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Research Article

Ultrasonic Microwave Assisted Extraction, Chemical Characterization and Antioxidant Activity of Polysaccharides from Fig Leaves

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Abstract: This study employed ultrasonic-microwave-assisted extraction (UMAE) to isolate water-soluble polysaccharides from fig (*Ficus carica* L.) leaves (FLPs). Polysaccharide yield was used as the primary index to evaluate extraction efficiency. The effects of material-liquid ratio, microwave power, extraction temperature, and extraction time on polysaccharide yield were systematically investigated through single-factor experiments, followed by response surface methodology optimization. Structural characterization and antioxidant activity of FLPs were subsequently evaluated. The optimal UMAE conditions were determined as: material-liquid ratio 1:51 (g/mL), microwave power 714 W combined with ultrasonic power 50 W, extraction temperature 63°C, and extraction time 18 min. Under these conditions, FLPs exhibited a molecular weight of 2.56×10^5 Da and consisted of rhamnose, arabinose, xylose, glucose, and galactose in a molar ratio of 9.97:7.86:0.78:25.61:5.05, respectively. FLPs demonstrated potent antioxidant activity: at 0.6 mg/mL concentration, the scavenging rates for superoxide anion, DPPH, and hydroxyl radicals were 73.21±1.34%, 76.77±1.31%, and 61.90±1.22%, respectively. These findings indicate that FLPs possess significant potential as a natural antioxidant and functional food ingredient. The optimized UMAE method provides an efficient approach for extracting bioactive polysaccharides from fig leaves, supporting their application in the food industry.

Keywords: Fig Leaves, Polysaccharides, Ultrasonic-Microwave Assisted Extraction, Response Surface Methodology, Antioxidant Activity, Structural Characterization, Natural Antioxidants, Functional Food

Introduction

In recent years, research on natural bioactive compounds has gained significant attention, particularly regarding their biological activities. Green coffee bean extract (GCBE) demonstrates multiple beneficial effects including anti-hyperglycemic activity through α-amylase inhibition and anti-hyperlipidemic properties via interference with lipid digestion and absorption. Furthermore, GCBE exhibits potent antioxidant capacity and demonstrates anti-inflammatory effects (Duangjai *et al*., 2024). Candlenut (*Aleurites moluccanus*) is a plant containing various bioactive compounds. The substances extracted from candlenut leaves with ethanol possess significant antioxidant and anti-inflammatory properties and have the potential to inhibit tumor necrosis factor-alpha (Zahara *et al*., 2024). Chitosan as a biodegradable polysaccharide exhibits enhanced adsorption properties due to its unique molecular structure. Furthermore, the antibacterial activity of chitosan is mainly due to the primary and secondary hydroxyl groups in its chemical configuration (Fatimah *et al*., 2024). Polysaccharides extracted from dandelion leaves have a protective effect on immune deficiency and oxidative stress,which can be a promising candidate as natural feed additives for chickens(Qiao *et al*., 2024).

Ficus carica L. is a deciduous plant belonging to the family Moraceae and Ficus, which thrives in tropical and temperate regions in most regions of China (Yang *et al*., 2023). Fig (*Ficus carica*) fruits tastes sweet with high nutritional and medicinal value. While extensive research has focused on fig fruits, particularly regarding processing

methods, bioactive compound extraction and functional applications, the potential benefits of fig leaves remain largely unexplored despite being frequently discarded as agricultural byproducts. In China, fig leaves are often used to treat diseases such as hemorrhoids, damp heat and diarrhea. Fig leaves contain various of beneficial components, including polysaccharides, flavonoids, volatile oils, vitamins, polyphenols, coumarins and so on (Alalwani *et al*., 2022; Wang *et al*., 2022). The pigments extracted from fig leaves could be used for dyeing cotton fabrics. The alcohol extract of fig leaves could be applied to the preservation of food (Yilmaz *et al*., 2022; Mouzahim *et al*., 2023). The volatile oil extracted from fig leaves exhibits strong insecticidal activity against nematodes and aphids (Li *et al*., 2021; Zhang *et al*.,2022; Gao *et al*.,2023). Therefore, the comprehensive utilization of fig leaves is attracting people's attention.

The extraction of polysaccharides is of vital importance for its practical application and research. In recent years, this has spurred extensive scientific investigations into extraction techniques for polysaccharides derived from a wide variety of plant and fungal sources. Many methods including hot water, ultrasound (Lin *et al*., 2018), enzyme (Wu *et al*., 2019; Li *et al*., 2016) and microwave (Chen *et al*., 2017) were used to extract polysaccharides from plants. Conventional hot-water extraction remains a classical method for polysaccharide isolation. However, this technique is characterized by notable limitations, including prolonged extraction durations and elevated temperature requirements, which may compromise thermolabile components (Chen *et al*., 2010). In recent years, microwave and ultrasonic technologies have been effectively used to extract the active ingredients of plants. Ultrasonic-wave has high penetration power causing cell fragmentation and can accelerate the rapid outflow of active ingredients, thereby shortening extraction time and improving extraction efficiency (Cui *et al*., 2018; Ma *et al*., 2017). Microwave generates electromagnetic radiation which can cause cell division, heat up the solvent rapidly and increase the dissolution rate of active ingredients (Shen *et al*., 2022). Consequently, the integration of ultrasonic and microwave-assisted extraction techniques offers a complementary approach that combines their respective advantages while mitigating individual limitations. Polysaccharides from fig leaves (FLPs) have many functions such as clearing free radicals, enhancing immunity, inhibiting bacteria and anti-inflammatory, antiviral, anti-tumor, sedative and hypnotic (Yang *et al*., 2009; Du *et al*., 2018; Zou *et al*., 2020). Ultrasonic-assisted extraction process of FLPs and its antioxidant activity were investigated (Wang *et al*. 2022). However, there is no report about ultrasonic-microwave assisted extraction (UMAE) of FLPs.

