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Shu-shan Jiao, Dong Li, Zhi-gang Huang, Zhen-Shan Zhang ...+3 more authors

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**Shun-shan Jiao,** *China Agricultural University*
**Dong Li,** *China Agricultural University*
**Zhi-gang Huang,** *Beijing Technology and Business University*
**Zhen-shan Zhang,** *China Agricultural University*
**Bhesh Bhandari,** *The University of Queensland*
**Xiao Dong Chen,** *China Agricultural University & Monash University*
**Zhi-huai Mao,** *China Agricultural University*

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# Optimization of Supercritical Carbon Dioxide Extraction of Flaxseed Oil Using Response Surface Methodology

Shun-shan Jiao, Dong Li, Zhi-gang Huang, Zhen-shan Zhang, Bhesh Bhandari, Xiao Dong Chen, and Zhi-huai Mao

## Abstract

The optimal conditions for the supercritical carbon dioxide (SC-$CO_2$) extraction of flaxseed oil from flaxseed were determined using response surface methodology (RSM). A second-order regression for rotation-orthogonal composite design was used to study the effects of three independent variables: extraction pressure (MPa), extraction temperature ($^{O}$C) and $CO_2$ flow rate (L/h) on the yield of flaxseed oil. The independent variables were coded at five levels and their actual values selected on the basis of preliminary experiments. The results indicated that the yield of flaxseed oil was beyond 29% at a probability of 95% in the range of extraction pressure: 38.6-42.3 MPa, extraction temperature: 52.3-57.0 $^{O}$C, and $CO_2$ flow rate: 27.8-31.2 L/h. The optimal extraction conditions were extraction pressure of 41 MPa, extraction temperature of 56 $^{O}$C and $CO_2$ flow rate of 31 L/h according to the analysis of response surface. In this condition, the experimental yield of flaxseed oil was 29.96%, which was close to the predicted value of 30.52%.

**KEYWORDS:** supercritical carbon dioxide, extraction, flaxseed oil, response surface methodology, optimization

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## 1. INTRODUCTION

Flaxseed also called linseed is the seed of the flax (*Limum usitatissimum*), which belongs to the flaxen family. Generally, flaxseeds are flat, oval, 4-6 mm long with a smooth, shiny surface (Z.S. Zhang et al., 2007). One thousand seed weight is about 5±1g, and contains about 40% oil, 30% dietary fiber, 20% protein, 4% ash, and 6% moisture (O.N. Tolkachev et al., 2000; J.E. Cacace & G. Mazza, 2006).

Flaxseed is abundant in many nutrients, such as polyunsaturated fatty acid, protein, and lignans (B. Wang et al., 2007). At present, flaxseed is mainly used to produce oil for a variety of industrial purposes (manufacturing of paints, varnishes, linoleum, printing inks, cosmetics, etc.) and oil-free meal for livestock (C. Acikgoz et al., 2004; M.W.Y. Chung et al., 2005). Flaxseed is also a popular ingredient for the use in various functional foods such as cereals, pancakes, muffins, pizza, and bread (Y. Wang et al., 2008). Flaxseed has already gained popularity in the health food market because of its reported health benefits and disease preventive properties (E.C.Y. Li-Chan & C.Y. Ma, 2002).

Supercritical fluid extraction (SFE) has attracted considerable attention in recent several decades as a promising alternative to conventional solvent extraction and mechanical pressing for extracting oils and other materials (X. Xu et al., 2007). SFE has been widely used since it enables the recovery of valuable food ingredients from natural matrices with high yield and the production of better quality products with improved functional or nutritional characteristics by operating under a wide range of conditions. In addition, easy and complete removal of the solvent from the final product makes it advantageous over conventional solvent extraction.

$CO_2$ is the most commonly used supercritical fluid in food industry because of its low critical temperature and pressure ($T_c$=31.1 $^oC$, $P_c$=7.28 MPa), non-toxic and non-flammable properties, and its availability in high purity with low cost. It is also an inert gas which does not react with the food constituents (S. Sonsuzer et al., 2004; S. Scalia et al., 1999). Supercritical carbon dioxide (SC-$CO_2$) was successfully used for the extraction of oils from oil seeds including parsley seed (V. Louli et al., 2004), cottonseed (P. Bhattacharjee et al., 2007), palm kernel (I.S.M. Zaidul et al., 2007) and rose geranium (P.B. Gomes et al., 2007).

The yield of flaxseed oil can be affected by various factors, such as extraction pressure, temperature time, $CO_2$ flow rate, particle size of flaxseed and moisture of the material. The traditional 'one factor at a time' approach to process optimization is time consuming and neglects the interaction of the factors.

