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Optimization of subcritical fluid extraction of seed oil from *Nitraria tangutorum* using response surface methodology

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ABSTRACT

Subcritical fluid extraction (SFE) technology was used to extract oil from *Nitraria tangutorum* seed. The best possible combination of extraction parameters was found using response surface methodology (RSM) in a three-variable, three-level Box-Behnken experimental design (BBD). The optimum extraction parameters were an extraction time of 40 min, an extraction pressure of 0.60 MPa, an extraction temperature of 44 °C and a raw material particle size of 0.45 mm. Conventional solvent extraction and supercritical CO_2 fluid extraction were comparatively used. The yield of seed oil obtained using SFE was 12.92%, which was similar to or higher than the other methods. The chemical compositions of the seed oil, determined by GC–MS, indicate that its unsaturated fatty acids content was 97%. SFE proved to be an effective technique for extracting oil from *N. tangutorum*.

1. Introduction

The genus *Nitraria* (Zygophyllaceae), comprising *ca.*15 species, is a kind of shrub with esculent berries. It is widely distributed in the Middle East, central Asia, and northwestern China. Among the *Nitraria* (*N.*) species, only *Nitraria tangutorum* Bobr. grows widely in China, notably in the desert areas of the Qinghai-Tibetan Plateau. Due to its natural ability to withstand wind and sand, it is often used for sand stabilization (Pan, Shen, & Chen, 1999; Wang, 2000). In addition, the fruits and seeds of *N. tangutorum* are often used by local residents to treat ailments of the spleen and stomach, indigestion, neurasthenia and colds, and its leaves are used in folk medicine as an antispasmodic, antineuropathic, and antiarrhythmic agent (Jiang, Zhang, & Wang, 1989). The fruits have anti-oxidative effects and decrease blood lipid levels (Suo, Wang, & Wang, 2004). However, the effective components of *N. tangutorum* have yet to be identified.

N. tangutorum seed oil is rich in unsaturated fatty acids, which play an important role in the regulation of a variety of physiological and biological functions in living organisms. Seed oil has been shown to improve immune response, reduce oxidants, and mitigate fatigue, so it has drawn increasing interest in recent years (Brondz, Olsen, Haapasalo, & Winkelhoff, 1991; Zhao et al., 2006). Generally, the traditional methods of extraction of *N. tangutorum* seed oil include expeller pressing and conventional organic solvent extraction methods, etc. The yield of the former method is lower. The latter method as the drawback that the oil must be heated to distill it and contains residual solvents, and at the same time the oil is oxidatively unstable, and is easily subject to rancidity during the separation process (Scalia, Giuffreda, & Pallado, 1999). Also supercritical fluid extraction is one of the newly emerging clean and environmentally friendly technologies for food and pharmaceutical products (Azevedo, Kopcak, & Mohamed, 2003). Among supercritical fluids, CO_2 is the most commonly used solvent for the extraction of oils from natural products. However, the efficiency of supercritical fluid extraction is hindered by the low solubility of the triglycerides in CO_2, and the high pressure and long extraction time required (Reverchon & Sesti Osséo, 1994).

Subcritical fluid extraction (SFE), also called pressurized low-polarity fluid extraction, is one of the most popular techniques which can overcome the defects of the conventional organic solvent extraction and expeller pressing methods. It is an excellent extraction that has numerous advantages such as lower operating temperature and pressures, shorter extraction time, environmental compatibility, good selectivity, one step from the extraction to the separation and avoidance of residual solvents (Herrero, Cifuentes, & Ibanez, 2006; Jiménez-Carmona & Luque de Castro., 1999; Rogelio & Luque de Castro, 2001). Different subcritical fluids have been used in SFE, but n-butane is used as the subcritical fluid mainly because it needs lower critical pressures and temperatures, and it

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has excellent dissolving power for lipophilic compound. Also this extractant is a low boiling point, inexpensive, colorless, and clean solvent that leaves no solvent residue in the product. SFE is rapidly emerging as a powerful means of extraction of solid samples, especially seeds oil. It can be considered a technological revolution in the extraction industry.

In this project, the subcritical fluid extraction parameters (extraction pressure, extraction temperature and raw material particle size) were optimized for the extraction of oil from *N. tangutorum* seeds via response surface methodology (RSM) with a three-variable-three-level Box-Behnken design (BBD) (Mostafa, 2011; Qiao et al., 2009; Zhang et al., 2009). The chemical compositions of seed oil were comprehensively analyzed by gas chromatography-mass spectrometry (GC–MS).

2. Materials and methods

2.1. Chemicals

Petroleum ether (30–60 °C, analytically pure) was obtained from the Dong Fang Hong Chemical Plant of Lin Bo (Zibo, China). *n*-Hexane, dichloromethane, methanol, boron trifluoride and ethyl-ether (all analytically pure) were purchased from Tianjin BASF Chemical Trade Co., Ltd. (Tianjin, China). N-butane was produced by Anyang Jing Hua Oil Engineering Co., Ltd. Other reagents were of analytical grade (Beijing Reagent Factory, Beijing, China).

