

Supercritical Extraction of Evening Primrose Oil: Experimental Optimization via Response Surface Methodology

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Supercritical carbon dioxide (CO₂) effective extraction parameters (pressure, temperature, static extraction time, and dynamic extraction time) of oil recovery from evening primrose seeds were optimized via response surface methodology (RSM). The results of this study indicated that the linear terms of static and dynamic time and the quadratics of temperature and pressure, as well as the interactions of temperature and static time, pressure and temperature had a significant effect on the oil recovery. The optimum extraction conditions of 14.2 MPa, 47.3°C, 30 min (static extraction time) and 150 min (dynamic extraction time) were obtained. Applying the optimum conditions, a mean experimental recovery of 92.98% (triplicate experiment) was achieved, which is well compatible with the RSM-predicted value (93.61%). The fatty acid composition of extracted evening primrose oil using supercritical CO₂ was compared with that obtained by Soxhlet method in which minor difference was observed. © 2011 American Institute of Chemical Engineers AIChE J, 57: 3378–3384, 2011

Keywords: supercritical CO₂ extraction, response surface methodology, evening primrose oil, fatty acids

Introduction

Evening primrose (*Oenothera biennis* L.) is cultivated as an oil seed crop, because its seeds are rich in oil containing γ -linolenic acid (GLA), which is used both as a nutritional and a medicinal supplement.^{1,2} At present, evening primrose oil (EPO) is one of the most important sources of GLA, which is in growing demand for its clinical and pharmaceutical applications.³ Diseases such as rheumatic arthritis, atopic eczema, cardiovascular disease, breast pain, and premenstrual syndrome could be influenced by GLA.⁴ The EPO is generally obtained by mechanical pressing followed by extraction with hexane.⁵ In the field of extraction processes, using supercritical fluids such as carbon dioxide (CO₂) over-

comes many drawbacks linked to the use of liquid organic solvents such as liquid hexane. Solvents commonly used present problems of toxicity and residual content in the final products after extraction. In addition to toxicity, there is also the problem of security during storage due to flammability. In this context, supercritical fluid extraction (SFE) especially supercritical CO₂ (SC-CO₂) extraction as an efficient extraction method has attracted attention during the last two decades, as its advantages include being nonexplosive, nontoxic, and available in high purity with low cost and nonsolvent residues.^{6,7} Besides, the extraction by supercritical CO₂ decreases the unit operation number in the process in comparison with classical processes using hexane. The CO₂ critical temperature is low (30.9°C), which is interesting for the extraction of thermolabile components. By varying pressure beyond its critical value (7.38 MPa), it is possible to control the solvent power to a large extent, permitting an important selectivity. The SC-CO₂ extraction has been widely adopted

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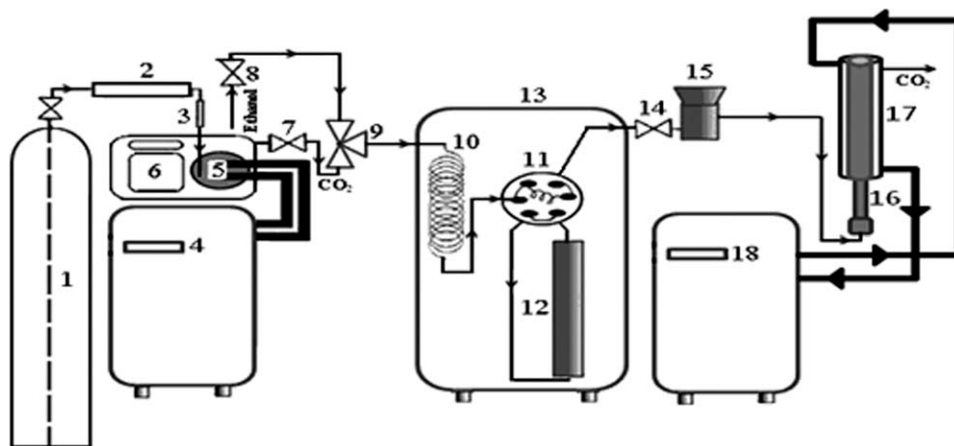


Figure 1. Schematic diagram of supercritical extraction system.

in the food processing industry such as extraction of essential oils^{8–13} from various plant materials.

