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Synthesis and antioxidant activity of selenium polysaccharide from *Lotus* root

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Abstract

In this study, *lotus* root polysaccharide was taken as raw material. The preparation technology of *its* polysaccharide selenium was established by response surface design, and its antioxidant activity in vitro was studied. Selenium content was taken as the detection index, and the effects of reaction time, the ratio of sodium selenite to the polysaccharide, and the volume fraction of nitric acid on selenium content were investigated. Through the response surface method, the optimal synthesis conditions of *lotus* root polysaccharide selenium were obtained as follows: reaction time of 5.36h, the ratio of sodium selenite and *lotus* root polysaccharide is 0.78, the volume fraction of nitric acid 0.47%, the selenium content of *lotus* root polysaccharide selenium 0.1106%. Selenium, a polysaccharide from *lotus* root, had a scavenging effect on ABTS radical and superoxide ion, and the maximum scavenging rate was 52.17% and 85.23%, respectively. Aa shows that the synthesized *lotus* root polysaccharide selenium can be used as an antioxidant or functional food.

Keywords: selenium polysaccharide; *Lotus* root; antioxidant.

Practical Application: The synthesized lotus root polysaccharide selenium can be used as an antioxidant or functional food.

1 Introduction

Polysaccharides are widespread substances in organisms. Polysaccharides are called polysaccharides only if they are composed of more than 10 monosaccharides. Polysaccharides are very important macromolecules in organisms and play a vital role in the operation of life activities (Song et al., 2021; Yu et al., 2018). Plant polysaccharides in polysaccharides play a particularly significant role in this respect. Plant polysaccharides, also known as plant polysaccharides, are polysaccharides formed by the metabolism and polymerization of at least 10 monosaccharides in plant cells. Plant polysaccharides have attracted more and more attention, and they play an important role in daily human life (Liu et al., 2015; Yin et al., 2019). Such as *Tremella* polysaccharides, *lotus* root polysaccharides, tea polysaccharides, *ginseng* polysaccharides, and so on are plant polysaccharides. Polysaccharides have immunomodulatory, antitumor, antiviral, and hypoglycemic effects, and *lotus* root polysaccharides also have inhibitory effects on human aging to a certain extent (Barbosa & de Carvalho Junior, 2021; Li & Huang, 2021). In addition, *lotus* root polysaccharides also have important effects such as antioxidation, reducing blood lipids, anti-radiation, antibacterial, anti-fatigue, and hypoglycemia (Hu et al., 2019; Yan et al., 2022).

Selenium is one of the essential nutrient elements for the human body, which can form glutathione peroxidase, protect cell tissue and maintain the function of the cell membrane (Steinbrenner et al., 2006). Selenium plays a good role in the antiaging of the human body. At the same time, selenium is an essential element in maintaining normal immune function and can enhance human immunity, resistance, anticancer, antitumor, and other effects. Furthermore, selenium can reduce blood viscosity, blood lipids, blood pressure, cholesterol, etc. At the same time, selenium participates in various metabolic processes in the body, which helps prevent cardiocerebrovascular diseases and detoxification (Lv et al., 2021). The study found that the content of selenium in the human body is closely related to cancer incidence (Bleys et al., 2008). Selenium is called by scientists as the "anticancer king" of trace elements in the human body.

Selenium polysaccharide is a kind of organic selenium compound that combinates polysaccharides with selenium and has the pharmacological activity of both selenium and polysaccharides (Huang et al., 2020; Liu et al., 2021; Shi et al., 2021). It is easy to be absorbed and used by the human body as a good selenium supplement. Moreover, selenium polysaccharides also have good antioxidation, which helps the human body scavenge free radicals and delay human aging (Liu et al., 2016). And selenium polysaccharides can block the damage of various carcinogenic factors to the human body, inhibit the proliferation of cancer cells, and have anticancer effects. The intake of selenium polysaccharides can effectively improve anemia caused by selenium deficiency, regulate symptoms such as Kaschin-Beck disease, hypertension, hyperlipidemia, and pancreatitis, enhance human immunity, and also improve the decline of immunity caused by lack of sleep and other symptoms. Therefore, selenium polysaccharides are widely used in immune regulation, antitumor, antioxidation, antiaging,

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and so on (Chen et al., 2022; Feng et al., 2022; Yang et al., 2021; Zhang et al., 2021). Due to the unique pharmacological activity, selenium polysaccharides from medicinal plants have gradually become the focus of research, showing great prospects in health care and medicine. This study will effectively support the utilization and development of functional foods and products related to selenium polysaccharides.

