Optimization of functional compounds extraction from *Ginkgo biloba* seeds using response surface methodology

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RESEARCH ARTICLE

Abstract

Ginkgo biloba seeds are important raw material for foods and medicines. A response surface method was used to obtain the following optimized extraction conditions for *Ginkgo biloba* seed extracts (GBSE) with the highest 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging ability: 0.08 g/mL material-to-liquid ratio, 70% ethanol concentration, 47°C extraction temperature and extraction time of 22 min. Fourier transform infrared spectroscopy revealed the polysaccharide structure of GBSE from three varieties of *Ginkgo biloba* seeds (Fozhi, Maling and Yuanling seed varieties). The extract yield, polysaccharides, total phenolics and total flavonoids in the three varieties were 5.77–6.11%, 11.45–364.69 mg/g, 22.34–25.54 mg/g and 14.87–16.47 mg/g, respectively. The GBSE has good antioxidant ability, including DPPH-reducing activity (1842.73–2616.00 micromol [mmol] Trolox Equivalents [TE]/gram [g]), ABTS (2,2-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid); 185.03–217.63 mmol TE/g) and ferric-reducing antioxidant power (FRAP; 220.46–230.77 mmol TE/g). This study provides a method for preparing GBSE with high antioxidant activity and improving the utilization value of *Ginkgo biloba* seeds.

Keywords: *Ginkgo biloba* seeds; response surface methodology; functional extracts; antioxidant activity

Introduction

Ginkgo biloba L. is the oldest species of trees that has existed on the earth for 200 million years (Liu *et al.,* 2021). As a traditional source of food, *Ginkgo biloba* (*G. biloba*) seeds are rich in proteins, starch, lipid, vitamins and other nutrients (Wang and Zhang, 2019), and are consumed by boiling, microwave heating, roasting and frying. *G. biloba* seeds also contain active substances such as polysaccharides, flavonoids and polyphenols (Mahadevan and Park, 2008) and are used in China as a traditional medicine to treat asthma, cough and allergic inflammation (Huang *et al.,* 2010). At present, the nutrient rich *G. biloba* seeds are mainly processed as typical foods such as various desserts, *G. biloba* seeds chicken stew, *G. biloba* seeds porridge and roasted or microwaved *G. biloba* seeds (Singh *et al.,* 2008; Yang *et al.,* 2011). However, its active ingredients and related products are less developed and utilized, and rough processing leads to weak economic benefits. Despite their abundant yield, *G. biloba* seeds have a low utilization rate, resulting in plenty of waste (Zou *et al.,* 2021b). Therefore, it is essential to seek new *G. biloba* seeds processing products, because improving the utilization rate of their functional components is essential for the comprehensive development of *G. biloba* seeds.

Preparation, characterization and functional evaluation of extracts derived from plant roots, leaves, flowers, fruits and seeds have received increasing attention (Elez Garofulić *et al.,* 2020; Fıçıcılar *et al.,* 2018; Galgano *et al.,* 2021). These extracts are mainly composed of plant metabolites, proteins and polysaccharides (Subaşı *et al.,* 2021; Campelo *et al.,* 2021). On the one hand,

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a multi-component extract is higher in functions than a single functional component such as antioxidation, anticancer and antibacterial activity (Bobinaitė *et al.,* 2013; Papoutsis *et al.,* 2021). On the other hand, the preparation method of a complex is convenient and straightforward. Previous studies have reported the extraction and preparation methods of single functional components (proteins, polysaccharides and flavonoids) in *G. biloba* seeds (Hu *et al.,* 2021). These processes cause loss of other active ingredients and a decrease in functionality. The ethanol extract of *G. biloba* leaves (Egb 761 [Ginkgo]) as a drug and dietary supplement has been developed and used in medicines and by the food industry (Chan *et al.,* 2007; Zhao *et al.,* 2012). The present study provides a reference for the multifunctional use of *G. biloba* seeds.

The effect of preparative conditions, including material-to-liquid ratio, solvent concentration, extraction temperature, and extraction time, on the content and activity of active ingredients of the extract are usually considered to obtain high-quality extracts (Nayak and Rastogi, 2013). First, the influence of one factor on the target value is considered by maintaining rest of the factors unchanged. Then, whether different aspects have an internal connection to the target value is determined. Response surface methodology (RSM) is usually used in optimization process because of its minimal number of experiments and the fastest experiment speed (Bezerra *et al.*, 2008). RSM results can intuitively reflect the influence of different factors and their interacting effect on response value. This method has been used for the extraction optimization of macromolecular substances, such as protein and polysaccharides, and other activity compounds such as phytochemicals or flavanols and polyphenols (Borges *et al.,* 2011; Lee and Yoon, 2021; Siddeeg *et al.,* 2015).