Response surface methodology (RSM) which takes interaction into consideration can reduce the times of experiments (C. Liyana-Pathirana & F. Shahidi, 2005; W.C. Lee et al., 2006). RSM uses an experimental design such as the central composite design to fit a model by least squares technique. If the proposed model is adequate, contour plots can be usefully employed to study the response and locate the optimum (I.S. Sanal, 2005). So it can be an effective method to optimize the conditions of SC-$CO_2$ extraction. RSM has been successfully employed to model and optimize SC-$CO_2$ extraction of oils from turmeric (G. Began et al., 2000) and apricot kernel (S.G. Ozkal et al., 2005).

The present study aimed at determining the effects of extraction pressure, extraction temperature and $CO_2$ flow rate on the yield of flaxseed oil as well as developing a second-order polynomial model equation that would predict the optimum conditions for total flaxseed oil yield using RSM. Finally, the optimal conditions were obtained by solving the prediction equation.

## 2. MATERIALS AND METHODS

### 2.1 Materials

Flaxseed used in the present study was grown in Neimenggu Province of China. The initial moisture content of the flaxseed powder determined by oven drying at 105±1 $^{o}$C for 24 h was 6.61% dry basis and the oil content was 43.62%. The $CO_2$ used in SC-$CO_2$ extraction was purchased from Pute Gas Co. (Beijing, China), which was more than 99% (w/w) pure.

### 2.2 Preparations of flaxseed powder

Flaxseeds were ground into small granules by using lipin pulverizer (RT-66S, Huanyatianyuan, Beijing, China), sieved and fractionated according to particle size by certified test sieves (Chunyao Apparatus Factory, Zhejiang Province, China). Sieving was performed by a shaker (Type 8411, Daoxuxingfeng Apparatus Factory, Zhejiang Province, China). The flaxseed powder passed through the sieves with an opening size of 0.90 mm and 0.45 mm. The distribution of the particle sizes was as follows: <0.45 mm (26.5%), 0.45-0.90 mm (50.2%), and >0.90 mm (23.3%). In the preliminary experiments, it was found that when other extractions conditions keep constant, the particle sizes of flaxseed powder between 0.45-0.90 mm extracted flaxseed oil more effectively. The flaxseed powder was easily condensed to agglomeration when the particle size less than 0.45 mm, and the extracted efficiency is very low when the particle size beyond 0.90 mm. So in this study, flaxseed powder of particle size between 0.45-0.90 mm was used in all the experiments.

### 2.3 Supercritical carbon dioxide extraction

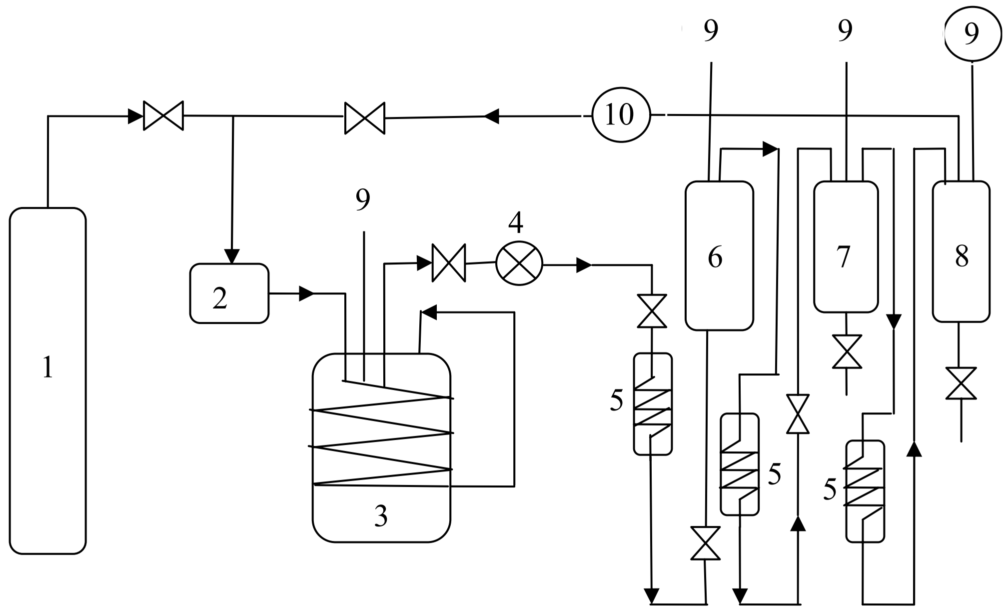


***Fig. 1.*** Schematic diagram of SC-$CO_2$ extraction apparatus: (1) $CO_2$ feed tank (2) Filter (3) Cold bath (4) Pump (5) Preheater (6) Extractor (7) Separator-1 (8) Separator-2 (9) Pressure manometer (10) Flow meter.