This study aims to develop an innovative FLPs extraction technology. The response surface methodology (RSM) was adopted to optimize the UMAE parameters to maximize the FLPs yield. Furthermore, the chemical characterization and antioxidant activity of FLPs were also evaluated. The results of this study will provide a scientific basis for the application of FLPs in the food industry.

Materials and Methods

Materials, Chemicals and Equipments

Fig leaves were provided Wuxi Lvyang Farm, China. DPPH was bought from Sigma Aldrich. Absolute ethanol, phenol, concentrated sulfuric acid, acetone, ether, salicylic acid, sucrose, hydrogen peroxide (30%) . All other chemicals are of analytical grade. The extraction of FLPs was performed using an ultrasonic-microwave synergistic extraction equipment (CW-2000). The maximum microwave power of this instrument is 800W with a frequency of 2450MHz. The fixed power of the ultrasonic transducer is 50W with a frequency of 40kHz.

Extraction of FLPs

The fresh fig leaves were thoroughly washed and subsequently dried at 60℃.The dried leaves were subsequently ground into a fine powder.The powder(2g) was homogenized with distilled water and extracted by an ultrasonic-microwave synergistic extraction equipment (CW-2000).Proteins were removed from the crude polysaccharides using the Sevage reagent (n-butanol:chloroform = 1:4, v/v). Then, the aqueous polysaccharides were precipitated with absolute ethanol at 4℃ for 24h.The obtained precipitates were washed successively with anhydrous ether and then freeze-dried to obtain FLPs (Fu *et al*., 2019; Xu *et al*., 2020).

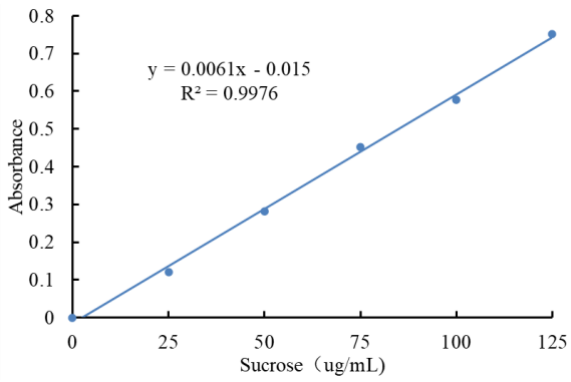


Fig. 1: The standard calibration curve for glucose

Quantification of FLPs Content

The polysaccharide content was determined spectrophotometrically using the phenol-sulfuric acid assay. The standard curve was presented in Fig. 1.

The yield of FLPs (Y) was calculated using the following Equation (1):

$$Y(\%) = \frac{m \times n}{m_0} \times 100\% \quad (1)$$

Where m is polysaccharide content calculated according to standard curve; n is dilution ratio; m_0 is weight of fig leaves powder (2g).

Single-Factor Experiments

The ultrasonic-microwave synergistic extraction system integrates both energy modalities simultaneously, with ultrasound generating cavitation effects and microwave inducing dielectric heating. This dual-mechanism approach significantly enhances extraction efficiency through complementary physical and thermal effects. In this study, we systematically evaluated the extraction efficiency of FLPs within the microwave power range under the condition of a constant ultrasonic frequency of 40 kHz and an ultrasonic power of 50W.

Effect of Extraction Time on the Yield of FLPs

According to the above extraction method, FLPs was extracted with distilled water as the solvent. Microwave power was 700W. Material-liquid ratio was 1:40 (g/mL) and extraction temperature was 50℃.To determine the optimal extraction time, we performed a time-course analysis evaluating FLPs yield across sequential time intervals (5, 10, 15, 20 and 25min). This systematic temporal assessment enabled precise characterization of extraction efficiency.

Effect of Extraction Temperature on the Yield of FLPs

FLPs was extracted using distilled water as the solvent under the conditions of microwave power of 700 W, extraction time of 10min and material-liquid ratio of 1:40 (g/mL). In order to systematically evaluate the influence of extraction temperature on the yield of FLPs, FLPs were extracted at 30, 40, 50, 60 and 70℃, respectively. Based on the effect of extraction time on the yield of *FLPs*, the optimal extraction time was determined.

Effect of Material-Liquid Ratio on the Yield of FLPs

FLPs was extracted using distilled water as the solvent under the conditions of microwave power of 700 W, extraction time of 10min and temperature of 50℃. In order to examine the influence of material-liquid ratio on the yield of FLPs, a series of material-liquid ratios (1:20, 1:30, 1:40, 1:50 and 1:60g/mL) were systematically evaluated. This systematic study establishes critical solvent usage, identifying the ideal material-to-liquid ratio that maximizes FLP yield while maintaining extraction efficiency.

Effect of Microwave Power on the Yield of FLPs

FLPs was extracted using distilled water as the solvent under the conditions of extraction time of 10min, temperature of 50℃and material-liquid ratio of 1:40 (g/mL). To investigate the influence of microwave power on the yield of FLPs, a power gradient study was conducted at 500, 600, 700, 800, and 900 W, respectively. This systematic evaluation of microwave power parameters provided essential insights into the energy-dependent extraction kinetics of FLPs.

Experimental Design of RSM

The combined effect of the four independent variables was investigated by using the four-factor, three-level Box-Behnken Design (BBD) system. The design Expert software (Trial version 8.0, Stat-Ease Inc., Minneapolis, MN, USA) is used for experimental design and subsequent statistical analysis. The independent variables including extraction time, temperature, material-liquid ratio and microwave power were designated as A, B, C, and D, respectively.

