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Optimization of ultrasonic-assisted extraction of polysaccharide from fig leaves and its antioxidant activity

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Abstract

Using fig leaves as raw materials, extraction time, extraction temperature, solid to liquid ratio, and ultrasonic power were selected as influencing factors to analyze their effects on the yield of fig leaf polysaccharides (FLPS). The response surface methodology was carried out to optimize the extraction parameters. Furthermore, the antioxidant activity of FLPS was studied. The experimental results show that the optimum extraction conditions are: extraction time of 121 min, extraction temperature of 72 °C, solid to liquid ratio of 1: 48 g/mL, and ultrasonic power of 250 W. In this case, the measured extraction rate of FLPS was 11.78%, which was in agreement with the theoretical extraction rate. Response surface analysis was used to accurately and reliably optimize the extraction of FLPS. The results of the antioxidant activity showed that FLPS had antioxidant activity. It has the best scavenging effect on ABTS radical cation ($ABTS^{+}\cdot$), and it also has ability to scavenge DPPH radical (DPPH·), hydroxyl radical (·OH), superoxide anion radical ($\cdot O_2^-$), and ferric reducing activity. This study laid a theoretical foundation for the further development and application of FLPS.

Keywords: fig leaf; polysaccharides; ultrasonic-assisted extraction; response surface methodology; antioxidant.

Practical Application: In this study, ultrasonic-assisted extraction of polysaccharides from fig leaves was optimized, and fig leaf polysaccharides were found to partly have the ability to scavenge DPPH·, ·OH, $\cdot O_2^-$, and $ABTS^{+}\cdot$ and to reduce Fe^{3+}. These results suggest that the FLPS have potential application as antioxidants in food.

1 Introduction

Fig is cultivated in all parts of China, and its application has a long history (Bey & Louaileche, 2015). Figs are rich in polysaccharides (Owino et al., 2004), phenol (Vinson et al., 2005), and flavonoids (Adiletta et al., 2019), and have antioxidant and immune activity (Ali et al., 2012; Lansky et al., 2008; Yang et al., 2009). The application of fig has a long history, and figs are often used to make wine, drinks, and jam, its root, leaves, fruit, stem can be used as medicine (Solomon et al., 2006), with spleen, stomach, lung cough, swelling pain function. However, after fruit picking of figs, fig leaves are often discarded as waste, resulting in insufficient utilization of fig leaves. Fig leaves contain a variety of chemicals: polysaccharides, plant fiber, vitamins, proteases, organic acids, flavonoids (Badgujar et al., 2014; Naghdi et al., 2016; Turkoglu et al., 2017), and have much functional activities (Jeong et al., 2009).

Zou et al. (2020) reported that the fig fruit polysaccharide components exhibited antioxidant and immunity enhancing activities. Fig polysaccharides can significantly improve the immune function of mice, and promote the recovery of immune function (Chen et al., 2015; Du et al., 2018; Yang et al., 2009). Du et al. (2018) found that a novel polysaccharide (FCPW80-2) isolated from fig had immunomodulatory activity. To our knowledge, there is no study on the antioxidant activity of FLPS. To further research polysaccharides, it is vital to optimize the process of FLPS and improve the extraction rate of FLPS. Ultrasonic-assisted extraction is one of the widely used methods in food processing (Lino et al., 2022; Scudino et al., 2020), and it is also used to extract active components (Guo et al., 2022; Meng et al., 2021; Song et al., 2020). To provide a reference for the use of FLPS, ultrasonic-assisted water extraction was used to extract polysaccharides from fig leaves. Based on the radical scavenging ability and ferric reducing activity, the antioxidation of FLPS in vitro was studied, which laid the foundation for the comprehensive utilization of fig leaves.

2 Materials and methods

2.1 Materials

Fig leaves were collected from Henan Institute of Science and Technology. 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH), 1,2,3-trihydroxy-benzene, and Potassium ferricyanide ($K_3[Fe(CN)_6]$) were purchased from Shanghai Aladdin Reagent Co., Ltd. Ferrous sulfate ($FeSO_4$), anthrone, sulfuric acid (> 95%), glucose, ethanol, ascorbic acid (vitamin C, Vc), and salicylic acid were purchased from Tianjin Komeo Chemical Reagent Co., Ltd. All the chemicals were the analytical grade.

