Original article

Process optimisation of microwave-assisted extraction of peony (*Paeonia suffruticosa Andr.*) seed oil using hexane–ethanol mixture and its characterisation

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Summary   Ethanol and hexane mixture agent microwave-assisted extraction (MAE) method was conducted to extract peony (*Paeonia suffruticosa Andr.*) seed oil (PSO). The aim of the study was to optimise the extraction for both yield and energy consumption in mixture agent MAE. The highest oil yield (34.49%) and lowest unit energy consumption (14 125.4 J g^{-1}) were obtained under optimum extraction condition: solid-liquid ratio 0.37 g mL^{-1}, extraction time 3.72 min, extraction temperature 80.92 °C, ethanol ratio 20.00%. GC–MS results showed that unsaturated fatty acids (UFAs) accounted for 88.60% of total fatty acids in PSO. Moreover, linolenic acid content of 37.35% was the highest UFA and caused PSO to possess good nutrition. PSO in DPPH radical scavenging experiment showed that IC$_{50}$ value of 28.80 ± 2.13 mg mL^{-1} exhibited strong antioxidant property. All experiments proved that mixed solvent MAE is an efficient and promising method to extract PSO. This method can effectively reduce the energy consumption and extraction time.

Introduction

Peony belongs to Paeoniaceae, genus Paeonia, and Sect. Moutan DC. The group is endemic to China. The peony seed oil content can reach around 40% (Ning *et al.*, 2015). Peony seed oil (PSO) contains various unsaturated fatty acids (UFAs), such as linoleic, oleic and linolenic acid. Notably, the linolenic acid content can reach more than 36% in total fatty acid (FA) content, and such high level of n-3 fatty acid is uncommon in seed oils (Li *et al.*, 2015). Linoleic acid, oleic acid and linolenic acid are responsible for antioxidant function, reduces blood fat and cholesterol and prevents head blood vessel diseases. Linolenic acid also has the effect of anti-inflammatory, improving intelligence and vision (Bourourou *et al.*, 2016), but it cannot be synthesised in the human body (Sevim *et al.*, 2013; Su *et al.*, 2015).

Therefore, linolenic acid external intake is necessary for people's health. Peony seed extract has potent anti-inflammatory activities (Kim *et al.*, 1999) and could protect HEK 293 cells from irradiation-induced DNA damage (He *et al.*, 2012).

Nowadays, the extraction methods for PSO mainly include organic solvent extraction, ultrasonic extraction, squeezing, microwave-assisted extraction (MAE) and super-critical carbon dioxide-assisted extraction (Balasubramanian *et al.*, 2010; Wang *et al.*, 2015; Franco-Vega *et al.*, 2016). MAE is widely used and easy to operate with low-energy consumption. These characteristics are caused by microwave form fluctuation and high frequency and penetration directly acting on the material molecules, thereby causing the molecules to occur as collision and friction in the polar motion, which occurs million times per second (Bouras *et al.*, 2015; Punegov *et al.*, 2015). Therefore, MAE can effectively destroy the cellular structure, including plant seeds, which are conducive to the oil release. To date, many studies have been conducted on the

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microwave extraction of active ingredient or essential oils in plants. Krishnan & Rajan (2015) utilised microwave to extract flavonoids from *Terminalia bellerica*. Hosseini *et al.* (2016) optimised the MAE of pectin from sour orange peel. Durmaz *et al.* (2015) used MAE to extract phenolic compounds from caper. In addition, Boukroufa *et al.* (2015) applied MAE to the biorefinery of orange peel waste for essential oil. Recently, the advantages of MAE to the extraction plant seed oil have been realised. Taghvaei *et al.* (2015) used microwave to extract cotton seed oil and subsequently evaluated it. Hence, MAE is proposed as an innovative method for the extraction of PSO.

Polar molecules can absorb microwave radiation energy well (Enomoto & Momose, 2005). On the contrary, non-polar molecules cannot, thereby many articles reported on a single polar molecule solvent as the extraction agent. Simić *et al.* (2016) applied ethanol solution as extraction solvent to extract total polyphenol compounds from chokeberries. Zhang *et al.* (2016) extracted the yellow horn seed oil via ethanol solution. Hexane is commonly used as oil extraction agent (Ferreira *et al.*, 2016; Khezeli *et al.*, 2016), but it is generally utilised for traditional extraction method because of its small polarity. Ethanol is a polar molecule solvent, which has a high dielectric constant, can reduce energy consumption. But it possesses a limited solubility to oil, so that the extraction rate is very low. To extract seed oil using MAE as a highly safe, low-cost method, ethanol and hexane mixture was used as the extracting agent. However, mixed solvent as MAE agent can change the dielectric constant of the agent so as to reduce the energy consumption and extraction time.

Thus, in this paper, we investigated the optimum preparation conditions of MAE. FA compositions, antioxidant activities and physicochemical properties of PSO were also detected. In addition, the cell structures of peony seed samples before and after extraction procedures were observed to confirm the extraction efficiency. This study provides technical support for the PSO processing and utilisation.

Material and methods

Sample preparations and reagents

Peony seeds were purchased from Shandong Province, Heze City. The seeds were new harvested in August and authenticated by the plant taxonomy professor Dr. Mao Zijun in our laboratory. After harvest, the seeds were grind slowly and dried at 60 °C for 12 h in a drier to 3% moisture content immediately. Afterwards, the dry seed powder was sieved through 20 mesh size standard mesh sieves. Finally, the powder was sealed into a plastic container and stored at 4 °C for oil extraction.

Hexane, petroleum ether, ethanol, ethyl acetate and acetone were all purchased from Fisher Scientific Co. (Waltham, USA). Hexane was chromatographic pure, and the others were analytically pure. Various FA standards and DPPH were obtained from Sigma–Aldrich Co. (St. Louis, USA). All other solvents and chemicals were obtained from Aladdin Reagent Co., Ltd. (Shanghai, China). Ultrapure water was prepared from a Milli-Q system at the 18.3 MX resistance (Millipore, MA).

MAE

MAE process

ETHOS EX microwave extraction system (Milestone Microwave Products Company, Sorisole, Italy) was used in this paper. Time, temperature, power and stirring rate were controlled through an electronic control panel, and the reaction energy consumption per second (P, J s^{-1}) could be read on the electronic control panel.