2 Materials and methods

2.1 Materials

Our lab extracted *lotus* root polysaccharides (LRP) (Yan et al., 2022). All other chemicals and solvents were of analytical grade.

2.2 Establish a selenium standard curve

Accurately weigh 50 mg selenium into the beaker, react with 3 mL mixed acid to clarify, and set the volume to 50ml with water. The above 1ml solution was taken and fixed volume in a 250 mL volumetric flask (selenium reference solution). The above selenium reference solutions 1 mL, 3 mL, 4mL, 5mL, 6 mL, 8 mL, 10 mL and 12 mL were added to 25 mL distilled water. Adjust the above solution to pH to 2 with ammonia water, add 2mL o-phenylenediamine solution (now used), shake, and stand for 2 hours (avoid light). Transfer the above solution into the liquid separation funnel and add 9.5 mL toluene, shake 2 min, static 5min, take the toluene layer, and fix the volume to 10mL. The standard curve of selenium can be obtained by checking the absorbance of 9 groups of data at 334nm.

2.3 Preparation of selenium polysaccharide from Lotus Root (LRPS)

A certain amount of polysaccharide was dissolved in the dilute nitric acid solution, stirring until all dissolved, added an appropriate mount of sodium selenite and barium chloride, then reacted in the water bath, cooled to room temperature after the reaction finished, finally we obtained the white milky polysaccharide selenium solution, then we adjusted pH to 6 with anhydrous sodium carbonate for getting the polysaccharide selenium by centrifugation, dialysis, and vacuum drying.

2.4 Determination of selenium in Polysaccharide selenium of Lotus Root

The general methods for determinating selenium content are spectrophotometry, graphite furnace atomic method, etc. Here spectrophotometry was taken to determine selenium content (Yang et al., 2020). The specific operation is as follows: take 20mg polysaccharides and polysaccharides selenium in the 50mL beaker, add 1mL concentrated sulfuric acid and 2mL concentrated nitric acid to clear, water bath at 90 °C for 2 hours, cool to room temperature after the reaction is over, add 25 mL distilled water, adjust pH to 2 with ammonia, add 2 mL 1% o-phenylenediamine test solution (now used), shake and stand for 2 hours, put in the 100 mL liquid separation funnel, add 9.5 mL toluene, extract static, separate toluene layer, use toluene to fix volume to 10mLfor getting the *lotus* root polysaccharide selenium reaction solution. Finally, the absorbance was measured at 334 nm.

2.5 Optimisation of LRPS synthesis and experimental design

Abs of LRPS was the detection index. Response surface methodology (RSM) was used to investigate the effects of the three variables. The levels and codes of SC% used in the Box-Behnken design (BBD) are shown in Table 1. The BBD and the results for LRPS synthesis are shown in Table 2.

2.6 Characterisation of LRP and CMLRP

(1) Fourier transform infrared spectroscopy (FTIR) analysis

20 mg dried LRPS were thoroughly mixed with 200 mg of KBr and uniformly ground, then the correct amount of fine powder was placed into a circular mold and pressed into a transparent circular sheet. The tableted powder was analyzed by Fourier transform infrared spectroscopy (FTIR; Spectrum400; PerkinElmer, USA) within the wavenumber range of 4000 to 500 cm^{-1}.

(2) Scanning electron microscopy (SEM) analysis

Bond the conductive film on the sample seat, evenly sprinkle a small amount of LRPS powder, gently blow the non adhered LRPS powder with an ear washing ball, and observe on the mirror after plating the conductive film.

Table 1. Levels and code of variables used in Box-Behnken design.

Variable	Symbols Coded	Coded levels -1 0 1		
Reaction time (h)	A	4	5	6
Na_2SeO_3:Polysaccharide	B	0.6	0.8	1.0
Nitric Acid(%)	C	0.3%	0.5%	0.7%

Table 2. Experimental Design and Results of LRPS Box-Behnken.