2.2. Materials

Sixty kilograms fresh *N. tangutorum* Bobr. fruits were collected from Delingha (Latitude: 37°12′ N, Longitude: 97°29′ E Altitude: 2860 m), Qinghai province, China. The fruits were hand-picked in August, 2010, then juiced to remove the seeds.

2.3. Sample preparation and autoclaving treatment

Fifteen kilograms dried *N. tangutorum* seeds were ground into powder in a cyclone mill and passed through a mesh sieve (aperture size, 40 mesh). The autoclaving treatment was carried out using the method described by Kasai et al. with some modifications (2003). The seed powder was dipped in 4 volumes of water and then stored overnight at 5 °C. After being filtered through Whatman No. 1 paper (Whatman-Xinhua Filter Papers Co., Zhejiang, China), 2 volumes of water was again added to the powder to promote adhesion between the *N. tangutorum* seeds cells to be transferred to the water. The powder was autoclaved at 121 °C for 12 min, then immediately depressurized to destroy the hard and compact honeycombed pericarp of seeds. The autoclaved seeds powder was filtered, air-dried for 24 h at 80 °C, and about thirteen kilograms of the dried product obtained and then stored at 4 °C for further use.

2.4. Supercritical carbon dioxide extraction

Extraction of supercritical carbon dioxide extraction (SCCE) was performed in a flow circulatory extraction apparatus (Ghoreishi & Sharifi, 2001; Mitra, Ramaswamy, & Chang, 2009). In all experiments, 500-g samples of powdered seeds were used. The extraction capacity was 1000 mL and the CO_2 flow rate was 77 L/hour. About 3.5 L carbon dioxide was pumped into the extractor from a 4.2 MPa pressurized bottle. Pressure was maintained at a constant of 25.3 MPa in the extractor, and at 6.0 and 4.4 MPa in separators I and II, respectively. The extractor and separators were jacketed to maintain constant temperatures at 50 °C and 55 °C, respectively. The oil was collected from the two separators every 20 min and the CO_2 was cooled and recycled into the extraction system. Oil samples were weighed and analyzed.

2.5. Soxhlet extraction

Soxhlet extraction (SE) is a traditional method for extracting fats and oils (Hawthorne, Grabanski, Martin, & Miller, 2000). Four grams of preprocessed seeds and 60 mL of ethyl ether were added to a Soxhlet extractor. The contents were rapidly heated to reflux for 3 h with vigorous stirring. After cooling, the contents were filtered. The ethyl ether was evaporated to dryness in a rotary vacuum evaporator at 52 °C, and the seeds oil was recovered.

2.6. Subcritical fluid extraction

Subcritical fluid extraction (SFE) was performed using the apparatus (AY Mantianxue Food Manufacturing Co., LTD, Henan province, China) shown in Fig. 1. A G445-5/6-13 pump (Beijing Huizhi Mechanical and Electrical Equipment Co., Ltd, China) with digital flow-rate and readouts was used to impel the n-butane extractant fluid through the system. The extraction capacity was 5000 mL and the maximum flow rate of the n-butane fluid was 80 L/hour. The extractor pressure was regulated at valves 2 and 4. The extractor also contained stainless steel filter plates to ensure that the plant material remained in the ends of the extraction chamber. This chamber had a stainless steel mezzanine and was located in an oven designed to build up to a proper temperature. It was controlled by a temperature controller: when the contents reached the separator, extracts were collected through valve 5. The extractant fluid became gaseous and reached the compression pump, enabling circulation of the extractant through valve 3.

For SFE, 500-g samples of pretreated seeds were placed on the extractor plates. About 1.0 L pure n-butane stored was pumped into the oven in the form of subcritical fluid. There it reached the fluid reservoir and passed through the extraction chamber containing the samples and a stainless steel plug inserted at the outlet end to protect the frit. The liposoluble extract reached the separator and, after passage through a valve, was collected in a vial. In kinetic experiments, a 50-min extraction was performed under optimum working conditions with the collection vial replaced at appropriate intervals.

2.7. Calculation of the extraction yield

Three times was carried out as the extraction times in the three different methods, respectively. The extraction yield was determined gravimetrically by the mass of extracted oil divided by the mass of *N. tangutorum* seed loaded in the extraction vessel, namely:

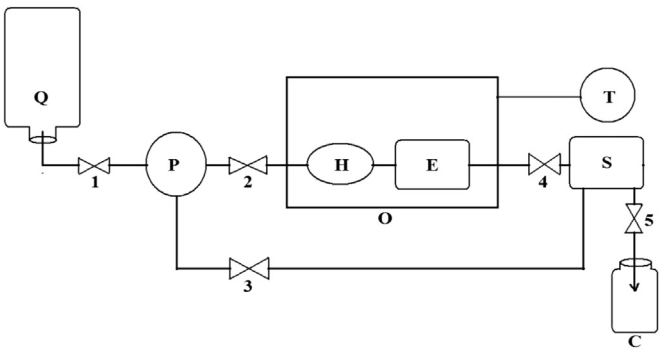

Fig. 1. Schematic diagram of the SFE apparatus. Q: normal butane bottle, P: compression pump, H: fluid reservoir, E: extractor, O: oven, T: temperature controller, S: separator, C: collector, 1–5: valves.