The efficiency of SC-CO₂ extraction can be affected by many factors including pressure, temperature, flow rate, static extraction time, and dynamic extraction time. To study the effect of multiple variables on output, the response surface method (RSM) is an effective technique.¹⁴ The RSM is a collection of mathematical and statistical techniques useful for the modeling and analysis of problems in which a response of interest is influenced by several variables and the objective is to optimize this response. The methodology involves three steps: (1) experimental design in which the independent variables and their experimental levels are set using well-established statistical experimental designs such as the central composite design; (2) response surface modeling through regression analysis; and (3) process optimization using the response surface models. The principles and applications of RSM have been well described.^{15,16} RSM is a faster and more economical method for gathering research results than classic one-variable-at-a-time or full-factors experimentation. RSM has been successfully used to model and optimize supercritical CO₂ extraction of oils from grape seed,¹³ cherry seed,¹⁷ walnut,¹⁸ apricot kernel,¹⁹ hazelnut,²⁰ rosemary,²¹ and rosehip seed.²²

In this study, the SC-CO₂ was used to extract the oil from the evening primrose seeds. The effect of important independent variables on extraction recovery such as supercritical fluid pressure and temperature, static time, and dynamic time were investigated in the range of 7.5–39.5 MPa, 33–61°C, 10–30 min, and 30–150 min, respectively, using RSM.

Experimental

Materials

The evening primrose seeds were obtained from Isfahan agricultural research center. Samples were vacuum dried at 60°C to a stable moisture content of less than 5%. As extraction kinetics in this study was controlled by the kernel particle size, an important sieving step was carried out to achieve reproducible extraction recovery in which the samples were passed through a sieve with mesh size of 18–35 (1–0.5 mm). Fatty acid methyl ester (FAME) standards were purchased

from Sigma. In Soxhlet extraction, *n*-hexane (99.6%, Merck) was used as the solvent. Industrial grade CO₂ (≥99%) was obtained from Zamzam (Isfahan, Iran).

Soxhlet extraction

For Soxhlet extraction, 3 g of milled seeds was weighted and set in a Soxhlet apparatus and then continuously extracted for 8 h at one time using *n*-hexane at 69°C. After extraction, the solvent was evaporated by rotary vacuum evaporator (30°C), and the extract was dried at 103°C to remove residual solvent until a constant weight.²³ The yield of oil was estimated gravimetrically. The results showed that Soxhlet extraction gave a yield of 32.45-g oil/100-g evening primrose seeds, which was considered as the total extractable oil content while calculating the oil recovery by SC-CO₂ extraction.

SFE: Apparatus

To carry out the objectives of this study, the supercritical extraction system shown in Figure 1 was used. This system operates at a temperature range of 25–250°C with a maximum pressure of 50 MPa. The system is constructed so that any noncorrosive or corrosive fluid can be used as the supercritical fluid. The experimental setup for the supercritical extraction system is composed of the following: (1) CO₂ cylinder (Zamzam), (2) molecular sieve column (Merck), (3) porous metal filter (Mott Metallurgical), (4) cooler circulator (Sina), (5) pump head cooler, (6) HPLC pump (Jasco), (7,8) valve (Shirvani), (9) three-way valve, (10) coil preheater, (11) injection valve (Rheodyne), (12) extraction column (Shirvani), (13) oven with PID temperature controller (Shimadzu, maximum temperature of 250°C), (14) valve (Shirvani), (15) back pressure regulator (Tescom), (16) solute collection vessel, (17) double pipe heat exchanger (Shirvani), and (18) cooler circulator (Sina).

Oil extraction by SC-CO₂

To increase the purity of the CO₂, which is stored in a CO₂ cylinder (1), it is passed through a column of molecular sieve beads (2), and metal porous filter (3). Then, CO₂ is

Table 1. Uncoded and Coded Levels of Independent Variables Used in the RSM Design

