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Optimization, purification and antioxidant potential of polyphenol ultrasonic-assisted extraction from pecan 'Shaoxing' green husk

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Abstract

In this study, ultrasonic-assisted extraction method was used to extract polyphenols from pecan 'Shaoxing' green husk. The optimization of extraction technology involved both single-factor and response surface methodology, while the purification technology was determined to refine the crude polyphenol extract. Assessment of radical scavenging activity of pecan 'Shaoxing' green husk polyphenols on 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,2'-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) (ABTS), hydroxyl radical (•OH), and superoxide anion ($O_2^{\bullet-}$) was conducted with L-ascorbic acid (Vc) as the control. Results showed that optimal extraction conditions, including ethanol solvent, material-liquid ratio of 1:15, 58% ethanol volume fraction, 60 min ultrasonic time, 160 W ultrasonic power, and 57 ℃ ultrasonic temperature, yielded a polyphenol content of 218.62 mg/g. Macroporous resin D-101 was selected for polyphenol purification with optimized parameters: 2 mg/mL loading concentration, pH = 4, 2 mL/min loading flow rate, elution with 70% ethanol volume fraction, and 3 mL/min elution flow rate. The purity of polyphenols increased from 31.45 to 69.34%. At the pecan 'Shaoxing' green husk polyphenol concentration of 0.9 mg/mL, DPPH, ABTS, $O_2^{\bullet-}$, and •OH radical scavenging activity were measured at 95.36, 99.4, 50.92, and 51.89%, respectively, indicating significant antioxidant activity. LC–MS analysis detected 24 polyphenol components in pecan 'Shaoxing' green husk, with relatively higher rutin and proanthocyanidin B2 contents compared to other components.

Keywords Pecan green husk, Polyphenols, Extraction and purification, Antioxidant activity, Compositional analysis

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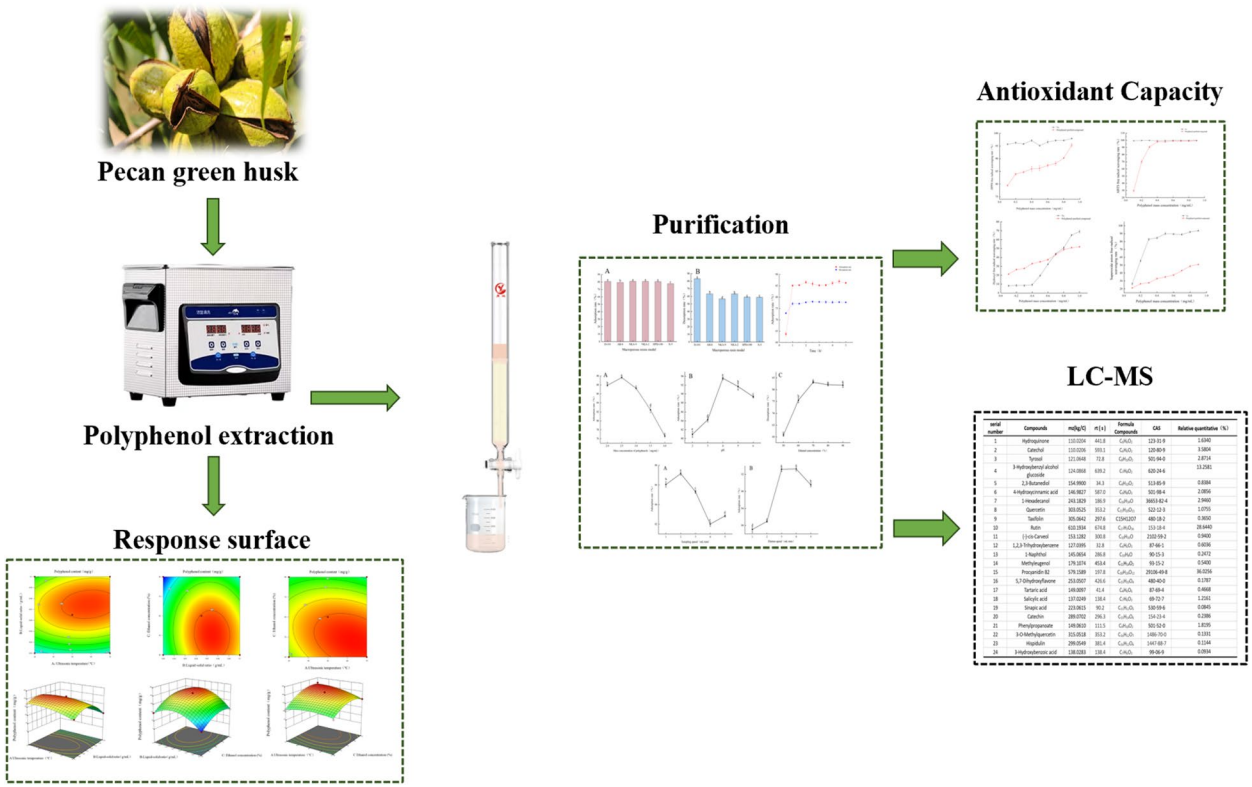
Graphical Abstract



Introduction

Pecan [*Carya illinoinensis* (Wagenh) K. Koch], native to the southern United States and Mexico, is a globally significant nut crop (Poletto et al., 2019). Introduced in the early twentieth century, pecan has a history of about 100 years in China (Jia et al., 2018). The pecan kernel is widely used in various foods due to its crisp and non-astringent taste (Wang et al., 2023; Zhao et al., 2023). Its green husk, often discarded as agricultural waste or burned, can negatively impact soil fertility and the environment (Wei et al., 2021). However, it is also used as a medicinal material, though its other food-related functions have not been thoroughly investigated.

Plant polyphenols, compounds with polyhydroxy structures, are found in plant roots, stems, leaves, flowers and fruits (Pinto et al., 2021). They possess beneficial physiological activities such as antioxidant (Faller et al. 2010), bacteriostatic (Stefanos et al., 2016), anti-inflammatory (Lee et al., 2018), anti-cancer (Zinov'Eva & Spasov, 2012), and anti-apoptotic effects (Rajha et al., 2023). Studies have shown that naphthoquinone, polyphenols, and other components extracted from pecan green husks may be used as fruit preservatives (Envelope et al., 2022; Flores-Estrada et al., 2020). The main methods used to evaluate the antioxidant activity include DPPH, ABTS, $O_2^{\bullet-}$, and •OH radical scavenging activity, total reducing power and so on (Li & Wang, 2017).

Advances in science and technology have led to improvements in plant polyphenol extraction methods, including solvent extraction (Naima et al., 2015), ultrasonic-assisted extraction (Lakka et al., 2020) microwave-assisted extraction (Asofiei et al., 2018), supercritical fluid extraction (Sarmento et al., 2008), and combined methods (Da Porto et al., 2015). Ultrasonic fragmentation and cavitation disrupt cell walls, promoting rapid extract release and complete dissolution in the solvent (Anaya-Esparza et al., 2018). This method is simple, efficient, and time-saving.

The polyphenol extract's purity can be enhanced through column chromatography to remove impurities present in the crude extract. The common materials employed for this purpose include silica gel, polyamide, and macroporous resin (Haider et al., 2009). Previous

studies (Riaz et al., 2020; Seker et al., 2019) indicated that macroporous resin is frequently utilized for purifying crude polyphenol extracts, owing to its selective adsorption function, primarily driven by van der Waals force or hydrogen bonding. The molecular sieve's property is determined by its porous structure nature. The effectiveness of the macroporous resin method lies in its robust adsorption capacity and renewability (Wang et al., 2020).

In this study, polyphenols were extracted from pecan 'Shaoxing' green husk using ultrasound-assisted extraction. The study investigates the impact of ultrasonic parameters such as time, temperature, and power, as well as the material-liquid ratio and solvent volume fraction on the final extraction yield. Subsequently, the optimal macroporous resin is selected for purifying the pecan 'Shaoxing' green husk polyphenols. Parameters such as sample concentration, sample flow rate, ethanol concentration, and elution flow rate during purification are examined. The aim is to determine the optimal extraction and purification techniques and assess the antioxidant activity of the purified pecan 'Shaoxing' green husk extract. This study intends to offer insights into the development and utilization of functional foods incorporating polyphenols from pecan 'Shaoxing' green husk. Research results may improve the utilization rate of pecan green husk and reduce the pollution of pecan green husk to environment.