A known amount of ground seed material was introduced into the microwave reactor (volume, 100 mL), as shown in Fig. S1. The reactor must be an even number and be installed symmetrically on the sample plate of microwave extraction system. Subsequently, 25 mL of extraction solvent and special rotor were added into the reactor. The microwave reactor should be assembled and airtight, the temperature sensor should be inserted in the No. 1 reactor and the door should be closed after installing all reactors. Temperature, time and power were set up, and extraction was started. After extraction, the samples were centrifuged at 1008 *g* and washed thrice with hexane. The supernatant was merged, and the PSO was collected using a rotary evaporation instrument. The oil samples were weighed, and the extraction yield is expressed as percent ratio (%, w/w). Each set of test was repeated four times to calculate the average yield.

The unit energy consumption E calculation formula is as follows:

$$E = \frac{P \times T}{M}$$

Where P: energy consumption per second (J s^{-1}), T: total time (s) and M: quality of PSO (g).

On the basis of our preliminary experiment results (data not shown), the particle size of 20 mesh and stirring speed at 200 rpm were optimal. Other significant parameters of the MAE process were performed under a range of conditions according to the experimental design.

MAE solvent screening

The reaction solvent system was controlled within 25 mL. Ethanol, ethyl acetate, hexane, hexane and

ethyl acetate, hexane and acetone, petroleum ether, and hexane and ethanol were used as extraction solvent. The microwave extraction operation was performed as described in Section 2.2.1 at the extraction time of 10 min, temperature of 70 °C and solid–liquid ratio of 0.25 g mL^{-1}. The final extraction solvent was determined through the comprehensive influence of yield and energy consumption to make sure it can get the highest yield and lowest energy consumption.

Preparation process optimisation

Single-factor experiment
Before the optimisation of MAE of PSO, the main influences of MAE process parameters were initially separately investigated in a single-factor experiment to limit the range of main influencing factors. When one factor was not studied, it was kept constant. Extraction temperature (°C), extraction time (min), ethanol ratio in extracting agent (%, w/w) and solid–liquid ratio between seed powder and extracting agent (%, w/v) were selected as independent variables, whereas PSO yield and unit energy consumption were selected as response. To investigate the effect of extracting each factor on the response value, the following values of extraction temperature (40, 55, 70, 85 and 100 °C), extraction time (2, 5, 10, 15, 20 and 25 min), ethanol ratio in extracting agent (0%, 2%, 8%, 14% and 20%, w/w) and solid–liquid ratio between seed powder and extracting agent (1:2, 1:5, 1:7, 1:9 and 1:11 g mL^{-1}, w/v) were used.

Response surface methodology (RSM) optimisation
RSM was applied to identify the optimum factors levels of four key independent variables, namely, extraction temperature (°C), extraction time (min), ethanol ratio in extracting agent (%, w/w) and solid–liquid ratio between seed powder and extracting agent (g mL^{-1}, w/v). On the basis of the single-factor experimental results, the factor levels corresponding to each independent variable were selected (Table S1). Box–Behnken Design (BBD) was used in optimisation design. The regression analysis was carried out to evaluate the response function as a quadratic polynomial for PSO yield and unit energy consumption to optimise the MAE process (Table S1). A total of 29 randomised experiments, including 24 factorial and 5 zero-point tests, were designed.

The factor levels were coded as −1 (low), 0 (central point) and 1 (high). Maximum information using minimum trials was obtained using experimental design based on mathematical rules. Regression analysis of the randomised experiments, which fit a second-order polynomial equation (quadratic model), was carried out according to the following quadratic polynomial, which was subsequently used to predict the optimum

conditions of the whole experiment (Wan et al., 2015; Gidwani & Vyas, 2016).

$$Y = \beta_0 + \sum_{j=1}^{k} \beta_j X_j + \sum_{j=1}^{k} \beta_{jj} X_j^2 + \sum \sum_{i<j} \beta_{ij} X_i X_j (k=4)$$

Where Y is the predicted response; β_0 is a constant coefficient; β_i, β_{ii} and β_{ij} are the coefficients of the linear, quadratic and interactive terms respectively; X_i and X_{ij} represent the coded independent variables; and k represents the number of variables. According to the ANOVA, the regression coefficients of the individual linear quadratic and interaction terms were determined. The obtained experimental data were compared with the values predicted by the regression model statistically using Design-Expert 8.0 (State-Ease, Inc., Minneapolis MN). ANOVA was deduced to test the model significance and suitability (Bhuyan et al., 2015; Gidwani & Vyas, 2016). All extraction processes and predicted responses were expressed as the means of yield (importance is 5) and unit energy consumption (importance is 3). Significance of model at $P < 0.05$ was considered as significant and highly significant levels. To compare and determine the optimal condition for extraction process, the optimal condition was performed thrice for confirmation. PSO detections were evaluated according to the optimum conditions selected via BBD.

Physicochemical index detection

Acid value, iodine value, peroxide and saponification of PSO were detected using AOCS Standard Methods (1997), in which the details were as follows. Acid and iodine values were carried out using Cd 3d-63(09) and Cd 1d-92(09) respectively. Peroxide and saponification were carried out using Cd 8-53(03) and Cd 3-25(13) respectively. Specific gravity was carried out using To 1a-64. The refractive index of PSO at 20 °C was measured using an Abbe refractometer (KEERTE Co., Shanghai, China).

Gas chromatography–mass spectrometer (GC–MS) analyses of FA compositions

The FA compositions of PSO were analysed using GC–MS. Prior to injection, the extracted oils were converted to their fatty acid methyl esters (FAMEs) according to the method reported by Garces & Mancha (1993), Park & Goins (1995). GC–MS analysis of FAMEs was performed using a 7890A-GC/5975-MS gas chromatography/mass spectrometer (Agilent, Santa Clara, USA), equipped with a HP-5MS capillary column (30 mm × 0.25 mm × 0.25 μm). The detail of the operating conditions was as follows. The GC detection conditions included the following: column

temperature, 150–240 °C; temperature rate, 5 °C min^{-1}; injection port temperature, 250 °C; precolumn pressure, 13.332 psi; sample quantity, 10 μL; and carrier gas, high-purity helium. The MS detection conditions were as follows: ionisation mode, EI; electron energy, 70 eV; transmission line temperature, 250 °C; ion source temperature, 230 °C; level 4 rod temperature, 150 °C; and quality scan range, 35–450 amu. The relative percentage compositions of oils were calculated from the total ion chromatograms via a computerised integrator. Wiley 7n.1 spectral library retrieval was used to retrieve results.