Coded variables levels (Z_i)	Pressure (X_1 , MPa)	Temperature (X_2 , °C)	Static time (X_3 , min)	Dynamic time (X_4 , min)
−2	7.5	33	10	30
−1	15.5	40	15	60
0	23.5	47	20	90
1	31.5	54	25	120
2	39.5	61	30	150

cooled down to 0°C in a cooler circulator/pump head cooler (4, 5), and subsequently charged by a feed pump (6). A valve (7) is placed at the effluent of the pump, and thus, the CO₂ stream is easily controlled and saved properly for further use. CO₂ is heated before entering the extraction column (12) using a coil preheater (10) that is placed in an oven (13). The stainless steel extraction column (height = 12.5 cm, inner diameter = 0.9 cm, and outer diameter = 1.3 cm) (12) fitted with porous metal filter at the inlet and outlet is manually charged with evening primrose seeds and glass beads (broken Pyrex laboratory glassware) with a mesh size of 18–35 in a ratio of 40–60% (w/w), respectively. First, CO₂ is charged into the extraction column while the valve (14) is closed, and the pump is set at the selected operating pressure and the desired temperature is obtained via the oven and preheater. After reaching the appropriate pressure and temperature in the column, the pump is turned off and isolated with a shut-off valve (7). Subsequently, a constant

static extraction time is allowed for SC-CO₂ to dissolve the oil. After carrying out the static extraction, the dynamic extraction with constant volumetric flow rate of CO₂ is started via opening the valve (14). At this stage, the system pressure is controlled and monitored by a back pressure regulator (15) and a high-pressure pump. By passing the CO₂ at a constant flow rate, the dissolved oil via static and dynamic extraction is discharged from the column, cooled (−15°C) via a 1-1 shell and tube heat exchanger (17, 18), trapped and collected via supercritical fluid expansion in the collection vessel (16). The whole system is washed with ethanol and subsequently purged with CO₂. The oil contained in the extracted samples was kept in the refrigerator for further GC-FID analysis. The oil recovery (R) was calculated using the following equation:

$$R = \frac{\text{weight of extracted oil}}{\text{mass of soxhlet extracted oil}} \times 100 \quad (1)$$

Experimental design

In this study, 2⁴ full factorial central composite rotatable design (CCRD) for four independent variables, each at five levels with eight axial points and seven replicates at the center points was used to fit second-order polynomial model. This procedure required 31 experimental runs. The axial points were added to the factorial design to estimate the model curvature. All experiments were carried out in

Table 2. Central Composite Rotatable Design and Values of Observed Response

Run	Coded variable				Process variable*				Oil recovery (%)
	Z_1	Z_2	Z_3	Z_4	X_1 (MPa)	X_2 (°C)	X_3 (min)	X_4 (min)	
1	−1	−1	−1	−1	15.5	40	15	60	78.98
2	1	−1	−1	−1	31.5	40	15	60	84.78
3	−1	1	−1	−1	15.5	54	15	60	84.04
4	1	1	−1	−1	31.5	54	15	60	83.73
5	−1	−1	1	−1	15.5	40	25	60	87.15
6	1	−1	1	−1	31.5	40	25	60	88.81
7	−1	1	1	−1	15.5	54	25	60	85.82
8	1	1	1	−1	31.5	54	25	60	82.74
9	−1	−1	−1	1	15.5	40	15	120	82.83
10	1	−1	−1	1	31.5	40	15	120	84.74
11	−1	1	−1	1	15.5	54	15	120	88.04
12	1	1	−1	1	31.5	54	15	120	86.69
13	−1	−1	1	1	15.5	40	25	120	89.21
14	1	−1	1	1	31.5	40	25	120	90.97
15	−1	1	1	1	15.5	54	25	120	90.17
16	1	1	1	1	31.5	54	25	120	87.43
17	−2	0	0	0	7.5	47	20	90	85.76
18	2	0	0	0	39.5	47	20	90	88.01
19	0	−2	0	0	23.5	33	20	90	82.06
20	0	2	0	0	23.5	61	20	90	86.53
21	0	0	−2	0	23.5	47	10	90	85.48
22	0	0	2	0	23.5	47	30	90	92.05
23	0	0	0	−2	23.5	47	20	30	85.76
24	0	0	0	2	23.5	47	20	150	91.09
25	0	0	0	0	23.5	47	20	90	90.75
26	0	0	0	0	23.5	47	20	90	90.01
27	0	0	0	0	23.5	47	20	90	88.29
28	0	0	0	0	23.5	47	20	90	89.61
29	0	0	0	0	23.5	47	20	90	88.54
30	0	0	0	0	23.5	47	20	90	89.77
31	0	0	0	0	23.5	47	20	90	89.37

* X_1 : Pressure; X_2 : Temperature; X_3 : Static time; X_4 : Dynamic time.