Materials and methods

Reagents and instruments

The primary reagents utilized are: ethanol, methanol (analytically pure, Sinopharm Chemical Reagents Co., Shanghai, China), phenol reagent, salicylic acid, ferrous sulfate, pyrogallol, sodium carbonate (analytically pure, Shanghai Macklin Biochemical Technology Co., Shanghai, China), DPPH (1,1-diphenyl-2-picrylhydrazyl), ABTS [2,2'azinobis(3-ethylbenzthiazoline-6-sulfonic acid)] (TCI (Shanghai) Development Co., Shanghai, China), Folin-Phenol (Beijing Solarbio Science & Technology Co., Beijing, China). Additionally, macroporous resin including models D-101, AB-8, NKA-9, NKA-2, HPD-100, X-5 (Zhengzhou Hechengxin Material Technology Co., Zhengzhou, China) was employed. Acetonitrile (analytically pur, Thermo Fisher Scientific), formic acid (LC–MS grade, TCI (Shanghai) Development Co., Ltd.), formic acid ammonium salt (analytically pure, Sigma Co., Shanghai, China).

The primary equipments comprise: DHG-9246A electrothermal constant temperature blast drying oven (Shanghai Jinghong Test Equipment Co., Shanghai, China), JP-040ST ultrasonic cleaning instrument (Shenzhen Jiemeng Cleaning Equipment Co., Shanghai, China), BHS-2 electric thermostatic water bath (Ningbo Qunan Experimental Instrument Co., Ningbo, China), 721 visible spectrophotometer (Shanghai Youke Instrument Co., Shanghai, China), MH-16KR micro high-speed freezing centrifuge (Shanghai Liuke Instrument Co., Shanghai, China). The LC analysis was performed on a Vanquish UHPLC System (Thermo Fisher Scientific, USA). Mass spectrometric detection of metabolites was performed on Orbitrap Exploris 120 (Thermo Fisher Scientific, USA) with ESI ion source.

Plant material

The experimental materials were gathered in Heyue Garden, Baoying County, Yangzhou, Jiangsu Province, China. The 'Shaoxing', an excellent pecan variety, widely popularized in China and extensively cultivated in Zhejiang, Jiangsu, and other regions, was chosen for the experiment. Pecan fruits were collected every seven days from September 26th to October 16th, 2022. Only fruits with full shape and devoid of obvious diseases and insect pests were screened. These selected fruits were placed in a biological ice box and promptly transported to laboratory. Subsequently, the pecan 'Shaoxing' green husks were peeled off, dried at 60 ℃ for 5 h through the electrothermal constant temperature blast drying oven, crushed, sifted through a 40-mesh sieve, and the resulting powders were sealed and stored at -20 ℃ before extraction.

Methods

Extraction of polyphenols from pecan 'Shaoxing' green husk

Extraction of polyphenols was performed by slightly modifying the method of Teh et al. (2014). 1 g of dried pecan 'Shaoxing' green husk powder was weighed by electronic balance, and 20 mL of 65% ethanol was added. After fully oscillating with a scroll oscillator, ultrasonic extraction was conducted at 50 ℃ with an ultrasonic power of 160 W for 50 min. Following extraction, the mixture underwent centrifugation at 3500 rpm for 10 min. The resulting pecan 'Shaoxing' green husk polyphenol extract was obtained after suction and filtration.

Single factor experiment on the extraction of polyphenols from pecan 'Shaoxing' green husk

The extraction method in the previous section was used to evaluate the polyphenol content of the 'Shaoxing' green pecan husk. The impact of various factors on extraction efficiency was examined, including extraction solvent, material-liquid ratio, extraction solution concentration, ultrasonic time, ultrasonic temperature, and ultrasonic power. The tested extraction solvent includes water, ethanol, and methanol. The material-liquid ratios of 1:10, 1:15, 1:20, 1:25, and 1:30 g/mL were used. The extraction solution concentrations of 45, 55, 65, 75, and

Table 1 Factors and levels of test

Level	Factor		
	A/ultrasonic temperature (℃)	B/ratio of material-liquid (g/mL)	C/ethanol concentration (%)
minimum	30	1:10	45
maximum	70	1:30	85
mean	50	1:20	65

85% were tested. The ultrasonic times of 30, 40, 50, 60, and 70 min were evaluated. Ultrasonic temperatures of 30, 40, 50, 60, and 70°C were used. Finally, ultrasonic power levels of 80, 120, 160, 200, and 240 W were assessed. Each experiment was repeated thrice.

Optimization scheme design of response surface test

Through the results of single factor experiment, the three most significant factors are selected for Box-Behnken design. The Design Expert 13 software (Stat-Ease Inc) was employed to determine the optimal extraction conditions for polyphenols from the pecan 'Shaoxing' green husk (Table 1).

Purification experiment of polyphenols extract

Purification experiment of polyphenols extract was performed by slightly modifying the method of Gao et al. (2018) and Cheng et al. (2021). The optimal purification conditions obtained in this section are applied to the purification after each extraction.

Pretreatment of macroporous resin

The macroporous resin pretreatment method was carried out according to the user manual for macroporous resin. Six kinds of macroporous resins, namely D-101, AB-8, NKA-9, NKA-2, HPD-100, and X-5, were soaked in a 95% ethanol solution. The solution was stirred until no bubbles were visible and then allowed to rest for 12 h, activating the macroporous resin. Finally, all the resins were rinsed with distilled water until no ethanol odor was detectable.

Selection of the best macroporous resin

Each fully activated macroporous resin (4 g) was placed into a conical bottle, and 20 mL of a known concentration of pecan 'Shaoxing' green husk polyphenol solution was added. After shaking for 24 h, the supernatant was collected to determine the polyphenol content. The adsorption rate was calculated using formula (1). Once the macroporous resin reached adsorption equilibrium, it was washed with distilled water, dried with filter paper, transferred to a new conical bottle, vibrated, and desorbed for 24 h. The supernatant was then analyzed for polyphenol content. The desorption rate is calculated according to formula (2).

$$\text{Adsorption rate (\%)} = \frac{B_0 - B_1}{B_0} \times 100\% \quad (1)$$

$$\text{Desorption rate (\%)} = \frac{B}{B_0 - B_1} \times 100\% \quad (2)$$

B_0 is the mass concentration of polyphenols in pecan 'Shaoxing' green husk, while B_1 is the content of polyphenols in the supernatant of conical bottles when the adsorption equilibrium is reached, B is the pecan 'Shaoxing' green husk polyphenol concentration in the desorption solution.

Static adsorption and desorption kinetics curve

The static adsorption and desorption kinetics curve is drawn by using the best macroporous resin selected and the method of determining the adsorption rate and resolution rate in the previous section. After 5 h of shock adsorption, samples of the supernatant were collected every 30 min. The adsorption rate was calculated using formula (1), and a static adsorption curve was plotted. Following adsorption equilibrium, the macroporous resin was subjected to vibration and desorption for five hours. Samples of the supernatant were collected every other 30 min to determine polyphenol content. The desorption rate was calculated using formula (2), and a dynamic desorption curve was generated.

Static adsorption and desorption

The effects of sample concentration, pH value and desorption solution concentration on the adsorption of macroporous resin were investigated (Table 2). Among them, the pH value of the sample is adjusted by 1mg/mL sodium hydroxide solution.

Table 2 Study on the effect pH, sample concentration and desorption solution concentration on both adsorption and desorption processes

Factor	Value				
pH	2	3	4	5	6
sample concentration (mg/mL)	2	2.5	3	3.5	4
desorption solution concentration (%)	50	60	70	80	90

Table 3 Study on the effect sample flow rate and elution flow rate on both adsorption and desorption processes

Factor	Value				
sample flow rate (mL/min)	1	2	3	4	5
elution flow rate (mL/min)	1	2	3	4	5

Dynamic adsorption and desorption

Furthermore, the effects of sample flow rate and elution flow rate on both adsorption and desorption processes were analyzed (Table 3).

Purity determination of polyphenols

After purification, the polyphenol solution was evaporated to no alcohol flavor, then dried in a freeze-drying box for 3 d, the dried sample was weighed, and the purity of polyphenol was calculated according to formula (3).

$$\text{Purity determination of polyphenols (\%)} = \frac{B \times V}{M} \quad (3)$$

B is the measured concentration of polyphenol purification solution (mg/mL), V is the liquid volume to be measured (mL), M is the quality of pecan 'Shaoxing' green husk after freeze-drying (g).