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2.2 Single factor test

After being washed and dried, the fig leaves were powdered. The dry powder of fig leaves was accurately weighed and extracted by ultrasonic-assisted extraction at a specific solid to liquid ratio, extraction temperature, and extraction time. Then the mixtures were centrifuged at 5000 rpm for 15 min. The supernatant was concentrated on a rotary evaporator at 60 °C and was decolorized by activated carbon. The concentrated liquid was precipitated with two times the volume of anhydrous ethanol overnight at 4 °C. Then, the precipitate was obtained by centrifugation at 5000 rpm for 10 min. The FLPS were dried at 50 °C.

The standard curve was drawn by the anthrone sulfuric acid method. Under the condition of ice bath, 4 mL anthrone sulfuric acid solution was added to 1 mL the standard glucose solution with different concentrations (0.02, 0.03, 0.04, 0.05, 0.06, 0.07 mg/mL), respectively. The mixtures were heated in water (100 °C) for 10 minutes. Then, after the temperature decreased to 25 °C, the mixtures were placed for 5 minutes, and the absorbance was measured at 620 nm (Equation 1).

$$\text{Yield of FLPS}(\%) = \frac{c \times v \times n}{m} \times 100 \quad (1)$$

c: concentration of FLPS solution (g/mL); v: volume of FLPS solution (mL); n: dilution multiple; m: mass of dry powder of fig leaf (g).

The effects of extraction time (30, 60, 90, 120, 150, 180, 210 min), extraction temperature (50, 60, 70, 80, 90 °C), solid to liquid ratio (1:10, 1:20, 1:30, 1:40, 1:50, 1:60) and ultrasonic power (150, 200, 250, 300 W) on the extraction rate of polysaccharides from fig leaves were investigated.

2.3 Response surface methodology

According to the results of the single factor experiment and the design principle of the Box-Behnken experiment, the response surface experiment was designed, and the scheme is shown in Table 1 and Table 2.

2.4 Determination of DPPH· scavenging ability

According to the method of Wang et al. (2022), the ability to scavenge DPPH· was determined. Firstly, the sample solution of FLPS of 2 mg/mL was prepared and diluted. 0.4 mmol/L DPPH was added to 2 mL the diluted sample, and then incubated in water bath at 30 °C for 30 minutes. The absorbance (A_1) was measured at 517 nm. Under the same experimental conditions, 2 mL DPPH solution was replaced by 2 mL distilled water, and the absorbance (A_2) was measured at 517 nm. Under the same experimental conditions, 2 mL of distilled water was used instead of 2 mL of sample, and the blank absorbance was detected at 517 nm (A_0). At the same time, different concentrations of Vc were used instead of the sample as the positive control. The following formula is the formula for calculating the DPPH· scavenging activity (Equation 2).

$$DPPH\cdot \text{ scavenging activity } (\%) = [A_0 - (A_1 - A_2)] / A_0 \times 100 \quad (2)$$

2.5 Determination of ·OH scavenging ability

According to the method of Sun et al. (2010), the ·OH scavenging ability was measured. 0.274 g $FeSO_4$ was dissolved in 300 mL distilled water. 0.249 g salicylic acid was dissolved in 300 mL anhydrous ethanol. 0.126 mL of 30% H_2O_2 was added to 200 mL distilled water. 2 mL H_2O_2, 2 mL 5 salicylic acid and 2 mL $FeSO_4$ solution were added to the 2 mL of different concentrations of polysaccharides, and incubated at 37 °C for 35 minutes. The absorbance (A_1) at 510 nm was determined. Under the same experimental conditions, 2 mL H_2O_2 was replaced with 2 mL distilled water, and the absorbance (A_2) was measured at 510 nm. Under the same experimental conditions, the 2 mL of sample was replaced by 2 mL of distilled water, and the absorbance was deceted at 510 nm. Under the same experimental conditions, different concentrations of Vc were used instead of the sample as the positive control. The ·OH scavenging activity was calculated as the following formula (Equation 3).

$$OH \text{ scavenging activity}(\%) = [A_0 - (A_1 - A_2)] / A_0 \times 100 \quad (3)$$

