Optimization of Ultrasonic-assisted Extraction and Fatty Acid Composition of Oil from *Paeonia suffruticosa* Andr. Seed

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Abstract: Response surface methodology (RSM) was applied to optimize the effects of extraction parameters including time, power, temperature and liquid-to-solid ratio on peony seed oil yield. Box-Behnken design (BBD) was employed for optimization of extraction parameters in oil yield that extracted assisting by ultrasonic while petroleum ether as solvent. The chemical composition of peony seed oil under optimal condition in ultrasonic-assisted extract method was analyzed by gas chromatography-mass spectrometry (GC-MS). The optimal conditions were that extraction time 45 min, extraction temperature 45℃, extraction power 90 W and liquid-to-solid ratio 7:1, respectively. Under this condition, the extraction yield value was 33.90% which was with 95% confidence level, hence indicated the reliability of RSM in optimizing ultrasonic-assisted extraction of oil from *Paeonia suffruticosa* Andr. seed. Three unsaturated fatty acid of peony oil such as n-3 α-linolenic acid (39.75%), n-6 linoleic acid (26.32%) and the oleic acid (23.66%), totally more than 89.00% was determined at optimum condition.

Key words: *Paeonia suffruticosa* Andr. seed oil, ultrasonic-assisted extraction, response surface optimization, GC-MS analysis

1 Introduction

*Paeonia suffruticosa* Andr. (peony) has long been known for its beautiful flowers, which are also applied in food, such as teas, cakes, and jam. Peony’s velamen usually presents in herb (named Mudanpi). Paeonol (2’-hydroxy-4’-methoxyacetophenone), a primary active component of the peony velamen, could improve the depression of the HMGB1–NF-κB P65 signaling pathway and serve as an essential treatment for acute lung injury\(^1\). And peony bud extract exerts an antibacterial effect in microbiology experiments. Extract fights against *Staphylococcus aureus* and *Escherichia coli* O157:H7 by decreasing bacterium’s membrane fluidity through reducing unsaturated fatty acids, which would destroy the cell membrane and inhibits the transcription of virulence factors\(^2\). Even though there are various ways of utilization of different parts of peony, people still miss peony seed. Peony seed has high polyunsaturated fatty acid (PUFA) content and oil yield, with high levels of α-linolenic acid (ALA) and low levels of n-6/n-3 fatty acids, which are uncommon situation in seed oils\(^3\). In view of basic needs of cell building and the difficulty of inversion between n-3 PUFAs and n-6 PUFAs, the lower n-6/ n-3 fatty acids rate oil has, the more benefit for body. Meanwhile, it’s also confirmed that ALA can be converted into eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) through desaturase in vivo with low conversion rate\(^4\). Besides, an in vitro assay confirmed that peony oil is a more potent scavenger of free radicals than extra virgin olive oil\(^5\). In 2011, the ninth document of the Ministry of Health issued in China listed purple peony and ‘Fengdan’ as a new food resource\(^6\). In addition, peony occupies a low acreage of cropland given that it is usually grown in mountainous regions and has a long cultivation history and a wide planting range in Heze, Shandong, Luoyang, Henan, and other places. Previous studies demonstrate that the peony can be developed as an emerging woody plant resource for dietary supplementation. Oil species are plants especially with oil contents of more than 8%\(^7\). Oil plants

Abbreviations: RSM - Response surface methodology, BBD - Box–Behnken design, GC-MS - gas chromatography-mass spectrometry, peony - *Paeonia suffruticosa* Andr., NIST98 MS library - NIST98 Mass Spectral Library, ANOVA - analysis of variance, adj \(R^2\) - Adjusted R-squared, pre \(R^2\) - Predicted R-squared.