Method for determination polyphenol content

Preparation of gallic acid standard curve

Polyphenol content determination was performed according to the method of Parys et al. (2009) with slight modification. To prepare the gallic acid standard solution, precisely 0.025 g of gallic acid standard sample was weighed and dissolved in distilled water and adjusted to a final volume of 100 mL. Subsequently, different volumes of the gallic acid standard solution (0, 1, 2, 3, 4, 5, 6, and 7 mL) were absorbed into eight 25 mL plug colorimetric tubes and diluted with distilled water. Eight washed test tubes each received 1 mL of the gallic acid solution and 1 mL of Folin-Ciocalteu phenol reagent. To this mixture, 3 mL of 12.5% sodium carbonate solution and 5 mL of distilled water were added. After incubation in a water bath at 30 ℃ for 1 h, absorbance was measured. The relationship between the mass concentration of gallic acid (x) and absorbance (y) followed the standard curve $y = 0.014x + 0.014$.

Determination of polyphenol content

According to the method in 'Extraction of polyphenols from 'Shaoxing' pecan green husk', polyphenols were extracted. The polyphenol content was calculated according to formula (4).

$$M = \frac{C \times V \times N}{m \times 1000} \quad (4)$$

M is the content of polyphenols (mg/g), C is the mass concentration of polyphenols in the solution to be measured (μg/mL), V is the volume of the liquid to be measured (mL), N is the dilution multiple, and m is the quality of the extraction raw material (g).

Antioxidant activity of pecan polyphenols extract

DPPH radical scavenging activity

Determination of DPPH radical scavenging activity was performed according to the method of Bans et al. (2011) with slight modification. Samples of varying concentrations (1 mL) were mixed with 1 mL DPPH solution, and after 30 min, absorbance (A_1) was measured at 517 nm. A_0 and A_2 were recorded with anhydrous ethanol replacing the sample and DPPH solution, respectively. Vc acted as the positive control. The clearance rate was calculated according to formula (5).

$$\text{DPPH radical scavenging rate (\%)} = \frac{A_0 - (A_1 - A_2)}{A_0} \times 100 \quad (5)$$

ABTS radical scavenging activity

Method for Determination of ABTS radical scavenging activity was performed according to the method of Dong et al. (2014) with slight modification. Configure 7.3 mmol/L ABTS reserve solution and 2.6 mmol/L $K_2S_2O_8$ reserve solution, take different concentrations of polyphenols extract of 0.2 mL and 3 mL ABTS reserve solution and fully mix them, stand in the dark for 5 min, and determine at 734 nm. The absorbance value is A_1, while the absorbance value measured by using anhydrous ethanol instead of the sample is A_0. The absorbance value measured by absolute ethanol instead of ABTS solution is A_2. Vc was used as a positive control. The clearance rate was calculated according to formula (6).

$$\text{ABTS radical scavenging rate (\%)} = \frac{A_0 - (A_1 - A_2)}{A_0} \times 100 \quad (6)$$

Hydroxyl radical scavenging activity

Determination of hydroxyl radical scavenging activity was performed according to the method of Ding et al. (2021) with slight modification. Polyphenol extract solutions (1 mL) were mixed with 1 mL of 9 mmol/L ferrous sulfate solution, 2 mL of 9 mmol/L salicylic acid–ethanol solution, and 2 mL of 3% hydrogen peroxide solution, then incubated at 37 ℃ for 1 h. The absorbance value (A_1) was determined at 510 nm, with A_2 measured using the

absorbance value of distilled water instead of hydrogen peroxide solution, and A_0 with distilled water instead of the sample. Vc served as the positive control. The clearance rate was calculated according to formula (7).

$$\text{Hydroxyl radical scavenging rate (\%)} = \frac{A_0 - (A_1 - A_2)}{A_0} \times 100 \quad (7)$$

Superoxide anion radical scavenging activity

Determination of Superoxide anion radical scavenging activity was performed according to the method of Ru et al. (2012) with slight modification. In a test tube, 4.5 mL Tris–HCl buffer was absorbed and incubated at 25 ℃ for 20 min. Then, 2 mL of polyphenol extract and 1 mL of 25 mmol/L pyrogallol solution were added and mixed. After 5 min at 25 ℃, one drop of 10 mmol/L hydrochloric acid solution was added to terminate the reaction. The absorbance value (A_1) was measured at 420 nm, with A_0 using distilled water instead of polyphenol extract, and A_2 using distilled water instead of pyrogallol solution. The clearance rate was calculated according to formula (8).

$$\text{Superoxide anion radical scavenging rate (\%)} = \frac{A_0 - (A_1 - A_2)}{A_0} \times 100 \quad (8)$$

LC–MS identification of polyphenols extracted from pecan 'Shaoxing' green husk

Pre-experimental pretreatment was performed according to the method of Vasilev et al. (2018). Accurately weigh an appropriate amount of sample into a 2 mL centrifuge tube, add 600 μL MeOH (Containing 2-Amino-3-(2-chloro-phenyl)-propionic acid (4 ppm)), vortex for 30 s. Add steel balls, placed in a tissue grinder for 60 s at 55 Hz. Room temperature ultrasound for 15 min centrifuge for 10 min at 12,000 rpm and 4 ℃, filter the supernatant by 0.22 μm membrane and transfer into the detection bottle for LC–MS detection.

Liquid chromatography conditions

The Vanquish UHPLC System (Thermo Fisher Scientific, USA) was used for the LC analysis. An ACQUITY UPLC ® HSS T3 (2.1 × 100 mm, 1.8 μm) column (Waters, Milford, MA, USA) was employed for chromatography, maintained at 40 ℃. The flow rate and injection volume were 0.3 mL/min and 2 μL, respectively. For LC-ESI (+)-MS analysis, mobile phases consisted of (B2) 0.1% formic acid in acetonitrile (v/v) and (A2) 0.1% formic acid in water (v/v). Gradient conditions were: 8% B2 (0 ~ 1 min), 8 ~ 98% B2 (1 ~ 8 min), 98% B2 (8 ~ 10 min), 98 ~ 8% B2 (10 ~ 10.1 min), and 8% B2 (10.1 ~ 12 min). For LC-ESI (-)-MS analysis, mobile phases were (B3) acetonitrile and (A3) ammonium formate (5 mM). Gradient conditions were: 8% B3 (0 ~ 1 min), 8 ~ 98% B3 (1 ~ 8 min, 98% B3 (8 ~ 10 min), 98 ~ 8% B3 (10 ~ 10.1 min), and 8% B3 (10.1 ~ 12 min) (Zelena et al., 2009).

Mass spectrum conditions

The Orbitrap Exploris 120 (Thermo Fisher Scientific, USA) with an ESI ion source was used for mass spectrometric detection of metabolites. Simultaneous MS1 and MS/MS acquisition in Full MS-ddMS2 mode, data-dependent MS/MS) was used. The parameters were: sheath gas pressure, 40 arb; aux gas flow, 10 arb; spray voltage, 3.50 kV [ESI (+)] and -2.50 kV ([ESI (-)]; capillary temperature, 325℃; MS1 range, m/z 100–1000; MS1 resolving power, 60,000 FWHM; data-dependent scans per cycle, 4; MS/MS resolving power, 15,000 FWHM; normalized collision energy, 30%; dynamic exclusion time, automatic (Want et al., 2013).

Statistic analysis

All experiments were conducted in triplicates. SPSS 26 (International Business Machines Corporation), EXCEL 2021 (Microsoft), and Origin 2021 (Origin Lab Corporation) software were utilized for single factor experiment, purification experiment of polyphenols extract and antioxidation test data processing and analysis in extraction process. The least significant difference method (LSD) was applied for comparison, with significant differences considered at $p < 0.05$.

LC–MS composition statistic analysis: The raw data were firstly converted to mzXML by MSConvert in ProteoWizard software package (v3.0.8789) and processed using R XCMS (v3.12.0) for feature detection, retention time correction and alignment. Key parameters settings were set as follows: ppm = 15, peak width = c (5, 30), mzdiff = 0.01, method = centWave. Then, the data is corrected by the area normalization method to eliminate systematic errors.

The metabolites were identified by accuracy mass and MS/MS data which were matched with HMDB (http://www.hmdb.ca), massbank (http://www.massbank.jp/), LipidMaps (http://www.lipidmaps.org), mzcloud (https://www.mzcloud.org), KEGG (https://www.genome.jp/kegg/). The molecular weight of metabolites was determined according to the m/z (mass-to-charge ratio) of parent ions in MS data. Molecular formula was predicted by adduct ion, and then matched with the database to realize MS identification of metabolites. At the same time, the MS/MS data from quantitative table of MS/MS data, were matched with the fragment ions and other information of each metabolite in the database, so as to realize the MS/MS identification of metabolites.