The experimental factors were evaluated at three coded levels: +1, 0, and -1. The specific values corresponding to these coded levels for each independent variable were presented in Table 1.

Chemical Composition Analysis and Determination of Molecular Weight of FLPs

The quantitative analysis of FLPs components was performed using established spectrophotometric methods. D-glucose was used as the standard and the phenol-sulfuric acid method was used to determine the total sugar content (Lin and Pomeranz, 1968). The protein was determined using the Bradford method (Bradford, 1976), with bovine serum albumin (BSA) as the standard. For uronic acid determination, the m-hydroxybiphenyl colorimetric method (Blumenkrantz and Gasboe, 1973) was employed with absorbance measurements taken at 525 nm with galacturonic acid as the calibration standard.

The analysis of monosaccharide composition was performed by a gas chromatograph (GC-14A, Shimadzu, Japan). The analytical procedure involved sequential

chemical derivatization steps. Initially, FLPs were hydrolyzed using trifluoroacetic acid (2mol/L) at 121℃for 1h in sealed tubes. The resulting monosaccharides were subsequently reduced to their corresponding alditols through reaction with hydroxylamine hydrochloride in pyridine (90℃, 30 min). The alditols were then acetylated using acetic anhydride at 90℃ for 30 min to form volatile alditol acetates. Chromatographic separation was carried out with an SPB-5 capillary column with the following temperature program. The initial temperature was 150℃. It increased to 190℃ at a rate of 10℃/min (maintained for 0.1min), and then increased to 240℃ at a rate of 2℃/min (maintained for 5 min). Quantification was performed using inositol as an internal standard and authentic monosaccharide standards. The relative percentage of each monosaccharide was determined based on peak area normalization using established response factors.

Table 1: Experimental design of independent variables and levels

Levels	Independent variable			
	A: Extraction time (min)	*B*: Extraction tempreture (℃)	*C*: Material-liquid ratio(g/mL)	*D*:Microwave power (W)
-1	10	50	1:40	600
0	15	60	1:50	700
1	20	70	1:60	800

High performance liquid chromatography (HPLC) coupled with an Evaporative Light Scattering Detector (ELSD) was used to determine the molecular weight distribution of FLPs. The analysis was performed on a ReproSil 200 SEC column (300×8 mm) following established protocols. A series of dextran standards with known molecular weights was used for molecular weight calibration.

Fourier Transform Infrared Spectroscopic Analysis of FLPs

The functional group analysis of FLPs was conducted using a Nicolet Nexus FT-IR spectrometer (Thermo Nicolet Nexus, USA). Spectroscopic-grade potassium bromide (KBr) powder was thoroughly homogenized with 1mg FLPs at a ratio of 1:100, and then pressed into transparent pellets. FT-IR spectra was acquired in transmission mode across the spectral range of 4000 to 400cm^{-1}.

Assay for Antioxidant Activities of FLPs

Analysis of the Scavenging Activity of Hydroxyl Radicals

The scavenging activity of hydroxyl radicals was analyzed based on the reported methods (Song *et al*., 2013). The reaction system consisted of 2 mL hydrogen peroxide solution (8.8 mmol/L), 2 mL salicylic acid-ethanol solution (9 mmol/L), and 2 mL ferrous sulfate solution (9 mmol/L), which were subsequently mixed with 2mL FLPs solutions of different concentrations (0.1, 0.2, 0.3, 0.4, 0.5 and 0.6 mg/mL).The reaction mixtures were incubated at 37℃for 60 min in a temperature-controlled water bath, followed by absorbance measurement at 510 nm (A_1) using a UV-Vis spectrophotometer(UV754N,Shenbei Scientific Instruments (Suzhou) Co., LTD, China). For background correction, an identical reaction mixture was prepared by replacing the salicylic acid-ethanol solution with distilled water, and the corresponding absorbance (A_2) was recorded. Additionally, a blank control was established using distilled water and its absorbance (A_0) was measured. The hydroxyl radical scavenging rate (RSA) was calculated by Equation (2) below:

$$\text{Hydroxyl RSA (\%)} = \left(1 - \frac{A_1 - A_2}{A_0}\right) \times 100\% \quad (2)$$

Analysis of the Scavenging Activity of DPPH Radicals

The scavenging activity of DPPH radicals was analyzed based on the method described by Li and Shah (Li *et al*., 2013). Briefly, 1 mL DPPH solution (0.35 mg/mL) was mixed with 1mL FLPs solution of different concentrations (0.1, 0.2, 0.3, 0.4, 0.5 and 0.6 mg/mL) respectively. The reaction mixtures were incubated in darkness for 30 min at room temperature, followed by absorbance measurement at 517 nm (A_1) using a UV-Vis spectrophotometer (UV754N, Shenbei Scientific Instruments (Suzhou) Co., LTD, China). A blank control was prepared by replacing the DPPH solution with ethanol and its absorbance (A_2) was recorded. Additionally, a negative control was established using distilled water instead of FLPs solution, and the corresponding absorbance (A_0) was determined. The DPPH radical scavenging rate (RSA) was calculated by Equation (3) below:

$$\text{DPPH RSA (\%)} = \left(1 - \frac{A_1 - A_2}{A_0}\right) \times 100\% \quad (3)$$

Analysis of the Scavenging Activity of Superoxide Anion Radicals

The scavenging activity of superoxide anion radicals was analyzed based on the pyrogallol autoxidation method (Li, 2012). The reaction system consisted of