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Accepted October 5, 2020 (received for review July 28, 2020)
Journal of Oleo Science ISSN 1345-8957 print / ISSN 1347-3352 online
http://www.jstage.jst.go.jp/browse/jos/ http://mc.manuscriptcentral.com/jjocs
are prolific in China and widely applied in biomass fuel, industrial processes, spices, medicine, and food. *Camellia oleifera*, *Vernicia fordii*, *Juglans*, and *Sapium sebiferum* are the four major woody oil crops in China. Among these species, *C. oleifera* and *Juglans* are edible oil plants. Using ultrasonic-assisted extraction, a study confirmed that the common oil yield of *C. oleifera* is between 16.25% to 35.49%, and the unsaturated fatty acid content of oil from this plant ranges from 83.56% to 91.50%\(^7\). The oil yield of *Juglans* is 45%–48%, and the unsaturated fatty acid content of its oil exceeds 90%\(^8\). Compared with common oil plant’s oil yield rate and unsaturated fatty acid content, it can be deduced that peony can be an eligible woody oil plant.

For the development of "green extraction" which determines to protect the environment and consumers, the extraction process as a unit operation would more focus on diminishing ecologic damage and material waste\(^9\). To higher extraction efficiency and lower energy consumption, the conventional extraction process, such as press and Soxhlet extraction, gradually replaced by innovative technologies. Three methods assisted by innovative technologies for oil extraction include ultrasonic-assisted extraction, microwave-assisted extraction, supercritical carbon dioxide fluid extraction. Supercritical carbon dioxide fluid extraction and provide the highest oil yield but limited by equipment\(^10\). Microwave-assisted and ultrasonic-assisted extraction method are more convenient than supercritical carbon dioxide fluid extraction and show higher oil yield than the conventional processes. Compared with the other two extraction processes, ultrasonic-assisted extraction is the most appropriate method given its faster and more effective energy use, high extraction yield, safety, complexity, and rapidity\(^11\). With the microscopic observations, the chain detexturation mechanism which ultrasound helps release the deeper substance had clarified in a special order following treating time: local erosion, shear forces, sonoporation, fragmentation, capillary effect, and detexturation is proved\(^12\). And ultrasound effect not limits to the surface but goes deeper to disruption at the tissue level, which helps release more target content. However, the fatty acid extracted by ultrasound usually shows slightly higher in peroxide value than conventional method\(^13,14\). It is proved that initiators, oxygen, enzymes, metals, light and temperature lead fatty acid to oxidation\(^15\). Avoiding reaction system attaching to oxygen and metal may be an effective method to diminish the degradation and enhance the quality of the oil.

Response surface methodology (RSM) is a collection of mathematical and statistical techniques for modeling and analysis of problems to optimize a response of interest that is influenced by several quantifiable variables (factors)\(^15\). There are many RSM methods, and the more commonly used are Box-Behnken Design (BBD) and Central Composite Design (CCD). BBD is suitable for three-level optimization experiments with 2 to 5 experimental factors, while CCD is generally suitable for three-level optimization experiments with three experimental factors. Compared with CCD, BBD is more suitable in employing for optimization of processes that sets 4 variables and gives its rational design and excellent analysis\(^16\). However, the precision of analysis result is easy affected by the optimal selection of factor’s level. It’s better to perform single-factor experiment in advance for optimal factor level selection. The single-factor experiment would keep one factor change in wider range while the other factors hold the line. And then finding the relationship between result and the variable for easy narrow the factor level.

In this study, a single-factor experiment was first performed in order to select suitable factor levels. The ultrasonic-assisted extraction method will be used, the seeds of the oil peony variety “Fengdan”\(^17\) are used as raw materials, and petroleum ether is used as the extractant. Based on the single-factor experiment, BBD will be used in a narrow level\(^18\). Using multiple quadratic regression equations to fit the relationship between response values and factors, using multiple quadratic equation models to determine the best extraction process for the four factors of time, temperature, power, and liquid-to-solid ratio; finally, GC-MS analyzes the composition of unsaturated fatty acids in crude oil made by optimal technology in order to provide technical support for the development and utilization of peony seed oil.