Results

Single factor test results

The factors affecting the extraction of polyphenols from the pecan 'Shaoxing' green husk were studied by single factor experiment, including extraction solvent, ratio of material-liquid ratio, alcohol concentration, ultrasonic time, ultrasonic power and ultrasonic temperature.

The polyphenol content of pecan 'Shaoxing' green husk extracted by water, methanol, and ethanol is 27.93, 56.69, and 58.97 mg/g, respectively. There is a significant difference in polyphenol extraction content between water and organic solvents, with ethanol exhibiting higher levels than water and methanol (Fig. 1). Consequently, ethanol emerges as the optimal extraction solvent.

The polyphenol content initially increases, then decreases with the rising material-liquid ratio. The highest polyphenol content is achieved at a material-liquid ratio of 1:15, after which it continuously decreases with the increasing material-liquid ratio (Fig. 2A). The effect of ethanol concentration on polyphenol extraction content follows a similar pattern, peaking at 55% ethanol concentration. Beyond this point, the polyphenol content decreases consistently (Fig. 2B). Ultrasonic treatment time significantly impacts polyphenol dissolution, reaching its peak at 60 min. At this point, polyphenols are mostly dissolved, and extending the ultrasonic time beyond 60 min results in decreased polyphenol content (Fig. 2C). The extraction content of polyphenols also initially increased then decreased within an ultrasonic temperature range of 30 ~ 70 ℃. The extraction efficiency peaks at 60 ℃, after which the polyphenols oxidize and decompose, reducing the extraction, reducing the extraction amount (Fig. 2D). Therefore, 60°C is the optimal ultrasonic temperature. Within the ultrasonic power range of 80–240 W, the polyphenol content initially increases, then decreases. The polyphenol content continuously rises with the increasing ultrasonic power, reaching its maximum at 160 W. Beyond this point, the extracted polyphenol content remains relatively stable (Fig. 2E).

Response surface test results

According to the single factor test results, ultrasonic temperature, material-liquid ratio, and ethanol concentration were chosen as response variables. The experiment was designed following the Box-Behnken Design principle. Table 4 presents the experimental design outcomes, while Table 4 illustrates the variance results.

Using Design Expert 13, analysis of Table 4 indicated that the regression equation for the extracted polyphenol content from 'Shaoxing' is as follows:

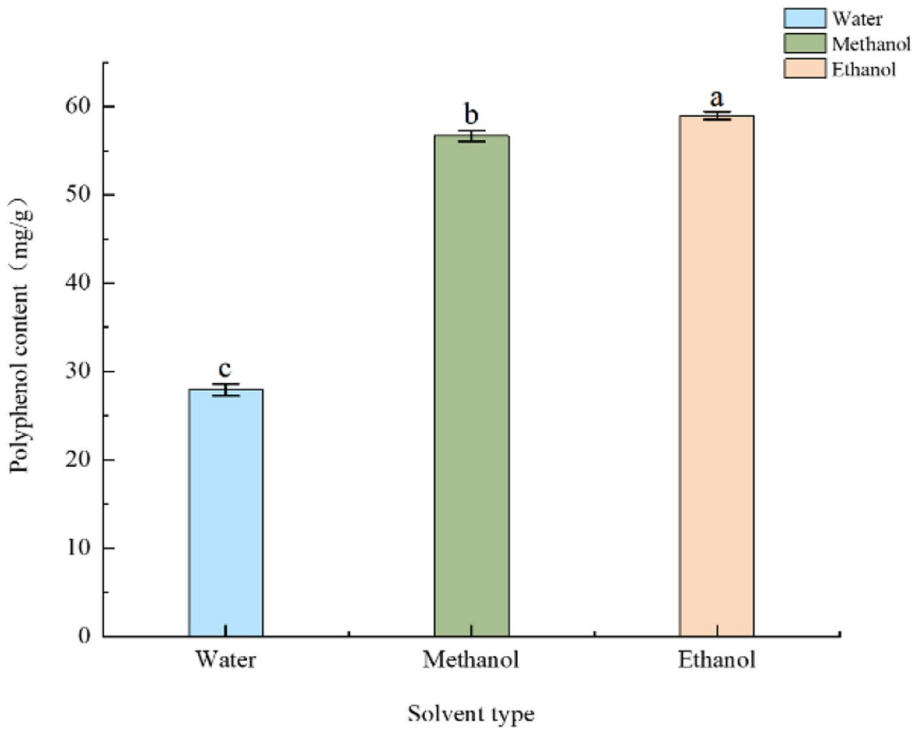


Fig. 1 Effect of solvent types on the pecan 'Shaoxing' green husk ultrasonic extraction of polyphenols quantity. Different lowercase letters indicate significant differences between treatments ($p < 0.05$)

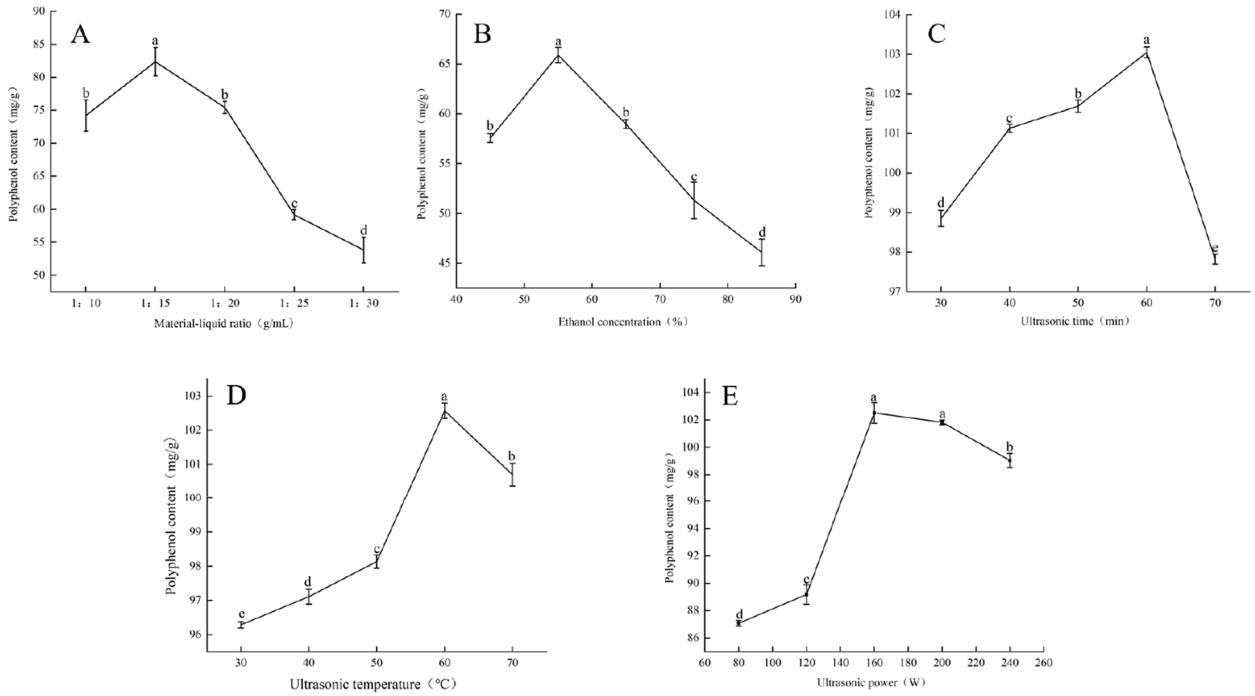


Fig. 2 Effects of different factors on ultrasonic extraction of polyphenols from pecan 'Shaoxing' green husk. **A** Effect of material-liquid ratio; **B** Effect of ethanol concentration; **C** Effect of ultrasonic time; **D** Effect of ultrasonic temperature; **E** Effect of ultrasonic power. Different lowercase letters indicate significant differences between treatments ($p < 0.05$)

Table 4 Response surface test results

Test number	A (ultrasonic temperature)	B (material -liquid ratio)	C (ethanol concentration)	Polyphenols content (mg / g)
1	50	0.030	85	53.93
2	30	0.065	45	154.10
3	70	0.065	85	114.64
4	70	0.065	45	216.79
5	50	0.065	65	207.68
6	30	0.030	65	91.79
7	50	0.065	65	201.07
8	50	0.100	45	169.11
9	50	0.065	65	185.89
10	50	0.100	85	99.82
11	70	0.100	65	172.14
12	70	0.030	65	91.61
13	50	0.065	65	207.50
14	30	0.100	65	138.93
15	50	0.030	45	95.89
16	30	0.065	85	115.00
17	50	0.065	65	205.54

$Y = 201.54 + 1.92A + 30.85B\text{-}31.56C + 8.35AB\text{-}15.76AC\text{-}6.83BC\text{-}16.24A^2\text{-}61.68B^2\text{-}35.17C^2$.