Antioxidation experiment

The DPPH radical scavenging experiments were detected according to the previous method reported by Molyneux (2004). The scavenging activities of both solvent extraction (100 g of peony seed powder and 1 L of hexane were mixed in a reaction for 24 h) and MAE PSO were determined through the same method. The antioxidant property of the two kinds of PSO was reported as IC_{50} values, which were calculated using the logarithmic regression curves for DPPH radical scavenging (mg mL^{-1}).

Scanning electron microscopy (SEM)

A Quanta-200 environmental scanning electron microscope system (FEI Company, USA) was used to examine the morphological alterations of peony seed samples before and after extraction. The samples were fixed on a specimen holder with an aluminium tape and sputtered with a thin layer of gold prior to examination under high-vacuum condition at an accelerating voltage of 12.5 kV.

Statistical analysis

Results were expressed as mean ± SD and analysed by SPSS 11.0 Statistical software through one-way analysis of variance (ANOVA). The significance of the differences was varied by the chi squared test. $P < 0.05$ was considered as a significant difference.

Results and discussion

Extraction agent selection results

The influence of different solvents on yield was investigated, and the results are shown in Fig. 1. Petroleum ether, hexane, and hexane and ethanol mixture showed the highest yield, and the difference was less than 1%. Thus, the three kinds of solvent extraction were further comparatively analysed, as shown in Table 1 (Jouyban *et al.*, 2004; Fakhree *et al.*, 2010). The higher

dielectric constant results in higher thermal efficiency of the microwave. The dielectric constant of hexane and ethanol mixture was the largest. Thus, the minimum unit energy consumption was 22176 J g^{-1}, and its yield was 24.35%. Hexane and ethanol mixture was used as microwave extraction agent. The traditional MAE process typically adopts single polarity molecules solvent as extracting agent. In this study, we used ethanol and hexane mixture as extraction agent, which makes the method highly safe and low-cost way. The existence of ethanol can effectively reduce the energy consumption and extraction time during extraction.

Single-factor experimental analysis

Temperature effect on yield and unit energy consumption
As shown in Fig. 2a, the overall trend between temperature yield and the unit energy consumption constantly increases with temperature rise persistently when the temperature is below 85 °C. When the temperature is 85–100 °C (extraction solvent boiling point limit), the yield starts to decline but is not inferior to 80 °C, and the unit energy consumption still keeps rising relatively. Therefore, we can obtain the highest yield of extraction when the temperature is between 70 and 100 °C. Thus, on principle of the BBD, we make the temperature factor range between 70 and 100 °C.
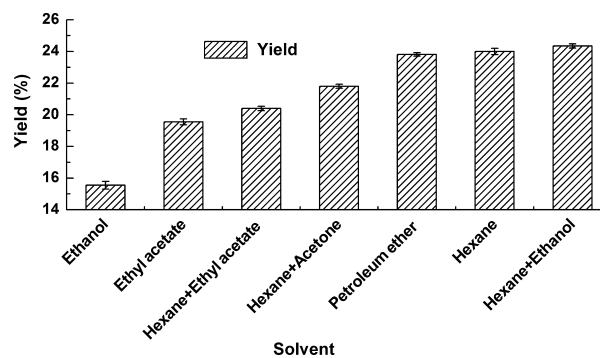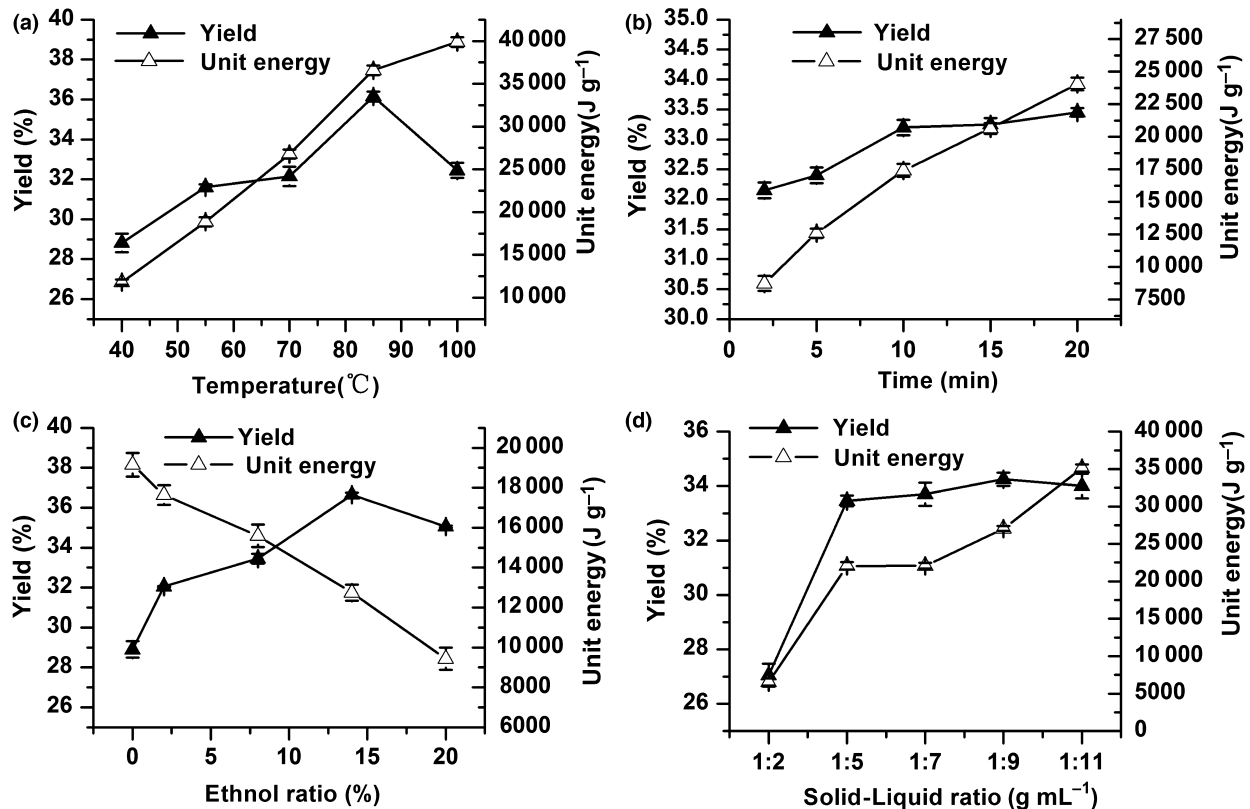

Figure 1 Effects of different solvents on yield.