### 2 Materials and Methods

#### 2.1 Plant material

Peony ('Fengdan', originating from Bozhou, Anhui) seeds were oven dried at 60°C for 72 h. Cores were separated from seeds by hand. The samples were smashed through a 60-mesh sieve into uniformly sized grains and stored at room temperature (25°C).

#### 2.2 Chemicals and reagents

Pure water was made by Barnstead in the laboratory. Petroleum ether (boiling range, 30-60°C), sulfuric acid, methanol, hexane, and anhydrous sodium sulfate were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China).

#### 2.3 Apparatus

A 200 W 40 kHz SB-5200DTD ultrasonic cleaner (Ningbo Scientz Biotechnology Co., Ltd., Ningbo, China) was used for oil extraction from peony seeds. The seeds were pulverized using a 14,000 W, 34,000 r/min multifunction pulverizer. GC-MS analysis was performed by gas chromatography–triple quadrupole mass spectrometer purchased from

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2.4 Ultrasound-assisted extraction

Ultrasound-assisted extraction was performed in a temperature-controlled ultrasonic cleaner following single-factor experiment table or BBD factor design table. With a view to actual input power from the device would converted to heat which is dissipated in the medium, the actual absorbing ultrasound power ought to be assessed by calorimetric measurements, calculated as shown in the Eq. (1) below:

\[ P = m \times c_p \times \frac{dT}{dt} \]

where \( c_p \) is the heat capacity of the solvent at constant pressure (J/g°C), \( m \) is the mass of solvent (g) and \( \frac{dT}{dt} \) is temperature rise per second.

However, the solvent used in this experiment is petroleum ether which is heterogeneous system that can’t determine the heat capacity. Therefore, the output power of the device is still used in the experimental data to represent the actual power.

The liquid-to-solid ratio was monitored under the table and was varied from 3:1 to 9:1. The mixture of peony seed powder and petroleum ether solvent was placed in a beaker and sealed with plastic wrap. The controlled ultrasonic cleaning instrument was set at a constant temperature of 45°C, the liquid-to-solid ratio of 1:5, and extraction time of 50 min. The effect of liquid-to-solid ratio (3:1, 4:1, 5:1, 6:1, 7:1, 8:1, and 9:1) on oil yield was determined with petroleum ether as the solvent under the following conditions: temperature of 45°C, power of 100 W, and extraction time of 50 min. The effect of time (20, 30, 40, 50, 60, and 70 min) on oil yield was determined with petroleum ether as the solvent under the following conditions: temperature of 45°C, power of 100 W, and liquid-to-solid ratio of 1:5.

2.5 Experimental design

2.5.1 Single-factor experimental design

The effect of temperature (30°C, 35°C, 40°C, 45°C, 50°C, 55°C, and 60°C) on oil yield was determined with petroleum ether as the solvent under the following conditions: liquid-to-solid ratio of 1:5, extraction time of 50 min, and power of 80 W. The effect of power (60, 80, 100, 120, 140, and 160 W) on oil yield was determined with petroleum ether as the solvent under the following conditions: temperature of 45°C, the liquid-to-solid ratio of 1:5, and extraction time of 50 min. The effect of liquid-to-solid ratio (3:1, 4:1, 5:1, 6:1, 7:1, 8:1, and 9:1) on oil yield was determined with petroleum ether as the solvent under the following conditions: temperature of 45°C, power of 100 W, and extraction time of 50 min. The effect of time (20, 30, 40, 50, 60, and 70 min) on oil yield was determined with petroleum ether as the solvent under the following conditions: temperature of 45°C, power of 100 W, and liquid-to-solid ratio of 1:5.

2.5.2 RSM design and data analysis

The extraction temperature, extraction power, liquid-to-solid ratio, and extraction time were selected as the variable factors and were represented as A, B, C, and D, respectively. Oil yield was used as the evaluation index. Based on the single-factor experiment results, three appropriate factor levels were selected and coded as −1, 0, and 1 (Table 1). A three-level, four-variable BBD was employed. A total of 29 experiments (Table 2) were required for the optimization of the extraction parameters. An efficient experimental process was used to obtain representative experimental data.