From Table 5, it is evident that the model's regression is highly significant ($P < 0.0001$), with a misfit item of 0.512, exceeding the significance threshold of 0.05. This indicates a strong model fit, capable of depicting the relationship between the response value and various factors. Primary items B and C, along with secondary factors B^2 and C^2, exhibited extremely significant differences. Primary item A, quadratic term A^2, and interaction item AC demonstrated significance. However, interaction item AB and BC showed no significance. The effects of the three factors on polyphenol extraction from pecan 'Shaoxing' green husk ranked as follows: ethanol concentration (C) > material-liquid ratio (B) > ultrasonic temperature (A).

From the stereo analysis diagram of the response surface, it is evident that ethanol concentration significantly impacts polyphenol extraction content. The contour map illustrates an approximate ellipse of the contour line, demonstrating the clear interaction between the two factors, with the interaction between ultrasonic temperature and ethanol concentration being particularly pronounced (Fig. 3). Model analysis

Table 5 Analysis of variance results

Source	Sum of Squares	df	Mean Square	F-value	*P*-value		Fit statistics
Model	42,406.94	9	4711.88	58.55	< 0.0001	**significant**	
A	1136.69	1	1136.69	14.12	0.0071		
B	7612.55	1	7612.55	94.59	< 0.0001		
C	7969.53	1	7969.53	99.03	< 0.0001		
AB	278.72	1	278.72	3.46	0.1051		
AC	993.83	1	993.83	12.35	0.0098		
BC	186.73	1	186.73	2.32	0.1715		
A^2	1110.03	1	1110.03	13.79	0.0075		
B^2	16,019.53	1	16,019.53	199.06	< 0.0001		
C^2	5207.16	1	5207.16	64.70	< 0.0001		
Residual	563.33	7	80.48				
Lack of Fit	228.97	3	76.32	0.9131	0.5102	**Not significant**	
Pure Error	334.36	4	83.59				
Cor Total	42,970.27	16					
R^2							0.9869
Adjusted R^2							0.9700
Predicted R^2							0.9026

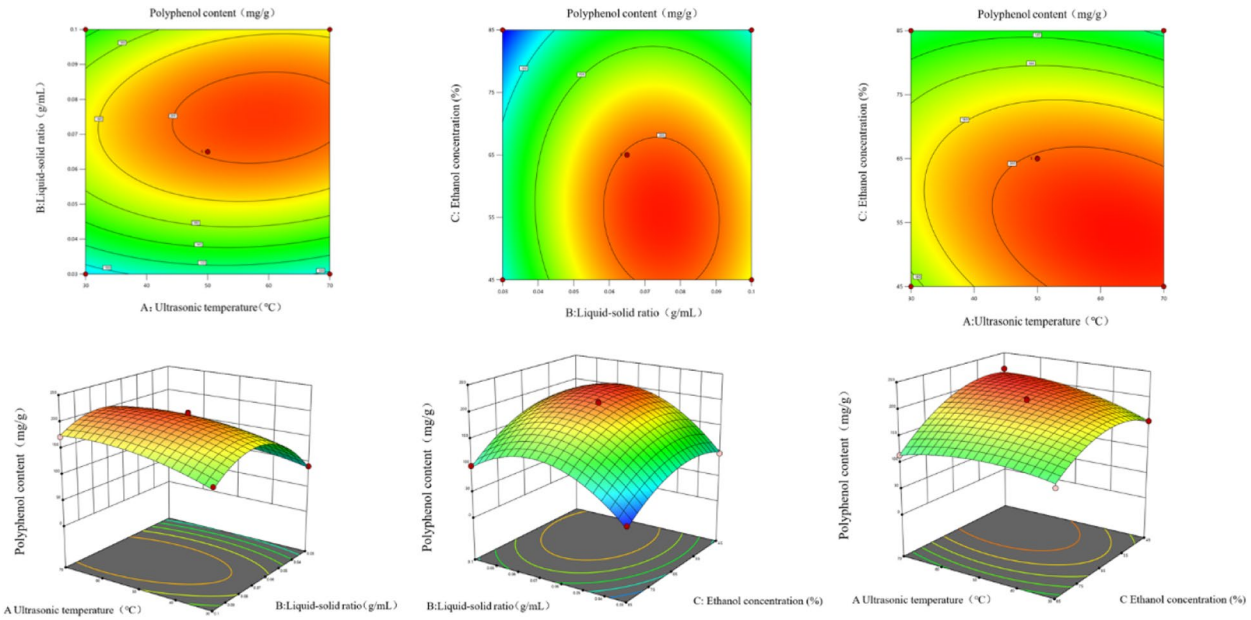


Fig. 3 Response Surface and Contour Map of interaction between two factors affecting pecan 'Shaoxing' green husk polyphenols extraction content

predicts the optimal extraction conditions for polyphenols from pecan 'Shaoxing' green husk as follows: material-liquid ratio of 0.067g/mL, ultrasonic temperature of 57.20 ℃, ethanol concentration of 58.34%, Combined with the model results, single factor test results and practical operation, the best technology for extracting polyphenols from pecan 'Shaoxing' green husk was obtained as follows: ethanol solvent, material-liquid ratio of 1:15, 58% ethanol volume fraction, 60 min ultrasonic time, 160 W ultrasonic power, and 57℃ ultrasonic temperature, yielded a polyphenol extraction of 218.62 mg/g.

Purification of polyphenols from pecan 'Shaoxing' green husk

Macroporous resin screening

Regarding adsorption rate, D-101, NKA-9, NKA-2, and HPD-100 macroporous resins exhibit significant differences from the other two macroporous resins, with NKA-9 showing the highest adsorption rate. In terms of desorption rate, D-101 exhibited a higher desorption rate compared to its four macroporous resin counterparts (Fig. 4). Considering these factors, D-101 macroporous resin was selected for purification testing.

Static adsorption kinetic curve

The left axis illustrates the adsorption test of pecan 'Shaoxing' green husk polyphenols by D-101 macroporous resin. The adsorption amount increases rapidly within 0 ~ 1 h, reaching dynamic equilibrium thereafter. Similarly, the desorption rate rises swiftly within 0 ~ 1 h, slows between 1 and 1.5 h, and stabilizes after 1.5 h. In summary, the optimal adsorption time for D-101 is 1 h, and the best desorption time is 2 h (Fig. 5).

Optimization of static adsorption conditions

Studying on optimization of static adsorption conditions by single factor test (Fig. 6), when the polyphenol mass concentration ranges from 2 to 2.5 mg/mL, the adsorption rate gradually increases. At a concentration of exactly 2.5 mg/mL, the adsorption rate of D-101 peaks at 89.65%. However, with mass concentrations exceeding 2.5 mg/mL, the adsorption rate began to decline (Fig. 6A). Polyphenol molecules exhibited micropolarity, which is influenced by pH (Fig. 6B). In the pH range of 2 ~ 6, the adsorption rate initially rises before decreasing again. Specifically, at pH = 4, the adsorption rate reaches the maximum of 93.55%, a significant difference. As for ethanol concentration, between 50 and 70%, the desorption rate increases with increasing ethanol concentration at ethanol concentration of 70%, the desorption rate peaked at 83.12%. Beyond 70% ethanol concentration, the change is not significant. Therefore, the optimal conditions for sample concentration are 2.5 mg/mL, pH 4, and an ethanol concentration of 70% during desorption (Fig. 6C).

Optimization of dynamic adsorption conditions

Studying on dynamic adsorption conditions by single factor test (Fig. 7), the adsorption rate of macroporous resin initially increased with the sample flow rate, peaking at 87.16% when the rate was 2 min/mL (Fig. 7A), followed by a decrease. Similarly, the desorption rate increased initially and then decreased with elution flow rate, with peaks at 93.19 and 93.26% when rates were 3 and 4 min/mL, respectively (Fig. 7B). Therefore, 2 and 3 min/mL were selected as the sample and elution flow rates, respectively, based on comprehensive consideration.

The pecan 'Shaoxing' green husk polyphenol solution with mass concentration of 2.5 mg/mL and pH of 4 was adsorbed by D-101 macroporous resin, then eluted with 70% ethanol solution, and the purified solution was obtained. After freeze-drying, the purity of polyphenol was calculated by formula, which was increased from 31.45 to 69.34%. The purification effect is good.