Table 3. Regression Coefficient of Polynomial Function of Response Surface of Evening Primrose Oil Recovery

Coefficient	Value	Standard error	<i>t</i> -value	<i>P</i> -value*
β_0	89.478	0.425	210.290	0.000
β_1	0.339	0.230	1.475	0.160
β_2	0.421	0.230	1.833	0.086
β_3	1.733	0.230	7.543	0.000
β_4	1.446	0.230	6.292	0.000
β_{11}	-0.829	0.211	-3.937	0.001
β_{22}	-1.476	0.211	-7.011	0.000
β_{33}	-0.359	0.211	-1.704	0.108
β_{44}	-0.444	0.211	-2.107	0.051
β_{12}	-1.163	0.281	-4.133	0.001
β_{13}	-0.528	0.281	-1.875	0.079
β_{14}	-0.281	0.281	-0.999	0.333
β_{23}	-1.321	0.281	-4.695	0.000
β_{24}	0.497	0.281	1.766	0.097
β_{34}	0.154	0.281	0.547	0.592

* $P < 0.001$ highly significant; $0.001 \leq P < 0.01$ significant; $P \geq 0.01$ not significant.

randomized order to minimize the effect of unexplained variability in the observed responses due to extraneous factors. Duplicate experiments were carried out at all designed points except at the central point (0, 0, 0, 0) where seven replications were performed to allow the estimations of pure error. The studied parameters and their concentration ranges were: pressure (X_1) from 7.5 to 39.5 MPa, temperature (X_2) from 33 to 61°C, static time (X_3) from 10 to 30 min, and dynamic time (X_4) from 30 to 150 min. The variables were coded according to the following equation:

$$Z_i = \frac{X_i - X_{0i}}{\Delta X_i} \quad i = 1, 2, 3, 4. \quad (2)$$

where Z_i is the coded value of the independent variable; X_i is its real value; X_{0i} is the its real value at the center point; and ΔX_i is the step change in the variable X_i . The coded and uncoded levels of the independent variables used in the RSM design are listed in Table 1. A second-order polynomial regression model was used to express R as a function of the independent variables as follows:

$$R = \beta_0 + \sum_i \beta_i X_i + \sum_i \beta_{ii} X_i^2 + \sum_i \sum_{j \neq i} \beta_{ij} X_i X_j \quad (3)$$

where R is the response variable (oil recovery %), β_0 is a constant and β_i , β_{ii} , β_{ij} are the linear, quadratic, and interactive coefficients, respectively. X_i and X_j are the levels of the independent variables. The responses obtained from the experimental design set (Table 2) were subjected to multiple

nonlinear regressions (Minitab software, version 15) to obtain the coefficients of the quadratic polynomial model. The validity of the model has been determined using the F -test with the Fisher–Snedecor tables, and the statistical significance has been calculated using the t -test with the Student tables.

Fatty acid composition analysis

FAMES were obtained and their analysis was performed using the described method.²⁴ The FAMES were analyzed using an Agilent 6890N GC–FID equipped with a fused silica capillary column (30 m \times 0.25 mm i.d., 0.32- μ m film thickness, Agilent Technologies, Folsom) coated with polyethylglycol. The 1- μ L sample was injected with a split ratio of 100:1 and the inlet temperature of 280°C. The oven temperature was initially set at 170°C for 14 min, then increased to 250°C at a rate of 10°C/min and kept at this temperature for 8 min. The detector temperature was set at 300°C. Nitrogen with a flow rate of 1.0 mL/min was used as the carrier gas. The FAME peaks were identified using FAME standards. Triplicate analysis was carried out for each sample. The composition of the fatty acids was calculated from their peak areas.

Results and Discussion

Model fitting and analysis of experimental data

As mentioned previously in the section of experimental design, an experimental procedure based on the CCRD was adopted for four independent variables (Table 1), which resulted in 31 experimental runs (Table 2). Table 2 also shows the observed oil recovery as the response variable. Multiple regression coefficients, obtained using a least squares technique to predict a second-order polynomial model for the oil recovery, are summarized in Table 3. For any of the terms in the model, a large absolute value of t and a small P -value would indicate more significant effects on the respective response variables. In this study, the model with $P < 0.001$, $0.001 \leq P < 0.01$, and $P \geq 0.01$ was considered as highly significant, significant, and insignificant, respectively. The second-order polynomial model used to express the oil recovery (R) as a function of independent variables (Eq. 3) is shown below (in terms of coded levels):

$$\begin{aligned} R = & 89.478 + 0.339Z_1 + 0.421Z_2 + 1.733Z_3 \\ & + 1.446Z_4 - 0.829Z_1^2 - 1.476Z_2^2 - 0.359Z_3^2 \\ & - 0.444Z_4^2 - 1.163Z_1Z_2 - 0.528Z_1Z_3 - 0.281Z_1Z_4 \\ & - 1.321Z_2Z_3 + 0.497Z_2Z_4 + 0.154Z_3Z_4 \end{aligned} \quad (4)$$