SC-$CO_2$ extraction of the flaxseed oil was carried out using HA220-50-06 extraction system (Hua'an Co., Ltd, Nantong city, Jiangsu province, China) which consisted of 5 L and 1 L extraction vessels. In this study, all experiments were carried out in the 1 L extraction vessel (350 mm long × 60 mm i.d.). ***Fig. 1*** is the schematic diagram of SC-$CO_2$ extraction apparatus. Ground flaxseed powder (250 g) was placed into the extraction vessel. After an initial air purge, a required value of extraction temperature was set according to the experimental design. When the temperature reached the given value, the liquefied $CO_2$ was pumped into the extraction vessel by a high pressure pump until the pressure reached the wanted value. Meanwhile, the flow rate of the $CO_2$ was regulated and maintained to a given value by adjusting the length of the pumping stroke. When $CO_2$ flow rate reached its desired value, the extraction was started. Then, the extraction process of flaxseed oil was timed and the oil was collected at certain time interval. The extraction pressure, temperature and $CO_2$ flow rate were controlled at an accuracy of ±0.5 MPa, ±0.5 $^{o}$C and ±1 L/h, respectively. In each extraction, oil was collected in separator-1 and water was recovered in separator-2 (***Fig. 1***). The oil samples were then weighed gravimetrically to obtain the yield (*M*). The yield of flaxseed oil (%) was calculated using the following equation:

$$\text{Yield of flaxseed oil (\%)} = \frac{\text{Weight of extracted flaxseed oil (g)}}{\text{Flaxseed powder (g)}} \times 100 = \frac{M}{250} \times 100 \quad (1)$$

## 2.4 Experimental design

For SC-$CO_2$ extraction, the independent variables which have significant effect on the yield consisted of extraction pressure, extraction temperature, $CO_2$ flow rate, extraction time and particle size of material. However, in the present work, particle size of flaxseed powder was kept a constant at 0.45-0.90 mm, since preliminary trials showed a particle size range of 0.45-0.90 mm to be the most suitable for an effective extraction. Also, the extraction time was kept constant at 180 minutes.

The effects of treatments (independent variables) on the yields of flaxseed oil in SC-$CO_2$ extraction were studied using RSM. RSM used as a generic means for optimization includes a group of empirical techniques to find the relationship between controlled experimental independent factors and the measured responses (dependent variables) according to one and more selected criteria (M.L.A. Teruel et al., 1997; M.G. Sajilata et al., 2008). Three independent variables of the design were extraction pressure ($X_1$), extraction temperature ($X_2$) and $CO_2$ flow rate ($X_3$) while the response variable was the yield of flaxseed oil. A three-factor and five-level second-order regression for rotation-orthogonal composite design consisting of 23 experimental runs was employed including nine replicates at the center point. Nine replicate runs at the central of the design were performed to allow the estimate of pure error (H.N. Sin et al., 2006). A second-order polynomial equation was used to express the yield of flaxseed oil ($Y$) as a function of the independent variables as follows:

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

Where $Y$ is the response, $\beta_0$ is the constant coefficient, $\beta_i$s are the linear coefficients, $\beta_{ii}$s are the quadratic coefficients, $\beta_{ij}$s are the interaction coefficients, and $X_i$ and $X_j$ are the coded values of independent variables. If the proposed model is adequate, as revealed by the diagnostic checking provided by an analysis of variance (ANOVA), contour plots can be usefully employed to study the response and locate the optimum. The practical yield was obtained under the optimal conditions. The experimental and predicted yields of flaxseed oil were compared in order to determine the validity of the model.

The second-order polynomial was fitted to the experimental data to obtain the regression equations using DPS data processing system (Version 6.55, Qiyi Tang, China). The statistical significance of the terms used in the regression equations was also examined. MATLAB® software (Version 7.0.1, Mathworks, America) was used to generate the response surfaces and contour plots while holding one variable constant in the second-order polynomial model (M. Wu et al., 2007). Frequency analysis was applied to determine the optimum ranges of extraction conditions (Z.F. Yuan & J.Y. Zhou, 2000).

## 3. RESULTS AND ANALYSIS

### 3.1 Fitting the model

Based on the preliminary experiments, the following values were selected as the ranges of independent variables, influencing the yield of flaxseed oil, (i) extraction pressure: 25-45 MPa, (ii) extraction temperature: 40-60 $^{o}$C, and (iii) $CO_2$ flow rate: 15-35 L/h.