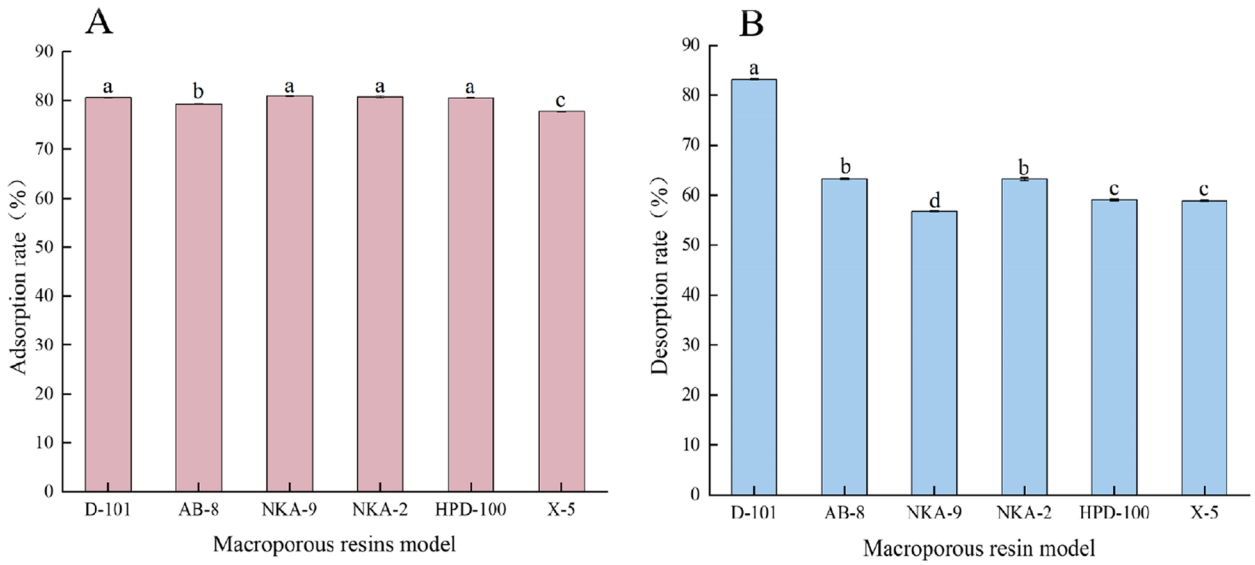


Fig. 4 Adsorption and desorption rates of different macroporous resins. **A** Adsorption rates of different macroporous resins; **B** Desorption rates of different macroporous resins. Different lowercase letters indicate significant differences between treatments ($p < 0.05$)

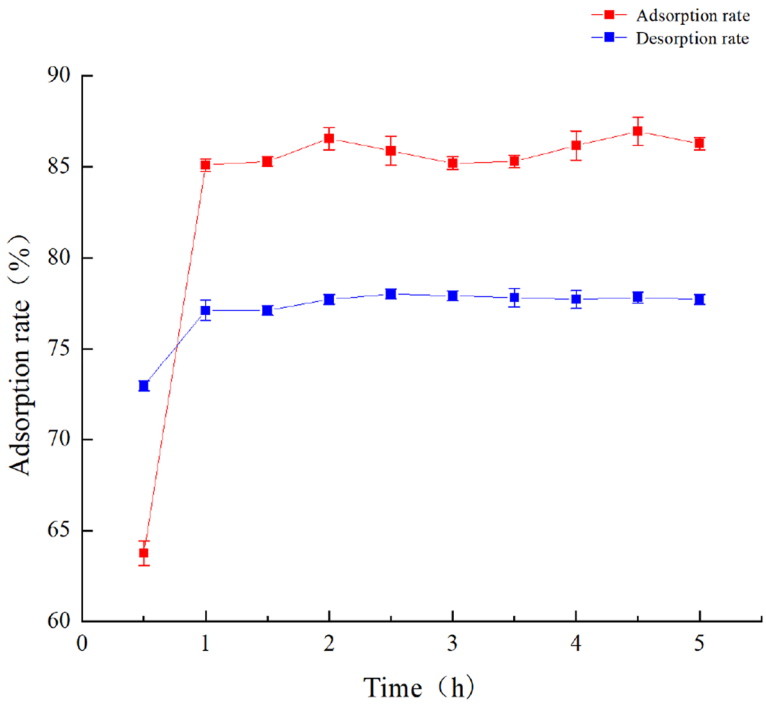


Fig. 5 Adsorption kinetics of polyphenols from pecan 'Shaoxing' green husk by D-101 microporous resin

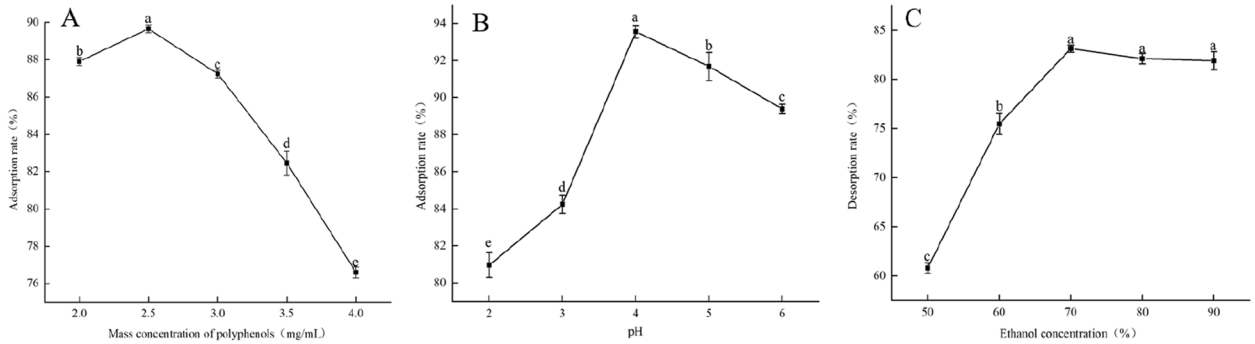


Fig. 6 Effects of different factors on static adsorption. **A** Effect of mass concentration polyphenols; **B** Effect of pH; **C** Effect of ethanol concentration. Different lowercase letters indicate significant differences between treatments ($p < 0.05$)

Determination of antioxidant activity

The DPPH radical scavenging activity of Vc remained consistently strong across a polyphenol concentration range of 0.1 ~ 0.9 mg/mL with minor variation. Conversely, the scavenging activity of pecan 'Shaoxing' green husk polyphenol extract showed an increasing trend within this range, reaching 95.36% at 0.9 mg/mL (Fig. 8A). In the range of 0.1 ~ 0.4 mg/mL, Vc exhibited stronger scavenging activity against ABTS radical cation compared to pecan 'Shaoxing' green husk polyphenols, while the latter showed an increasing trend in scavenging activity. Vc and pecan 'Shaoxing' green husk polyphenols exhibited comparable scavenging abilities towards ABTS radical cation within the range of 0.5 ~ 0.9 mg/mL (Fig. 8B). Within the range of 0.1 ~ 0.6 mg/mL, pecan 'Shaoxing' green husk polyphenols demonstrate superior scavenging activity to Vc, whereas Vc exhibited slightly stronger scavenging activity within the 0.7 ~ 0.9 mg/mL range (Fig. 8C). Vc displays stronger scavenging activity against superoxide anion radicals within the range of 0.1 ~ 0.9 mg/mL compared to pecan 'Shaoxing' green husk polyphenols, with the latter showing an increasing trend in scavenging activity (Fig. 8D).