This study aimed to prepare *G. biloba* seeds with high 2-diphenyl-1-picrylhydrazyl (DPPH)-reducing activity. RSM was performed to optimize the preparation of *G. biloba* seed extracts (GBSE) with maximum DPPH-reducing activity under practical operating conditions (material-to-liquid ratio, ethanol concentration, extraction temperature, and extraction time). Furthermore, structure, composition and antioxidant activity of the extracts prepared from different varieties of *G. biloba* seeds (Maling, Fozhi and Yuanling varieties) were calculated and compared.

Materials and methods

Plant Materials

Fresh *G. biloba* seeds (Maling, Fozhi and Yuanling varieties) were purchased from Pizhou, Jiangsu Province, China. The seeds were collected after removing episperm, mesosphere and endopleura. The seeds were then freeze-dried (Christ plus freeze drier, Germany), pulverized and filtered using 80-mesh sieves.

Chemicals and reagents

Analytical grade sodium acetate, potassium persulfate, KCl, $AlCl_3$, $FeSO_4$, $FeCl_3 \cdot 6H_2O$, Na_2CO_3, NaOH, ethanol and acetic acid were obtained from Sinopharm Chemical Reagent Co. Ltd. (Beijing, China). Folin–Ciocalteu reagent was obtained from Yuanye Co. Ltd. (Shanghai, China). Gallic acid, ABTS (2,2-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)), DPPH, Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) and TPTZ (2,4,6-tri(4-pyridyl)-1,3,5-triazine; purity: ≥99.0%) were bought from Sigma-Aldrich Co. Ltd. (Shanghai, China).

Experimental design of response surface methodology

Effects of different extraction factors on the DPPH scavenging capacity of G. biloba *seed extracts*

Single-factor experiment was performed to determine the lower (-1) and upper levels (1) of RSM design variables for preparing GBSE with high DPPH scavenging ability (Gammoudi *et al.,* 2021). The four variable parameters were as follows: material-to-liquid ratio (0.2, 0.1, 0.06, 0.05, 0.04 and 0.02 g/mL), ethanol concentration (40, 50, 60, 70, 80 and 90% v/v), extraction temperature (30, 40, 45, 50, 55, 60 and 70°C) and extraction time (10, 20, 30, 40, 50, 60 and 70 min).

The extraction experiment was carried out using the above parameters, and the fourth factor was adjusted by keeping the other three factors unchanged. Extracts were shaken in a water bath. Different extraction parameters were set, and centrifugation was performed after extraction (4,000 r/min, 10 min, Sigma, model 2-16K, Germany). The supernatant (2 mL) was blended with 2 mL of 0.1-mol/L DPPH solution to avoid light penetration for 30 min at 517 nm (721G-100, Lichen, Shanghai, China) to determine light absorption value (Brand-Williams *et al.,* 1995). Results were expressed per gram (g) of *G. biloba* seeds to micromol (mmol) Trolox equivalent (TE).

Box–Behnken experimental design

After single factor test, material-to-liquid ratio, ethanol concentration, and extraction time and temperature were chosen as test factors to carry out 4^3-level Box–Behnken experiment. The DPPH scavenging ability of the extracts was used as response value to optimize extraction conditions. The factor level coding is shown in Table 1. Significance of the model and lack of fit were used to examine its applicability in predicting the DPPH

scavenging capacity under different extraction conditions (Javanmardi *et al.,* 2021). R^2, adjusted R^2 and predicted R^2 values were evaluated for predictability of the model (Ray *et al.,* 2020). The R^2 were calaculated based on the following equation:

$$R^2 = 1 - \frac{\sum_{i=1}^{n}(x_i - x_{if})^2}{\sum_{i=1}^{n}(x_i - x_y)^2}$$

Where, x_i is predicted value; x_{if} is the experimental or actual value; x_y is the mean of experimental value, and n is the number of observations.

The factors studied were expressed as a mathematical model by using the following second-order polynomial equation (Jabbar *et al.,* 2015):

$$Y = \alpha_0 + \sum_{i=1}^{n}\alpha_i X_i + \sum_{i=1}^{n}\alpha_{ii} X_i^2 + \sum_{i\neq j=1}^{n}\alpha_{ij} X_i X_j,$$

where Y is the investigated response, α_0 is the intercept, n is the number of factors analyzed (1–4), and α_i, α_{ii} and α_{ij} are linear, quadratic and interactive model coefficients, respectively. X_i and X_j indicate levels of independent parameters.

Preparation of *G. biloba* seed extracts

Three varieties of *G. biloba* seeds, including Fozhi, Maling and Yuanling, were used to prepare extracts. After preparation under optimal conditions, the extracts were concentrated and freeze-dried to calculate yield (%), and stored at –20°C for further analysis.

Fourier transform infrared (FT-IR) spectroscopy of *G. biloba* seed extracts

The FT-IR spectrum of GBSE was recorded on a Vertex 80V FT-IR spectrometer (BRUKER, Germany) at room temperature (Zou *et al.,* 2021a). The samples were mixed with potassium bromide (KBr; 1:100, g:g), ground and pressed into a tablet form prior to measurement. Determination conditions of RSM design variables were as follows: scanning wave number from 400 cm^{-1} to 4,000 cm^{-1}, scanning instances: 64 times, and resolution of 4 cm^{-1}.