$$\text{The extraction yield}(\%) = (\text{mass of extracted oil/mass of dried material}) \times 100\%$$

2.8. Experimental design and statistical analysis

On the basis of single-factor experimentation, preliminary proper ranges of raw material particle size, extraction time, extraction pressure and extraction temperature were determined. A three-variable-three-level BBD (software Design-Expert 7.0.1.0, Stat-Ease, Inc, Minneapolis, U.S.) was used to determine the best combination of extraction variables for the production of seeds oil (Wanasundara & Shahidi, 1996; Wang, Sun, Cao, Tian, & Li, 2008; Yu, Dandekar, Toledo, Singh, & Patil, 2007;). Based on single-factor experiments, the key variables were determined to be extraction pressure (MPa, X_1), extraction temperature (°C, X_2) and raw material particle size (mm, X_3). Table 1 details the BBD matrix and response values carried out for developing the model. The whole design consisted of 17 experimental points carried out in random order. Five replicates (treatments 13–17) at the center of the design were used for estimating of a pure error sum of squares.

Regression analysis was performed for the experimental data and was fitted into an empirical second-order polynomial model, as shown in the following equation:

$$Y = \beta_0 + \sum_{i=1}^{3} \beta_i X_i + \sum_{i=1}^{3} \beta_{ii} X_i^2 + \sum_{i=1}^{2} \sum_{j=i+1}^{3} \beta_{ij} X_i X_j$$

Here, β_0, β_i, β_{ii}, and β_{ij} are the regression coefficients of variables for intercept, linear, quadratic, and interaction terms, respectively, and X_i and X_j are independent variables ($i \neq j$). The coefficients of the second polynomial model and the responses obtained from each set of experimental design were subjected to multiple nonlinear regressions using Design-Expert software. The fitness of the polynomial model equation is expressed by the coefficient of determination R^2, and its statistical significance was confirmed by F-test at a probability (P) of 0.001, 0.01, or 0.05. The significances of the regression coefficients were also confirmed by F-test.

2.9. GC–MS analysis

The KOH-methanol methyl esterification method was employed directly. *N. tangutorum* seed oil (0.2 g) was added into a 10 mL test tube equipped with a stopper and mixed with 0.5 mol mL^{-1} KOH-methanol solution (1 mL). Then, the test tube with the stopper was placed in a water bath at 40 °C, and shaken for 30 min. Then n-hexane (1 mL) was added and the mixture kept in a water bath at 20 °C and shaken for 10 min. After cooling water was added to 10 mL and the mixture was extracted for 1 min. A small portion of anhydrous sodium sulfate was added to the liquid supernatant that was obtained after centrifugation for 10 min at a rotation speed of 12,000 r min^{-1}. The filtered liquid was prepared for GC–MS sample by 100 times dilution.

An HP 6890 gas chromatograph equipped with an HP 7683 auto injector and an HP 5973 MSD (Agilent Technologies, Palo Alto, CA, U.S.) were used for GC–MS analysis. Gas chromatographic separation was carried out with an HP-5 capillary column (30 m × 0.25 mm i.d., film thickness 0.25 μm). The samples (1 μL) were injected manually in the pulsed splitless mode. The pulse time was 1.5 min, the pressure was 210 kPa, and the injector and MSD ion source temperature were set at 250 °C and 230 °C, respectively. The column temperature was increased from 80 °C to 290 °C at 4 °C/min. After that, temperature was kept constant for 30 min. Helium (purity of 99.999%) was used as a carrier gas at a flow rate of 1.2 mL/min. The mass spectrometer was operated in electron-impact ionization (EI) mode with 70 eV energy.

The identification of oil components was based on matching their recorded retention indices and mass spectra with those in NIST (National Institute of Standards and Technology) library data provided by the GC–MS software.

Table 1
BBD matrix and the response values for the oil yield of *N. tangutorum* seeds.

Run	Independent variable			Oil yield (%)	
	X_1 (pressure, MPa)	X_2 (temperature, °C)	X_3 (particle size, mm)	Experimental	Predicted
1	−1 (0.45)	−1 (40)	0 (0.5)	12.37	12.347
2	+1 (0.65)	−1 (40)	0 (0.5)	12.69	12.773
3	−1 (0.45)	+1 (60)	0 (0.5)	12.21	12.113
4	+1 (0.65)	+1 (60)	0 (0.5)	12.44	12.447
5	−1 (0.45)	0 (50)	−1 (0.3)	11.94	11.983
6	+1 (0.65)	0 (50)	−1 (0.3)	12.48	12.417
7	−1 (0.45)	0 (50)	+1 (0.7)	10.87	10.917
8	+1 (0.55)	0 (50)	+1 (0.7)	11.30	11.243
9	0 (0.55)	−1 (40)	−1 (0.3)	12.39	12.353
10	0 (0.55)	+1 (60)	−1 (0.3)	12.11	12.147
11	0 (0.55)	−1 (40)	+1 (0.7)	11.36	11.307
12	0 (0.55)	+1 (60)	+1 (0.7)	10.93	10.953
13	0 (0.55)	0 (50)	0 (0.5)	12.53	12.710
14	0 (0.55)	0 (50)	0 (0.5)	12.78	12.710
15	0 (0.55)	0 (50)	0 (0.5)	12.88	12.710
16	0 (0.55)	0 (50)	0 (0.5)	12.65	12.710
17	0 (0.55)	0 (50)	0 (0.5)	12.70	12.710