The second-order polynomial Eq. (3), which includes all interaction terms, was used to calculate predicted response (y):

\[ y = b_0 + \sum_{i=1}^{4} b_i x_i + \sum_{i=1}^{4} b_{ij} x_i^2 + \sum_{i<j}^{4} b_{ij} x_i x_j \]

where \( y \) is the response, \( b_0 \) is the offset term, \( b_i \) is the linear effect, \( b_{ij} \) is the squared effect, \( b_{ij} \) is the interaction effect, and \( x_i \) and \( x_j \) are independent variables.

Data were analyzed by Design Expert (10.0.7), and coefficients were interpreted using F-test. Analysis of variance (ANOVA), regression analysis, and plotting of response surface plots were conducted to examine the significance of the data and the quality of model fit.

2.6 Pretreatment: methyl esterification

Long-chain fatty acids are unstable at high temperatures and are easily lost during the analysis, so they cannot be analyzed directly through GC-MS unless they are derivatized into volatile methyl esters. In order to get stationary phase and clear peak, before analyzing the components presented in the table below:

### Table 1 Independent variable and their level for BBD.

| Factor | Level | Temperature /°C | Power /W | Liquid-to-solid ratio /mL/g | Time /min |
|--------|-------|-----------------|----------|-----------------------------|-----------|
| A      | −1    | 35              | 30       | 6:1                         | 30        |
| B      | 0     | 42.5            | 40       | 7:1                         | 40        |
| C      | 1     | 50              | 50       | 8:1                         | 50        |

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of fatty acids and fats, long-chain fatty acids are reacted with methanol and transformed into fatty acid methyl esters to reduce the boiling point and improve their stability. About Feng Yin’s research, peony seed oil was pre-treated through sulfuric acid–methanol esterification. Peony seed oil (1 g) was placed in a conical flask and added with 10 mL of sulfuric acid–methanol (volume ratio 1:10, v/v). The mixture rested for 10 min at room temperature. An equivalent volume of hexane was added for extraction. The remainder was extracted with an equivalent volume of hexane. The two extracts were combined and washed with pure water several times to layer the product. The extract was dried with anhydrous sodium sulfate. After filtration and centrifugation (5,000 r/min, 10 min), the supernatant was subjected to GC-MS analysis.

### 2.7 GC-MS analysis

Chromatography was performed with HP-5 capillary column (30 mm × 0.25 mm, 0.33 m). The initial temperature was held at 140°C for 5 min, then increased at the rate of 4°C/min to the final temperature of 240°C, and held for 25 min. The carrier gas was high-purity helium, and the

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### Table 2  BBD of factors with coded value.

| Run | Factors | Experimental oil yield (%) |
|-----|---------|---------------------------|
|     | A  | B  | C     | D    |                          |
| 1   | -1 | 0  | 0     | 1    | 29.06                    |
| 2   | 0  | 1  | 0     | 1    | 31.80                    |
| 3   | 0  | 0  | -1    | 1    | 28.75                    |
| 4   | 0  | -1 | -1    | 0    | 27.67                    |
| 5   | 0  | 1  | 0     | -1   | 25.58                    |
| 6   | 1  | 0  | 0     | -1   | 26.09                    |
| 7   | 0  | 0  | 0     | 0    | 33.55                    |
| 8   | 1  | -1 | 0     | 0    | 29.36                    |
| 9   | 0  | 0  | 0     | 0    | 32.31                    |
| 10  | 1  | 0  | 1     | 0    | 30.52                    |
| 11  | -1 | 0  | 0     | -1   | 27.35                    |
| 12  | 1  | 0  | 0     | 1    | 31.31                    |
| 13  | 0  | 0  | 1     | -1   | 26.11                    |
| 14  | 0  | 1  | 1     | 0    | 31.29                    |
| 15  | 0  | 0  | 1     | 1    | 31.28                    |
| 16  | 1  | 0  | -1    | 0    | 27.41                    |
| 17  | -1 | 0  | -1    | 0    | 27.39                    |
| 18  | 0  | -1 | 0     | -1   | 26.81                    |
| 19  | 0  | 1  | -1    | 0    | 27.56                    |
| 20  | 1  | 1  | 0     | 0    | 31.93                    |
| 21  | 0  | 0  | 0     | 0    | 33.36                    |
| 22  | 0  | 0  | 0     | 0    | 33.29                    |
| 23  | -1 | 1  | 0     | 0    | 28.87                    |
| 24  | 0  | 0  | -1    | -1   | 27.22                    |
| 25  | 0  | -1 | 0     | 1    | 29.58                    |
| 26  | 0  | 0  | 0     | 0    | 33.54                    |
| 27  | -1 | 0  | 1     | 0    | 27.65                    |
| 28  | -1 | -1 | 0     | 0    | 28.39                    |
| 29  | 0  | -1 | 1     | 0    | 27.65                    |
flow rate was 2.25 mL/min. The inlet temperature was 250°C, the sample volume was 1 μL, and the split ratio was 50:1.