Table 4. ANOVA of the Fitted Quadratic Polynomial Model of SFE Extraction

Source	Degree of freedom	Adjusted sum of squares	Adjusted mean squares	<i>F</i> -value	<i>P</i> -value*
Regression	14	266.122	19.009	15.000	0.000
Linear	4	129.299	32.325	25.510	0.000
Square	4	77.186	19.296	15.230	0.000
Interaction	6	59.637	9.939	7.840	0.000
Residual error	16	20.278	1.267	—	—
Lack of fit	10	15.944	1.594	2.210	0.172
Pure error	6	4.334	0.722	—	—
Total	30	286.400	—	—	—

* $P < 0.001$ highly significant; $0.001 \leq P < 0.01$ significant; $P \geq 0.01$ not significant.

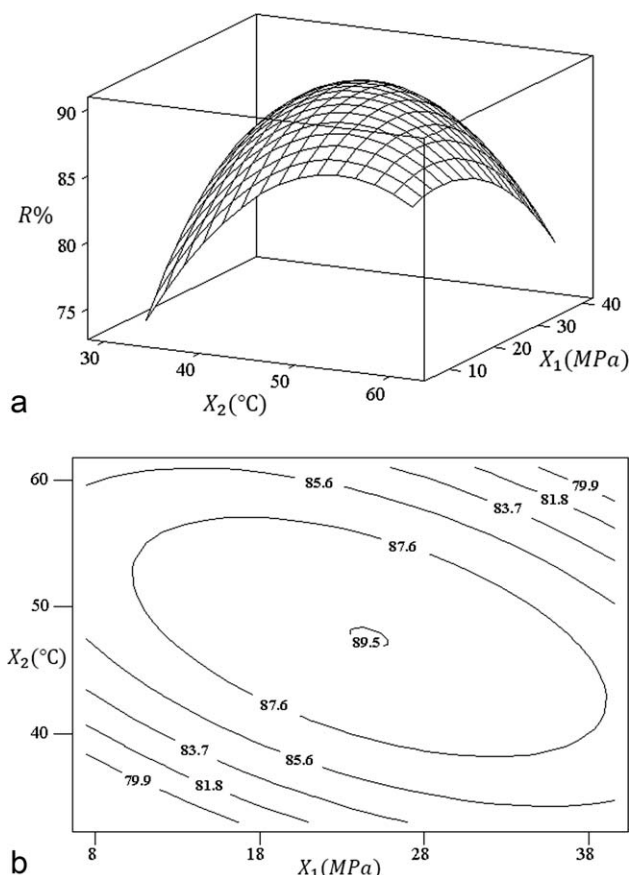


Figure 2. Response surface (a) and contour (b) plots for oil recovery (R) as a function of pressure (X_1) and temperature (X_2) at fixed static (20 min) and dynamic (90 min) time.

From Table 3 and Eq. 4, it was observed that the factors with the high significant effect on the oil recovery were the linear terms of static and dynamic time ($P < 0.001$), followed by the quadratic term of temperature ($P < 0.001$) and the interactions between temperature and static time ($P < 0.001$). The quadratic term of pressure and the interactions between pressure and temperature also had significant effects on the oil recovery ($P < 0.01$).

The analysis of variance (ANOVA) for the response surface model is given in Table 4. These results show that the model predicted for the oil recovery was suitable as indicated by ANOVA lack-of-fit analysis ($P > 0.01$: nonsignificant). The regression model for the oil recovery was highly significant ($P < 0.001$). The coefficient of determination (R^2) and adjusted coefficient of determination ($\text{Adj.}R^2$) were 92.9 and 86.7%, respectively. These values indicate that the model adequately represented the real relationship between the parameters chosen and 92.9% of the variations could be explained by the fitted model.