Table 1. Independent variables and their levels used in response surface analysis.

Independent variables	Coded symbols	Level		
		–1	0	1
Material-to-liquid ratio (G/mL)	X_1	0.04	0.07	0.1
Ethanol concentration (%)	X_2	60	70	80
Extraction temperature (°C)	X_3	40	45	50
Extraction time (min)	X_4	10	20	30

Determination of primary functional compounds in *G. biloba* seed extracts

Determination of polysaccharide: The phenol–sulfuric acid method was applied to determine polysaccharide content in three varieties of GBSE (Azeem *et al.,* 2018). In brief, 1 mL of GBSE solution (1 mg/mL) was added with 1.0 mL of 6% phenol and 5.0 mL of concentrated sulfuric acid. After shaking and cooling, the absorption value was measured at 490 nm after coloration in a boiling water bath for 15 min. Glucose was used as a standard, and the total sugar content in GBSE was expressed as mg glucose/g of GBSE.

Determination of total phenolics: The Folin–Ciocalteu method was used to determine total phenolic content in three varieties of GBSE (Liu *et al.,* 2017). In brief, 1 mL of GBSE water solution (1 mg/mL) reacted with 5.0-mL Folin–Ciocalteu reagent and shaken for 10 min. The mixture was added with 10 mL of 10% Na_2CO_3 solution and allowed to stand for 60 min at room temperature. The supernatant was obtained and centrifuged at 3,500 r/min for 10 min, and the absorption value was determined at 765 nm. The total phenolic content in GBSE was calculated using gallic acid as a standard and expressed as mg gallic acid equivalent (GAE)/g of GBSE.

Determination of total flavonoids: The method described by Osae *et al.* (2019) was applied to detect total flavonoid content in the three varieties of GBSE. In brief, 0.5 mL of extracted solution (1 mg/mL) was mixed with 0.15 mL of $NaNO_2$ solution (5%, m/v) and allowed to stand for 6 min. The mixture was incubated with 0.15 mL of $AlCl_3$ solution (10%, m/v) for 6 min and added with 4% NaOH solution (2 mL). The reaction solution was diluted to 5 mL using distilled water and allowed to stand for 15 min. Absorbance at 510 nm was determined by a spectrophotometer. The total flavonoid content in GBSE was expressed as mg rutin equivalents (mg RE)/g of GBSE.

Determination of antioxidant capacity of *G. biloba* seed extracts

Determination of DPPH scavenging ability: A total of 2.0 mL GBSE solution (1 mg/mL) was mixed with 4.0 mL of

100 mmol/L DPPH solution (Brand-Williams *et al.,* 1995). The reaction was carried for 30 min in darkness at room temperature and the absorbance was recorded at 517 nm.

ABTS determination: Fresh ABTS solution was obtained by reaction of the same amounts of 2.45 mmol/L potassium persulfate solution and 7 mmol/L ABTS in dark at room temperature for 16 h (Re *et al.,* 1999). Prior to determination, ABTS solution was diluted with 80% ethanol to an absorption value of 0.70 ± 0.01 at 734 nm. In addition, 3.6 mL of ABTS solution was added to 0.4 mL of GBSE solution. After incubation for 6 min, the absorbance was measured at 734 nm.

Determination of ferric-reducing antioxidant power (FRAP): FRAP reagent was obtained by mixing 40 mmol/L TPTZ solution with 20 mmol/L $FeCl_3 \cdot 6H_2O$ solution and 300 mmol/L sodium acetate buffer (pH 3.6) in a ratio of 1:1:10 (Benzie and Strain, 1996). Approximately 0.2 mL of GBSE solution was mixed with 5 mL of FRAP reagent and incubated at 37°C for 30 min. The absorbance was measured at 593 nm. DPPH, ABTS and FRAP were expressed as: mmol TE per gram of GBSE.

Statistical analysis

All data were expressed as mean ± standard deviation (SD). Duncan's multiple range test and *t*-test were performed on the data using one-way ANOVA in the SPSS 20.0 software (SPSS Inc., Chicago IL, USA). Significant level of differences between different samples was determined at $P < 0.05$. Design–Expert version 8.0.6, DX8 (R2014a, The Mathworks Inc., Natick, USA) was used to optimize the extraction process.