Run	A	B	C	Abs
1	5	0.6	0.7	0.375
2	5	0.6	0.3	0.506
3	4	0.6	0.5	0.469
4	6	0.6	0.5	0.556
5	5	0.8	0.5	0.731
6	4	0.8	0.3	0.425
7	5	0.8	0.5	0.776
8	5	0.8	0.5	0.814
9	4	0.8	0.7	0.344
10	5	0.8	0.5	0.791
11	6	0.8	0.7	0.464
12	5	0.8	0.5	0.774
13	6	0.8	0.3	0.651
14	4	1	0.5	0.395
15	6	1	0.5	0.561
16	5	1	0.7	0.332
17	5	1	0.3	0.358

(3) Antioxidant activity of LRPS

The ABTS and superoxide anion radical scavenging activity were investigated as described by Yan and Yu (Yan et al., 2022; Yu et al., 2022). We prepared the solutions of LRPS at concentrations of 1, 2, 4, and 8 mg/mL for analysis of antioxidant activities.

2.7 Statistical analyses

Data from triplicate assays were subjected to ANOVA to identify significant changes in treatment response. We considered the differences obviously at $P < 0.05$ and highly significant at $P < 0.01$. Data are presented as the mean ± SEM unless otherwise stated.

3 Results and discussion

3.1 Establishment of selenium standard curve

The selenium standard curve Figure 1 was obtained by measuring the absorbance of selenium solution with a concentration of 0.4,0.8,1.2,2.4,3.2,4 μg/mL at 334 nm.

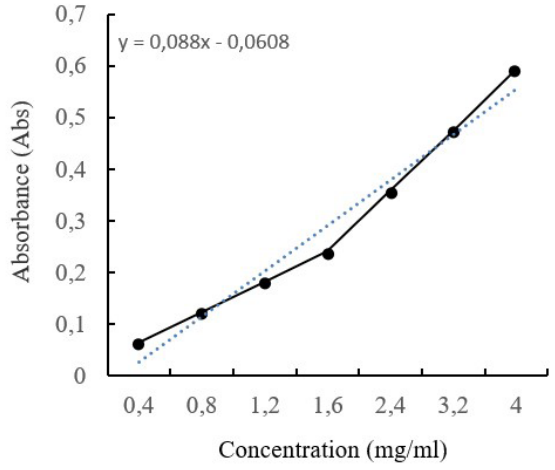

Figure 1. Establishment of selenium standard curve.

3.2 Response surface analysis of CMLRP

In the single factor experiment, we used the control variable method to investigate the effects of reaction time, Na_2SeO_3:Polysaccharide, and volume fraction of nitric acid (%) on the selenium content (SC%) of selenium polysaccharides, then we researched the optimum values of three single factors.

As shown in Figure 2A, when sodium selenite: lotus root polysaccharide was 0.8 and nitric acid concentration was 0.5%, the selenium content increased at first. It then decreased with the reaction time, and the absorbance of LRPS reached the maximum when the reaction time was 5 h. We concluded that when time is a single variable, 5h is the best condition. In Figure 2B, The nitric acid concentration is 0.5%, and the reaction time is 5h. The absorbance of LRPS increases and reaches the maximum at first, then decreases when the ratio of the sodium selenite to *lotus* root polysaccharide is 0.8. Therefore, as a single variable, 0.8 of the ratio is the best condition. Also, in Figure 2C, when fixing the reaction time 5h and the ratio of sodium selenite to lotus root polysaccharide as 0.8, with the concentration of nitric acid increasing to 0.5%, the absorbance of LRPS increases to the maximum first, then decreasing. It can be seen that the nitric acid concentration of 0.5% is the best.

The results of 17 trial points tested in a random order based on the BBD design, including design and experimental values, are presented in Table 2. The Abs of the LRPS can be fitted into the following second-order polynomial Equation 1:

$$Abs = 0.42+0.059A+0.49B+0.056C+0.02AB-0.013AC+0.013BC-0.1A^2-0.18B^2-0.051C^2 \quad (1)$$

The model results were analyzed by variance analysis, and the results are shown in Table 3. The F value of the regression equation is 45.39, $P < 0.001$, which shows that the experimental model fitted by the response surface method is very significant. According to the regression analysis in Table 3, there was no significant effect except for AB, AC, and BC. The effect of the B-mass ratio was significant, A-time and C-nitric acid concentrations were highly influential, and the others were highly significant. The model established in the

