.77X_2^2 - 1.63X_3^2 \quad (7)$$

The results of the regression analysis and analysis of variance were used for fitting the model given in Table 3. The p value was used to test the significance of the coefficient. The corresponding variables become more effective when the p value becomes smaller, and the p value can be used to check the interaction strength of the combined factors [28]. The R^2, R^2_{adj}, and coefficient of variation (CV %) were calculated to check the model adequacy.

According to Table 3, the p value of the model is p (0.0007) < 0.001, which suggests that the predicted quadratic response surface model was fitted significantly. The independent variable of the water/raw material ratio (X_1), ultrasound time (X_2), the quadratic term of the water/raw material ratio (X_1^2), ultrasound time (X_2^2), ultrasound power (X_3^2), and interaction term (X_2X_3) proved that the variables had a significant effect on the yield of ASP because its p value was less than 0.05. The ultrasound power (X_3) and the interaction term (X_1X_2), (X_1X_3) were not significant. The p value of the lack of fit was 0.0754, implying that the lack of fit was not significant relative to the pure error of the model. The determination coefficient (R^2) and the adjusted determination coefficient (R^2_{adj}) were 0.9534 and 0.8935, respectively, verifying that the model was markedly significant and reasonable. A low CV value (2.57) clearly indicates that the model is reproducible and reliable [35]. Therefore, the regression model is satisfactory.

Table 3 – ANOVA for response surface quadratic model.

Source	Sum of squares	*df*	Mean square	*F*	*p* Prob > *F*
Model	37.20	9	4.13	15.91	0.0007[a]
X_1	3.11	1	3.11	11.98	0.0105[a]
X_2	5.18	1	5.18	19.95	0.0029[a]
X_3	1.02	1	1.02	3.91	0.0886[c]
X_1X_2	0.90	1	0.90	3.47	0.1046[c]
X_1X_3	0.91	1	0.91	3.51	0.1031[c]
X_2X_3	1.80	1	1.80	6.91	0.0340[a]
X_1^2	8.37	1	8.37	32.23	0.0008[a]
X_2^2	2.48	1	2.48	9.55	0.0175[a]
X_3^2	11.12	1	11.12	42.81	0.0003[a]
Residual	1.82	7	0.26		
Lack of fit	1.44	3	0.48	5.07	0.0754[c]
Pure error	0.38	4	0.095		
Cor total	39.02	16			
R^2	0.9534				
R^2_{adj}	0.8935				
Adeq precision	10.805				
CV %	2.57				

[a] Significant at $p < 0.05$.
[c] Not significant.
ANOVA = analysis of variance; CV = coefficient of variation; *df* = degrees of freedom.