3. Results and discussion

3.1. Effects of extraction pressure

To investigate the effects of extraction pressure on the oil yield of *N. tangutorum* seeds, the extraction process was carried out using different extracting pressures: 0.25, 0.35, 0.45, 0.55 and 0.65 MPa. Other extracting parameters were fitted as follows: raw material particle size 0.9 mm, extraction temperature 40 °C, and extraction time 60 min. As shown in Fig. 2A, the seeds oil yield increased slowly with increasing pressure, ultimately reaching a maximum at 0.55 MPa. These results also indicate that extraction pressure has a significant positive effect on oil yield when it is below 0.55 MPa. The effect is not significant when extraction pressure is above 0.55 MPa. Therefore, 0.55 MPa was selected as the central point of extraction pressure in the RSM experiments because higher pressures can cause equipment problems, waste energy, and increase costs.

3.2. Effects of extraction temperature

The effects of extraction temperature on the seed oil yield were studied at 20, 30, 40, 50 and 60 °C (in all cases, pressure was maintained at 0.55 MPa, raw material particle size was 0.9 mm, and extraction time was 60 min). As shown in Fig. 2B, the oil yield increased with increasing temperature, ultimately reaching a maximum at 50 °C. The weakness of the relationship between oil yield and temperature is due to retrograde solubility. The density of the subcritical butane decreased with increasing temperature and served to decrease the solubility of the seeds oil, but the pressure of the solute in subcritical butane mixtures increased concomitantly with an increase in temperature, thus improving solubility.

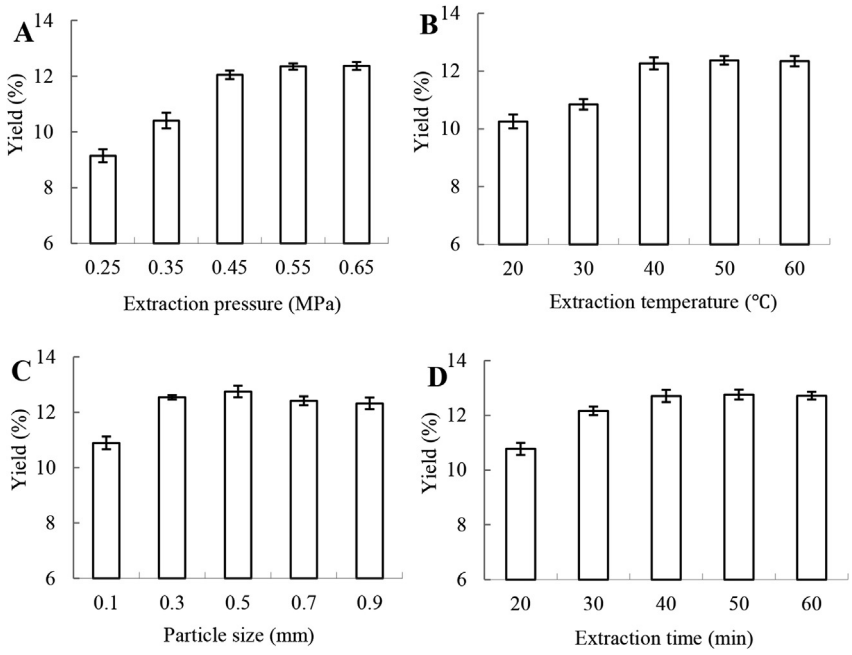

Fig. 2. Effects of (A) extraction pressure, (B) extraction temperature, (C) raw material particle size, and (D) extraction time on the oil yield of *N. tangutorum* seeds.

3.3. Effects of raw material particle size

Raw material particle size is another factor that influences the extraction efficiency and selectivity of fluids. In this work, we evaluated the effects of particle size on extraction efficiency at a given pressure (0.55 MPa), temperature (50 °C) and extraction time (60 min). As can be seen in Fig. 2C, there was an increasing trend in oil yield from 0.1 to 0.5 mm and a decreasing trend from 0.5 to 0.9 mm. Statistical analysis showed that significant differences existed between 0.1 and 0.3 mm and between 0.1 and 0.5 mm ($P < 0.05$), but there was no significant difference between 0.3, 0.5, 0.7, and 0.9 mm ($P > 0.05$). The smaller the particle size, the higher the oil yield. However, we could not use the smallest samples because these cannot form a sheet and increase mass transfer resistance. We selected 0.5 mm as the central point for raw material particle size in the RSM experiments.