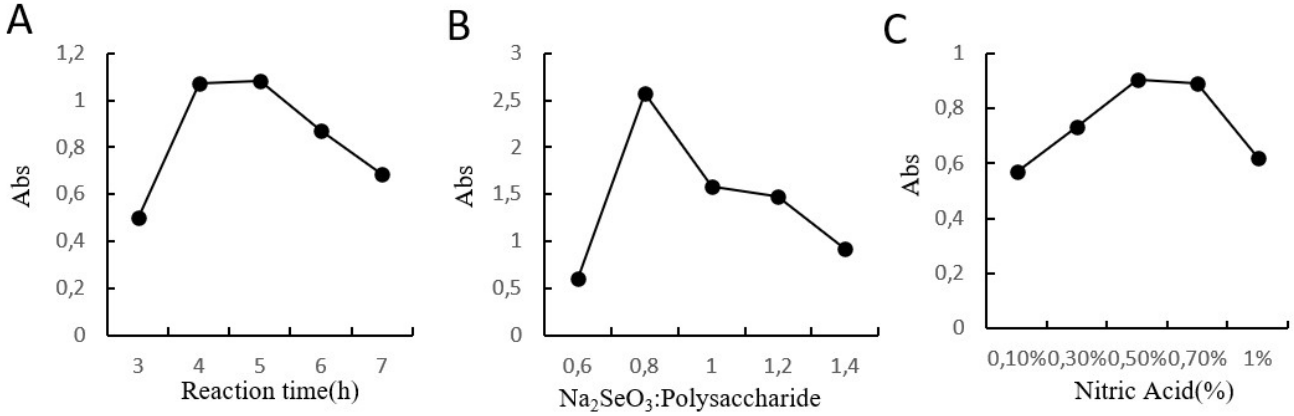

Figure 2. The effect of single factor on Abs of LRPS. (A) Reaction time single-factor experiment results; (B) Single-factor experiment results of Na_2SeO_3:Polysaccharide; (C) Reaction Nitric Acid(%) single factor experiment results.

Table 3. Analysis of variance of the experimental results of the LRPS.

Variables	Sum of squares	df	Mean square	*F*-value	*p*-Value
Model	0.48	9	0.053	45.39	<0.0001***
A	0.045	1	0.045	38.42	0.0004 **
B	8.450E-003	1	8.450E-003	7.24	0.0311 *
C	0.023	1	0.023	19.34	0.0032 **
AB	1.560E-003	1	1.560E-003	1.34	0.2856
AC	2.809E-003	1	2.809E-003	2.41	0.1648
BC	2.756E-003	1	2.756E-003	2.36	0.1683
A^2	0.044	1	0.044	37.41	0.0005 ***
B^2	0.14	1	0.14	116.98	<0.0001 ***
C^2	0.18	1	0.18	150.60	<0.0001 ***
Residual	8.173E-003	7	1.168E-003		
Lack of fit	4.482E-003	3	1.494E-003	1.62	0.3188
Pure error	3.691E-003	4	9.227E-004		
Correlation total	0.49	16			
$R^2 = 0.9832$	$R^2_{Adj} = 0.9615$	$R^2_{Pred} = 0.8403$	Adeq precisior = 16.936		

*P<0.05. ** P<0.01. ***P<0.001. df: degrees of freedom.

experiment was highly significant (P < 0.001), but the misfit (0.3352 > 0.05) was not significant. These data show that the response surface model established in this experiment is feasible for optimizing the preparation process of lotus root polysaccharide selenium. From the above Figures, we can see that the signal-to-noise ratio (AdeqPrecisior) of the model is 16.936, a relatively high used to predict the experimental results with the model. On the other hand,, the model correction decision coefficient R2Adj is 0.9615, indicating that the model can correctly predict the response value of 96.15%. Also, the determination coefficient of the model is relatively high, which suggests that the model fits well. The model can be used to analyze and predict the influence of various factors in the synthesis process on the preparation of *lotus* root polysaccharide selenium. Similarly, from the above table, it can be seen that the difference between R2Pred=0.8403 and R2D0.9832 is slight, and the response surface regression equation is reliable. As a result, the regression model shown was used to guide further experiments, including determining the two-factor effect of the model. The results of these analyses are shown in Figure 3.