Table 1 The dielectric constant and unit energy consumption of different solvents

Extraction solvent	Petroleum ether	Hexane	Hexane+Ethanol
Permittivity	1.8	1.9	2.796
Energy per (J g^{-1})	39 316	35 047	22 176
Yield (%)	23.81	24	24.35

$\varepsilon = \varphi_A\varepsilon_A + \varphi_B\varepsilon_B$ (φ_A and φ_B are the volume fractions of solvents).

Figure 2 Single-factor experiment result analysis diagram. (a) Effect of temperature on yield and unit energy consumption. (b) Effect of time on yield and unit energy consumption. (c) Effect of ethanol ratio on yield and unit energy consumption. (d) Effect of the solid–liquid ratio on yield and unit energy consumption.

Time effect on yield and unit energy consumption
As shown in Fig. 2b, the yield increases with the increase in time, which started at 10 min. Thereafter, the yield remained unchanged. With the increase in time, the unit energy consumption continued to increase. To preserve the principles of low-energy consumption and high yield, the time factor used was in the range of 2–10 min.

Ethanol ratio in extracting agent effect on yield and unit energy consumption
Figure 2c shows the total variation trend of the ethanol ratio on the yield and unit energy consumption. The yield curve increased until the ethanol ratio was 14%, and relatively small reduction was observed at the ratio of 20%. The highest yield was obtained when the ethanol ratio was in the range of 8–20%. With the increase in volume of ethanol, the unit energy consumption showed a significant downward trend. On the basis of the above results and BBD principle, we controlled the ethanol ratio factor range at 8–20%. The ethanol ratio was optimised in the extracting agent to examine its effect on yield and unit energy consumption, in the premise of ensuring the high yield

of seed oil. Increased ethanol content can reduce energy consumption and extraction time obviously.

Solid–liquid ratio between seed powder and extracting agent effect on yield and unit energy consumption
When the mixing amount of the extraction solvent was 25 mL, Fig. 2d shows that the solid–liquid ratio in the range of 1:2–1:5 g mL^{-1} of the yield and unit energy consumption presented a linear upward trend. At the solid–liquid ratio of 1:5–1:11 g mL^{-1}, the yield changes remained relatively stable, and only a modest increase was observed. When the solid–liquid ratio was 1:5–1:7 g mL^{-1}, the unit energy consumption trend was similar, but a significant increase was observed at 1:7–1:11 g mL^{-1} unit energy consumption curve. Thus, the yield and unit energy consumption demands were combined according to the BBD method, and we controlled the solid–liquid ratio range at 1:2–1:7 g mL^{-1}.

Optimisation of PSO preparation

BBD model establishment
The BBD model and corresponding results of RSM experiments are shown in Table S2. The multiple

regression analysis on the experimental data showed that the model for the predicted response could be expressed through the quadratic polynomial equations (in the form of coded values).

$$Y_1 = 34.26 - 1.33X_1 + 0.46X_2 + 0.76X_3 + 1.06X_4 - 5.35X_1^2 - 1.06X_2^2 - 2.28X_4^2$$

$$Y_2 = 43154.83 + 20360.33X_1 + 13371.61X_2 - 13975.22X_3 + 2646.58X_4 + 5224.40X_1X_2 + 3408.48X_1X_3 + 5298.35X_2X_4 + 4249.65X_1^2 - 5317.80X_2^2 - 3622.51X_4^2$$

The ANOVA results were analysed to evaluate the effects of the variables and their possible interactions (Table S3). Coefficients of the corresponding result model were evaluated via regression analysis to test for their significance. The insignificant factors were excluded from the data via backward elimination ($P < 0.05$). The ANOVA results of the yield were presented as the response model. The P-value of the adjusted model ($P < 0.0001$) implied that the mode was significant, but the P-value of the lack of fit ($P = 0.0514$) was insignificant. Thus, the significance of the model indicated that the model was well adapted to the response. The R^2 was 0.9029, which meant that only 9.71% of the total variations were not explained by the BBD mode. For a good statistical model, the adjusted R^2 (R^2_{adj}) should be compared with R^2. In this model, R^2_{adj} was 87.06%, which was relatively close to the R^2 of 90.29%. The regression coefficient from the experimental data and adjusted one was reasonably close, which indicated a high degree of correlation between the observed and predicted results. The ANOVA results suggested that the mode is ideal.

The remaining data were analysed in the same manner, with the ANOVA results of unit energy consumption as the response model (Table S4). The P-value of the adjusted model ($P < 0.0001$) implied that the mode was significant, but the P-value of the lack of fit ($P = 0.0889$) was insignificant. Therefore, the significance of the model indicated that the model was well adapted to the response. The R^2 was 0.9859, which meant that only 1.41% of the total variation was not explained by the BBD mode. For a good statistical model, the adjusted R^2 (R^2_{adj}) should be compared with R^2. In this model, R^2_{adj} was 97.81%, which was relatively close to R^2 of 98.59%. The regression coefficient from the experimental data and adjusted one was reasonably close, which indicated a high degree of correlation between the observed and predicted results. The ANOVA results suggested that the mode is ideal.

2D and 3D response surface plots
As shown in Figs 3 and 4, the influence of the different varying influencing factors on the response values

(average size) is visualised on the 2D contours and 3D response surface plots. Six 2D contours and 3D plots were formed for the responses.

Figure 3a shows the combined effect of time and temperature on yield. When the extraction temperature was below 82–86 °C, the yield increased with the increase in temperature, and a high yield was obtained. When the temperature was higher than 82–86 °C, the yield showed a slow decline with the increase in the temperature, until it eventually reached the boiling point of solvent extraction. This result demonstrated that at the same extraction temperature, the maximum yield was obtained at around 6–8 min. As shown in Fig. 3b, the combined effect of ethanol ratio and temperature on yield is visible. Yield increased with the increase on the ethanol amount, and the highest yield was obtained at 20% solid–liquid ratio. As shown in Fig. 3c, when the solid–liquid ratio was in the range of 0.32–0.38 g mL^{-1}, the maximum yield maximum was obtained. Yield decreased with the increase in solid–liquid ratio, which was larger than 0.38 g mL^{-1}. As shown in Fig. 4a–c, with the increase in time, the temperature and feed rate factors increased, and the unit energy consumption showed a rising trend. By contrast, the unit energy consumption showed slowly declining trend with the increase in ethanol rate.

Considering various influencing factors interaction for the two response values, namely yield and energy consumption, the model gradually optimised the most suitable extraction condition: solid–liquid ratio: 0.37 g mL^{-1}; extraction time: 3.72 min; extraction temperature: 80.92 °C; and ethanol ratio: 20.00%. The highest oil yield of 34.49% and lowest energy consumption of 14125.4 J g^{-1} were obtained.