Results and discussion

Effects of different extraction conditions on the DPPH scavenging ability of *G. biloba* seed extracts

As shown in Figure 1, four independent variables, including material-to-liquid ratio, ethanol concentration, extraction temperature and extraction time, had significant effects on the DPPH scavenging ability of *G. biloba* seed extracts. Figure 1A shows that with increase in material-to-liquid ratio, the DPPH scavenging ability of GBSE first increased and then decreased. When the material-to-liquid ratio was 0.1, the DPPH scavenging ability reached the highest value of 101.68 mmol TE/g. Therefore, the material-to-liquid ratio ranging from 0.04 to 0.1 was chosen for RSM assays (Table 1). The material-to-liquid ratio influences the efficiency of extraction process (Marincaș *et al.,* 2018). A high ratio of solvent-to-raw material allows the material to disperse easily in solvent and improves the dissolution of active ingredients from raw material (Prakash-Maran *et al.,* 2013). The DPPH scavenging ability of GBSE decreased gradually with increase in ethanol concentration. The highest value of DPPH (104.72 mmol TE/g) was obtained when the ethanol concentration was 70% (Figure 1B). According to the principle of similar compatibility, active ingredients in the raw material can be effectively extracted by employing the appropriate polarity of extraction solvent (Kaanin-Boudraa *et al.,* 2021). These results were in agreement with the research results obtained by Hassan *et al.* (2020). According to the obtained values, ethanol concentrations of 60–80% were selected for RSM experiments. The DPPH scavenging ability increased with increase in extraction temperature from 30 to 45°C but decreased slightly when extraction temperature increased from 50 to 70°C (Figure 1C). The maximum DPPH scavenging activity of 104.23 mmol TE/g was obtained at 40°C. Appropriate temperature accelerates dispersion of solid substances in liquids (Corrales *et al.,* 2009). However, excessive extraction temperature degrades bioactive compounds in food materials (Dahmoune *et al.,* 2013). The maximum DPPH scavenging ability of GBSE at 105.10 mmol TE/g was obtained when the extraction time was 30 min, and no significant difference was found with other extraction periods (Figure 1D). Extraction period had a significant effect on yield of active ingredients and extraction efficiency (Wang *et al.,* 2008). Therefore, extraction time was set ranging from 10 to 30 min for RSM study.

Establishment of regression equation and ANOVA

According to the single-factor experiment, material-to-liquid ratio, ethanol concentration, extraction temperature and extraction time significantly affect the DPPH scavenging activity of GBSE. An experiment with a 4^3-factor design (a total of 27 experiments) was performed to determine the most critical factors influencing the response (DPPH scavenging activity (Y) of GBSE). The four factors considered were material-to-liquid ratio (X_1), ethanol concentration (X_2), extraction temperature (X_3) and extraction time (X_4). Linear, interactive and quadratic effects of each independent variable on the DPPH scavenging activity of GBSE were given by the following equation:

$$\text{DPPH scavenging activity (Y)} = 105.27 + 8.28X_1 + 0.17X_2 + 8.37DX_3 + 5.36X_4 + 6.88X_1X_2 - 1.69X_1X_3 + 0.71X_1X_4 + 9.77X_2X_3 + 1.60X_2X_4 - 1.75X_3X_4 - 24.37X_1^2 - 22.46X_2^2 - 10.66X_3^2 - 14.82X_4^2.$$

Independent variables exhibited linear effects on the DPPH scavenging activity in test array during extraction. Determination coefficient of Y model ($R^2 = 0.9476$) was close to 1, indicating that the F-test was