The relationship between the coded variables ($X_i$) and natural variables ($x_i$) is defined as in the following equation.

$$X_i = \frac{x_i - [x_{\max} + x_{\min}]/2}{[x_{\max} - x_{\min}]/2\alpha} \qquad (3)$$

Where $X_i$s are the dimensionless coded values of the natural variable $x_i$, while the $x_{max}$ and $x_{min}$ are the maximum and minimum values of the natural variable, and $\alpha$=1.682.

***Table 1*** Independent variables and their coded and actual values used for the optimization.

| Independent variable | Units | Symbol | Coded levels | | | | |
|---|---|---|---|---|---|---|---|
| | | | -α | -1 | 0 | 1 | α |
| Extraction pressure | MPa | $x_1$ | 25 | 29 | 35 | 41 | 45 |
| Extraction temperature | $^{o}C$ | $x_2$ | 40 | 44 | 50 | 56 | 60 |
| $CO_2$ flow rate | L/h | $x_3$ | 15 | 19 | 25 | 31 | 35 |

The level of factors and the results of coding are shown in ***Table 1***. The design arrangement and the experimental results of the optimization design are shown in ***Table 2***. Multiple regression coefficients were calculated by DPS software and are summarized in ***Table 3***. As shown in ***Table 3***, most coefficients are significant at different levels except $\beta_2$ and $\beta_2\beta_3$. Taking the coefficients into the generalized model (***Eq. 2***), a second-order polynomial equation for the yield of flaxseed oil was obtained for the coded unit as shown in ***Eq. 4***:

$$Y = 28.847 + 3.136X_1 - 0.517X_2 + 2.346X_3 + 1.139X_1X_2 - 1.009X_1X_3 + 0.331X_2X_3 - 2.026X_1^2 - 0.690X_2^2 - 1.224X_3^2 \quad (4)$$

The F-test and p value listed in ***Table 3*** indicated that the extraction pressure ($X_1$), $CO_2$ flow rate ($X_3$) and the quadratic term of extraction pressure ($X_1^2$) had the largest effect on the yield of flaxseed oil, followed by the quadratic term of $CO_2$ flow rate ($X_3^2$), interaction of the extraction pressure, extraction temperature ($X_1X_2$), interaction of the extraction pressure, $CO_2$ flow rate ($X_1X_3$) and quadratic term of extraction temperature($X_2^2$).

The predicted model was rearranged by eliminating the terms which were not significant in the second-order polynomial model. Finally the model was reduced to the following equation:

$$Y = 28.847 + 3.136X_1 + 2.346X_3 + 1.139X_1X_2 - 1.009X_1X_3 - 2.026X_1^2 - 0.690X_2^2 - 1.224X_3^2 \quad (5)$$

***Table 2*** The arrangement and response of the three-factor, five-level second-order regression for rotation-orthogonal composite design.

| Trial | Arrangement of the design | | | | | | | | | Response |
|---|---|---|---|---|---|---|---|---|---|---|
| | $X_1$ | $X_2$ | $X_3$ | $X_1X_2$ | $X_1X_3$ | $X_2X_3$ | $X_1$' | $X_2$' | $X_3$' | $Y$ |
| 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0.406 | 0.406 | 0.406 | 29.42 |
| 2 | 1 | 1 | -1 | 1 | -1 | -1 | 0.406 | 0.406 | 0.406 | 25.63 |
| 3 | 1 | -1 | 1 | -1 | 1 | -1 | 0.406 | 0.406 | 0.406 | 28.73 |
| 4 | 1 | -1 | -1 | -1 | -1 | 1 | 0.406 | 0.406 | 0.406 | 25.53 |
| 5 | -1 | 1 | 1 | -1 | -1 | 1 | 0.406 | 0.406 | 0.406 | 23.96 |
| 6 | -1 | 1 | -1 | -1 | 1 | -1 | 0.406 | 0.406 | 0.406 | 15.40 |
| 7 | -1 | -1 | 1 | 1 | -1 | -1 | 0.406 | 0.406 | 0.406 | 27.09 |
| 8 | -1 | -1 | -1 | 1 | 1 | 1 | 0.406 | 0.406 | 0.406 | 20.59 |
| 9 | -1.682 | 0 | 0 | 0 | 0 | 0 | 2.234 | -0.594 | -0.594 | 17.52 |
| 10 | 1.682 | 0 | 0 | 0 | 0 | 0 | 2.234 | -0.594 | -0.594 | 29.74 |
| 11 | 0 | -1.682 | 0 | 0 | 0 | 0 | -0.594 | 2.234 | -0.594 | 27.27 |
| 12 | 0 | 1.682 | 0 | 0 | 0 | 0 | -0.594 | 2.234 | -0.594 | 27.55 |
| 13 | 0 | 0 | -1.682 | 0 | 0 | 0 | -0.594 | -0.594 | 2.234 | 22.93 |
| 14 | 0 | 0 | 1.682 | 0 | 0 | 0 | -0.594 | -0.594 | 2.234 | 28.87 |
| 15 | 0 | 0 | 0 | 0 | 0 | 0 | -0.594 | -0.594 | -0.594 | 28.73 |
| 16 | 0 | 0 | 0 | 0 | 0 | 0 | -0.594 | -0.594 | -0.594 | 28.66 |
| 17 | 0 | 0 | 0 | 0 | 0 | 0 | -0.594 | -0.594 | -0.594 | 28.72 |
| 18 | 0 | 0 | 0 | 0 | 0 | 0 | -0.594 | -0.594 | -0.594 | 28.93 |
| 19 | 0 | 0 | 0 | 0 | 0 | 0 | -0.594 | -0.594 | -0.594 | 29.01 |
| 20 | 0 | 0 | 0 | 0 | 0 | 0 | -0.594 | -0.594 | -0.594 | 28.72 |
| 21 | 0 | 0 | 0 | 0 | 0 | 0 | -0.594 | -0.594 | -0.594 | 28.71 |
| 22 | 0 | 0 | 0 | 0 | 0 | 0 | -0.594 | -0.594 | -0.594 | 29.17 |
| 23 | 0 | 0 | 0 | 0 | 0 | 0 | -0.594 | -0.594 | -0.594 | 28.80 |