According to the response surface optimization results, the best synthesis process of *lotus* root polysaccharide selenium was 5.38h, the ratio of sodium selenite to polysaccharide was 0.78, and the nitric acid concentration of 0.47%. The expected absorbance under these conditions and the predicted absorbance of LRPSwere determined to be 0.797, and the selenium content was 0.206%. To further verify the reliability of the response surface optimization experimental model, the absorbance values 0.695, 0.678, 0.826, and 0.868 were obtained through four groups of parallel experiments. The average value is 0.767, which is 96.20%, consistent with the theoretical value of 0.797, and the corresponding error is 3.79%. Thus we concluded model is highly compatible and reliable and can be used to synthesize lotus root polysaccharide selenium.

3.3 Characterisation and analysis of LRPS

The FTIR spectra of LPRS are shown in Figure 4A. The polysaccharides were analyzed with infrared spectroscopy to investigate the characteristic organic groups in the two samples,. The existing selenium forms in selenium polysaccharides may be Se-H and Se-O. LRPS has a strong absorption peak at 3432.7, which is caused by the formation of molecular health and internal hydrogen bond of-OH on polysaccharides, and 2928 is a stretching vibration absorption peak of-CH3 which belongs to a medium-strong peak. Compared with the FITR spectrum of LRP, selenium may exist in the form of Se-H on the branched-chain of water-soluble polysaccharides and produce a stretching peak at 1150.55cm-1. The XRD spectra of LPRS are shown in Figure 4B. Compared to LRP, a comparison of our XRD spectra confirmed that the LRPS formed in our experiments were nanocrystals, as evidenced by the peaks at 2θ values of 25.22°, 29.20°, 31.30°, 41.81°, 47.06 °, and 53.72°.

The SEM of LPRS is shown in Figure 5. Figure 5A-C is 500 times (100 μm), 200 times (50 μm), and 400 times (50 μm) of LRPS, respectively. According to the SEM image, LRPS is irregular and loose, the composition is trivial and weird, and the surface is uneven.

3.4 Antioxidant activities of LRP and CMLRP

As shown in Figure 6A, the clearance rate of *lotus* root polysaccharide selenium against scavenging superoxide ion increased to the maximum of 85.23% at first and then decreased, which shows that *lotus* root polysaccharide selenium has a strong scavenging effect on superoxide ion. However, with the further increase of the concentration of *lotus* root polysaccharide selenium solution, the clearance rate decreased obviously. We speculated that the clearance rate decreased because the concentration of *lotus* root polysaccharide selenium solution was too high.

As shown in Figure 6B, the scavenging rate of lotus root polysaccharide selenium to ABTS free radical showed an