4.5mL Tris-HCl buffer (50mmol/L, pH 8.2) mixed with 2mL FLPs solution of different concentrations (0.1, 0.2, 0.3, 0.4, 0.5 and 0.6 mg/mL) respectively. The reaction was initiated by adding 0.4mL pyrogallol solution (25mmol/L), followed by incubation at 25℃for 5min. The absorbance measurement at 325nm (A_1) using a UV-Vis spectrophotometer (UV754N, Shenbei Scientific Instruments (Suzhou) Co., LTD, China) was performed. A blank control was prepared using distilled water instead of polysaccharide solution and its absorbance (A_0) was recorded. The superoxide anion radical scavenging rate (RSA) was calculated by Equation (4) below:

$$\text{Superoxide anion RSA (\%)} = \left(1 - \frac{A_1}{A_0}\right) \times 100\% \quad (4)$$

Statistical Analysis

All experimental measurements were conducted three times.The results were expressed as mean ± Standard Deviation (SD). Statistical analysis was performed using SAS software (Version 8.1, SAS Institute Inc.). The differences between sample groups were analyzed by one-way analysis of variance.The difference was statistically significant at $p<0.05$.

Results and Discussion

Single-Factor Experiments

In order to optimize the extraction parameters for FLPs, a comprehensive investigation was conducted using FLP yield (Y) as the primary response variable. The study systematically examined four critical factors including extraction time, extraction temperature, material-liquid ratio and microwave power to determine their individual and interactive effects on extraction efficiency.

Effect of extraction time on the yield of FLPs was presented in Fig. 2

(A). Within the first 10min, the yield of FLPs increased rapidly and reached 12.4±0.12%. After 10 min, the yield of FLPs began to increase gradually. As illustrated in Fig 2(A), the optimal extraction time was 10min. Effect of temperature on the yield of FLPs was illustrated in Fig. 2(B). Temperature had obvious effect on the yield of FLPs. The yield of FLPs increased from 30 to 60℃, reaching 13.8±0.16% at 60℃. When the extraction temperature was 70℃, the yield of FLPs decreased slowly. Effect of material-liquid ratio on the yield of FLPs was presented in Fig. 2(C). The optimal material-liquid ratio was 1:50(g/mL). At this optimal ratio, the yield of FLPs reached 13.2±0.15%. Effect of microwave power on the yield of FLPs was presented in Fig. 2(D). The yield demonstrated a positive correlation with microwave power, reaching its maximum value of 13.5±0.15% at 700W, beyond which no significant improvement in extraction efficiency was observed.

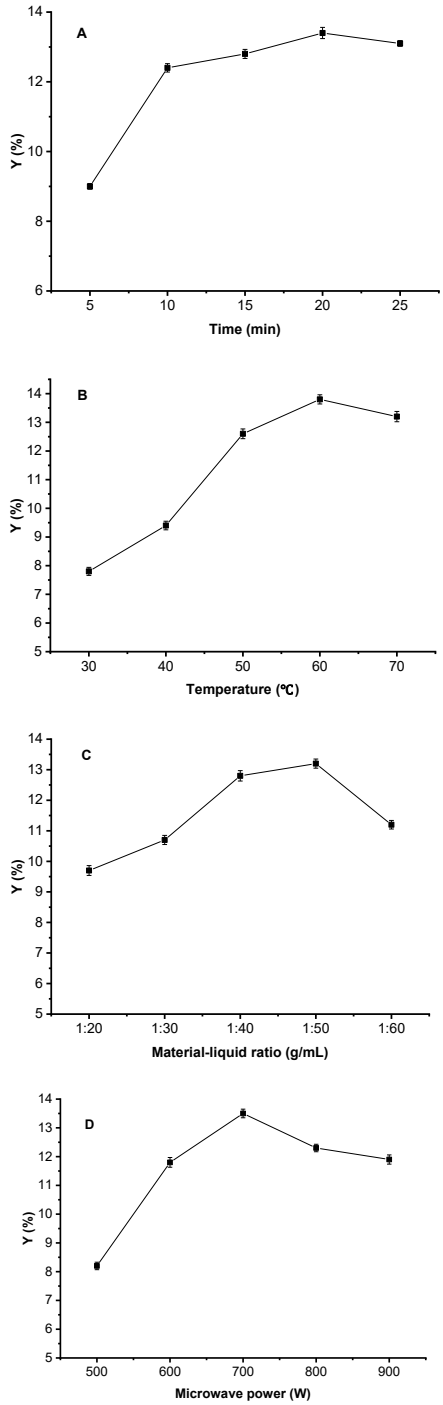


Fig. 2: Effect of extraction time (A), temperature (B), material-liquid ratio (C) and microwave power (D) on the yield of FLPs. Values were presented as means ±SD (n=3).

Box-Behnken Design and Analysis

Based on the results of single-factor experiments, the extraction conditions for FLPs were further optimized through RSM. Specifically, four parameters was optimized through 29 experimental runs using a Box-Behnken design (BBD). The results were presented in Table 2. With the help of multiple regression analysis, the relationship between the response value and each factor was established as a second-order polynomial equation:

$$Y(\%)=14.32+0.57\times A+0.86\times B+0.049\times C+0.46D+0.015\times A\times B+0.093\times A\times C-0.56\times A\times D+0.16\times B\times C+0.29\times B\times D-0.073\times C\times D-0.51\times A^2-1.70\times B^2-0.73\times C^2-0.83\ D^2$$

The results of the model statistical analysis were presented in Table 3. The regression model demonstrated excellent predictive capability with a coefficient of determination (R^2) of 0.9679, indicating that 96.79% of the total variation was attributed to the independent variables. The model showed highly significant predictive capability ($F = 17.14$, $p<0.0001$), confirming that the regression explained substantially more variance than would be expected by chance alone. The non-significant lack-of-fit test ($F= 2.24$) suggested the model adequately described the experimental data without systematic bias, as the residual variance was comparable to pure experimental error. According to the model, the terms A, B, D, B^2, C^2 and D^2 were highly significant ($P<0.001$), while the interaction term AD and the quadratic term A^2 were significant ($P<0.05$).