3.4. Effects of extraction time

Fig. 2D shows the effects of extraction time on oil yield when other factors are set as follows: extraction pressure, 0.55 MPa; extraction temperature, 50 °C; raw material particle size, 0.5 mm. There was an increasing trend in oil yield accompanying increases in extraction time, but there was no significant difference ($P > 0.05$) between 30, 40, 50, and 60 min. Taking yield and processing costs into consideration, 40 min were sufficient for the extraction of seed oil. Forty minutes were selected as the extraction time for the next experiments.

3.5. Response surface optimization of subcritical fluid extraction condition

Subcritical fluid extraction parameters were optimized using response surface methodology (RSM) according to the Box-Behnken design (BBD). The range and center point values of three independent variables were based on the results of the single-factor experiments. The response values (oil yield) for different experimental combinations are given in Table 1. It can be seen from Table 1 that there is considerable variation in oil yield depending upon extraction conditions. The regression coefficients of the intercept, linear, quadratic, and interaction terms of the model were calculated using the least square technique. They are presented in Table 2. It was evident that all linear parameters and two quadratic parameters (extraction pressure and raw material particle size) were significant ($p < 0.05$ or $p < 0.01$). All interaction parameters were insignificant ($p > 0.1$). These results indicate that the effects of extraction pressure and raw material particle size were the major contributing factors to the oil yield.

The application of RSM offered, based on parameter estimates, an empirical relationship between the experimental variables and response. By employing multiple regression analysis on the experimental data, the predicted response Y for oil yield can be obtained by the following second-order polynomial equation: $Y = 12.71 + 0.19X_1 - 0.14X_2 - 0.56X_3 - 0.023X_1X_2 - 0.027X_1X_3 - 0.037X_2X_3 - 0.17X_1^2 - 0.12X_2^2 - 0.90X_3^2$, where X_1, X_2, and X_3 are in terms of coded factors of the test variables, extraction pressure, extraction temperature, and raw material particle size, respectively.

The analysis of variance for the experimental results of the BBD are also shown in Table 2. The Model F-value 49.93, implied that the model was valid. The quality of the model can be confirmed by the determination coefficients (R^2) and the multiple correlation coefficients (R). The closer the values of R are to 1, the better the correlation between experimental and predicted values (Pujari & Chandra, 2000). In this experiment, the coefficient of determination (R^2) of the model was 0.9847, which indicated good agreement between the experimental and predicted values of oil yield. The results of analysis of error indicated that the lack of fit was insignificant ($p > 0.05$). The F-value (0.64) and P-value (0.6285) implied that the lack of fit, relative to the pure error, was not significant. It indicated that the model equation was adequate for predicting the yield of seeds oil under any combination of values of the variables. The coefficient of variation (C.V.) was below 5%, indicating that the model was reproducible (Mason, Gunst, & Hess, 1989; Wanasundara & Shahidi, 1996). The model's predicted residual sum of squares (PRESS), a measure of how a particular model fits each point in the design, was 0.65. The value of pred R^2 (0.9043) is

Table 2
Estimated regression coefficients for the quadratic polynomial model and the analysis of variance (ANOVA) for the experimental results.

Parameter	Coefficient estimate	Standard error	Sum of squares	DF	Mean square	F-value	Prob > F
Model			6.64	9	0.74	49.93	<0.0001
Intercept	12.71	0.054		1			
X_1	0.19	0.043	0.29	1	0.29	19.56	0.0031
X_2	−0.14	0.043	0.16	1	0.16	10.62	0.0139
X_3	−0.56	0.043	2.49	1	2.49	168.36	<0.0001
X_1X_2	−0.023	0.061	2.025E-003	1	2.025E-003	0.14	0.7221
X_1X_3	−0.027	0.061	3.025E-003	1	3.025E-003	0.20	0.6645
X_2X_3	−0.037	0.061	5.625E-003	1	5.625E-003	0.38	0.5567
X_1^2	−0.17	0.059	0.11	1	0.11	7.79	0.0269
X_2^2	−0.12	0.059	0.056	1	0.056	3.79	0.0927
X_3^2	−0.90	0.059	3.37	1	3.37	228.50	<0.0001
Residual			0.10	7	0.015		
Lack of fit			0.034	3	0.011	0.64	0.6285
Pure error			0.070	4	0.017		
R^2	0.9847		Adj R^2	0.9649			
C.V.%	1.00		Pred R^2	0.9043			
PRESS	0.65		Adeq Precision	19.875			

in reasonable agreement with the adj R^2(0.9649). The value of adeq precision measures the signal to noise ratio. A ratio greater than 4 is desirable (Vohra & Satyanarayana, 2002). The experimental ratio of 19.875 indicates an adequate signal. In summary, the ANOVA of quadratic regression model demonstrated that the model was significant, and the Fisher's *F*-test had a high model *F*-value (49.93) and a very low *P*-value ($P < 0.0001$). The BBD showed that polynomial regression model well matched with the experimental results. So, this model can be used to navigate the design space.