Mass spectrometry was performed under the following conditions: ion source for EI source, ion source temperature of 230°C, electronic energy of 70 eV, and mass scanning range of 40–450 m/z.

Quantitative analysis was performed by referring to the NIST98 MS library and consulting the relevant literature. The composition and relative percentage content of the fatty acids of peony seed oil were analyzed.

3 Results and Discussion

3.1 Single-factor experiment

The first single-factor experiment set temperature as the variable that change in the range of 30, 35, 40, 45, 50, 55, 60°C when liquid-to-solid ratio was 5:1, time was 50 min, and power was 80 W.

As shown in Fig. 1 (A), the oil yield of peony seed first increased from 28.55% to 33.05% when the temperature rose from 30°C to 45°C, and then decreased from 33.05% to 29.78% when the temperature rose from 45°C to 60°C. The oil yield peaked at 45°C. At 30°C–45°C, higher temperatures reduced oil viscosity and facilitated the movement of oil molecules from the seeds. Thereby extraction accelerated and oil yield increased. However, high temperatures also promoted solvent volatilization which induced the oil–solvent contact time decrease. Moreover, heat may induce oil denaturation. These all effects eventually led to a decrease in oil yield at 45–60°C.

The second single-factor experiment set power as variable that change in the range of 60, 80, 100, 120, 140, 160 W when liquid-to-solid ratio was 5:1, time was 50 min, and the temperature was 45°C.

Figure 1 (B) shows that the highest oil yield of 33.39% was obtained when the power was set at 80 W. The oil yield of peony seed first increased at 60–80 W and then decreased at 80–160 W with increasing power. With increasing power, the cavitation effect and mechanical action intensified, which led to the increased frequency of blasting and an increase in oil dissolution. However, when the power exceeded 80 W, the thermal effect promoted the decomposition and degeneration of fatty acids as well as peony seed oil. That would reduce the oil yield.

The third single-factor experiment set liquid-to-solid ratio as variable that changes in the range of 3:1, 4:1, 5:1, 6:1, 7:1, 8:1, 9:1 when power was 100 W, time was 50 min, and the temperature was 45°C.

As shown in Fig. 1 (C), the maximum oil yield of 31.09% was obtained when the liquid-to-solid ratio was 7:1. When the liquid-to-solid ratio was 3:1–7:1, the oil yield steadily increased. The increase in the difference in oil concentration between peony grains and solvent increased the diffusion rate, which resulted in the increase in the oil yield. Furthermore, increasing the extraction solvent was conducive to increasing the contact area between the solvent and the peony grains, facilitating the extraction of oil.