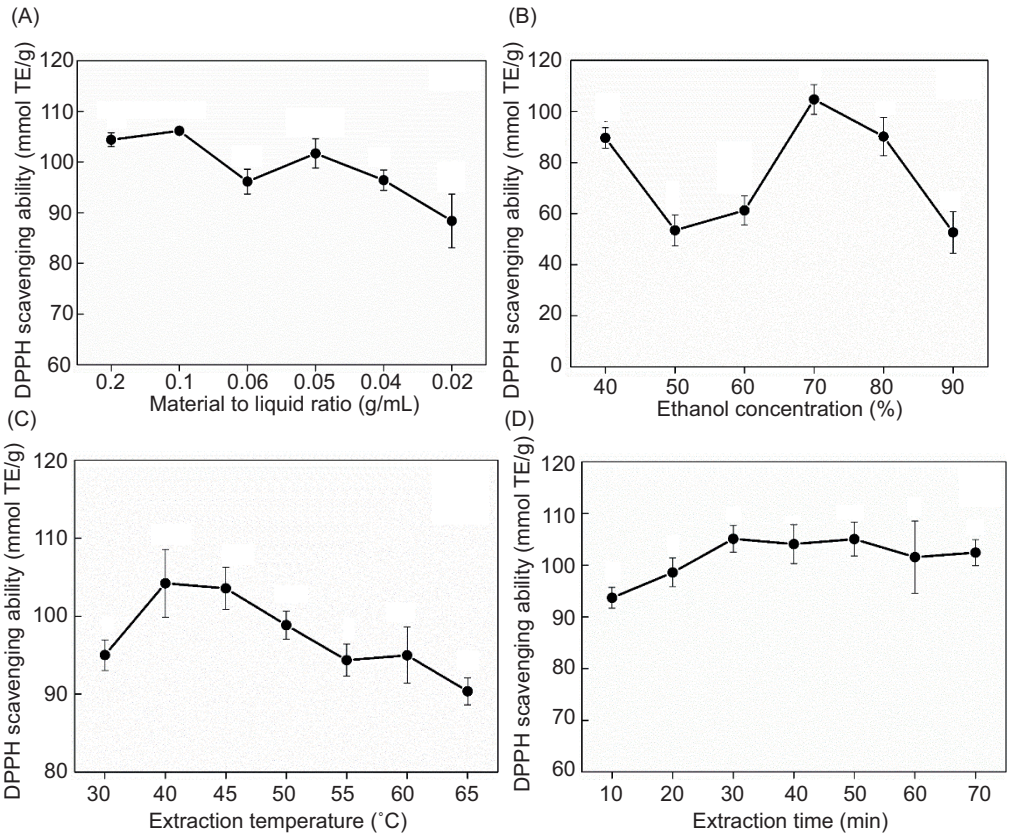

Figure 1. Results of single-factor experiments for functional compounds from *G. biloba* seeds (A) Material-to-liquid ratio. (B) Ethanol concentration. (C) Extraction temperature. (D) Extraction time. Each value represents the mean of three replicates, and error bars indicate standard deviation (±SD). Different letters indicate significant differences between different extraction conditions ($P < 0.05$).

not significant ($P > 0.05$), and a valid correlation existed between predicted and actual values shown in Table 2. Table 3 summarizes ANOVA and regression coefficients of the investigated model. The significance of the model ($P < 0.001$) and the pure error were significant. *P*-value for lack of fit was not significant (0.911 > 0.05). R^2, adjusted-R^2, predicted-R^2 and adequate precision values in this model were 0.9478, 0.8953, 0.8134 and 14.407, respectively. These values indicated that the model could be used to predict DPPH scavenging activity under different extraction conditions (Javanmardi *et al.,* 2021; Shen *et al.,* 2021). Material-to-liquid ratio (X_1), extraction temperature (X_3) and extraction time (X_4) significantly influenced the DPPH scavenging activity of GBSE ($P < 0.001$). However, ethanol concentration (X_2) significantly influenced Y at $P < 0.05$. Owing to quadratic effects, these four factors (X_1^2, X_2^2, X_3^2 and X_4^2) significantly affected the DPPH scavenging activity of GBSE.

Different independent variables interact to affect the extraction yields of response in the extraction of active substances (Jabbar *et al.,* 2015). Figure 2 shows the effects of independent variables and their mutual interaction on the DPPH scavenging activity of GBSE. Interaction of ethanol concentration with material-to-liquid ratio ($X_1 X_2$) and extraction time ($X_2 X_4$) was highly significant at $P < 0.05$. Kaanin-Boudraa *et al*. (2021) reported that ethanol concentration and material-to-liquid ratio could significantly affect the extraction of total phenolics from *Citrus paradisi* peels. X_1X_3, X_1X_4, X_2X_3 and X_3X_4 had no significant effects at *P* values of 0.8141, 0.5791, 0.6008 and 0.5662 respectively. These results indicated a clear secondary relationship of four factors with the DPPH scavenging activity of GBSE.

Optimization of interactions between the factors of response surface

Three-dimensional (3D) response surfaces were used to evaluate relationship between experimental levels of investigated factors and response (Triveni *et al.,* 2001). The 3D response surface graphs demonstrated significant ($P < 0.05$) and positive interactive effects of

Table 2. Box–Behnken design results with the obtained responses and predicted values for DPPH scavenging ability of GBSE.

	Independent variables				DPPH scavenging ability (mmol TE/g)	
Run	X_1 (G/mL)	X_2 (%)	X_3 (˚C)	X_4(min)	Experimental	Predicted
1	0.1	80	45	20	79.19	73.78
2	0.07	70	45	20	96.67	105.27
3	0.1	70	40	20	68.56	71.86
4	0.04	60	45	20	53.85	56.87
5	0.07	70	45	20	106.56	105.27
6	0.1	70	45	30	76.70	80.45
7	0.07	60	40	20	71.41	73.38
8	0.07	60	45	30	75.91	71.59
9	0.07	60	50	20	66.57	70.58
10	0.1	70	50	20	85.62	85.21
11	0.1	60	45	20	64.94	59.68
12	0.07	80	40	20	53.87	54.18
13	0.07	80	45	10	58.81	61.21
14	0.07	80	50	20	88.11	90.46
15	0.07	70	50	10	87.52	84.57
16	0.04	70	50	20	77.25	72.03
17	0.07	60	45	10	63.50	64.07
18	0.04	70	45	10	52.59	53.17
19	0.04	80	45	20	40.59	43.45
20	0.07	70	50	30	89.55	91.77
21	0.07	80	45	30	77.61	75.12
22	0.1	70	45	10	64.27	68.31
23	0.07	70	40	30	77.98	78.54
24	0.07	70	45	20	98.30	105.27
25	0.04	70	40	20	53.41	51.90
26	0.07	70	40	10	68.94	64.32
27	0.04	70	45	30	62.17	62.45