Verification of the predictive model
The reliability of the theoretical model was verified using three parallel experiments under optimised preparation conditions. A yield of 34.06 ± 0.46% and unit energy consumption of 14283.7 ± 246.76 J g^{-1} were obtained from three parallel experiments ($P < 0.05$), which were only 0.43% and 158.3 J g^{-1} different from the value forecasted (34.49% and 14125.4 J g^{-1}) by the regression model. Therefore, the PSO extraction conditions achieved by RSM were reliable and practical. Moreover, the optimised yield of PSO via MAE was higher than 25% of the oil yield obtained via ultrahigh pressure-assisted extraction (Wang *et al.*, 2015).

Physicochemical properties

The physicochemical properties of PSO are illustrated in Table 2. No significant differences ($P < 0.05$) were observed for the refractive index, acid or saponification, iodine and peroxide values of either method.
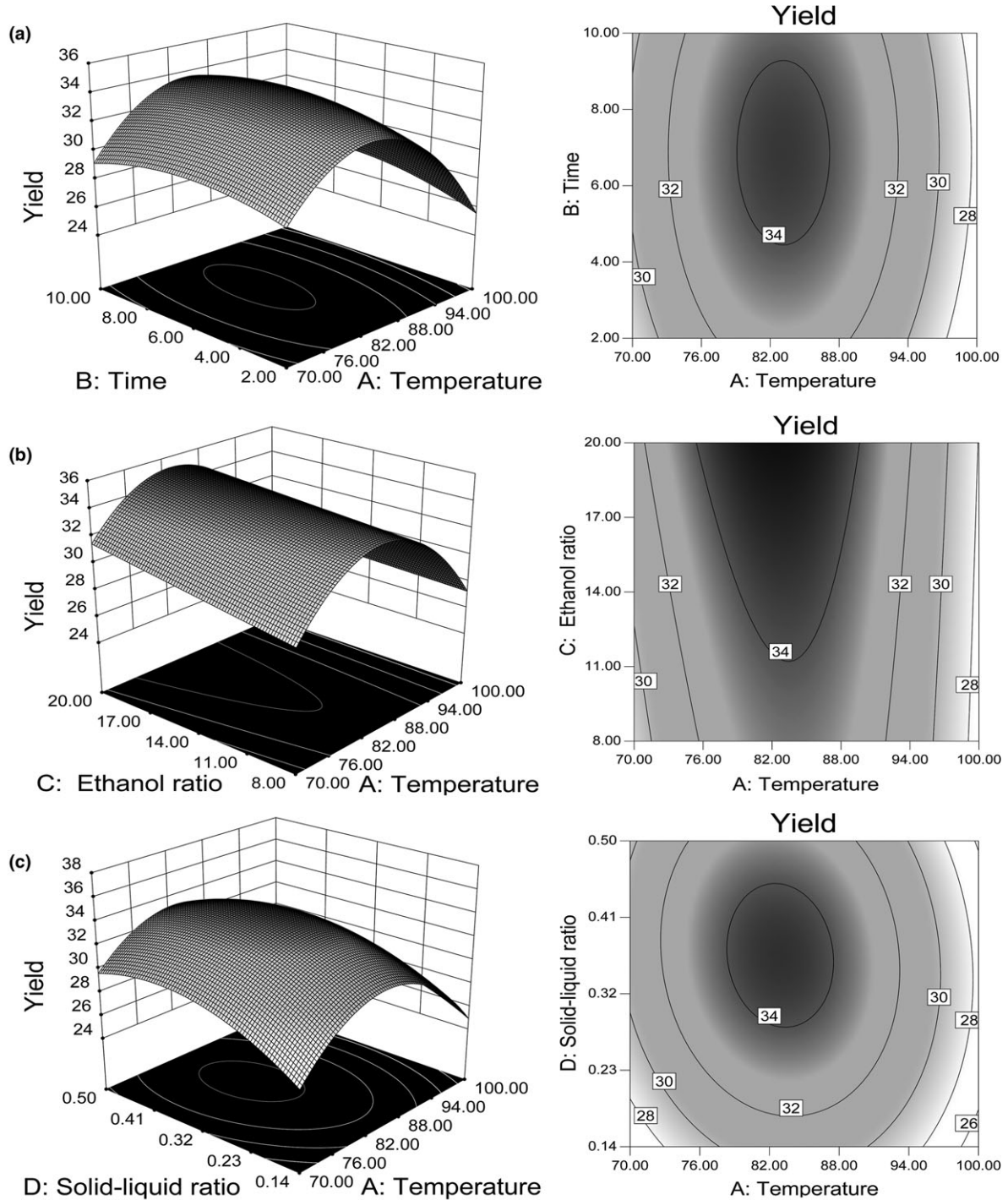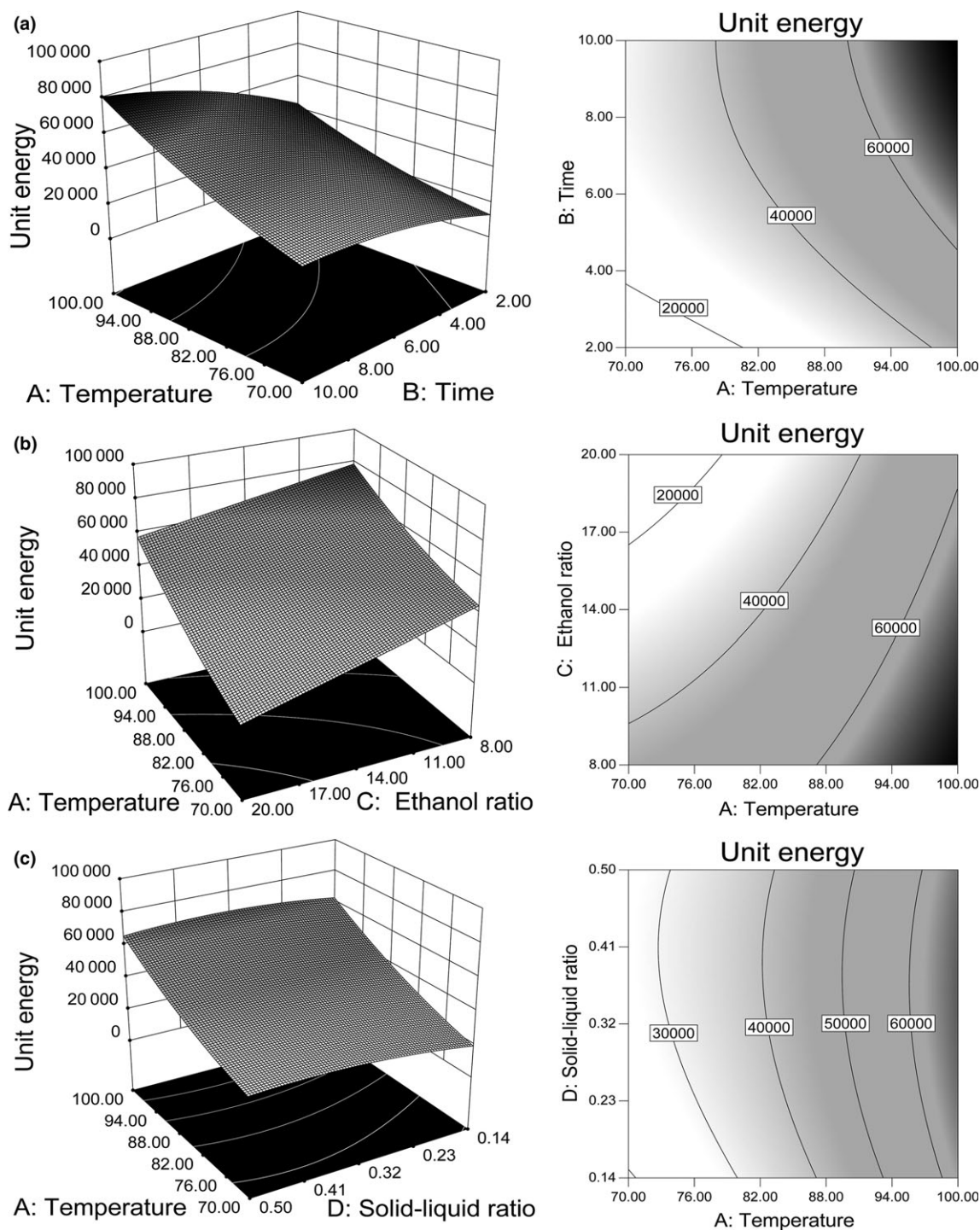
Figure 3 2D and 3D plots presenting the correlative effects of temperature and time (a), temperature and ethanol ratio (b) and temperature and solid–liquid ratio (c) on yield.