***Table 3*** Regression coefficient of the predicted second-order polynomial model for the response of the yield of flaxseed oil ($Y$).

| | $\beta_0$ | $\beta_1$ | $\beta_2$ | $\beta_3$ | $\beta_1\beta_2$ | $\beta_1\beta_3$ | $\beta_2\beta_3$ | $\beta_1{}^2$ | $\beta_2{}^2$ | $\beta_3{}^2$ |
|---|---|---|---|---|---|---|---|---|---|---|
| $\beta_i$ | 28.847 | 3.136 | -0.517 | 2.346 | 1.139 | -1.009 | 0.331 | -2.026 | -0.690 | -1.224 |
| $F_i$ | | 142.252[c] | 3.866 | 79.637[c] | 10.991[b] | 8.625[a] | 0.930 | 68.238[c] | 7.504[a] | 24.452[b] |

[a] Significant at 0.05

[b] Significant at 0.01

[c] Significant at 0.001

Afterwards, the interaction analysis was carried out with the natural variables and the following equation was obtained.

$$Y = -108.732 + 3.643x_1 + 0.824x_2 + 3.126x_3 + 0.032x_1x_2 - 0.029x_1x_3 - 0.057x_1^2 - 0.02x_2^2 - 0.035x_3^2 \quad (6)$$

This predicted polynomial model was used to obtain the response surfaces and contour plots for all the interactions among the extraction pressure, the extraction temperature, and the $CO_2$ flow rate.

## 3.2 Analysis of response surface

The best way to visualize the effect of the independent variables on the dependent ones is to draw surface response plots of the model. Three-dimensional response surfaces of the extraction pressure, the extraction temperature, and the $CO_2$ flow rate were obtained by varying two variables within the experimental range and holding the other one constant at the central point. The response surfaces are shown in ***Figs. 2, 3, and 4***.

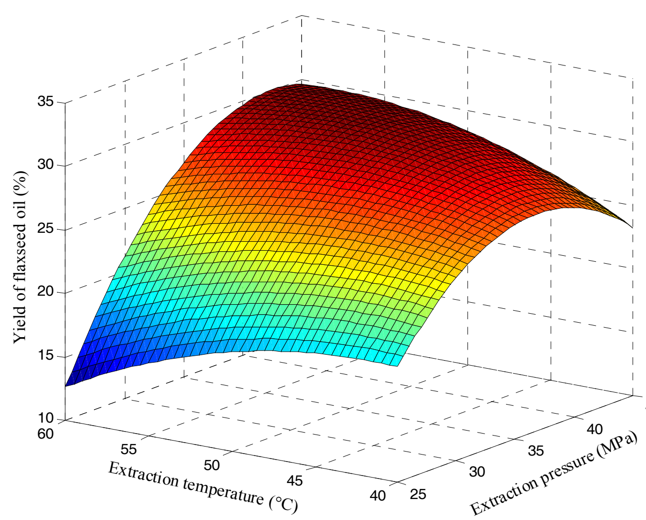


***Fig. 2.*** Response surface showing the effect of extraction pressure and extraction temperature on the yield of flaxseed oil.