2.6 Determination of $\cdot O_2^-$ scavenging ability

6 mL of 0.2 mol/L Tris-HCl buffer solution (PH 8.2) was added to 2 mL polysaccharides solution, respectively. The mixtures were incubated at 37 °C for 30 minutes, then 2 mL of 7 mmol/L 1,2,3-trihydroxy-benzene hydrochloric acid solution was added. After being incubated for 6 minutes, the absorbance (A_1) was measured at 325 nm. Under the same experimental conditions, 2 mL of distilled water replaced 2 mL of sample, and the absorbance (A_0) was measured under 325 nm. Under the same experimental conditions, different concentrations of Vc were used instead of the sample as the positive control. The $\cdot O_2^-$ scavenging activity was calculated as the following formula (Equation 4).

$$O_2^- \text{ scavenging activity}(\%) = (A_0 - A_1) / A_0 \times 100 \quad (4)$$

2.7 Determination of $ABTS^+\cdot$ scavenging ability

According to the method of Li et al. (2021), the ability to scavenge $ABTS^+\cdot$ of FLPS was detected. The 50 mL of 3.5 mmol/L ABTS

Table 1. Factor and level of response surface test.

Level	Factor			
	A	B	C	D
	Extraction time (min)	Extraction temperature (°C)	Solid to liquid ratio (g/mL)	Ultrasonic power (W)
-1	90	60	1:40	200
0	120	70	1:50	250
1	150	80	1:60	300

Table 2. Box-Behnken design and the yields of fig leaf polysaccharides.

Number	A	B	C	D	Yield of polysaccharides (%)
1	-1	-1	0	0	9.14
2	1	-1	0	0	9.59
3	-1	1	0	0	9.43
4	1	1	0	0	9.64
5	0	0	-1	-1	9.52
6	0	0	1	-1	9.24
7	0	0	-1	1	10.48
8	0	0	1	1	8.39
9	-1	0	0	-1	8.70
10	1	0	0	-1	9.86
11	-1	0	0	1	9.52
12	1	0	0	1	9.09
13	0	-1	-1	0	9.54
14	0	1	-1	0	10.57
15	0	-1	1	0	8.97
16	0	1	1	0	8.74
17	-1	0	-1	0	8.44
18	1	0	-1	0	9.08
19	-1	0	1	0	7.28
20	1	0	1	0	7.60
21	0	-1	0	-1	10.38
22	0	1	0	-1	10.56
23	0	-1	0	1	10.83
24	0	1	0	1	10.80
25	0	0	0	0	11.66
26	0	0	0	0	11.71
27	0	0	0	0	11.76
28	0	0	0	0	11.79
29	0	0	0	0	11.86

was mixed with the 50 mL of 1.225 mmol/L potassium persulfate, and stored at room temperature for 17 hours to get the $ABTS^{+}\cdot$ reserve solution. The $ABTS^{+}\cdot$ reserve solution was diluted until the absorbance was 0.692 at 734 nm, and the $ABTS^{+}\cdot$ determination solution was obtained. 2 mL of $ABTS^{+}\cdot$ determination solution was added to 2 mL of different concentrations of polysaccharides solution. The absorbance (A_1) was measured at 734 nm. Under the same experimental conditions, 2 mL phosphate buffer (pH 7.4, 10 mmol/L) was used instead of $ABTS^{+}\cdot$ solution, and the absorbance (A_2) at 734nm was measured. Under the same experimental conditions, 2 mL distilled water was used instead of polysaccharides, and the absorbance was deteced at 734 nm. At the same time, different concentrations of Vc were used instead of the sample as the positive control. The $ABTS^{+}\cdot$ scavenging activity was calculated as the following formula (Equation 5).

$$ABTS^{+}\cdot \ scavenging \ activity(\%) = [A_0 - (A_1 - A_2)] / A_0 \times 100 \quad (5)$$

2.8 Determination of ferric reducing activity

According to the method of Kan et al. (2015), the ferric reducing activity of FLPS was measured. The was absorbed, and 2 mL of phosphate buffer (0.2 mol/L, PH 6.6) and 2 mL of $K_3[Fe(CN)_6]$ were added to 2 mL of different concentrations of polysaccharides, then incubated at 50 °C for 25 min. 2 mL of trichloroacetic acid was added to the mixtures, then centrifuged at 3000 rpm/min for 10 min. 0.4 mL of ferric chloride and 2 mL of distilled water were added to 2 mL of the supernatant, and incubated for 15 min, and the absorbance was detected at 700 nm. At the same time, different concentrations of Vc were used instead of the sample as the positive control.