GC–MS analysis of FA composition of PSO

As shown in Fig. 5, five main FA components, two saturated FAs and three UFAs were identified. Among them, palmitic acid C16:0 (7.83%) and stearic acid C18:0 (2.60%) were the main saturated FAs. Linolenic acid C18:3 (37.35%), linoleic acid C18:2 (27.65%) and oleinic acid C18:1 (23.60%) were the main UFAs

Figure 4 2D and 3D plots presenting the correlative effects of temperature and time (a), temperature and ethanol ratio (b) and temperature and solid–liquid ratio (c) on unit energy consumption.

(Table 3). UFAs accounted for 88.60% of the total FA. Remarkably, the linolenic acid is a kind of essential FA that cannot be synthesised by the human body. The lack of this acid in the human body will result in lipid metabolism disorder, decreased immunity, vision loss, atherosclerosis and other symptoms. As reported

by Ning *et al.* (2015) linolenic acid in 40 days after flowering of PSO can reach more than 40%, but the oil yield is low, which is less than 5%. However, the linolenic acid in later than 60 days after flowering of PSO shows an average value of 22.26%, which is considerably less than 37.35%. Therefore, PSO via MAE is rich in linolenic acid; thus, it can offer health benefits and ensure the intake of linolenic acid.

Antioxidant activities

As shown in Fig. 6, the antioxidant activities of oils prepared with solvent extraction and MAE were assessed using DPPH radical scavenging assay, which is commonly used to test plant oil antioxidant capacity. DPPH radical scavenging activities increased with the increasing concentration of the extracted oils. However, MAE oil exhibited stronger antioxidant capacity in scavenging the DPPH radicals ($P < 0.05$, IC_{50} 28.80 ± 2.13 mg mL^{-1}) compared with the solvent extraction oil ($P < 0.05$, IC_{50} 61.62 ± 1.97 mg mL^{-1}).

Morphological characteristics

As illustrated in Fig. 7, the structural changes in samples before and after extraction were examined via SEM to understand the extraction effect of the MAE of oils from peony seeds. Figure 7a shows that the intact oil cells were presented on the surface of seed tissues before extraction. MAE caused significant

structural changes to the seed tissues, where most cells were broken, disorganised and incomplete (Fig. 7b). According to the SEM, mass transfer was affected by both microwave and chemical affinity between extraction solvents and oils in the seeds. Therefore, the destruction of cell structure with the help of organic extracting agent and microwave high-frequency vibration was beneficial for releasing oils, which were previously bound in the peony seed cell structure.

Table 3 GC–MS analysis of fatty acid composition of MAE peony seed oil

Fatty acid composition	Molecular formula	Molecular weight	Microwave extracted (%)
Palmitic acid	$C_{16}H_{32}O_2$	256.42	7.83
Stearic acid	$C_{18}H_{36}O_2$	284.48	2.60
Oleinic acid	$C_{18}H_{34}O_2$	282.47	23.60
Linoleic acid	$C_{18}H_{32}O_2$	280.44	27.65
Linolenic acid	$C_{18}H_{30}O_2$	278.43	37.35

Table 2 Physicochemical properties of peony seed oil

Item	Value
Acid value (mg KOH per g oil)	2.27
Iodine value (g I$_2$/100 g oil)	168.53
Peroxide value (bmeq kg^{-1})	1.71
Saponification value (mg KOH g^{-1} oil)	182.41
Refractive index (25 °C)	1.472
Specific gravity (25 °C, g mL^{-1})	0.973
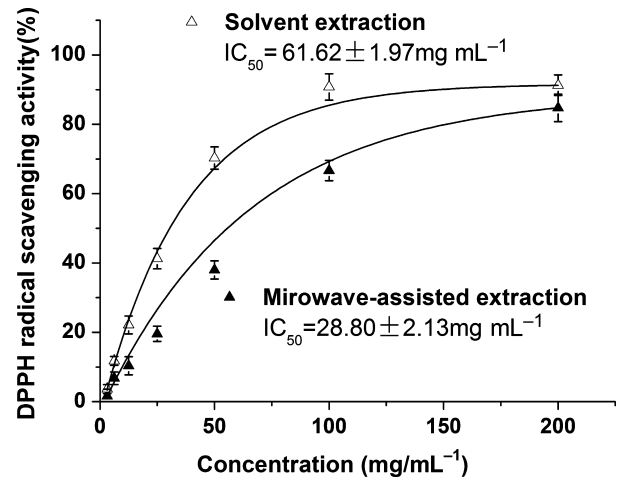
Figure 6 Antioxidant activities of the MAE PSO and the solvent extraction of PSO via DPPH radical scavenging assay.