Fig. 1 Effect of temperature (A), ultrasonic power (B), liquid-to-solid ratio (C), and time (D) on the extraction yield of oil from seeds of P. suffruticosa.
peony grains. Continue to increase the amount of solvent, the concentration of oil and fat in the solution drops, resulting in excess leaching agents, which goes against the concept of green extraction. And because of the large amount of leaching agent, multiple suction and distillation are required, which is easy to cause oil loss during operation. Thus, the ideal range selected for the liquid-to-solid ratio was 6:1–8:1.

The fourth single-factor experiment set time as variable that change in the range of 20, 30, 40, 50, 60, 70 min when power was 100 W, time was 50 min, and liquid-to-solid ratio was 5:1.

The effect of time on oil yield is shown in Fig. 1. The maximum oil yield was 30.62 when the extraction time was 40 min. The oil yield increased gradually as the extraction time was extended from 20 min to 40 min. Under prolonged extraction time, the physicochemical properties of oil changed due to the thermal effects of ultrasonic waves. This effect led to a reduction in the oil content. Hence, appropriately extending the solid–liquid contact time is conducive to the full dissolution of fatty acids.

3.2 Optimization of ultrasonic-assisted extraction

3.2.1 Model fitting

Among the 29 experiments, including five replicates (Table 2), experiment 7 (extraction temperature 42.5°C, extraction power 40 W, liquid-to-solid ratio 7:1, extraction time 40 min) provided the highest oil yield (ranging from 25.58% to 33.55%). The influence of each parameter and interaction effect was analyzed by Design Expert (10.0.7). First, the experimental data were fitted with a significant test with models, including linear, 2FI, quadratic, and cubic models. As shown in Table 3, the quadratic polynomial model was highly significant and rigorous for explaining the relationship between the response and each parameter. ANOVA (Table 4) showed that the quadratic polynomial model was highly significant with a low p-value (<0.0001). The lack-of-fit test, which was applied to select the model with a nonsignificant lack-of-fit, showed nonsignificance for a value greater than 0.05. The F-value of 2.06 implied that the model was significant, and the analysis showed that the model was significant.

![Table](image)

### Table 3 Data for proving to fit the quadratic model.

| Source  | Sequential p-value | Lack of fit p-value | Adjusted R-squared | Predicted R-squared | Pure error |
|---------|--------------------|---------------------|--------------------|--------------------|------------|
| Quadratic | <0.0001            | 0.2539              | 0.9241             | 0.8074             | Suggested  |

### Table 4 ANOVA for the quadratic polynomial model.

| Source  | Sum of squares | Degrees of freedom | Mean squares | F-value | p-value probability > F | Significance |
|---------|----------------|--------------------|--------------|---------|-------------------------|--------------|
| model   | 165.69         | 14                 | 11.84        | 25.36   | <0.0001                 | **           |
| A       | 5.21           | 1                  | 5.21         | 11.17   | 0.0048                  | *            |
| B       | 4.78           | 1                  | 4.78         | 10.23   | 0.0054                  | *            |
| C       | 6.02           | 1                  | 6.02         | 12.90   | 0.0029                  | *            |
| D       | 42.64          | 1                  | 42.64        | 91.37   | <0.0001                 | **           |
| AB      | 1.09           | 1                  | 1.09         | 2.34    | 0.1484                  |              |
| AC      | 2.03           | 1                  | 2.03         | 4.35    | 0.0558                  |              |
| AD      | 3.08           | 1                  | 3.08         | 6.60    | 0.0223                  | *            |
| BC      | 3.52           | 1                  | 3.52         | 7.53    | 0.0158                  | *            |
| BD      | 2.98           | 1                  | 2.98         | 6.38    | 0.0243                  | *            |
| CD      | 3.31           | 1                  | 3.31         | 7.10    | 0.0185                  | *            |
| A²      | 27.22          | 1                  | 27.22        | 58.32   | <0.0001                 | **           |
| B²      | 23.50          | 1                  | 23.50        | 50.36   | <0.0001                 | **           |
| C²      | 45.62          | 1                  | 45.62        | 97.77   | <0.0001                 | **           |
| D²      | 43.75          | 1                  | 43.75        | 93.76   | <0.0001                 | **           |
| Residual| 6.53           | 14                 | 0.47         |         |                         |              |
| Lack of Fit | 5.47       | 10                 | 0.55         | 2.06    | 0.2539                  | Not significant |
| Pure Error | 1.06         | 28                 | 0.27         |         |                         |              |
| Cor Total | 172.23       | 28                 |              |         |                         |              |
that the lack of fit was not significant relative to pure error. The low value of the pure error indicated the satisfactory reproducibility of data from ANOVA and the determination coefficient. Furthermore, the high values of \( \text{adj } R^2 \) indicated a high correlation. The \( \text{adj } R^2 \) of the quadratic polynomial model was the highest among the models, and pre \( R^2 \) was close to \( \text{adj } R^2 \) (\( \text{adj } R^2 \)- pre \( R^2 < 0.2 \)). Only 8.59\% of the total variation was not explained by the model, indicating that most of the process can be explained by the selected regression model\(^2\). C.V.% was 2.32\%, which was less than 10\% and demonstrated the reliability and accuracy of the experiment. The signal-to-noise ratio was measured with adequate precision (14.932), and a value greater than 4 was desirable and indicated an adequate signal that the model could be used to navigate the design space.