Optimization of extraction operating conditions

Based on the above model (Eq. 4), the optimal conditions for oil recovery were calculated to be 14.2 MPa, 47.3°C, 30 min (static extraction time), and 150 min (dynamic extrac-

tion time) with 93.61% oil recovery. The model suitability was authenticated by performing triplicate independent experiments at the optimal conditions giving the extraction recovery of 92.98%, which was very compatible with the model prediction.

Response surface analysis

Three-dimensional response surface and contour plots were plotted according to Eq. 4 to study the interactions among the various selected factors and to determine their optimum values for attaining the maximum oil recovery. The plots were generated by plotting the response using the z -axis against two independent variables while keeping the other two independent variables at their zero level. Figure 2 shows the effect of pressure and temperature on the oil recovery at the fixed static and dynamic extraction time of 20 and 90 min, respectively. The trend of oil recovery vs. pressure is due to a dual effect. On one hand, increasing the pressure causes higher SC- CO_2 density and, therefore, improves the solubility of oil and leads to higher recovery. On the other hand, increasing the pressure reduces the oil diffusivity and convective mass transfer coefficient, resulting in a lower recovery. As shown in Figure 2 in the early extraction stage with low operating temperature, a significant oil recovery enhancement was observed with increasing pressure, because the oil diffusivity and mass transfer coefficient reduction is less effective than that of increase in solvent density. The similar phenomenon for the extraction of grape seed oil by supercritical CO_2 and ethanol modifier was reported.¹³ At higher operating temperature, the oil recovery reaches a steady behavior at a certain pressure value (14.2 MPa). Beyond that a sharp reduction in oil recovery is observed, which is due to the fact that the trend of dual effect with respect to diffusivity, convective mass transfer coefficient, and density is reversed.

Similarly, temperature also displays a complex effect on the extraction. First, the temperature elevation decreases the density of CO_2 , leading to the reduction in the solvent power to dissolve the solute. Second, the temperature rise increases the vapor pressure of the solutes, causing higher oil solubility in SC- CO_2 . Consequently, the solubility of the solute is likely to decrease, stay constant, or increase with the temperature rise at constant pressure, which depends on whether the solvent density or the solute vapor pressure effect is the predominant one. As demonstrated in Figure 2, increasing the extraction temperature, up to 47.3°C, favors the extraction recovery because of increasing the vapor pressure of oil, whereas higher temperature lowers the recovery because of decreasing the solvent density. This is the direct effect of well-known phenomenon of retrograde solubility in which the maximum recovery is usually observed when the system reaches a point that the effect of solute vapor pressure equals to solvent density. Similar phenomena were also reported for the extraction of other lipids via SC- CO_2 .^{6,25,26}

Figure 3 shows the three-dimensional response surface and contour plots for the oil recovery as a function of static and dynamic extraction time at constant pressure and temperature of 23.5 MPa and 47°C, respectively. Pourmortazavi and Hajimirsadeghi²⁷ reported that 10–20 min static extraction before dynamic process improved the SFE recovery.

Samples were held in the static extraction mode in the range of 10–30 min, followed by a dynamic extraction in the range of 30–150 min. Especially, when a liquid entrainer is used, static extraction is necessary, because it can allow the solvent penetration into the sample matrix. The results of this study indicate that static extraction longer than 25 min did not significantly increase extraction recovery due to gradual depletion of mass transfer driving force. In other words, recovery enhancement occurred with a moderate slope at this stage in comparison with sharp increasing slope for the static time of less than 25 min. Using dynamic process after static extraction causes higher mass transfer driving force at the beginning and provides a suitable condition for extraction. This continues up to 130 min but further increase in dynamic time resulted in little change in the oil recovery due to the gradual disappearance of the solid matrix oil.

Fatty acid composition of extracted oils

Table 5 presents the fatty acid composition of the extracted oil by SC-CO₂ at 14.2 MPa, 47.3°C with static and dynamic time of 30 and 150 min and its comparison with the conventional Soxhlet method using *n*-hexane. Not much difference is observed in the fatty acid composition of the extracted oil by the two methods. Table 5 showed that the fatty acids in the EPO were dominated by polyunsaturated

Table 5. Fatty Acid Composition of Evening Primrose Seed's Oil (g/100 g) Extracted by *n*-Hexane and Supercritical Carbon Dioxide