2.9 Statistical analysis

All experiments were performed with three biological replicates, and the data were shown as mean ± standard deviation. One-way analysis of variance (ANOVA) was used to compare the differences between the treatments. Statistical analysis was obtained by SPSS 26.0 (SPSS Inc., Chicago, IL, USA).

3 Results and discussion

3.1 The results of single factor test

Standard curve

The concentration of FLPS in the crude extract of fig leaves was determined by the standard curve. As shown in Figure 1A,

the standard curve formula was Y = 10.494 * X - 0.0174 with a coefficient of determination (R^2) of 0.9991, indicating that X had a good linear relationship with Y in the range of 0.02-0.07 mg/mL.

Effect of extraction time on the yield of FLPS

The extraction time had a significant influence on the yield of FLPS. As shown in Figure 1B, the extraction times were from 30 to 120 min, the yield of FLPS increased with the extraction time, and the highest yield was 10.99% at 120 min. The yield of polysaccharides decreased with the extraction time from 120 to 210 min. The reason may be that the longer the extraction time, the polysaccharides will dissolve more fully. Still, the long extraction time may induce the degradation of the polysaccharides by oxidation or degradation (Chen & Xue, 2019). So, the yield of FLPS decreased. Therefore, 120 min was selected as the optimum extraction time.

Effect of extraction temperature on the yield of FLPS

As shown in Figure 1C, the extraction temperature significantly affected the yield of FLPS. With the extraction temperatures in the range of 50 to 70 °C, the yield of FLPS increased as the temperature rose. The largest yield of FLPS was 10.21% at 70 °C. With the extraction temperatures in the range of 70 to 90 °C, the yield of polysaccharides decreased with rising temperature. The reason may be that the high temperature will reduce the stability of the polysaccharides, and the heating will destroy its structure, so the yield of polysaccharides decreased (Wu et al., 2020). The optimum extraction temperature was 70 °C.

Effect of solid to liquid ratio on the yield of FLPS

As shown in Figure 1D, the solid to liquid ratio significantly affected the yield of FLPS. The FLPS yield ratio increased significantly with the increase of solvent water consumption, and there was no significant difference between the yield at 1:50 and the yield at 1:60 ($P > 0.05$). The reason may be that FLPS were easily soluble in water, and the FLPS yield increased with the increase of water consumption. At the same time, when other impurities dissolve, the existence of impurities would occupy the polysaccharides' dissolution space. It was also recognized that higher consumption of water might improve the reaction between the FLPS and water, inducing the degradation of FLPS (Cho et al., 2019). And the high consumption of water would increase the generation of wastewater in the extraction process, and increase the extraction cost. Hence, the optimum solid to liquid ratio was 1:50.

Effect of ultrasonic power on the yield of FLPS

As shown in Figure 1E, with the ultrasonic power in the range of 150 to 250 W, the yield of FLPS increased with the increasing ultrasonic power, and the highest yield was 5.05% at 250 W. Compared with the yield of polysaccharides at 250 W, the yield decreased at 300 W. This excellent extraction efficiency by ultrasonic-assisted extraction is mainly attributed to its mechanical effects, which greatly facilitate mass transfer between immiscible phases (Zhong & Wang, 2010). But when the ultrasonic power is too high, it would produce high temperature and high pressure, and the polysaccharides molecule would be decomposed. Then, the yield of polysaccharides would be reduced. Therefore, 250 W was selected as the optimum ultrasonic power.