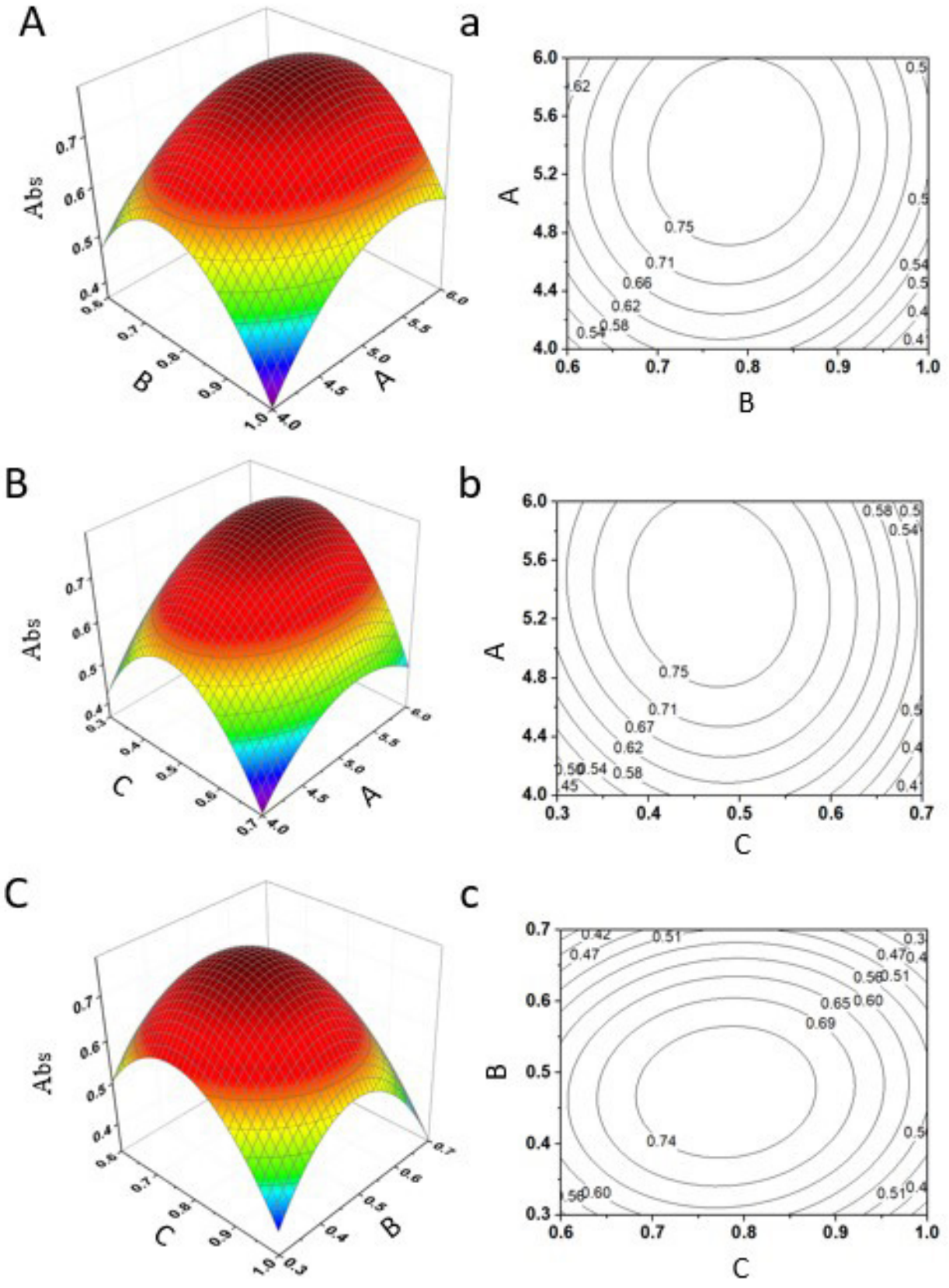

Figure 3. Response surface plots showing the effect of Abs on LRPS. (A, a) Response surface map and contour map of time (A) and Na_2SeO_3:Polysaccharide (B). (B, b) Response surface map and contour map of time (A) and Nitric Acid(%) (C). (C, c) Response surface map and contour map of Na_2SeO_3:Polysaccharide (B) and Nitric Acid(%) (C).

upward trend, with a minimum scavenging rate of 32.88% and a maximum scavenging rate of 52.17%. As shows that it has an apparent scavenging effect on ABTS free radicals. At the same time, it also indicates that the scavenging rate of lotus root polysaccharide selenium to ABTS is related to the concentration. The higher the concentration, the greater the scavenging rate.