Optimization of Extraction Process of FLPs

To comprehensively evaluate the interaction effects between experimental factors and identify optimal extraction conditions for FLPs, the generation of three-dimensional response surface plots and corresponding contour plots was carried out using the Design-Expert software (version 8.0). The 3D response surfaces illustrating the interactive relationships between variable pairs were presented in Fig. 3 (A-F). The corresponding two-dimensional contour plots which facilitate the identification of optimal regions were shown in Fig. 3(a-f). These graphical representations were derived from the regression model and provide valuable insights into factor interactions and their combined effects on FLP yield. The 3D response surfaces and contour plots revealed that the interactions between extraction temperature and time, as well as between microwave power and extraction time were significant, indicating that extraction temperature, extraction time, and microwave power had substantial impacts on the extraction rate of FLPs.

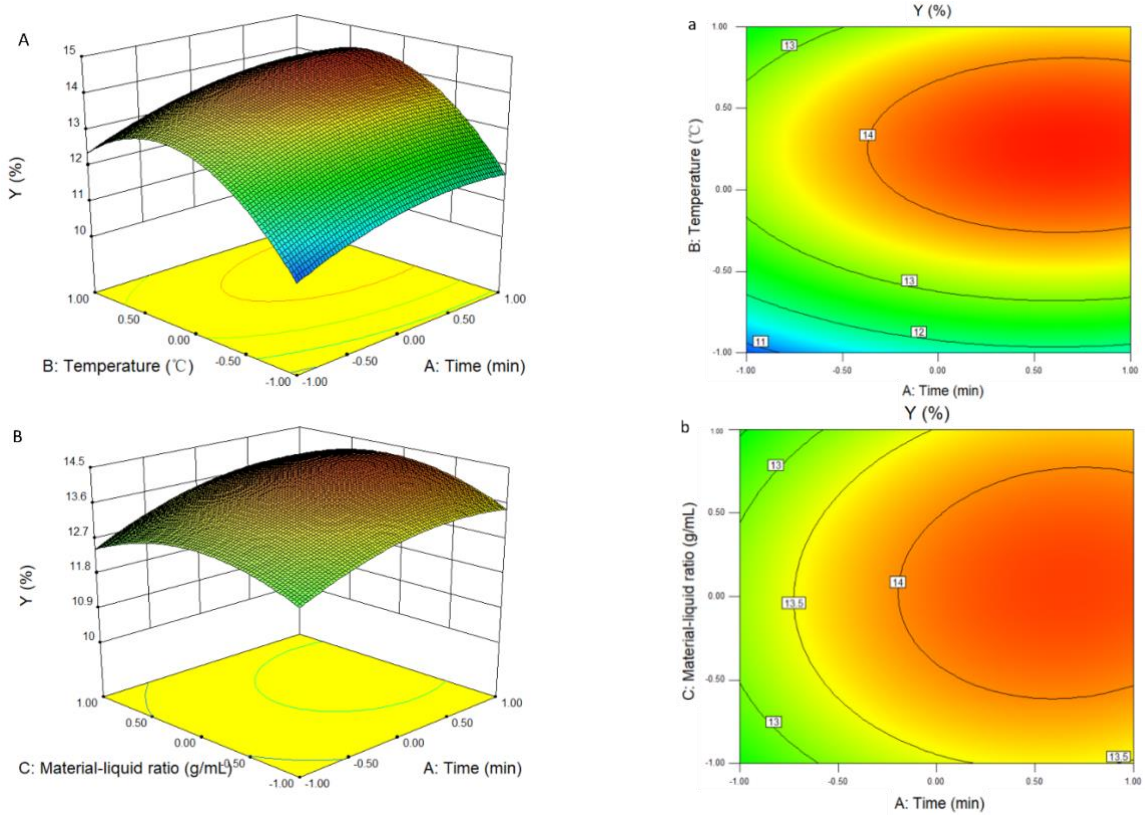
Table 2: Experimental design and results for RSM

Runs	*A*	*B*	*C*	*D*	Y(%)
1	0	0	0	0	14.32
2	0	0	1	-1	12.72
3	-1	0	0	-1	11.32
4	0	1	1	0	13.26
5	0	1	0	1	13.58
6	0	0	1	1	13.06
7	0	-1	0	-1	10.46
8	0	0	-1	1	13.17
9	1	0	1	0	13.49
10	-1	0	0	1	13.32
11	1	-1	0	0	11.74
12	-1	1	0	0	12.66
13	0	1	0	-1	11.58
14	0	-1	-1	0	10.74
15	1	0	0	-1	13.66
16	1	0	-1	0	13.87
17	-1	0	-1	0	12.72
18	1	0	0	1	13.42
19	0	-1	1	0	11.25
20	0	0	-1	-1	12.54
21	-1	-1	0	0	10.92
22	0	0	0	0	14.32
23	0	1	-1	0	12.12
24	0	0	0	0	14.32
25	1	1	0	0	13.54
26	0	-1	0	1	11.29
27	0	0	0	0	14.32
28	0	0	0	0	14.32
29	-1	0	1	0	11.97