***Fig. 2*** shows the effect of the extraction pressure and temperature on the yield of flaxseed oil at a constant $CO_2$ flow rate of 25 L/h. As shown in ***Table 3***, the yield of flaxseed oil was found to be a function of the linear and quadratic effects of extraction pressure. The linear and quadratic effects were both positive at low pressure levels, which resulted in a curvilinear increase with the extraction pressure (***Fig. 2***). This was most likely due to the improvement of oil solubility resulted from the increased $CO_2$ density with the rise of pressure (X. Xu et al., 2007). When the extraction pressure was over about 41MPa, there was a little decrease on the yield of flaxseed oil. This was probably a reflection of the increased repulsive solute-solvent interactions resulting from the highly compressed $CO_2$ at high pressure levels (T. Clifford, 1999).

The linear effect of extraction temperature was insignificant ($p>0.05$), however, the quadratic term of extraction temperature was found to be significant ($p<0.05$). At low pressure levels, the oil yield decreased with the rise of temperature, most likely due to the reduced density of $CO_2$ at higher temperatures. But at higher pressures, the oil yield increased with the rise of temperature. The crossover pressure, beyond which the effect of temperature on the oil yield began to reverse, was about 35 MPa. This crossover phenomenon was also reported for the SC-$CO_2$ extraction of apricot kernel oil (S.G. Ozkal et al., 2006) and carotenoid (M. Sun & F. Temelli, 2006).

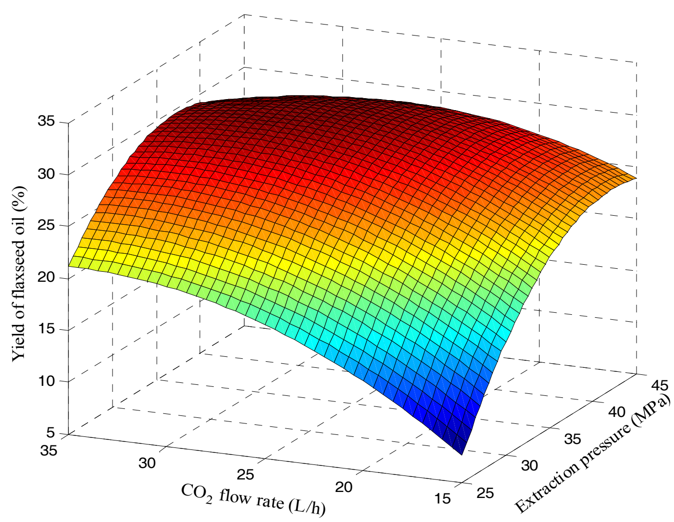


***Fig. 3.*** Response surface showing the effect of extraction pressure and $CO_2$ flow rate on the yield of flaxseed oil.

***Fig. 3*** shows the effects of the extraction pressure and the $CO_2$ flow rate on the yield of flaxseed oil at fixed extraction temperature of 50 $^oC$. The yield of flaxseed oil was found to be a function of the linear and quadratic effects of $CO_2$ flow rate. The linear and quadratic effects were both positive, which resulted in a curvilinear increase with $CO_2$ flow rate for most of the extraction process employed (***Fig. 3***). Increasing the flow rate of $CO_2$ can increase the mass transfer velocity which results in shortening of the extraction time and improving the extraction process. But the ***Fig. 3*** shows that when the extraction pressure and $CO_2$ flow rate were both at high level, the yield of flaxseed oil had a little decrease. When the level of $CO_2$ flow rate were excessively high, the time of SC-$CO_2$ in the extraction vessel would decrease, thus the limited contact time between solvent and SC-$CO_2$ may restrict the improvement of the extraction yield of flaxseed oil. The interaction between extraction pressure and $CO_2$ flow rate also had significant effect ($p$<0.05) on the yield of flaxseed oil (***Table 3***).

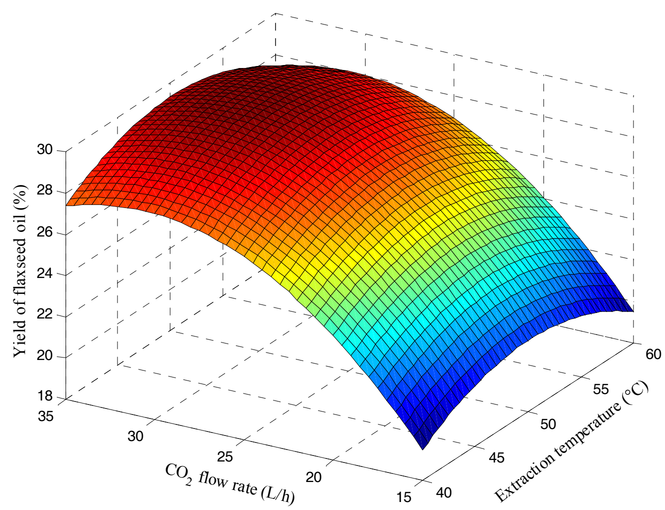


***Fig. 4.*** Response surface showing the effect of extraction temperature and $CO_2$ flow rate on the yield of flaxseed oil.