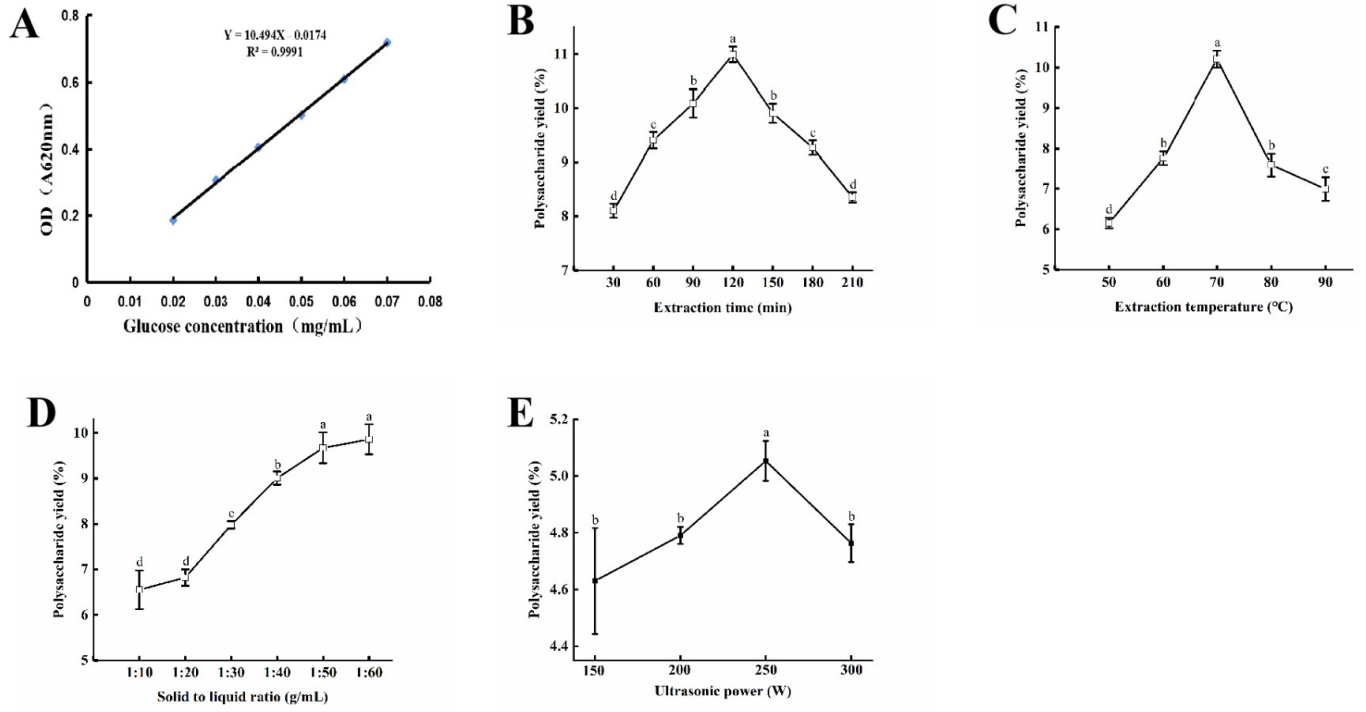

Figure 1. Standard curve (A). Effect of extraction time (B), extraction temperature (C), solid to liquid ratio (D), and ultrasonic power (E) on the yield of FLPS. The different lowercase letters mean the statistical difference in the same picture ($P < 0.05$).

3.2 The results of response surface methodology

Prediction model and statistical analysis

The data as described in Table 2, were analyzed with the Design-Expert 10.0.7 software. The regression equation was shown as below (Equation 6). The yield of

$$FLPS = 11.76 + 0.20 * A + 0.11 * B - 0.62 * C + 0.073 * D - 0.058 * AB - 0.082 * AC - 0.40 * AD - 0.31 * BC - 0.052 * BD - 0.45 * CD - 1.85 * A^2 - 0.50 * B^2 - 1.79 * C^2 - 0.6 * D^2 \quad (6)$$

Table 3 showed the ANOVA results. The *P* value (< 0.0001) of the model indicated that the model was significant. The *P* value of the lack of fit was 0.16 > 0.05, which suggested that the model had good reliability. The R^2 was 0.9958, which indicated that there was a high degree of correlation between the experimental yields and the predicted yields (Wu et al., 2020). Therefore, the model can be used to predict the optimum parameters of the FLPS extraction.

Analysis of response surface

The response surface plot is the imagic display of the regression equation (Feng et al., 2022). As shown in Figure 2, the more significant the interaction between the factors, the greater the slope of the response surface. The corresponding contour plot can also reflect the interaction between the factors, the ellipse represents a significant interaction, and the circle indicates a relatively weak interaction. Therefore, we could learn that the interactions between extraction time and ultrasonic power, extraction temperature and solid to liquid ratio, and solid to liquid ratio and ultrasonic power were significant, and the interactions between extraction time and extraction temperature, extraction time and solid to liquid ratio, and extraction temperature and ultrasonic power were not significant, which was consistent with the analysis results in Table 3.