Fatty acids	Extraction medium	
	<i>n</i> -Hexane	SC-CO ₂
Palmitic acid, C16:0	7.12	6.83
Stearic acid, C18:0	2.93	2.07
Oleic acid, C18:1 (n-9)	8.14	8.26
Vaccenic acid, C18:1 (n-7)	0.73	0.69
Linoleic acid, C18:2 (n-6)	71.56	72.35
γ -linolenic acid, C18:3 (n-6)	8.21	8.51
Eicosanoic acid, C20:0	0.42	0.37
Other fatty acids	0.89	0.92
MUFA	8.87	8.95
PUFA	79.77	80.86
UFA	88.64	89.81
SFA	10.47	9.27

fatty acids (PUFA), which accounted for more than 79% of the total fatty acids. Saturated fatty acids were the second most abundant at ~10%, whereas the content of monounsaturated fatty acids was less than 9% of the total fatty acids. The main PUFAs in the EPO were linoleic acid (about 72%), followed by GLA and oleic acid (about 8%). The aforementioned results are in agreement to a great extent with the data reported by Christie.²⁸

Conclusions

RSM was successfully applied for optimizing supercritical CO₂ extraction of EPO. The linear terms of static and dynamic time and the quadratics of pressure and temperature, significantly affected the oil recovery. In addition, the interactions between temperature and static time, and pressure and temperature, had a significant effect on the oil recovery. The parametric analysis was adopted to find the optimum operating conditions of 14.2 MPa, 47.3°C, 30 min, and 150 min (static and dynamic). The experimental oil recovery of 92.98% was achieved using the optimum conditions, which is compatible with the predicted value of 93.61%.

Acknowledgments

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Notation

ANOVA = analysis of variance
 EPO = evening primrose oil
 FAME = fatty acid methyl ester
 GLA = γ -linolenic acid
 PUFA = polyunsaturated fatty acids
 R = oil recovery (%)
 RSM = response surface methodology
 SC-CO₂ = supercritical carbon dioxide
 SFE = supercritical fluid extraction
 X_{0i} = real value of i th variable at the center point
 X_1 = real value of pressure (MPa)
 X_2 = real value of temperature (°C)
 X_3 = real value of static extraction time (min)
 X_4 = real value of dynamic extraction time (min)
 Z_1 = coded value of pressure

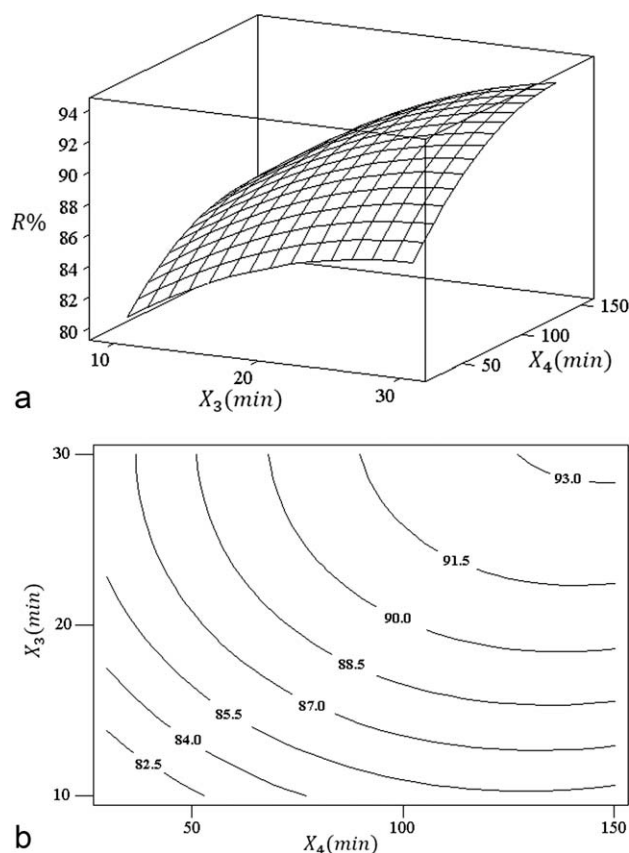


Figure 3. Response surface (a) and contour (b) plots for oil recovery (R) as a function of static time (X_3) and dynamic time (X_4) at fixed 23.5 MPa and 47°C.

Z_2 = coded value of temperature
 Z_3 = coded value of static extraction time
 Z_4 = coded value of dynamic extraction time

Greek letters

β_0 = intercept
 β_i = linear coefficient
 β_{ii} = squared coefficient
 β_{ij} = interaction coefficient

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