In ***Fig. 4***, the effects of extraction temperature and $CO_2$ flow rate on the yield of flaxseed oil are presented at a constant extraction pressure of 35 MPa. The yield of flaxseed oil was not affected significantly by extraction temperature. The linear effect of extraction temperature was insignificant ($p$>0.05) while the quadratic term was significant ($p$<0.05), which is observed in the nature of the curve as shown in ***Fig. 4***. With the increase in extraction temperature, the yield of flaxseed oil increased when the extraction temperatures were kept at levels under 50 $^{o}$C, but it decreased when the extraction temperatures were at higher levels than 50 $^{o}$C. The extraction temperature had a two-side effect on the yield of flaxseed oil in the SC-$CO_2$ extraction. One effect was, by increasing extraction temperature, the solute vapor pressure goes up, the heat transfer between the $CO_2$ molecules strengthened and the diffusion coefficient increased. These conditions facilitate $CO_2$ to penetrate through the deep surface of material and improve the extraction rate. The second effect is the influence of temperature on the density of fluid $CO_2$ with the increase in temperature, $CO_2$ fluid density decreases which results to decrease in its solvent effect. This makes the solubility of oil to decrease, consequently reduces the extraction rate (R.M. Liu et al., 2002). The interaction effect between extraction temperature and $CO_2$ flow rate was insignificant ($p$>0.05) (***Fig. 4***).

### 3.3 Optimization of extraction conditions

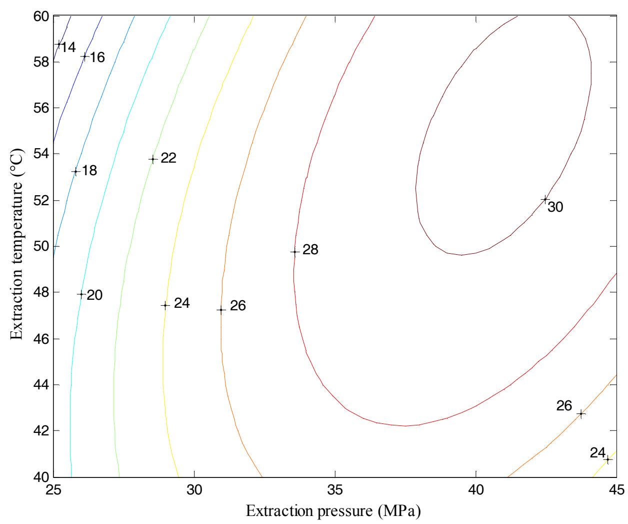


***Fig. 5***. Contour plot showing the effect of extraction pressure and extraction temperature on the yield of flaxseed oil.

***Fig. 5*** shows the contour plots for the response of extraction pressure and extraction temperature to the yield of flaxseed oil. The contours indicated that enhanced extraction pressure can increase the yield of flaxseed oil. However, when the extraction pressure exceeded 41 MPa, the yield of flaxseed oil started to decrease. In other words, it was not advisable to use very high extraction pressure when extracting flaxseed oil from flaxseed. Not only a high extraction pressure will decrease the efficiency of extraction process, it also decreases the life of

SC-$CO_2$ extraction equipment. The optimum extraction condition for the yield of flaxseed oil was the extraction pressure of 38-42 MPa and the extraction temperature of 50-57 $^oC$.