3.2.2 Response analysis of oil yield

The effects of ultrasonic-assisted extraction parameters such as extraction time, extraction power, extraction temperature, and liquid-to-solid ratio on oil yield from peony were investigated. The predicted code equation in terms of coded factors could be employed to predict the response at the coded levels for each factor\(^3\) and identify the relative effect of the factors through comparison of factor coefficients. The significance of each coefficient was determined based on \( F \)-values and \( p \)-values\(^4\). Large \( F \)-values and small \( p \)-values indicate the high significance of the corresponding coefficients\(^5\). The response surface analysis of data using Eq. (3) fitted with the quadratic model with a good regression coefficient \( R^2 = 0.9241 \).

\[
Y = 33.21 + 0.66 \times A + 0.63 \times B + 0.71 \times C + 1.89 \times D + 0.52 \times A \times B + 0.71 \times A \times C + 0.88 \times A \times D + 0.94 \times B \times C + 0.86 \times B \times D + 0.91 \times C \times D - 2.05 \times A^2 - 1.90 \times B^2 - 2.65 \times C^2 - 2.60 \times D^2
\]

As shown in Table 4, the \( p \)-values of \(< 0.0001 \) indicated

\[ \text{Fig. 2} \quad \text{3D response surface and contour plots: (A) Power & Temperature interaction; (B) Time & Liquid-to-solid ratio interaction; (C) Temperature & Liquid-to-solid ratio interaction; (D) Time & Temperature interaction; (E) Liquid-to-solid ratio & Power interaction; (F) Power & Time interaction.} \]

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that the model was highly significant, and that $D$, $A^2$, $B^2$, $C^2$, and $D^2$ had a highly significant effect ($p$-value less than 0.0001) on oil yield. $A$, $B$, $C$, $AD$, $BC$, $BD$, and $CD$ had a significant effect ($p$-value less than 0.05) on the results. Only $AB$ had a reduced effect ($p$-value more than 0.1) on ultrasonic-assisted oil extraction. The influence of each factor on the yield of peony seed oil conformed to the following order based on $F$-values: $D$ (extraction time) $> C$ (liquid-to-solid ratio) $> A$ (extraction temperature) $> B$ (extraction power).

The relationship between the extraction parameters and oil yield was investigated using response surface plots. Figure 2 shows the mutual interaction among extraction time, extraction power, extraction temperature, and liquid-to-solid ratio on oil yield.

Figure 2 (A) presents the mutual interaction between extraction temperature and power when the liquid-to-solid ratio and time were fixed at 7:1 and 40 min, respectively. When the temperature was fixed, the oil yield increased first but decreased later with increasing power. Temperature also exhibited quadratic effect on the oil yield when the power was fixed. A peak was observed within the middle values of time and power. High temperatures led to the evaporation of the solvent, thereby decreasing the contact time of the oil and consequently the oil yield.