Optimization of parameters and verification of the model

According to the regression model, the optimal parameters were determined: extraction time of 121.27 min, extraction temperature of 71.64 °C, solid to liquid ratio of 1:47.97 g/mL, and ultrasonic power of 255.79 W, and the predicted maximum yield of polysaccharides was 11.86%. Considering the actual situation, the parameters were adjusted as extraction time of 121 min, extraction temperature of 72 °C, solid to liquid ratio of 1:48 g/mL, and ultrasonic power of 250 W. The mean value of 11.78% was gained, which was in agreement with the predicted value. The above results confirmed that the regression model was competent for reflecting the expected optimization, and the model was satisfactory and precise.

3.3 Antioxidant activity of FLPS

DPPH· scavenging ability

The DPPH· scavenging activity of FLPS was shown in Figure 3A. The alcohol solution of DPPH is dark purple with a maximum absorption peak at 517 nm, and the DPPH· scavenger would scavenge the DPPH· and reduce the absorbance of the solution (Sah et al., 2015). As shown in Figure 3A, the DPPH· scavenging activity increased with the concentration of FLPS. When the concentration of FLPS was 2 mg/mL, the DPPH· scavenging ability reached 84.78%. Vc was used as the positive control, and the DPPH· scavenging ability was between 94.44-95.52%, without significant differences between the different concentrations.

Table 3. ANOVA for the response surface quadratic model.

Source	Sum of squares	DF	Mean Square	F Value	*P* Value	Significance
Model	43.29	14	3.09	238.11	< 0.0001	**
A	0.46	1	0.46	35.32	< 0.0001	**
B	0.14	1	0.14	10.59	0.0058	**
C	4.60	1	4.60	354.20	< 0.0001	**
D	0.064	1	0.064	4.90	0.0440	*
AB	0.013	1	0.013	1.02	0.3294	
AC	0.027	1	0.027	2.06	0.1731	
AD	0.63	1	0.63	48.57	< 0.0001	**
BC	0.40	1	0.40	30.46	< 0.0001	**
BD	0.011	1	0.011	0.82	0.3803	
CD	0.82	1	0.82	62.89	< 0.0001	**
A^2	22.17	1	22.17	1707.26	< 0.0001	**
B^2	1.59	1	1.59	122.46	< 0.0001	**
C^2	20.73	1	20.73	1596.54	< 0.0001	**
D^2	2.32	1	2.32	178.50	< 0.0001	**
Residual	0.18	14	0.013			
Lack of fit	0.16	10	0.016	2.68	0.1776	
Pure error	0.024	4	5.909E-003			
Cor Total	43.47	28				
	R^2 = 0.9958	R^2_{Adj} = 0.9916				

DF: Degree of Freedom; R^2: determination coefficient; R^2_{Adj}: adjusted R^2. **The difference is extremely significant $P < 0.01$; *The difference was significant $0.01 < P < 0.05$.