The three-dimensional (3D) response surface and two-dimensional (2D) contour plots that are the graphical representations of regression equation obtained from the calculated response surface are indicated in Fig. 3. They provide a means of visualizing the relationship between the responses and experimental levels of each variable and the type of interactions between the two test variables. The shapes of the contour plots, circular and elliptical, indicate whether the mutual interactions between the variables were significant or not. Circular contour plots indicate that the interactions between the corresponding variables are negligible, while elliptical contour plots indicate that the interactions between the corresponding variables are significant (Muralidhar, Chirumamila, Marchant, & Nigam, 2001). In this study, three independent response surface plots and their respective contour plots were generated using Design-Expert as shown in Fig. 3. The interactions between two variables and their optimum ranges can be seen. All the mutual interactions between the test variables were found to be insignificant. The predicted oil yield was 12.70% and lay in the following ranges of the examined variables: extraction pressure 0.54–0.65 MPa, extraction temperature 40–52 °C, and raw material particle size 0.34–0.55 mm. The optimum values of the test variables were extraction pressure, 0.61 MPa; extraction temperature, 43.80 °C; and raw material particle size, 0.44 mm. Under these conditions, the maximum predicted oil yield was 12.90%, which was slightly higher than that obtained from plots analysis.

The trial experiments were conducted under optimized conditions. Taking convenience into account, the optimum experimental parameters were determined as follows: extraction pressure, 0.60 MPa; extraction temperature, 44 °C; raw material particle size, 0.45 mm. To compare the predicted results (12.90%) with experimental values, rechecking was performed using deduced optimal conditions. The mean value of 12.92% ($n = 3$), obtained from experiments, showed the validity of this RSM model because the differences between 12.90% and 12.92% ($n = 3$) were not significant ($p > 0.05$). The strong correlation between experimental and predicted results confirmed that the response model was accurate and adequate to reflect the expected optimization of the oil extraction process.

3.6. Comparison with conventional extraction techniques

For the three extraction methods (subcritical fluid extraction, supercritical carbon dioxide extraction and Soxhlet extraction), the routine conditions were used as described in the experimental section. The extraction experiments performed on *N. tangutorum* seeds using different methods showed that the highest oil yield (12.92 g/100 g seeds) was attained in the case of subcritical fluid extraction (SFE) with n-butane solvent under the following conditions: solid to solvent ratio (0.5 g/mL), low pressure (0.60 MPa) and temperature (44 °C) with a short extraction time (40 min). However, traditional Soxhlet extraction (SE) performed with a long extraction time (180 min) and high temperature yielded only 9.45 g of oil from 100 g of seeds. Supercritical carbon dioxide extraction (SCCE) produced 11.01 g oil per 100 g of seeds, and the extraction pressure (25.3 MPa) was very high. From the extraction yields, it can be seen that SFE had the highest extraction yield. Moreover, SFE did not use toxic organic solvents and performed under lower pressure, temperature and amount of solvent. Therefore, SFE seems to be the best method for *N. tangutorum* seeds oil extraction from the aspect of oil yield and extraction efficiency. All these characteristics of SFE make it very popular in the functional food, medicine, and health product fields.

3.7. Fatty acid composition analysis of N. tangutorum seed oil

Fatty acid composition analysis was carried out with GC–MS for the oils obtained by Soxhlet extraction (SE), supercritical carbon dioxide extraction (SCCE), and subcritical fluid extraction (SFE). As shown in Table 3, all the oils extracted were rich in unsaturated fatty acids (linoleic acid and oleic acid making up from 96.91% to 97.92% of total fatty acids), and relatively low in saturated fatty acids. The main component of these seed oils was linoleic acid, and the concentration of linoleic acid was between 78.70% and 80.23%. There were no significant differences among the seed oils obtained using these three extraction methods.

4. Conclusion

The performance of the subcritical fluid extraction of seeds oil from *N. tangutorum* was studied with a statistical method based on