Table 3: ANOVA for response surface quadratic model of FLPs yields

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	F-value	P-value
Model	35.42	14	2.53	17.14	<0.0001**
A	3.72	1	3.72	25.19	0.0002**
B	8.69	1	8.69	58.85	<0.0001**
C	0.029	1	0.029	0.20	0.6343
D	2.58	1	2.58	17.45	0.0009**
AB	$9.025*10^{-3}$	1	$9.025*10^{-3}$	0.061	0.8083
AC	0.034	1	0.034	0.23	0.6376
AD	1.25	1	1.25	8.50	0.0113*
BC	0.099	1	0.099	0.67	0.4260
BD	0.34	1	0.34	2.32	0.1501
CD	0.021	1	0.021	0.14	0.7115
A^2	1.15	1	1.15	7.82	0.0143*
B^2	16.77	1	16.77	113.62	<0.0001**
C^2	2.77	1	2.77	18.74	0.0007**
D^2	3.67	1	3.67	24.83	0.0002**
Residual	2.07	14	0.15		
Lack of fit	1.75	10	0.18	2.24	0.2269
Pure error	0.31	4	0.078		
Total	37.49	28			
R^2	0.9449				

Notes: * indicates a significant difference (p<0.05). ** indicates an extremely



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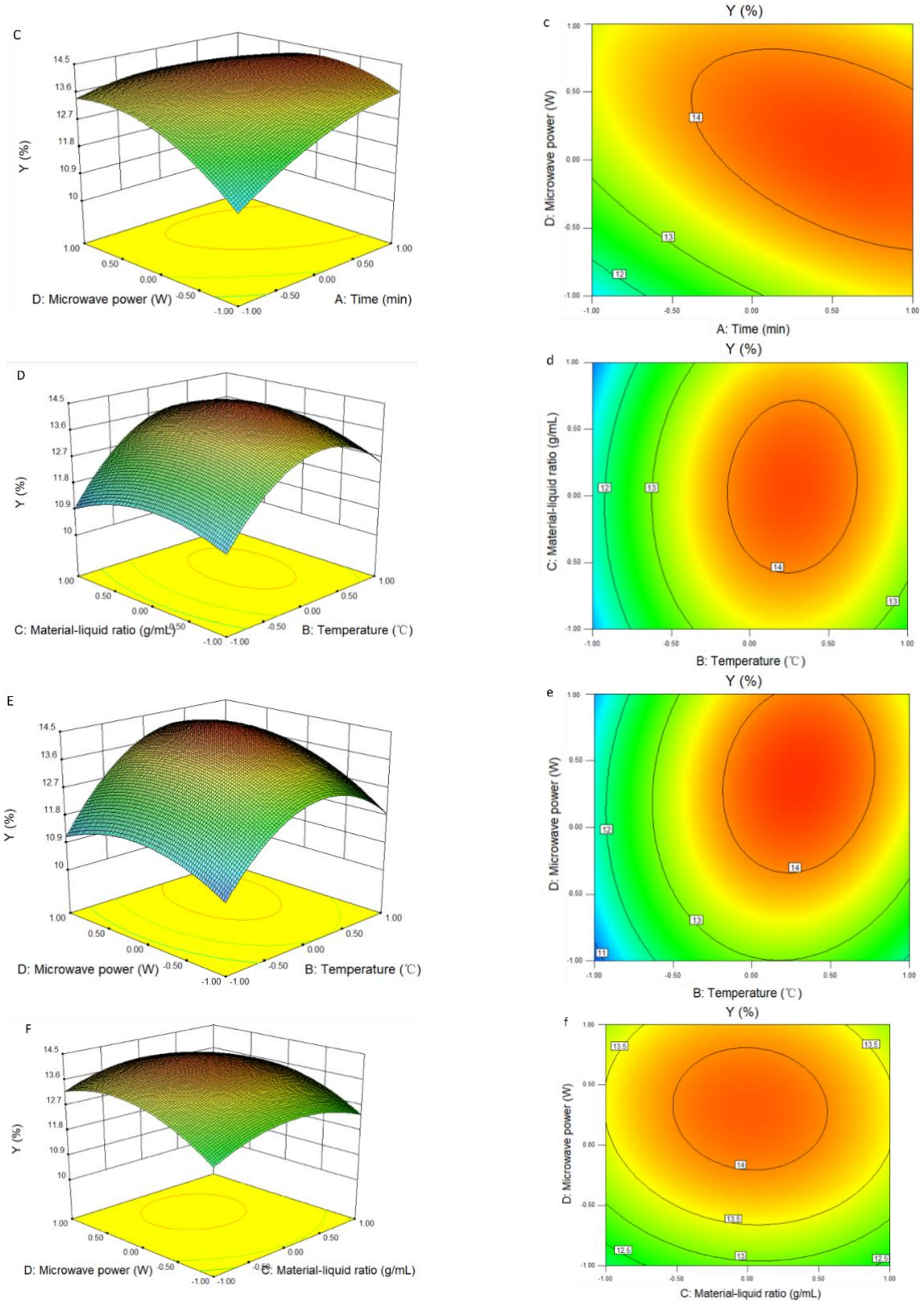

Fig. 3: Response surface plots of effect of the interaction of each factor on the yield of FLPs

Through comprehensive model optimization analysis, the optimal extraction parameters for maximizing FLP yield were determined as follows: extraction time was 18min, temperature was 63℃, material-liquid ratio was 1:51 (g/mL) and microwave power was 714W. The model predicted a maximum FLPs yield of 14.48% under these conditions. Experimental validation through triplicate independent trials yielded an actual FLPs production of 14.36±0.23%. The relative deviation between the predicted value (14.48%) and the actual yield was 0.84%, confirming that this mathematical model can effectively simulate and accurately predict the experimental results under actual conditions. These results confirmed the reliability and predictive capability of the developed model for optimizing FLP extraction process.