Note: The experimental values are mean ± SD of three replicates.

material-to-liquid ratio (X_1), ethanol concentration (X_2), extraction temperature (X_3) and extraction time (X_4) on the DPPH scavenging activity of GBSE (Figures 3A–F). As shown in Figure 3A, interaction between ethanol concentration and material-to-liquid ratio significantly affected the DPPH scavenging ability of GBSE as depicted by the elliptical shape of contour plot (Triveni *et al.,* 2001). When the extraction temperature was 40°C, extraction time was 30 min and material-to-liquid ratio was constantly changed, the DPPH scavenging ability of GBSE first increased and then decreased with increase in ethanol concentration. Figure 3e shows the significant influence of ethanol concentration and extraction time on the DPPH scavenging activity of GBSE at a certain material-to-liquid ratio and extraction temperature. Therefore, preparation of highly active GBSE required suitable material-to-liquid ratio, ethanol concentration, and extraction time and temperature.

Model optimization and validation

Design-Expert 8.0.6.1 (Stat-Ease Inc., Minneapolis, MN, USA) was used to optimize the extraction procedure that maximized the DPPH scavenging activity of GBSE. The optimal preparative conditions of GBSE with maximized DPPH scavenging activity were as follows: material-to-liquid ratio 0.08 g/mL, ethanol concentration 71.29%, extraction temperature 47.12°C and extraction time 21.97 min. The predicted value of DPPH scavenging activity of GBSE was 108.24 ± 0.05 mmol TE/g. A verification experiment using the material-to-liquid ratio

Table 3. ANOVA and regression coefficients of the extraction conditions model for response variable (DPPH radical scavenging ability).

Source of variation	Coefficient	DF	Mean square	*F*-value	*P*-value
Model		14	644.30	18.10	<0.0001**
X_1	8.28	1	823.50	23.13	0.0003**
X_2	0.17	1	0.34	0.011	0.0239*
X_3	8.37	1	344.30	9.67	0.0077**
X_4	5.36	1	840.91	23.62	0.0003**
X_1X_2	6.88	1	189.27	5.32	0.0370*
X_1X_3	–1.69	1	2.04	0.057	0.8141
X_1X_4	0.71	1	11.48	0.32	0.5791
X_2X_3	9.77	1	10.21	0.29	0.6008
X_2X_4	1.60	1	381.95	10.73	0.0055**
X_3X_4	–1.75	1	12.29	0.35	0.5662
X_1^2	–24.37	1	3851.23	108.17	<0.0001**
X_2^2	–22.46	1	3273.01	91.93	<0.0001**
X_3^2	–10.66	1	1423.87	39.99	<0.0001**
X_4^2	–14.82	1	737.17	20.71	0.0005**
Residual		14	35.60		
Lack of fit		10	23.75	0.368	0.9111
Pure error		4	65.23		
R^2		0.9476			
R^2 (adjusted)		0.8953			
R^2 (predicted)		0.8134			
Adequate precision		14.407			
CV (%)		7.92			

Note: *P < 0.05: significant difference; **P < 0.0001: very significant difference. DPPH: 2,2-diphenyl-1-picrylhydrazyl

of 0.08 g/mL, ethanol concentration of 71%, extraction temperature of 47°C and extraction time of 22 min was conducted to ensure the feasibility of optimized procedure. Three repeated experiments were carried out based on the above conditions, and the obtained DPPH scavenging activity of GBSE was 107.97 ± 0.91 mmol TE/g. This value was close to the theoretically predicted value. Therefore, the Box–Behnken model successfully optimized GBSE extraction conditions and provided a correct and reliable prediction (Zhang *et al.,* 2013).

FT-IR spectra of GBSE

The FT-IR spectra of GBSE for Fozhi, Maling and Yuanling varieties were recorded at 400–4,000 cm^{-1} wavelengths to characterize molecular characteristics of GBSE and determine their structures (Figure 3). The FT-IR spectra of three GBSE varieties were similar. Figure 3 shows the FT-IR spectra of specific polysaccharides from the extracts of three GBSE varieties (Zou *et al.,* 2021a). The absorption peak of C = O was located near 592.68 cm^{-1}. The absorption peak at 1052.08 cm^{-1} was O-H variable angle and C-O stretching vibration on the carboxyl group. The peak of 1403.72 cm^{-1} was the C-H variable angle vibration peak. The C-O bond vibration of carboxylate occurred near 1589.47 cm^{-1}. The absorption peak near 2936.47 cm^{-1} was derived from the stretching of C–H on polysaccharides (Li *et al.,* 2021). GBSE demonstrated strong and broad absorption peaks at around 3361.84 cm^{-1}, which was due to O-H stretching vibration (Yan *et al.,* 2015).