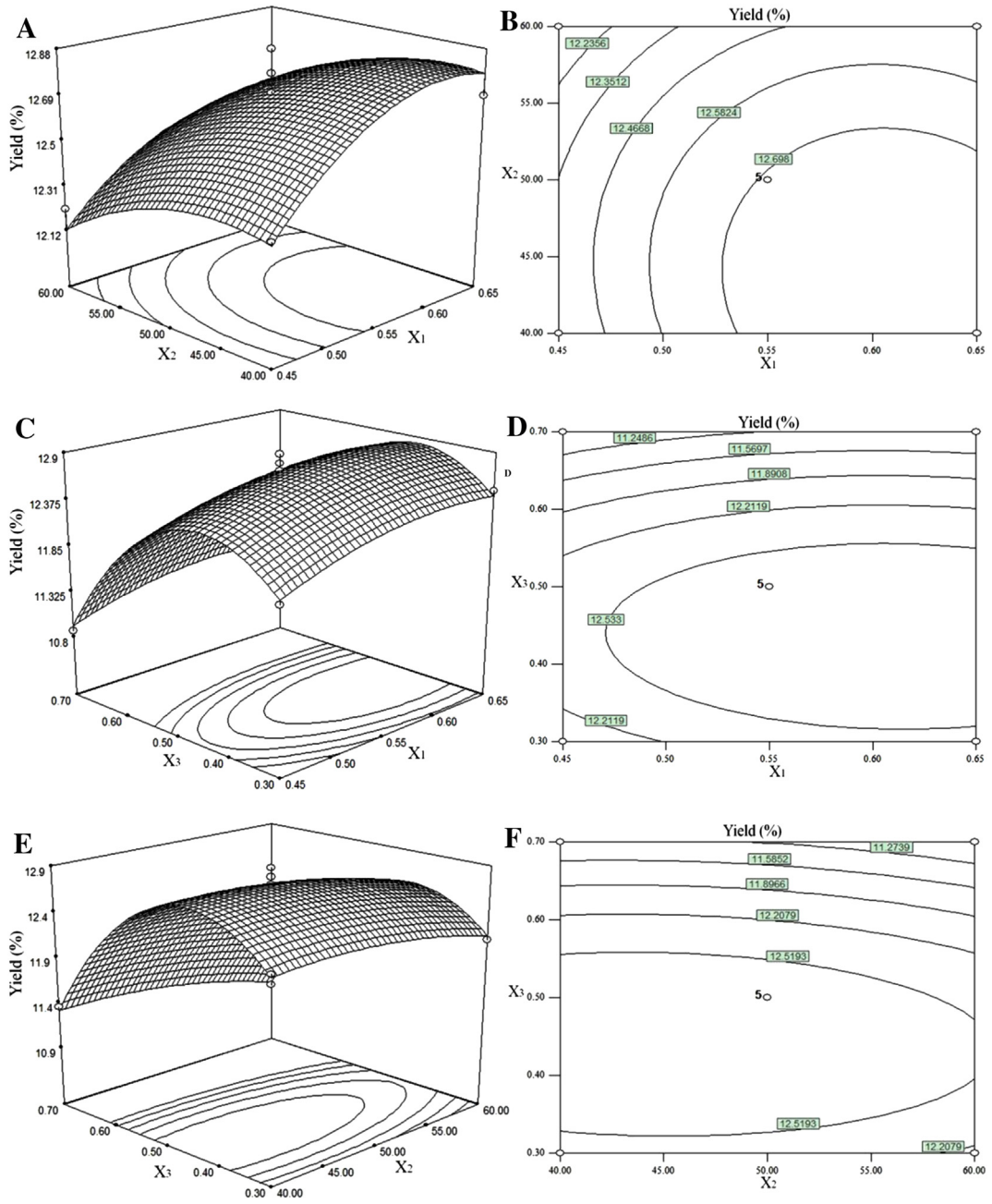

Fig. 3. Response surface plots (A, C and E) and contour plots (B, D and F) of the oil yield affected by extraction pressure (X_1), extraction temperature (X_2), and raw material particle size (X_3).

Table 3
Fatty acid composition of the *N. tangutorum* seed oils extracted via different procedures.[a]

Fatty acid	SE (%)	SCCE (%)	SFE (%)
Palmitic acid	1.920	1.264	1.493
Linoleic acid	78.698	80.227	79.349
Trans-oleic acid	17.972	17.561	17.833
Cis-oleic acid	0.241	0.136	0.227
Stearic acid	0.425	0.306	0.466
8,10-Dimethoxy-octadecanoic acid	0.465	0.389	0.385
Eicosanoic acid	0.084	0.035	0.095
Doeicosanoic acid	0.115	0.043	0.090
Tetracosanoic acid	0.079	0.040	0.061

[a] Results are expressed as % over the total content (relative content); SE, Soxhlet extraction; SCCE, supercritical carbon dioxide extraction; SFE, subcritical fluid extraction.

the response surface methodology (RSM) in order to identify and quantify the variables which may maximize the yield of oil. The experiment results showed that the optimal conditions for the production of seeds oil were as the following: extraction time 40 min, extraction pressure 0.60 MPa, extraction temperature 44 °C, and raw material particle size 0.45 mm. By comparing these three extraction methods, the subcritical fluid extraction (SFE) had higher oil yield and efficiency than the Soxhlet extraction and supercritical carbon dioxide extraction. Also the SFE was very applicable to industrial products regarding chemical compositions and extraction for natural medicines or foods. The established technique could be hopefully applied to the extraction and analysis of liposoluble components from plants, fungi, medicines and biochemistry samples.

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References

Azevedo, A., Kopcak, U., & Mohamed, R. S. (2003). Extraction of fat from fermented Cupuacu seeds with supercritical solvents. *The Journal of Supercritical Fluids, 27*, 223–237.

Brondz, I., Olsen, I., Haapasalo, M., & Winkelhoff, A. J. V. (1991). Multivariate analyses of fatty acid data from whole-cell methanolysates of Prevotella, Bacteroides and Porphyromonas spp. *Journal of General Microbiology, 137*, 1445–1452.

Ghoreishi, S. M., & Sharifi, S. (2001). Modeling of supercritical extraction of mannitol from plane tree leaf. *Journal of Pharmacology and Biomedical Analysis, 24*, 1037–1048.

Hawthorne, S. B., Grabanski, C. B., Martin, E., & Miller, D. J. (2000). Comparisons of Soxhlet extraction, pressurized liquid extraction, supercritical fluid extraction and subcritical water extraction for environmental solids: recovery, selectivity and effects on sample matrix. *Journal of Chromatography A, 892*, 421–433.