The mutual interaction between extraction time and liquid-to-solid ratio when the power and temperature were fixed at 40 W and 42.5°C, respectively, is shown in Fig. 2 (B). The extraction time and the liquid-to-solid ratio exhibited a quadratic effect on the oil yield. The peak in the response surface plots of the mutual interaction of time with the liquid-to-solid ratio was found under the condition of extraction time of 40 min and the liquid-to-solid ratio of 7:1. These values are all in the middle of the range. Further increasing or decreasing the extraction time and power reduced the oil yield. Hence, extraction time and ultrasonic...
power had a significant effect on the oil extraction yield.

Highly elliptical contour maps are indicative of highly significant interactions between two factors\(^2\). The interaction of temperature and power had a weak significant effect, whereas the other factors had significant interaction effects, thereby confirming the ANOVA results.

3.2.3 Optimization of extraction parameters and validation of models

The optimum conditions for the ultrasonic-assisted extraction of oil from peony seeds were determined through the steepest ascent search starting at a point within the model concentration range. The optimal conditions were given by Design Expert (10.0.7) as follows: temperature of 45.51°C, power of 88.76 W, the liquid-to-solid ratio of 7.36:1, and extraction time of 45.66 min. The predicted oil yield under the above conditions was 34.14%.

Given the restriction of the virtual conditions, the process parameters were adjusted to the temperature of 45°C, power of 90 W, the liquid-to-solid ratio of 7:1, and time of 45 min. Under these conditions, the actual oil yield was 33.90%, which is within the 95% confidence interval of the predicted oil yield. Consequently, the conditions reformulated from the model were considered to be feasible and proved the reliability and accuracy of the model.

3.3 Chemical profile of the oil

Before detection, the extracted peony seed oil was stabilized through methyl esterification pretreatment. The fatty acid composition of the ultrasonic-assisted extracted oil was detected through GC-MS. The chemical composition of the main fatty acids of the peony seed oil was identified using information retrieved from the NIST 98 Mass Spectral Library and relevant literature. The relative contents of the main fatty acids were detected through the normaliza-
4 Conclusion

RSM was successfully employed to optimize the ultrasonic-assisted extraction of oil from peony seeds. The optimal conditions including extraction power, extraction time, extraction temperature, and liquid-to-solid ratio were determined using the regression equation and a highly significant fitted model that can be used to predict oil yield. Compared with the oil yield of peanuts, olives, and rapeseed, the oil yield of peony resembles the standard of the oil yield of conventional edible oil. Otherwise, the output of peony seeds per unit area can also meet the demand for oil. The GC-MS results revealed that the main chemical components of peony seed oil were unsaturated fatty acids, including n-3 α-linolenic acid (39.75%), n-6 linoleic acid (26.32%), and oleic acid (23.66%). The ratio of n-6/n-3 of peony seed oil was 0.66 which was between 0.4 and 1.6. These results are in line with the Chinese peony seed industry standards issued in 2014. This standard state that the content of alpha-linolenic acid should not be less than 38%.

Table 5  Fatty acid composition of the extracted seed oil.

| Number | Compound         | Chemical formula | Relative content |
|--------|------------------|------------------|------------------|
| 1      | Palmitic acid    | C_{16}H_{32}O_{2} | 7.73%            |
| 2      | Stearic acid     | C_{18}H_{32}O_{2} | 2.54%            |
| 3      | Oleic acid       | C_{18}H_{34}O_{2} | 23.66%           |
| 4      | n-6 Linoleic acid| C_{18}H_{32}O_{2} | 26.32%           |
| 5      | n-3 α-Linolenic acid| C_{18}H_{30}O_{2} | 39.75%           |

Acknowledgments

This work was supported by the Ningbo Science and Technology Enrichment Project (No. 2016C10012).

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