Figure 5 Gas chromatography–mass spectrometer analyses on the fatty acid compositions of the microwave-assisted extraction (MAE) peony seed oil (PSO).
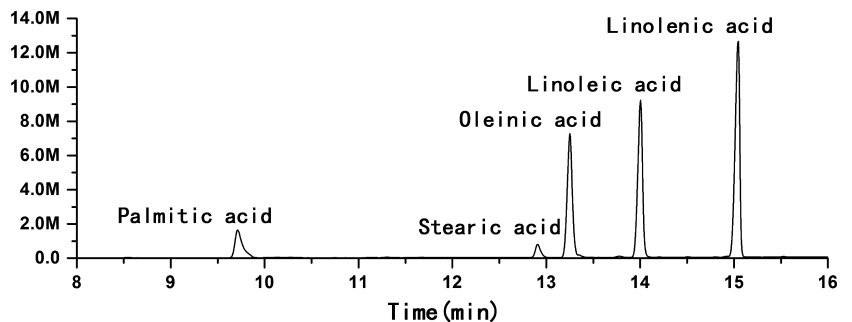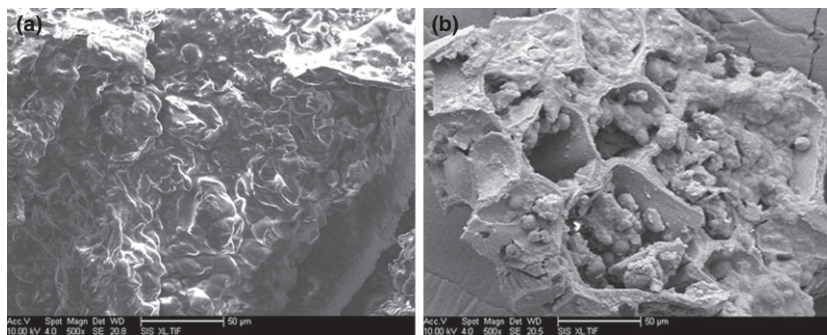
Figure 7 Scanning electron micrographs of peony seed samples: non-extraction sample (a); MAE sample (b).

Conclusions

MAE using ethanol and hexane mixture as extraction agent can effectively reduce the energy consumption and extraction time during PSO extraction. The highest oil yield of 34.49% and lowest energy consumption of 14125.4 J g^{-1} were obtained through the following optimised extraction conditions: solid–liquid ratio: 0.37 g mL^{-1}; extraction time: 3.72 min; extraction temperature: 80.92 °C; and ethanol ratio: 20.00%. A yield of 34.06 ± 0.46% and unit energy consumption of 14283.7 ± 246.76 J g^{-1} were obtained from three parallel experiments. The physicochemical properties were also detected. The acid value was 2.27 mg KOH per g oil; the saponification value was 182.41 mg KOH per g oil; the iodine value was 168.53 g I$_2$/100 g oil; and the peroxide value was 1.71 bmeq kg^{-1}. In addition, GC–MS results showed that UFAs accounted for 88.60% of total FAs in PSO. The linolenic acid content (37.35%) was the highest in UFA; the linoleic acid amount was 27.65%; and the oleinic acid content was 23.60%, which guaranteed that PSO possesses physiological effects of oil. MAE oil also exhibited prominent efficacy for DPPH radical scavenging, which showed low IC$_{50}$ value ($P<0.05$, IC50 28.80 ± 2.13 mg mL^{-1}). In addition, the SEM results demonstrated that the destruction of cell structure with the help of organic extracting agent and microwave high-frequency vibration was beneficial for an efficient significant increase in the release of oils in the peony seeds. Overall, the results suggested that mixed solvent MAE can produce high-quality PSO for healthy food field and is a promising environment-friendly technology for seed oil extraction.

Acknowledgment

Conflict of Interest

The authors declare no conflict of interest.

References

Balasubramanian, S., Allen, J.D., Kanitkar, A. & Boldor, D. (2010). Oil extraction from Scenedesmus obliquus using a continuous microwave system–design, optimization, and quality characterization. *Bioresource Technology*, **102**, 3396–3403.

Bhuyan, D.J., Vuong, Q.V., Chalmers, A.C., van Altena, I.A., Bowyer, M.C. & Scarlett, C.J. (2015). Microwave-assisted extraction of Eucalyptus robusta leaf for the optimal yield of total phenolic compounds. *Industrial Crops and Products*, **69**, 290–299.

Boukroufa, M., Boutekedjiret, C., Petigny, L., Rakotomanomana, N. & Chemat, F. (2015). Bio-refinery of orange peels waste: a new concept based on integrated green and solvent free extraction processes using ultrasound and microwave techniques to obtain essential oil, polyphenols and pectin. *Ultrasonics Sonochemistry*, **24**, 72–79.

Bouras, M., Chadni, M., Barba, F.J., Grimi, N., Bals, O. & Vorobiev, E. (2015). Optimization of microwave-assisted extraction of polyphenols from Quercus bark. *Industrial Crops and Products*, **77**, 590–601.

Bourourou, M., Heurteaux, C. & Blondeau, N. (2016). Alpha-linolenic acid given as enteral or parenteral nutritional intervention against sensorimotor and cognitive deficits in a mouse model of ischemic stroke. *Neuropharmacology*, **108**, 60–72.

Durmaz, E., Sumnu, G. & Sahin, S. (2015). Microwave-assisted extraction of phenolic compounds from caper. *Separation Science and Technology*, **50**, 1986–1992.

Enomoto, K. & Momose, T. (2005). Microwave Stark decelerator for polar molecules. *Physical Review A*, **72**, 913–922.

Fakhree, M.A.A., Delgado, D.R., Martínez, F. & Jouyban, A. (2010). The Importance of dielectric constant for drug solubility prediction in binary solvent mixtures: electrolytes and zwitterions in water + ethanol. *Aaps Pharmscitech*, **11**, 1726–1729.