Functional compounds and antioxidant capacity of GBSE

Key functional compounds and antioxidant activity of GBSE prepared from Fozhi, Maling and Yuanling varieties are shown in Table 4. Maximum extraction yield of Fozhi and Yuanling varieties was significantly higher than that of Maling variety. Maximum polysaccharide contents in GBSE were found in Maling and Yuanling varieties at 364.69 mg/g and 363.72 mg/g, respectively. Polysaccharides determined in *G. biloba* seeds have

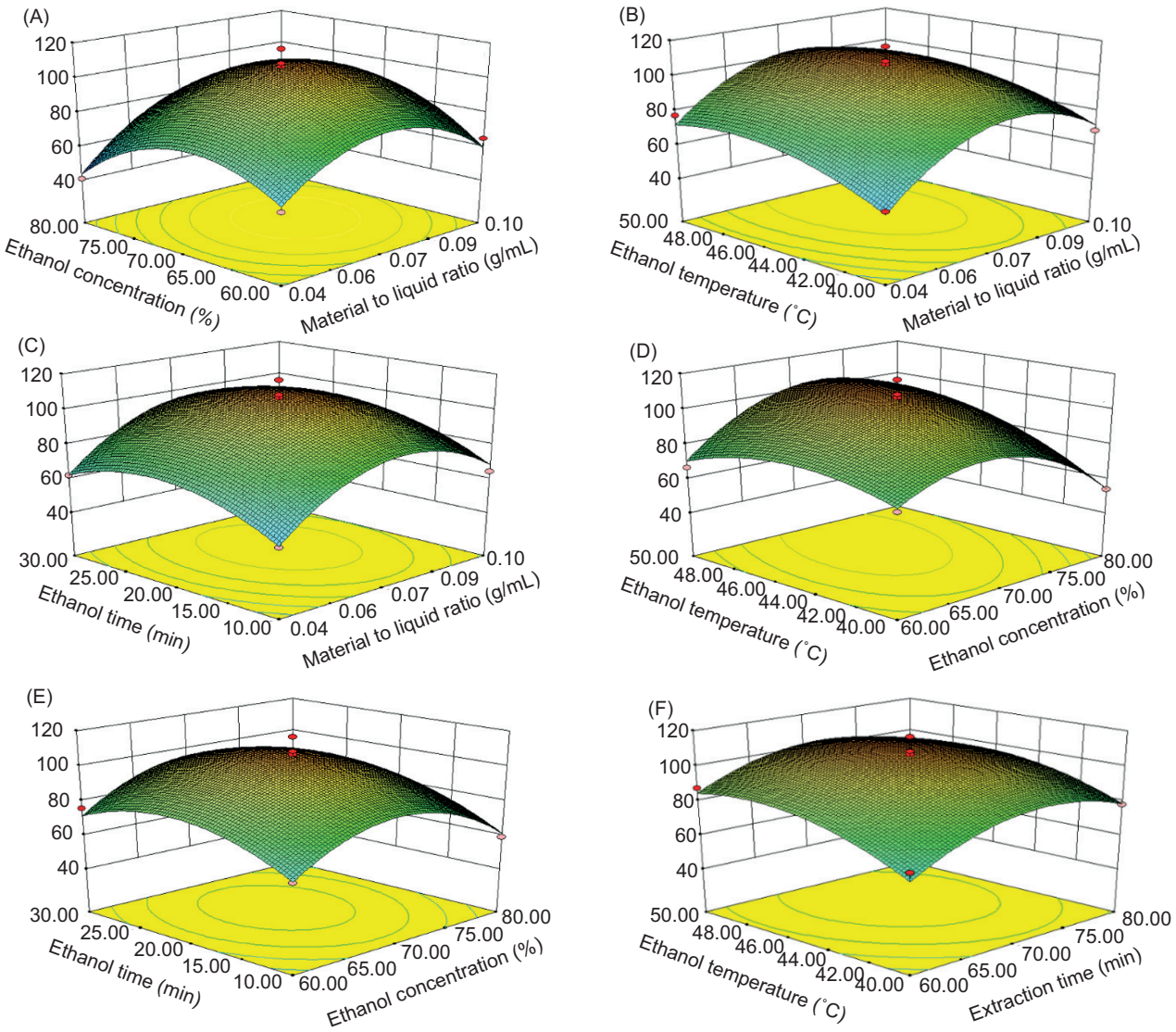

Figure 2. Response surface analysis for the DPPH scavenging ability of GBSE with respect to: (A) Material-to-liquid ratio and ethanol concentration. (B) Material-to-liquid ratio and extraction temperature. (C) Material-to-liquid ratio and extraction time. (D) Ethanol concentration and extraction temperature. (E) Ethanol concentration and extraction time. (F) Extraction temperature and extraction time. DPPH: 2,2-diphenyl-1-picrylhydrazy.