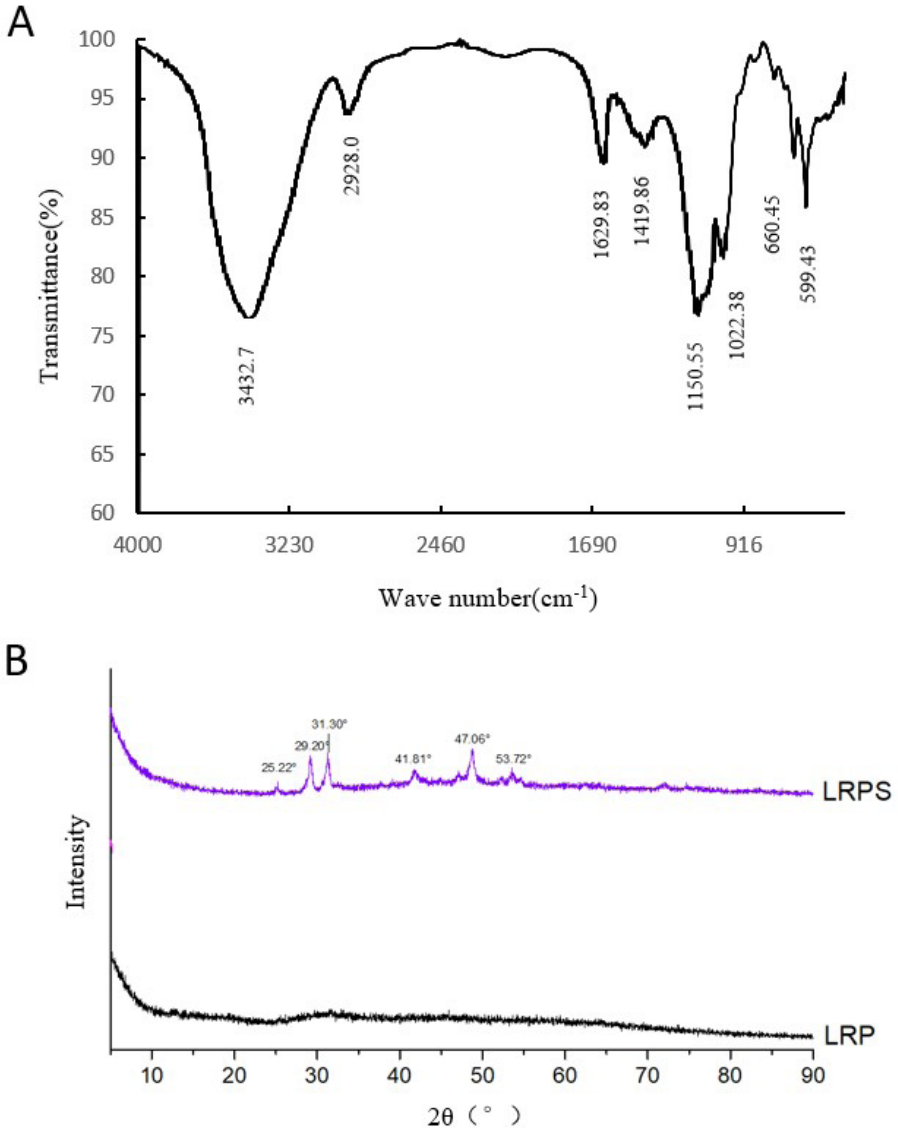

Figure 4. Characterization analysis of LRPS. (A) FTIR spectra of LRPS. (B). XRD spectra of LRP and LRPS.

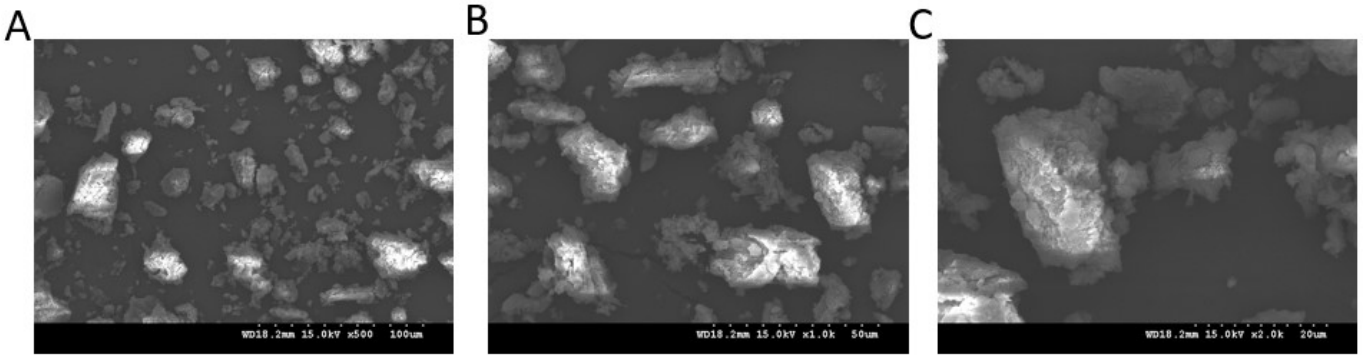

Figure 5. Morphology of LRPS and CM-LRP observed by SEM at different magnifications. A1, A2, and A3 SEM spectra of LRPS.