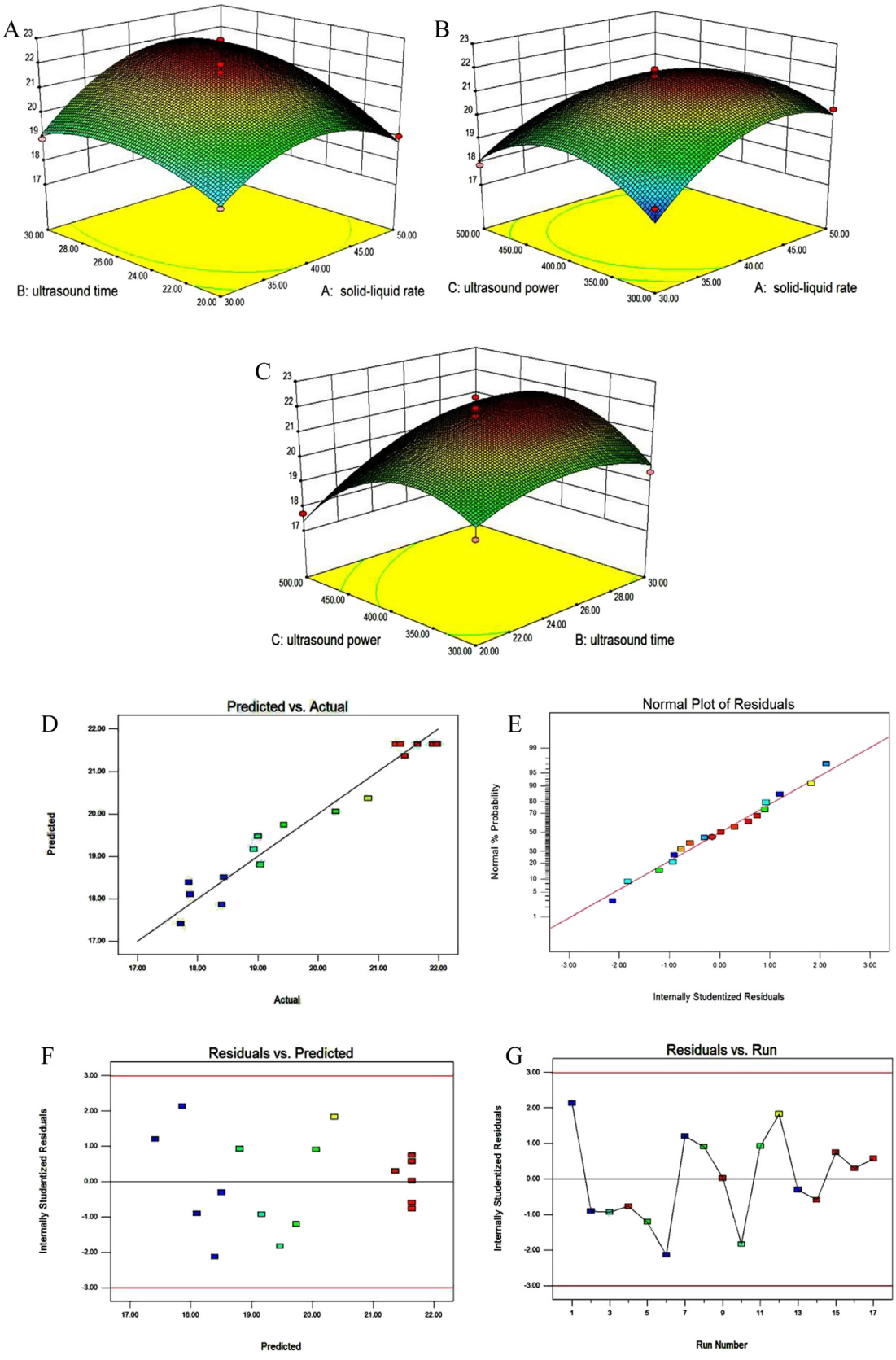

Figure 2 – Response surface figure (3D) and diagnostics diagrams for the model adequacy. (A) Solid–liquid rate and ultrasonic time. (B) Solid–liquid rate and ultrasonic power. (C) ultrasonic time and ultrasonic power. (D) Predicted versus actual. (E) Normal plot of residuals. (F) Residuals versus predicted. (G) residuals versus run numbers.

3.3.2. *Optimization of extraction conditions of ASP*

The optimal values of the variables were obtained by the regression equation. The three-dimensional response surface figures were acquired using Design-Expert 8.0.5.0. The influence on ASP yield by the water/raw material ratio, ultrasound power, and ultrasound time is displayed in Figure 2. In the response surface figures, the yield of ASP was acquired using the two continuous variables whereas the other variable was kept in 0 levels.

In Figure 2A, the extraction yield of ASP was obtained with a different water/raw material ratio and ultrasonic time at ultrasonic power of 0 levels and increased with the increase in water/raw material ratio and ultrasonic time; the maximum value of ASP yield was 21.65%. Figure 2B shows that the incremental tendency of the water/raw material ratio was more obvious than that of ultrasonic power. It attested that the water/raw material ratio exerts a far greater impact on ASP yield than ultrasonic power. Figure 2C displays the response surface figures of the ASP yield at diverse ultrasonic time and ultrasonic power. It was clear that the yield of ASP increased with the increase in ultrasonic time and ultrasonic power in the beginning, but when ultrasound power was increased further, the yield of ASP reduced visibly. It was consistent with single factor tests.