a good antioxidant activity (Wang and Zhang, 2019). As a major component, polysaccharides play an essential role in the biological function of GBSE. The highest total phenolics were recorded in the GBSE prepared from Maling variety (25.54 mg/g) and the lowest was determined for Fozhi variety (22.34 mg/g). Total flavonoid contents of GBSE from its three varieties ranged from 14.87 mg/g to 16.47 mg/g, and the lowest lavonoid content was obtained from Fozhi variety. The antioxidant activity of *G. biloba* seeds was limited by their low flavonoid content (Shen *et al.,* 2020). Through optimized extraction conditions, the effective enrichment of flavonoids became conducive to GBSE functioning. GBSE may also contain small amounts of proteins, peptides and other unknown components (Huang *et al.,* 2010). Antioxidant activity of the three varieties of *G. biloba* seeds, including DPPH, ABTS and FRAP, was also investigated. Fozhi variety had the highest DPPH-reducing ability at 2,616.00 mmol TE/g. Yuanling variety had the highest ABTS value at 217.63 mmol TE/g. No significant difference was observed between Fozhi and Yuanling varieties concerning FRAP, and its value was significantly higher than that of Maling variety. Differences in the quality of *G. biloba* seeds were established between different varieties, and this was the main reason for variation in the composition and functioning of GBSE (Gong *et al.,* 2019). These results indicated that GBSE had potential antioxidant activity.

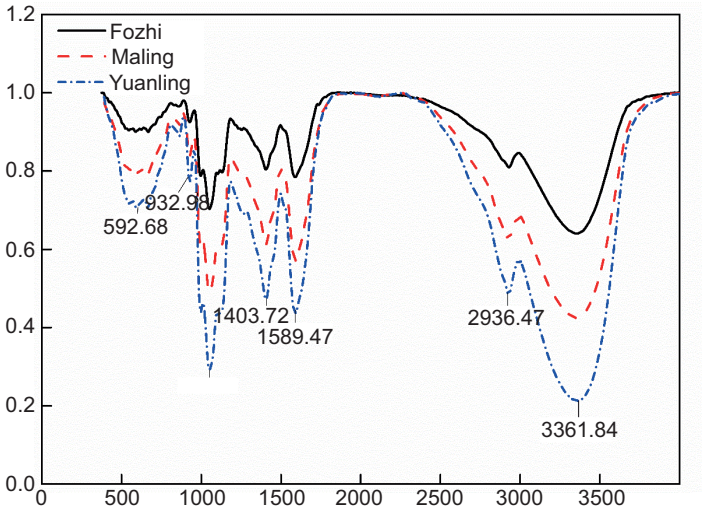

Figure 3. Fourier infrared absorption spectra of three different varieties of GBSE.

Table 4. Composition and antioxidant capacity of GBSE prepared from different varieties.

Sample	Fozhi	Maling	Yuanling
Yield (%)	6.16 ± 0.06^{a}	5.77 ± 0.13^{b}	5.92 ± 0.31^{a}
Total sugar (mg/g)	311.45 ± 6.06^{b}	364.69 ± 4.98^{a}	363.72 ± 19.49^{a}
Total polyphenols (mg/g)	22.34 ± 0.56^{c}	25.54 ± 0.84^{a}	24.26 ± 0.05^{b}
Total flavonoids (mg/g)	14.87 ± 0.25^{c}	16.47 ± 0.89^{a}	15.20 ± 0.37^{b}
DPPH (mmol TE/g)	2009.13 ± 122.35^{b}	2616.00 ± 14.68^{a}	1842.73 ± 24.47^{c}
ABTS (mmol TE/g)	185.03 ± 10.10^{b}	194.79 ± 8.30^{b}	217.63 ± 12.16^{a}
FRAP (mmol TE/g)	220.46 ± 0.09^{b}	230.47 ± 0.09^{a}	230.77 ± 0.05^{a}

Note: The values are mean ± SD of three replicates. Different superscript letters ($^{a–c}$) in the same line show significant difference at $P < 0.05$. GBSE: *Ginkgo biloba* seed extracts.

Conclusion

GBSE had the highest DPPH-reducing ability if prepared under the following extraction conditions: material-to-liquid ratio of 0.08 g/mL, ethanol concentration of 70.0%, extraction temperature of 47°C and extraction time of 22 min. Thus, the response surface method model indicated that material-to-liquid ratio and extraction time and temperature had a meaningful effect on the DPPH-reducing activity of GBSE. In addition, material-to-liquid ratio, ethanol concentration, and extraction time and temperature showed significant interaction. GBSE samples from the three varieties of *G. biloba* seeds (Fozhi, Maling and Yuanling) extracted under optimal conditions contained polysaccharides, total phenolics and total flavonoids. These substances had important contributions to the antioxidant activity of GBSE. Furthermore, the prepared GBSE also had good antioxidant capacity, including DPPH, ABTS, and FRAP. This work successfully optimized the preparative conditions of GBSE by applying response surface method and enriching the functional components of *G. biloba* seeds. Hence, GBSE could become an alternative functional food in the food processing industry.

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