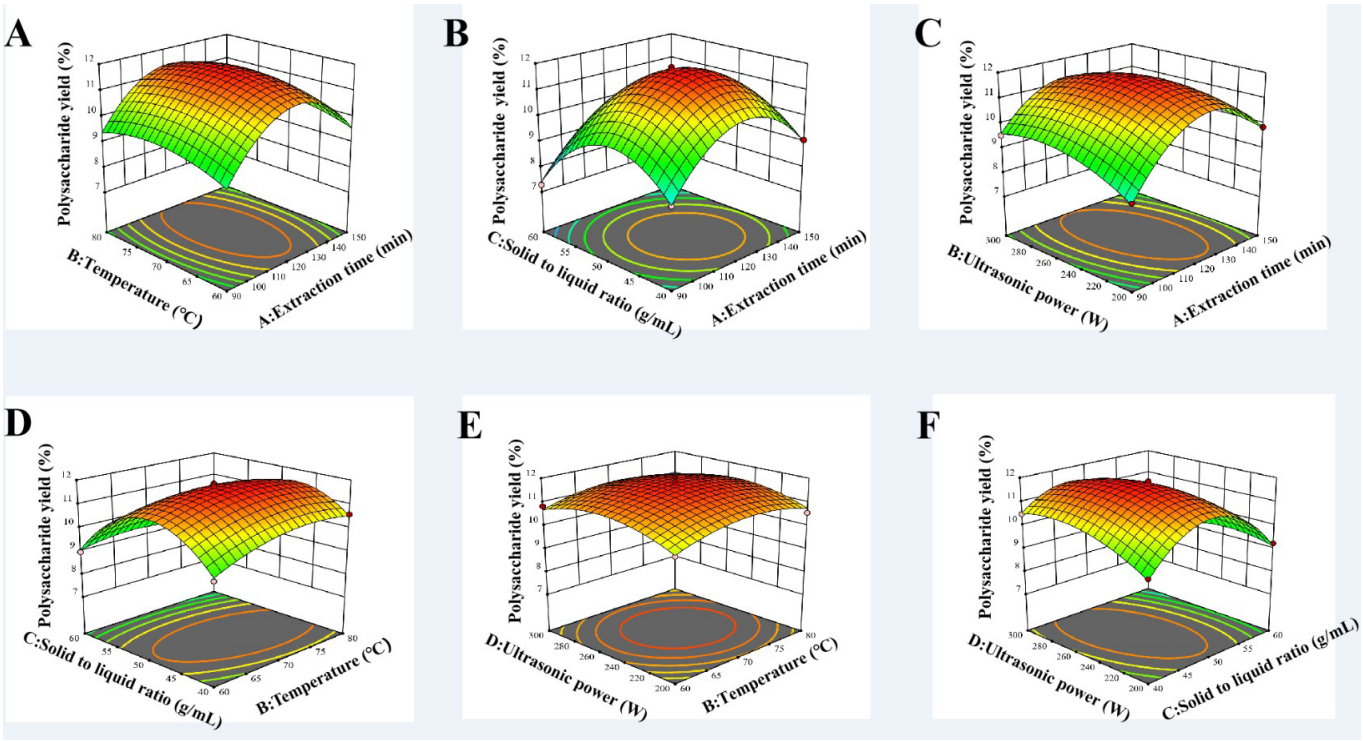

Figure 2. Response surface plots for FLPS yield. (A) extraction time and temperature; (B) extraction time and solid to liquid ratio; (C) extraction time and ultrasonic power; (D) temperature and solid to liquid ratio; (E) temperature and ultrasonic power; (F): solid to liquid ratio and ultrasonic power.