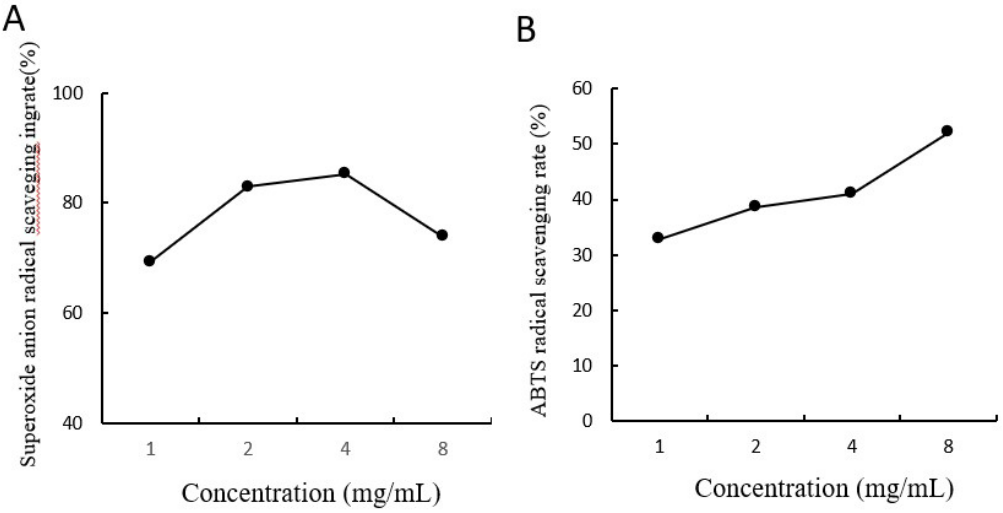

Figure 6. Antioxidant capacity of LRPS (A) Superoxide anion radical scaveging ingrate (%); (B) ABTS radical scavenging rate (%).

4 Conclusion

Selenium is an essential micronutrient for animals and plants. Selenium from animals or plants is mainly existed in organic-selenium form, including selenoprotein, selenium polysaccharides, and selenium tRNA. It can form active centers of some oxides, promote the decomposition of peroxides in the body, and protect the structure and function of cell membranes. Many diseases in the human body are seriously related to selenium deficiency (Chen & Berry, 2003). Because selenium cannot be synthesized in the body, it is common in the human body and must be ingested from foods that lack selenium. Human intake of selenium is mainly in the form of organic selenium. Selenium polysaccharides, including natural selenium polysaccharides from plants or synthetic derivatives, contain both selenium and polysaccharides and belong to organic selenium compounds. Studying its action mechanism further improves the utilization of selenium and polysaccharides and broadens the channels of obtaining selenium polysaccharides (Huang et al., 2020). Therefore, selenium modification has become a hot spot in the research field of polysaccharides. It was found that the covalent combination of selenium and polysaccharides had a significant protective effect on cadmium-induced toxicity (Zhou et al., 2020). The nanoparticles formed by sugar and selenium have antitumor activity by inhibiting the proliferation and migration of tumor cells and the angiogenesis of zebrafish embryos (Zhang et al., 2021). It was found that higher selenium content could effectively improve the antitumor activity of selenium polysaccharides in vitro (Zhu et al., 2020). Similarly, polysaccharides from selenium-enriched mycelia of mulberry yellow (PSeP) decreased the level of reactive oxygen species (ROS), the activity of myeloperoxidase (MPO) and the content of malondialdehyde (MDA), and increased the activities of glutathione peroxidase (GSH-Px) and catalase (CAT) (Luo et al., 2021). Se-Le-30, an analog of lentinan, could significantly inhibit the proliferation of human peripheral blood mononuclear cells stimulated by anti-CD3 antibody or allogeneic stimulation, down-regulate the ability of CD3+T cells to produce tumor necrosis factor (TNF)-α, and significantly reduce the cytotoxic activity of natural human killer (NK) cells (Kaleta et al., 2021).

This experiment synthesized *l*otus root polysaccharide selenium using lotus root polysaccharide as experimental raw material. The optimum conditions of single factor were as follows: reaction time 5h, sodium selenite: polysaccharide 0.8, nitric acid concentration 0.5%. The polysaccharide selenium of lotus root can be obtained by water bath at 70°C. The synthesis process of lotus root polysaccharide selenium was established with the help of response surface software Design-Expert. The optimum conditions for preparing lotus root polysaccharide selenium were as follows: reaction time 5.38h, sodium selenite: polysaccharide 0.78, nitric acid concentration 0.47% (volume fraction), and the accuracy of response surface design was verified. The polysaccharides of lotus root selenium ABTS radical and superoxide radical have an obvious scavenging effect, which also provides a specific reference value for the study of antioxidation of lotus root polysaccharide selenium.

Competing interests

The authors have declared that no competing interest exists.

Data availability

The data used to support the findings of this study are included in the article.

Author contributions

Dr. Zhang XF designed the study. Yan YY, Wang Q, Sun LH, and Zhang XF collected data. All authors agreed to the final version.

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