Ferreira, A.F., Dias, A.P.S., Silva, C.M. & Costa, M. (2016). Effect of low frequency ultrasound on microalgae solvent extraction: analysis of products, energy consumption and emissions. *Algal Research-Biomass Biofuels and Bioproducts*, **14**, 9–16.

Franco-Vega, A., Ramirez-Corona, N., Palou, E. & Lopez-Malo, A. (2016). Estimation of mass transfer coefficients of the extraction process of essential oil from orange peel using microwave assisted extraction. *Journal of Food Engineering*, **170**, 136–143.

Garces, R. & Mancha, M. (1993). One-step lipid extraction and fatty acid methyl esters preparation from fresh plant tissues. *Analytical Biochemistry*, **211**, 139–143.

Gidwani, B. & Vyas, A. (2016). Preparation, characterization, and optimization of altretamine-loaded solid lipid nanoparticles using Box-Behnken design and response surface methodology. *Artificial Cells Nanomedicine and Biotechnology*, **44**, 571–580.

He, C.N., Zhang, Y.C., Peng, Y., Yang, J.S. & Xiao, P.G. (2012). Monoterpene glycosides from the seeds of *Paeonia suffruticosa* protect HEK 293 cells from irradiation-induced DNA damage. *Phytochemistry Letters*, **5**, 128–133.

Hosseini, S.S., Khodaiyan, F. & Yarmand, M.S. (2016). Optimization of microwave assisted extraction of pectin from sour orange peel and its physicochemical properties. *Carbohydrate Polymers*, **140**, 59–65.

Jouyban, A., Soltanpour, S. & Chan, H.K. (2004). A simple relationship between dielectric constant of mixed solvents with solvent composition and temperature. *International Journal of Pharmaceutics*, **269**, 353–360.

Khezeli, T., Daneshfar, A. & Sahraei, R. (2016). A green ultrasonic-assisted liquid-liquid microextraction based on deep eutectic solvent for the HPLC-UV determination of ferulic, caffeic and cinnamic acid from olive, almond, sesame and cinnamon oil. *Talanta*, **150**, 577–585.

Kim, H. J., Chung, S. K. & Choi, S. W. (1999). Lipoxygenase inhibitors from *Paeonia lactiflora* seeds. *Preventive Nutrition & Food Science*, **4**, 163–166.

Krishnan, R.Y. & Rajan, K.S. (2015). Microwave assisted extraction of flavonoids from Terminalia bellerica: study of kinetics and thermodynamics. *Separation & Purification Technology*, **157**, 169–178.

Li, S.S., Yuan, R.Y., Chen, L.G., Wang, L.S., Hao, X.H., Wang, L.J., Zheng, X.C. & Du, H. (2015). Systematic qualitative and quantitative assessment of fatty acids in the seeds of 60 tree peony (*Paeonia section Moutan DC.*) cultivars by GC-MS. *Food Chemistry*, **173**, 133–140.

Molyneux, P. (2004). The use of the stable free radical diphenylpicrylhydrazyl (DPPH) for estimating antioxidant. *Songklanakarin Journal of Science & Technology*, **26**, 211–219.

Ning, C.L., Jiang, Y., Meng, J.S., Zhou, C.H. & Tao, J. (2015). Herbaceous peony seed oil: a rich source of unsaturated fatty acids and gamma-tocopherol. *European Journal of Lipid Science and Technology*, **117**, 532–542.

Park, P.W. & Goins, R.E. (1995). *In situ* preparation of fatty acid methyl esters for analysis of fatty acid composition in foods. *Journal of Food Science*, **59**, 1262–1266.

Punegov, V.V., Kostromin, V.I., Fomina, M.G., Zaynullin, V.G., Yushkova, E.A., Belyh, D.V., Chukicheva, I.U. & Zaynullin, G.G. (2015). Microwave-assisted extraction of hypericin and pseudohypericin from *Hypericum perforatum*. *Russian Journal of Bioorganic Chemistry*, **41**, 757–761.

Sevim, D., Senol, F.S., Gulpinar, A.R., Orhan, I.E., Kaya, E., Kartal, M. & Sener, B. (2013). Discovery of potent *in vitro* neuroprotective effect of the seed extracts from seven *Paeonia L.* (peony) taxa and their fatty acid composition. *Industrial Crops and Products*, **49**, 240–246.

Simić, V. M., Rajković, K. M., Stojičević, S. S., Veličković, D. T., Nikolić, N. Č., Lazić, M. L. & Karabegović, I.T. (2016). Optimization of microwave-assisted extraction of total polyphenolic compounds from chokeberries by response surface methodology and artificial neural network. *Separation & Purification Technology*, **160**, 89–97.

Su, J.H., Wang, H.X., Ma, C.Y., Lou, Z.X., Liu, C.X., Rahman, M.T., Gao, C.Z. & Nie, R.J. (2015). Anti-diabetic activity of peony seed oil, a new resource food in STZ-induced diabetic mice (vol 6, pg 2930, 2015). *Food & Function*, **6**, 3400–3400.

Taghvaei, M., Jafari, S.M., Nowrouzieh, S. & Alishah, O. (2015). The influence of cooking process on the microwave-assisted extraction of cotton seed oil. *Journal of Food Science and Technology-Mysore*, **52**, 1138–1144.

Wan, P., Yang, X.M., Cai, B.N., Chen, H., Sun, H.L., Chen, D.K. & Pan, J.Y. (2015). Ultrasonic extraction of polysaccharides from Laminaria japonica and their antioxidative and glycosidase inhibitory activities. *Journal of Ocean University of China*, **14**, 651–662.

Wang, C.Z., Xu, L., Wu, Q., Zhou, Z.K., Ren, X.C. & Yang, R. (2015). The importance of ultrahigh pressure processing over the quality of the extracted oil from peony seeds (*Paeonia suffruticosa Andr.*). *Industrial Crops and Products*, **76**, 1142–1147.

Zhang, D.Y., Yao, X.H., Luo, M., Zhao, C.J. & Fu, Y.J. (2016). Optimization of negative pressure cavitation-microwave assisted extraction of yellow horn seed oil and its application on the biodiesel production. *Fuel*, **166**, 67–72.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Experimental design levels of selected variables.

Table S2. The experimental design layout and corresponding responses for BBD based on RSM for MAE peony seed oil.

Table S3. Analysis of variance (ANOVA) for the yields obtained by using MAE.

Table S4. Analysis of variance (ANOVA) for the unit energy consumption obtained by using MAE.

Figure S1. Schematic of microwave extraction apparatus.