Table 4: Monosaccharide composition of FLPs[a]

Sample	Rhamnose	Arabinose	Xylose	Glucose	Galactose
FLPs	9.97	7.86	0.78	25.61	5.05

[a] Monosaccharides were given in mass ratio.

Table 5: Chemical composition and molecular weight of FLPs

Polysaccharide Sample	*Protein (%)*	*Total carbohydrate (%)*	*Uronic acid (%)*	*Molecular weight (Da)*
FLPs	2.55±0.36	82.19±1.88	5.85±1.12	2.56×10^5

Monosaccharide Composition and Chemical Characterization of FLPs

Gas chromatographic profile of FLPs was shown in Fig. 4. Monosaccharide composition of FLPs was presented in Table4.The monosaccharide composition analysis of FLPs revealed the presence of rhamnose, arabinose, xylose, glucose and galactose in a molar ratio of 9.97:7.86:0.78:25.61:5.05. Chemical composition and molecular weight of FLPs were presented in Table5. Molecular weight of FLPs was 2.56×10^5Da. Quantitative chemical analysis revealed FLPs contained a total carbohydrate content of 82.19±1.88% (w/w), a uronic acid residue content of 5.85±1.12% (w/w) and a protein content of 2.55±0.36% (w/w).

Fourier Transform Infrared Spectroscopic Analysis of FLPs

The fourier transform infrared spectroscopic characteristics of FLPs was presented in Fig.5. The spectrum revealed several characteristic absorption bands corresponding to specific functional groups. A broad and intense absorption band centered at 3394cm^{-1} was the characteristic of the stretching vibration of O-H from hydroxyl groups, indicating the presence of extensive hydrogen bonding networks. Two distinct peaks were observed at 2937cm^{-1} and 1383cm^{-1}, which belong to the stretching vibration and the bending vibration of C-H bonds (Chen *et al*., 2015; Singthong *et al*., 2004). The presence of esterified carboxyl groups was evidenced by absorption bands at 1740 cm^{-1} (C = O stretching) and 1254 cm^{-1} (C-O stretching) (Monsoor *et al*., 2001). Additionally, carboxyl groups were identified through asymmetric and symmetric stretching vibrations at 1637 cm^{-1} and 1417 cm^{-1}, respectively (Chen and Xue, 2019). These spectral features collectively confirmed the polysaccharide nature of FLPs and provided valuable structural information regarding their functional group composition.

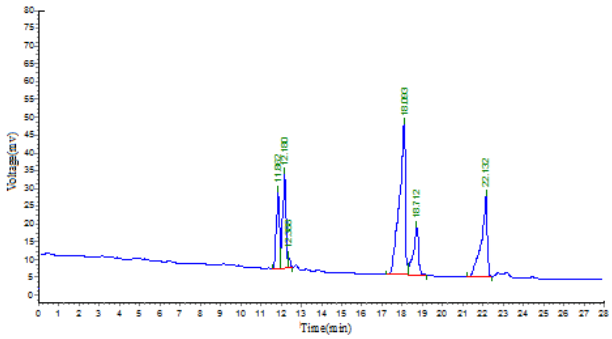

Fig. 4: Gas chromatographic profile of FLPs with acid hydrolysis and acetylation

Antioxidant Activities of FLPs

Compelling scientific evidence has firmly established that chronic overproduction of free radicals contributes significantly to the pathogenesis of numerous diseases, including cancer, metabolic disorders (hyperlipidemia and diabetes mellitus), cardiovascular diseases, hepatic cirrhosis, and immune system dysfunction (Valko *et al*., 2007; Wang *et al*., 2023). Extensive research has established that antioxidants effectively attenuate free radical-mediated cellular damage through their potent reactive oxygen species (ROS) scavenging activity. The polysaccharides called UAEE-GMRP-1A, UAEE-GMRP, CM-30 and Ac-30 had a good scavenging effect on hydroxyl radicals (Tang and Huang, 2024). A novel polysaccharide SLMP1-1 from *Schizochytrium limacinum meal* exhibits significantly stronger antioxidant activity, which has a scavenging effect on DPPH scavenging activity (Zhang *et al*., 2024). The polysaccharide PEP-1 from *Phascolosoma esculentas* demonstrated stronger in vitro antioxidant activity, which had scavenging activities against hydroxyl radical and DPPH radicals (Zhou *et al*., 2024). The antioxidant of FLPs was evaluated in comparison with ascorbic acid (Vc) shown in Fig. 6. The superoxide

anion radical scavenging assay (Fig.6A) revealed a concentration-dependent antioxidant activity, with FLPs demonstrating significant scavenging capacity. When the concentration was 0.6mg/mL, the scavenging ability (RSA) of FLPs for superoxide anion radicals was 73.21±1.34%. Parallel concentration-dependent patterns emerged in DPPH and hydroxyl radical assays at this concentration (Fig.6B and Fig.6C). When the concentration was 0.6mg/mL, the DPPH RSA of FLPs reached 76.77±1.31% and the hydroxyl RSA of FLPs reached 61.9±1.22%. Compared with the antioxidant of FLPs by Jiang *et al*. (2023), FLPs in the present study demonstrated significantly higher superoxide radical scavenging rate, reaching 58.89±1.16% at a concentration of 0.4 mg/mL. The results of Jiang *et al*. indicated that the scavenging rate of superoxide radical by FLPs at a concentration of 0.4 mg/mL was only about 20%. However, comparative evaluation of hydroxyl radical and DPPH radical scavenging activities revealed comparable efficacy between our FLPs and FLPs reported by Jiang *et al*. (2023) at equivalent concentrations. These differences may be attributed to variations in raw material sources, extraction methodologies, polysaccharide composition and structural characteristics (Marrelli *et al*., 2014).