Herrero, M., Cifuentes, A., & Ibanez, E. (2006). Sub- and supercritical fluid extraction of functional ingredients from different natural sources: plants, food-by-products, algae and microalgae: a review. *Food Chemistry, 98*, 136–148.

Jiang, Z. J., Zhang, G. J., & Wang, J. H. (1989). Flavonoid constituents from the seeds of *Nitraria Tangutorum* Bobr. *Acta Botanica Sinica, 31*, 241–243.

Jiménez-Carmona, M. M., & Luque de Castro, M. D. (1999). Isolation of *Eucalyptus* essential oil for GC-MS analysis by extraction with subcritical water. *Chromatographia, 50*, 578–582.

Kasai, N., Imashiro, Y., & Morita, N. (2003). Extraction of soybean oil from single cells. *Journal of Agricultural and Food Chemistry, 51*, 6217–6222.

Mason, R. L., Gunst, R. F., & Hess, J. L. (1989). *Statistical design and analysis of experiments: With applications to engineering and science*. In *Wiley series in probability and mathematical statistics*. New York: John Wiley and Sons.

Mitra, P., Ramaswamy, H. S., & Chang, K. S. (2009). Pumpkin (*Cucurbita maxima*) seed oil extraction using supercritical carbon dioxide and physicochemical properties of the oil. *Journal of Food Engineering, 95*, 208–213.

Mostafa, K. (2011). Optimization of process variables for essential oil components from *Satureja hortensis* by supercritical fluid extraction using Box-Behnken experimental design. *Journal of Supercritical Fluids, 55*, 944–948.

Muralidhar, R. V., Chirumamila, R. R., Marchant, R., & Nigam, P. (2001). A response surface approach for the comparison of lipase production by *Candida cylindracea* using two different carbon sources. *Biochemical Engineering Journal, 9*, 17–23.

Pan, X. L., Shen, G. M., & Chen, P. (1999). A preliminary research of taxonomy and systematics of genus *Nitraria*. *Acta Botony Yunnanica, 3*, 287–295.

Pujari, V., & Chandra, T. S. (2000). Statistical optimization of medium components for enhances riboflavin production by a UV-mutant of *Eremothecium ashbyii*. *Process Biochemistry, 36*, 31–37.

Qiao, D. L., Hu, B., Gan, D., Sun, Y., Ye, H., & Zeng, X. X. (2009). Extraction optimized by using response surface methodology, purification and preliminary characterization of polysaccharides from *Hyriopsis cumingii*. *Carbohydrate Polymers, 76*, 422–429.

Reverchon, E., & Sesti Osséo, L. (1994). Comparison of processes for the supercritical carbon dioxide extraction of oil from soybean seeds. *Journal of the American Oil Chemists' Society, 71*, 1007–1012.

Rogelio, S. A., & Luque de Castro, M. D. (2001). Continuous subcritical water extraction as a useful tool for isolation of edible essential oils. *Food Chemistry, 75*, 109–113.

Scalia, S., Giuffreda, L., & Pallado, P. (1999). Analytical and preparative supercritical fluid extraction of Chamomile flowers and its comparison with conventional methods. *Journal of Pharmaceutical and Biomedical Analysis, 21*, 549–558.

Suo, Y. R., Wang, H. L., & Wang, H. Q. (2004). Research on decreasing blood lipid and anti-oxidative effect of fruit of *Nitraria Tangutorum* Bobr. from Qaidam Basin. *Natural Product Research Development, 1*, 54–58.

Vohra, A., & Satyanarayana, T. (2002). Statistical optimization of the medium components by response surface methodology to enhance phytase production by *Pichia anomala*. *Process Biochemistry, 37*, 999–1004.

Wanasundara, P. K. J. P. D., & Shahidi, F. (1996). Optimization of hexametaphosphate-assisted extraction of flaxseed proteins using response surface methodology. *Journal of Food Science, 61*, 604–607.

Wang, N. (2000). A review of *Nitraria* source and development. *Shanxi Forest Science and Technology, 1*, 17–18.

Wang, J., Sun, B. G., Cao, Y. P., Tian, Y., & Li, X. L. (2008). Optimization of ultrasound-assisted extraction of phenolic compounds from wheat bran. *Food Chemistry, 106*, 804–810.

Yu, J., Dandekar, D. V., Toledo, R. T., Singh, R. K., & Patil, B. S. (2007). Supercritical fluid extraction of limonoids and naringin from grapefruit (*Citrus paradisi Macf.*) seeds. *Food Chemistry, 105*, 1026–1031.

Zhang, Q. A., Zhang, Z. Q., Yue, X. F., Fan, X. H., Li, T., & Chen, S. F. (2009). Response surface optimization of ultrasound-assisted oil extraction from autoclaved almond powder. *Food Chemistry, 116*, 513–518.

Zhao, X. E., Li, Y. L., Suo, Y. R., Shi, Y. W., Chen, X. M., Zhang, H. F., et al. (2006). Determination of free fatty acids from soil and bryophyte by HPLC with fluorescence detection and identification with mass spectrometry. *Chinese Journal of Analytical Chemistry, 34*, 150–154.