3.3.3. *Model adequacy diagnostics*

Model adequacy diagnostics is necessary to check the applicability of the model to the real system. Four diagnostics diagrams for model adequacy are shown in Figure 2. Figure 2D shows the predicted and the actual experimental values. The spots of the predicted and actual values showed normal distribution and are close to the 45° line, attesting that the model has a good adaptation. Figure 2E shows a normal % probability plot of residuals for the normality assumption; the residual plot that approached a straight line proved that the normality assumption was appropriate. The internally studentized residuals versus predicted values are displayed in Figure 2F. The plots of the internally studentized residuals dispersed randomly showed that the original variance was constant for all values [35]. The internally studentized residuals versus experimental run numbers are shown in Figure 2G, and all the points are located within a limited range. All data indicated that the response surface model was applied to the ASP extraction, and the model was significant and accurate.

3.3.4. *Validation of the model*

The optimum extraction conditions for the extraction of ASP consisted of a water/raw material ratio of 43.31 mL/g, an ultrasonic time of 28.06 minutes, and an ultrasonic power 396.83 W, which were calculated using the second-order quadratic equation of the model by solving the regression equation and analyzing the response surface. The maximum predicted value of ASP yield was 21.99%.

In order to validate the predicted value of ASP yield and the applicability of the model, three parallel tests were implemented under the aforementioned optimum extraction conditions. The yield of ASP was $21.89 \pm 0.21\%$ ($n = 3$), which approached the predicted value of 21.99%. The results indicated that the RSM was appropriate for optimizing the conditions for ASP extraction, and the regression model was accurate and applicable for extracting ASP. The investigation showed that the extraction yield was less than 6% when hot water extraction was used and only 15.2% when the microwave-assisted extraction method was used [1,27,36].

3.4. *Antioxidant activity of polysaccharide*

Oxidation is a chemical reaction that transfers electrons from oxide atoms to oxidants and can engender harmful compounds (radicals). Radicals can damage the tissues and cells, and result in chronic diseases and aging effects. Many kinds of polysaccharides have antioxidant activity, such as POJ-U1a of *Ophiopogon japonicus* [37], polysaccharides of *Schisandra sphenanthera* [38], and polysaccharides of *Cyclocarya paliurus* (Batal.) Iljinskaja [24]. In this study, the scavenging activity of ASP at 25 μg/mL, 50 μg/mL, 100 μg/mL, 200 μg/mL, 400 μg/mL, and 800 μg/mL against superoxide anion radicals, hydroxyl radicals, and DPPH with ascorbic acid (Vc) as a positive control are shown in Figure 3. ASP was the *Angelica sinensis* polysaccharide in optimal extraction parameters.

A certain number of superoxide anion radicals exist in the human body, and when they combine with hydroxyl radicals the result can damage the cell DNA and destroy the function of the human body [39]. Figure 3A displays the scavenging activity of superoxide anion radicals. The scavenging activity of superoxide radicals is dependent on the concentration of polysaccharides. The minimum scavenging activity of superoxide anion radicals was 7.53% at 25 μg/mL, and the maximum was 23.74% at 800 μg/mL. The increase of scavenging effect on hydroxyl radicals increased slightly. Therefore, the result suggested that ASP has potential scavenging activity of superoxide radicals.

Hydroxyl radical is known as an important reactive oxygen species with a strong ability of oxidizability and can kill red blood cells, and degrade DNA and cell membranes [40]. The scavenging effect of hydroxyl radicals is shown in Figure 3B. The scavenging activity of hydroxyl radicals increased as the concentration of polysaccharides increased, and the increment was conspicuous. The scavenging activity was 10.18% at 25 μg/mL and 11.22% at 50 μg/mL. But when the concentration of polysaccharides was 400 μg/mL, the scavenging activity of hydroxyl radicals in ASP was 48.36% and increased dramatically. When the concentration of polysaccharides was increased to 800 μg/mL, the scavenging activity of hydroxyl radicals in ASP was close to that of ascorbic acid (81.34%), and the highest scavenging activity was 61.45%. This proved that ASP has a remarkable scavenging capability of superoxide radicals in high concentration.