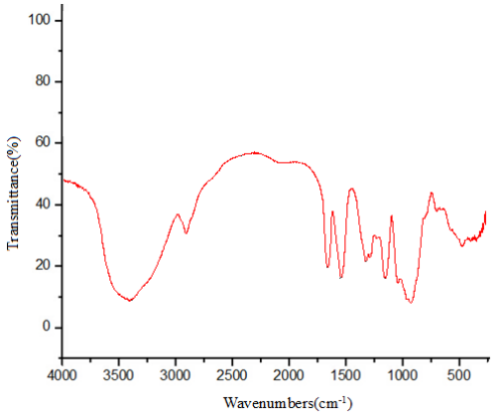

Fig. 5: Fourier transform infrared spectra of FLPs

Conclusion

In the present study, the optimization of extraction process for FLPs was carried out using UMAE. Characterization and antioxidant activity of FLPs were investigated. The optimum conditions for UMAE of FLPs were obtained as follows. The material-liquid ratio was 1:51(g/mL), microwave power was 714W assisted with ultrasonic power of 50W, extraction temperature was 63°C and extraction time was 18min. Under these optimal conditions, the FLPs yield achieved 14.36±0.23%. Analysis of the composition of FLPs revealed that it was mainly composed of rhamnose, arabinose, xylose, glucose

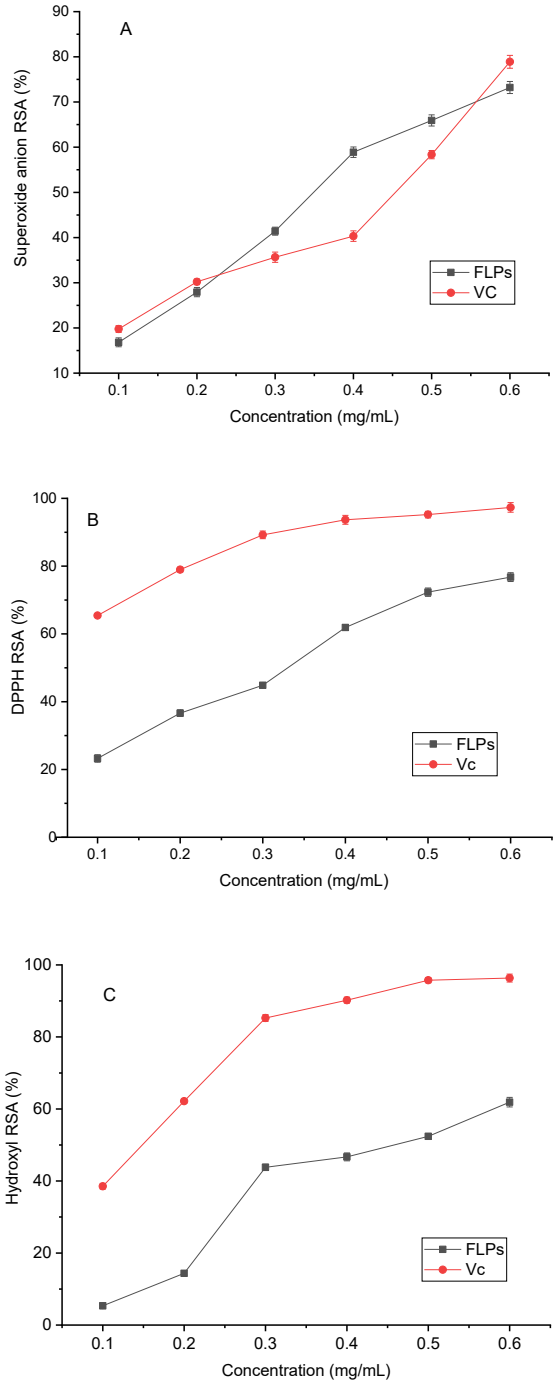

Fig. 6: The antioxidant activity of FLPs at various concentrations

and galactose, with a molecular weight of 2.56×10^5Da. Through superoxide anion radical, DPPH radical, and hydroxyl radical scavenging assays, it was found that FLPs exhibited good antioxidant activity in vitro. FLPs could be a beneficial source of organic antioxidants and

functional foods. The results will have certain significance for the value-added and comprehensive utilization of discarded fig leaves. In the future, it is very important to explore the industrial application of UMAE in the commercial field and expand its application in natural substance extraction. Meanwhile, the potential impact of UMAE on the environment deserves further attention. In addition, the bioavailability and metabolic pathways of FLPs need further research. It is necessary to further study the bioavailability and metabolic pathways of FLPs and explore the purification, structure, antioxidant mechanism and other biological activities of FLPs such as anti-inflammatory effect, which will lay a theoretical foundation for its application in more fields.

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Authors Contributions

Baikangzhe Zheng: Conceptualization, formal analysis, writing-original draft.
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Yi Zhou, Min Zhang and Shiwen Pan: Investigation,validation , methodology.
Jingjing Liu: Methodology and data curation.
Rong Gao: Methodology and resources.
Yingyun Peng, Dongxing Zhu and Yiyong Chen: Funding acquisition, supervision, project administration, writing-review and edited.

Ethics

This article is original and contains unpublished material. The corresponding author confirms that all of the other authors have read and approved the manuscript and no ethical issues involved.

Competing Interest

The authors declare that they have no competing interest.

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