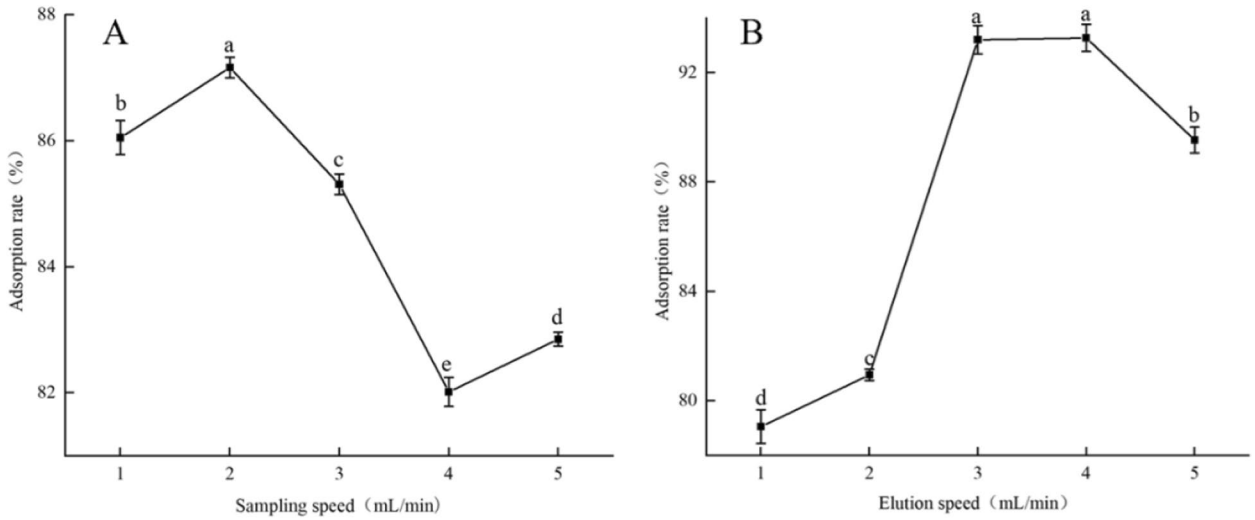


Fig. 7 Effects of different factors on dynamic adsorption. **A** Effect of sampling speed; **B** Effect of elution speed. Different lowercase letters indicate significant differences between treatments ($p < 0.05$)

LC–MS composition analysis

LC–MS combines the characteristics of liquid chromatography (LC) and mass spectrometry (MS). In the LC–MS system, the samples are first separated by liquid chromatography and then sent to the mass spectrometer for quality analysis. The combination of the two methods for qualitative and quantitative analysis of samples.

From Table 6, Analysis via high-performance liquid chromatography reveals the presence of 24 polyphenols in pecan 'Shaoxing' green husk extract. These include hydroquinone, catechol, tyrosol, 3-hydroxybenzyl alcohol glucoside, 2,3-butanediol, 4-hydroxycinnamic acid, 1-hexadecanol, quercetin, taxifolin, rutin, (-)-cis-carveol, 1,2,3-trihydroxybenzene, 1-naphthol, methyleugenol, procyanidin B2, 5,7-dihydroxyflavone, tartaric acid, salicylic acid, sinapic acid, catechin, phenylpropanoate, 3-O-methylquercetin, hispidulin, and 3-hydroxybenzoic acid. The relative content of rutin and proanthocyanidin B2 significantly exceeds that of other polyphenols.

Discussion

This study utilized ultrasound-assisted extraction to obtain polyphenols from pecan 'Shaoxing' green husk. The extraction content was compared using water, methanol, and ethanol, with ethanol yielding the highest polyphenol content. Previous studies compared the effects of 50% methanol solution, 50% ethanol solution, 50% acetone solution, and water solution on the extraction of *Matricaria pubescens* phenols. The polyphenol content of 50% methanol solution and 50% ethanol solution was the highest (Metrouh-Amir et al., 2015). This aligns with our findings, suggesting that the polyphenol extract from pecan' Shaoxing' green husk contains numerous polar substances.

The extraction concentration of polyphenols is affected by multiple factors. For instance, ultrasonic power directly impacts cavitation; increased ultrasonic power enhanced cavitation, but excessive power may damage the polyphenol molecular structure and lower the extraction rate (López-Téllez & Cañizares-Macías, 2024). The ultrasonic process also generates heat, which can decompose polyphenols at high temperatures. Prolonged ultrasonic time benefits polyphenol precipitation, but extended durations can increase impurities and reduce the extraction rate (Deng et al., 2024). Conversely, higher material-liquid ratios and ethanol concentrations decrease polyphenol extraction rates. It is hypothesized that these conditions facilitate impurity dissolution, hindering polyphenol extraction. Additionally, they raise experimental costs and complicate subsequent extraction and filtration processes.

Based on the results of a single-factor experiment, three key variables were chosen: ethanol concentration, material-liquid ratio, and ultrasonic temperature. Through response surface optimization, the sequence of factors influencing extraction content was determined as follows: ethanol concentration-gt; ratio of material to liquid-gt; ultrasonic temperature. Following response surface optimization, polyphenols from pecan 'Shaoxing' green husk were extracted under the optimal conditions. The polyphenol content was measured at 218.62 mg/g, closely aligning with predicted values, affirming the reliability of the model.

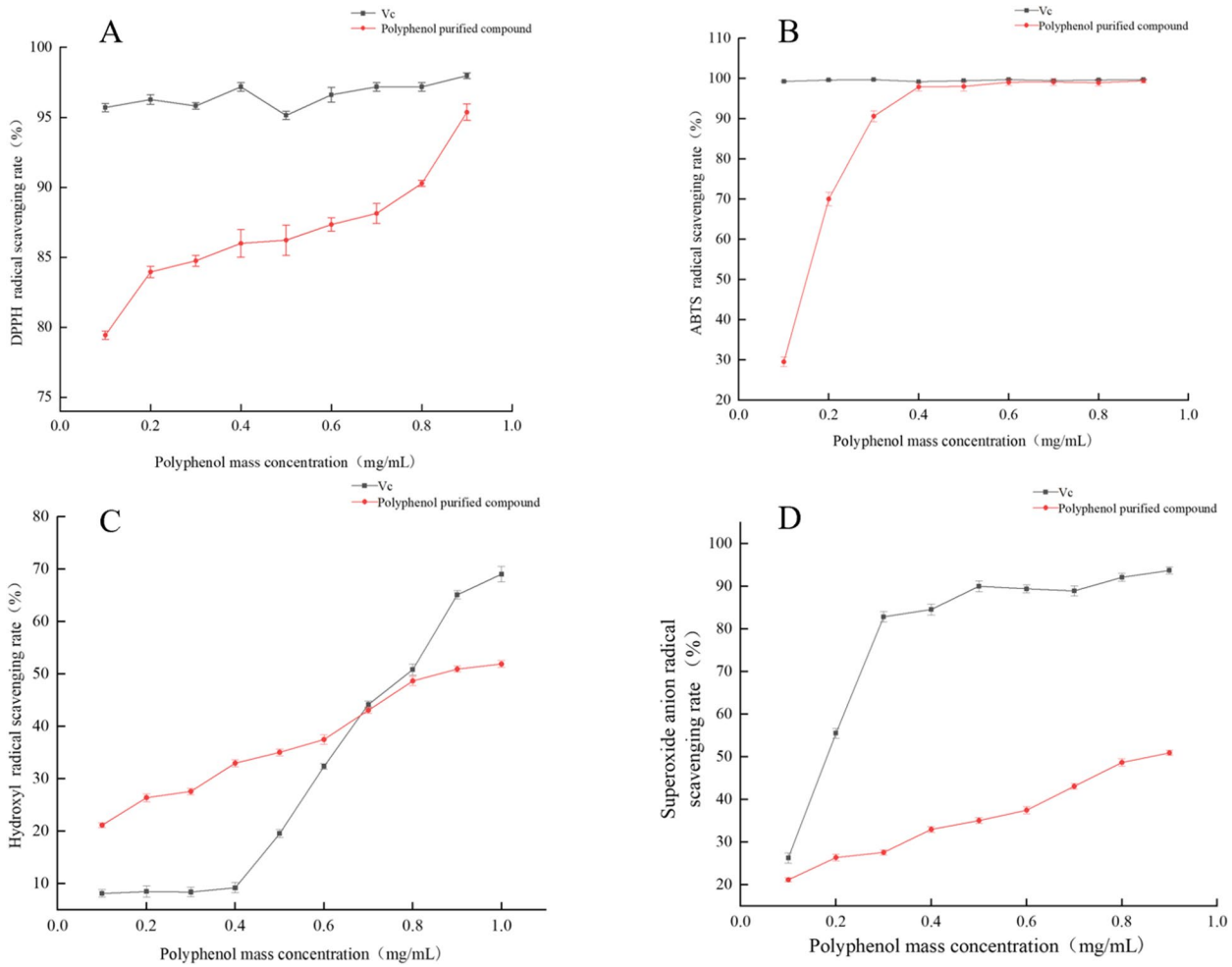


Fig. 8 Scavenging activity of pecan 'Shaoxing' green husk polyphenol extract and Vc to four types of free radicals. **A** DPPH scavenging activity (**B**) ABTS scavenging activity (**C**) •OH scavenging activity (**D**) $O_2^{\bullet-}$ scavenging activity

In this experiment, the optimal adsorption effect was determined among six types of macroporous resins: non-polar D-101, HPD-100, and X-5 resins, polar NAK-9 and NAK-2 resins, and weak polar AB-8 resins. These six resins are commonly utilized for purifying polyphenols (Hou & Zhang, 2021; Yang et al., 2024; Zhang et al., 2022). However, due to the diverse nature and complex structure of polyphenols extracted from various plants, appropriate resin selection is essential for purification. The experimental results show that D-101, NAK-9, NAK-2, and HPD-100 exhibited similar effectiveness in polyphenol extraction. Notably, D-101 demonstrates significantly superior desorption compared to other resins. This suggests that D-101 resin is better than other resins in separating and extracting organic molecules with low water solubility, such as pecan husk polyphenols, owing to its weak polarity. D-101 resin is a non-polar resin and has better adsorption and desorption of polyphenols (Yu et al., 2019). Moreover, previous studies, like Wang et al. (2015) have also utilized D-101 resin for polyphenol solution purification. During purification process optimization, it was observed that higher sample concentrations and excessive acidity adversely affect polyphenol adsorption. Additionally, during elution, higher ethanol concentrations result in more impurity elution, making it less likely for macroporous resin adsorption (Xi et al., 2015). Investigation into sample and elution flow rates led to the selection of flow rates capable of fully absorbing polyphenol solutions for experimentation.