DPPH is a stable radical that centers on nitrogen and shows a maximum absorption at 517 nm in methanol [38,40]. Figure 3C shows the DPPH radical scavenging activity of different concentrations of polysaccharides. The scavenging activity of DPPH was similar to that of superoxide anion radicals, which rose indistinctly as the polysaccharides' concentration increased. The scavenging activity of DPPH was lower than that of superoxide anion radicals at a different concentration. The DPPH radical scavenging activity increased from 4.40% to 19.26% when the concentration of ASP increased from 25 μg/mL to 800 μg/mL. When the concentration of ASP was 200 μg/mL, the scavenging activity was

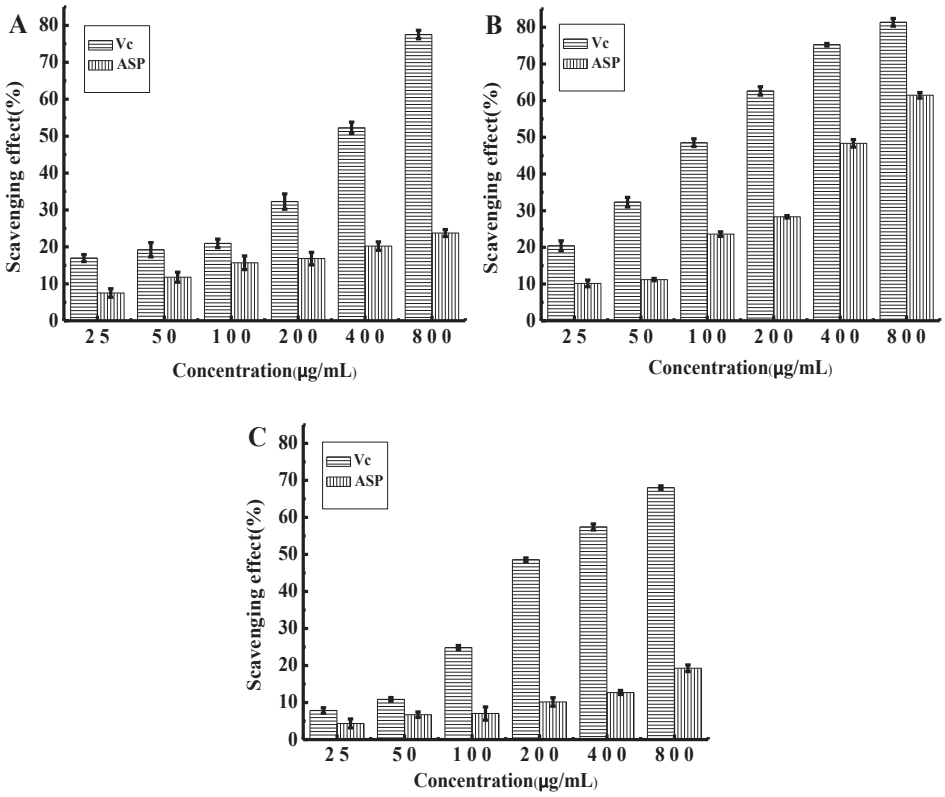

Figure 3 – Scavenging activity of (A) ASP on superoxide anion radicals, (B) hydroxyl radicals, and (C) DPPH. Values are means ± SD ($n = 3$). ASP = *Angelica sinensis* polysaccharide; DPPH = 2,2-diphenyl-1-picry-hydrazyl; SD = standard deviation.

only 10.17%, and it was less than the scavenging activity of superoxide anion radicals and hydroxyl radicals, whose scavenging activity exceeded 11% at 50 μg/mL. The results indicated that the scavenging activity of DPPH in ASP was low compared with that of ascorbic acid.

4. Conclusion

The optimal polysaccharide extraction conditions of water/raw material ratio (43.31 mL/g), ultrasound time (28.06 minutes), and ultrasound power (396.83 W) were obtained using the RSM of BBD based on single factor experiments and orthogonal test. The maximum extraction yield was 21.89 ± 0.21% under the optimum extraction conditions, which is close to the predicted yield of 21.99%. The RSM of BBD was able to successfully optimize the extraction conditions of ASP, and the regression model was applicable for extracting ASP. The antioxidant activity of superoxide anion radicals, hydroxyl radicals, and DPPH *in vitro* assays indicated that ASP has antioxidant activities that increased with the increasing concentration of the polysaccharide solution. Thus, these results suggested that polysaccharide from *A. sinensis* should be explored as a novel and potential natural antioxidant for use in functional or medicinal medicine. Further studies on purification and structure identification are in progress to study the polysaccharide thoroughly.

Conflicts of interest

All authors declare no conflicts of interest.

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