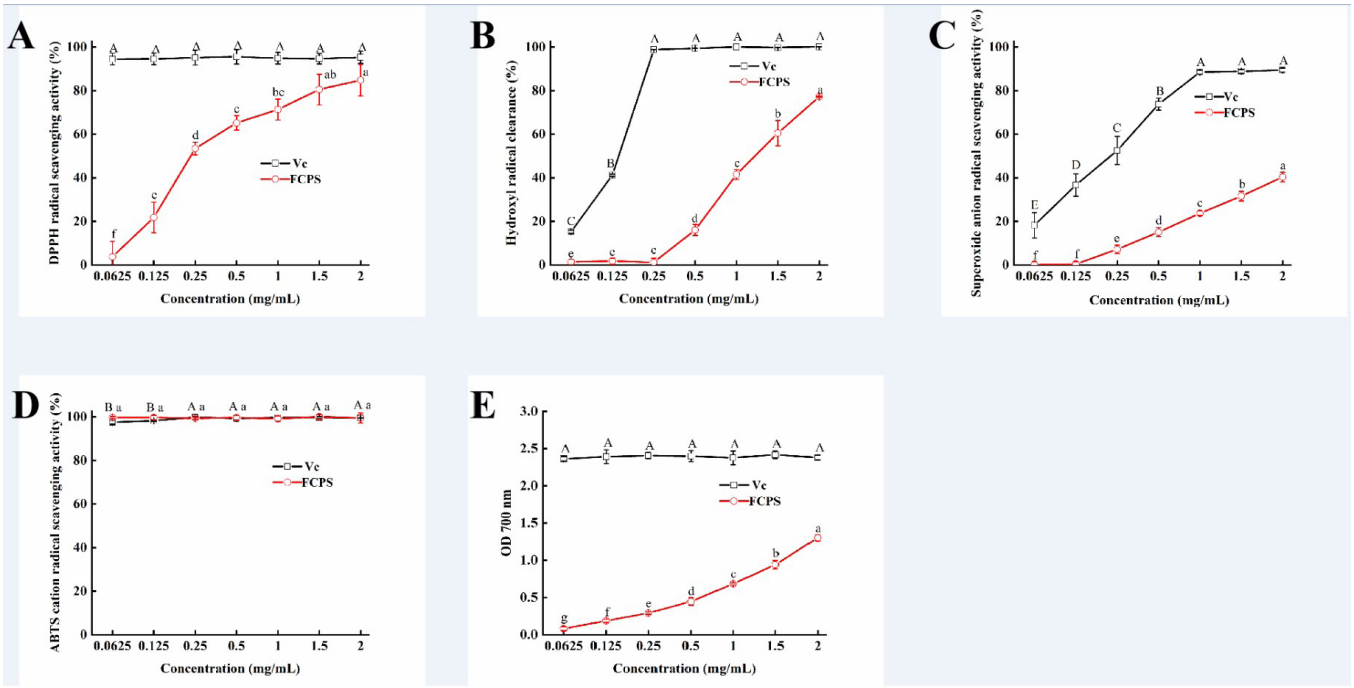

Figure 3. Antioxidant activity of FLPS. (A): DPPH radical scavenging activity, (B): Hydroxyl radical scavenging ability, (C): Superoxide anion radical scavenging activity, (D): ABTS cation radical scavenging activity, (E): Ferric reducing activity. The different lowercase letters mean the statistical difference in the activity of FLPS in the same picture ($P < 0.05$). The different uppercase letters mean the statistical difference in the activity of Vc in the same picture ($P < 0.05$).

·OH scavenging ability

The ·OH scavenging activity of FLPS was shown in Figure 3B. We could learn that the ·OH scavenging activity of FLPS increased with the increase of the FLPS concentration. When the concentration of FLPS was 2 mg/mL, the ·OH scavenging ability reached 77.21%. With the concentration of Vc in the range of 0.0625 mg/mL to 0.25 mg/mL, the ·OH scavenging activity was significantly enhanced with the increasing concentration. With the concentration of Vc in the range of 0.25 mg/mL to 2.5 mg/mL, there was no significant change, and the ·OH scavenging activity reached 100%.

$\cdot O_2^-$ scavenging ability

The $\cdot O_2^-$ scavenging activity of FLPS was shown in Figure 3C. With the concentration of FLPS in the range of 0.0625 mg/mL to 2 mg/mL, the $\cdot O_2^-$ scavenging activity increased with the increase of the concentration, and the $\cdot O_2^-$ scavenging activity was up to 40.32% at the concentration of 2 mg/mL. The results showed that FLPS had $\cdot O_2^-$ scavenging effect, but the scavenging activity was weaker than that of Vc.

$ABTS^{+}\cdot$ scavenging ability

The $ABTS^{+}\cdot$ scavenging ability of FLPS was shown in Figure 3D. The FLPS and Vc had remarkable $ABTS^{+}\cdot$ scavenging ability. The $ABTS^{+}\cdot$ scavenging ability of FLPS at 0.0625 mg/mL and 0.125 mg/mL were higher than that of Vc, and the $ABTS^{+}\cdot$ scavenging ability of FLPS was above 99% at 0.0625 mg/mL and 0.125 mg/mL.

Ferric reducing activity

The results are shown in Figure 3E. The increased absorbance indicated an increase in the ferric reducing activity (Zhang et al., 2015). As shown in Figure 3E, the OD 700 nm increased significantly with the concentration of FLPS, and OD 700 nm reached 1.30 at 2 mg/mL of the FLPS. With Vc in the range of 0.0625 mg/mL to 2 mg/mL, OD 700 nm was above 2.36 without a significant difference. The FLPS had a weaker ferric reducing power compared with Vc.

4 Conclusion

The optimal parameters were determined, and the optimal yield of FLPS was 11.78%. Further, the antioxidant activity of FLPS was studied, and FLPS were found to partly have the ability to scavenge DPPH·, ·OH, $\cdot O_2^-$, and $ABTS^{+}\cdot$ and to reduce Fe^{3+}. Therefore, we can conclude that FLPS have good antioxidant activity. These results suggest that the FLPS have potential application as antioxidants in food.

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