Polyphenols exhibit potent antioxidant activity. Testing their scavenging activity against DPPH, ABTS, and $O_2^{\bullet-}$, we found that pecan 'Shaoxing' green husk displays similar scavenging abilities to Vc for DPPH and ABTS, while its $O_2^{\bullet-}$ scavenging activity is moderate. Similarly, the areca seed polyphenol extract shows scavenging abilities equivalent to Vc for DPPH and ABTS. However, its •OH scavenging activity is comparatively weak (Saito et al., 2008), aligning with our study's findings. The

Table 6 Qualitative analysis by LC–MS

Serial number	Compounds	mz(kg/C)	rt (s)	Formula Compounds	CAS	Relative quantitative (%)
1	Hydroquinone	110.0204	441.8	$C_6H_6O_2$	123–31-9	1.6340
2	Catechol	110.0206	593.1	$C_6H_6O_2$	120–80-9	3.5804
3	Tyrosol	121.0648	72.8	$C_8H_{10}O_2$	501–94-0	2.8714
4	3-Hydroxybenzyl alcohol glucoside	124.0868	639.2	$C_7H_8O_2$	620–24-6	13.2581
5	2,3-Butanediol	154.9900	34.3	$C_4H_{10}O_2$	513–85-9	0.8384
6	4-Hydroxycinnamic acid	146.9827	587.0	$C_9H_8O_3$	501–98-4	2.0856
7	1-Hexadecanol	243.1829	186.9	$C_{16}H_{34}O$	36,653–82-4	2.9460
8	Quercetin	303.0525	353.2	$C_{21}H_{20}O_{11}$	522–12-3	1.0755
9	Taxifolin	305.0642	297.6	C15H12O7	480–18-2	0.3650
10	Rutin	610.1934	674.8	$C_{27}H_3O_{16}$	153–18-4	28.6440
11	(-)-cis-Carveol	153.1282	300.8	$C_{10}H_{16}O$	2102–59-2	0.9400
12	1,2,3-Trihydroxybenzene	127.0395	32.8	$C_6H_6O_3$	87–66-1	0.6036
13	1-Naphthol	145.0654	286.8	$C_{10}H_8O$	90–15-3	0.2472
14	Methyleugenol	179.1074	453.4	$C_{11}H_{14}O_2$	93–15-2	0.5400
15	Procyanidin B2	579.1589	197.8	$C_{30}H_{26}O_{12}$	29,106–49-8	36.0256
16	5,7-Dihydroxyflavone	253.0507	426.6	$C_{15}H_{10}O_4$	480–40-0	0.1787
17	Tartaric acid	149.0097	41.4	$C_4H_6O_6$	87–69-4	0.4668
18	Salicylic acid	137.0249	138.4	$C_7H_6O_3$	69–72-7	1.2161
19	Sinapic acid	223.0615	90.2	$C_{11}H_{12}O_5$	530–59-6	0.0845
20	Catechin	289.0702	296.3	$C_{15}H_{14}O_6$	154–23-4	0.2386
21	Phenylpropanoate	149.0610	111.5	$C_9H_{10}O_2$	501–52-0	1.8195
22	3-O-Methylquercetin	315.0518	353.2	$C_{16}H_{12}O_7$	1486–70-0	0.1331
23	Hispidulin	299.0549	381.4	$C_{16}H_{12}O_6$	1447–88-7	0.1144
24	3-Hydroxybenzoic acid	138.0283	138.4	$C_7H_6O_3$	99–06-9	0.0934

determination of *Platycladus orientalis* (L.) Franco leaf polyphenol extract revealed varying scavenging abilities against DPPH, ABT S, •OH, and $O_2^{\bullet-}$. While DPPH and ABTS scavenging abilities were weaker than Vc, •OH and $O_2^{\bullet-}$ scavenging abilities were stronger (Ren et al., 2019).

Analysis of pecan 'Shaoxing' green husk identified 24 polyphenols, with the relative contents of rutin and proanthocyanidin B2 being predominant, which was consistent with (Lujan et al., 2021)'s findings, who identified proanthocyanidins B2 as the main compound. Proanthocyanidin B2 is a super antioxidant. Studies have shown that proanthocyanidin B2 may treat diabetic peripheral neuropathy (Zhang et al., 2018). Rutin has been proven to have a variety of pharmacological activities, including antioxidant, vascular protection and anticancer activities. Some studies have shown that the extracts of *Phoradendron serotinum* and *Croton lechleri* have cytotoxicity in vitro and anti-tumor effect in vivo, and the main component is rutin (Alonso-Castro et al., 2013). Other polyphenols tested, such as Gallic acid and catechin, have good antioxidant, antibacterial and anti-tumor activities. The effects of different concentrations of Gallic acid and catechin on the growth and metabolism of *Lactobacillus Hilgadii* 5w (a wine spoilage lactic acid bacterium) were reported (Alberto et al., 2004). With the increasing application of plant polyphenols in food additives, medicine, skin care and other industries, the study of polyphenols in the pecan 'Shaoxing' green husk is helpful to the development and utilization of pecan 'Shaoxing' green husk. For example, pecan 'Shaoxing' green husk polyphenols extract has antioxidant activity and can be used in food preservation.

Conclusion

The optimum conditions of ultrasonic extraction of polyphenols were determined through single-factor and response surface optimization tests. Ethanol served as the best extraction solvent, with a material-liquid ratio of 1:15 and an ethanol concentration of 58%. Ultrasound parameters included a 60 min duration, a power output of 160 W, and a temperature of 57 ℃. Under these conditions, polyphenol content reached 218.62 mg/g. By comparing the adsorption and desorption rates of six different macroporous resins, D-101, AB-8, NKA-9, NKA-2, HPD-100, and

X-5, the D-101 resin was chosen for purifying the pecan 'Shaoxing' green husk polyphenol solution. Following process optimization, the purification protocol included a sample concentration of 2 mg/mL, pH at 4, and a flow rate of 2 mL/min. Elution utilized ethanol at a concentration of 70%, with a flow rate of 3 mL/min. The purity of polyphenols increased from 31.45 to 69.34%.

The polyphenols from pecan 'Shaoxing' green husk demonstrated scavenging activity against free radicals DPPH, ABTS, $O_2^{\bullet-}$, and •OH. At a polyphenol concentration of 0.9 mg/mL, maximum scavenging activity for DPPH, ABTS, $O_2^{\bullet-}$, and •OH was achieved, reaching 95.36, 99.4, 50.92, and 51.89%, respectively. The scavenging activity of pecan 'Shaoxing' green husk polyphenols against DPPH, ABTS, and •OH radicals were similar to that of Vc, although slightly weaker against $O_2^{\bullet-}$ radicals compared to Vc solution. Therefore, the polyphenol extract from pecan 'Shaoxing' green husk has strong antioxidant activity, which provides a basis for the subsequent development and utilization of its antioxidant activity.

The polyphenols in pecan 'Shaoxing' green husk were identified using LC–MS, revealing 24 main polyphenols, notably including rutin and proanthocyanidin B2.

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Not applicable.

Authors' contributions
Chunhua Zhou and Tian Hu designed this experiment. Tian Hu performed the experiments, analyzed the data, organized the figures. Fei Wang performed parts of the experiments, Zhe Zhao and Kaifeng Hu analyzed the data. Tian Hu wrote and revised the manuscript, and Chunhua Zhou critically revised the manuscript.

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Data availability
All data generated or analyzed during this study are included in this published article.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors have no known conflicts of interest to declare.

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