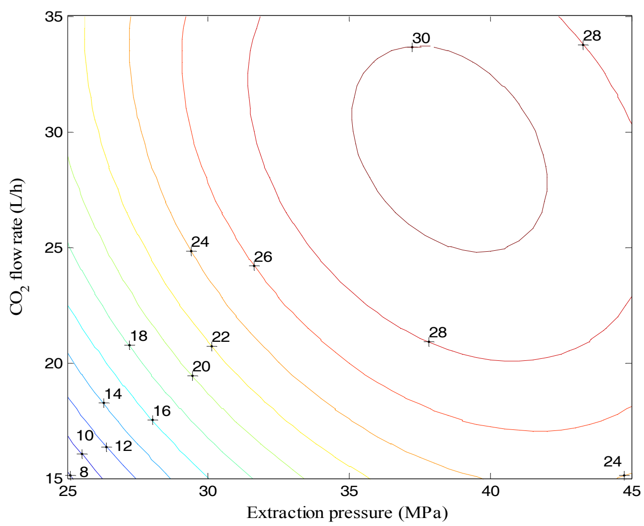


***Fig. 6.*** Contour plot showing the effect of extraction pressure and $CO_2$ flow rate on the yield of flaxseed oil.

***Fig. 6*** shows the contour plots for the response of extraction pressure and $CO_2$ flow rate to the yield of flaxseed oil. It was observed that increasing either extraction pressure or $CO_2$ flow rate, the extraction rate of flaxseed oil could increase when both of them were maintained relatively at low levels. On the contrary, when both of them were at high levels, such as extraction pressures exceeding 41MPa and $CO_2$ flow rates over 30 L/h, the yield of flaxseed oil had a little reduction. The maximum yield of flaxseed oil was obtained at extraction pressure of 35-41 MPa and $CO_2$ flow rate of 25-32 L/h.

Contour plot for the response of extraction temperature and the $CO_2$ flow rate to the yield of flaxseed oil is presented in ***Fig. 7***. As shown in ***Fig. 7***, the yield of flaxseed oil increased when the extraction temperature was below 50 $^oC$, but it decreased when the extraction temperature exceeded 50 $^oC$. Meanwhile, the yield of flaxseed oil increased with the increase in $CO_2$ flow rate up to about 32 L/h, and then decreased with the increase in $CO_2$ flow rate. The ranges of extraction temperature from 44 $^oC$ to 56 $^oC$ and $CO_2$ flow rate from 27 L/h to 32 L/h were considered to be the most suitable for flaxseed oil extraction.

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With the limits of experimental conditions, the optimal conditions obtained using MATLAB® software, were as follows: the extraction pressure of 41 MPa, the extraction temperature of 56 °C, and the $CO_2$ flow rate of 31 L/h. The model showed that the maximum yield of flaxseed oil was 30.52% at this point.

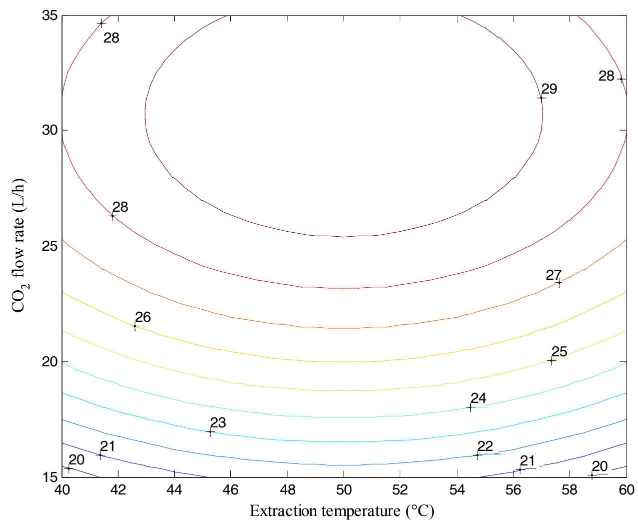


***Fig. 7.*** Contour plot showing the effect of extraction temperature and $CO_2$ flow rate on the yield of flaxseed oil.

### 3.4 Frequency analysis

***Table 4*** The quantity and frequency of each factors in the 15 schemes which the yield of flaxseed oil beyond 29%.

| Factors / Coding | Frequency of extraction pressure ($X_1$) | Frequency of extraction temperature ($X_2$) | Frequency of $CO_2$ flow rate ($X_3$) |
|---|---|---|---|
| -1.682 | 0 | 0 | 0 |
| -1 | 0 | 1 | 0 |
| 0 | 4 | 4 | 5 |
| 1 | 7 | 6 | 8 |
| 1.682 | 4 | 4 | 2 |
| Average value($\overline{X}$) | 0.915 | 0.719 | 0.758 |
| 95% confidence interval ($X_i$) | 0.602-1.229 | 0.387-1.177 | 0.464-1.051 |
| Parameter interval ($x_i$) | 38.6-42.3 | 52.3-57.0 | 27.8-31.2 |

A three-factor and five-level experiment design can take out $5^3$=125 arrangements. There were 15 arrangements in which the yields of flaxseed oil were beyond 29%, which occupied 12% in all arrangements. The results of frequency analysis are presented in ***Table 4***.

Flaxseed oil was extracted under the conditions listed in the last row of ***Table 4***. The conditions were the extraction pressures ranging from 38.6 MPa to 42.3 MPa, the extraction temperatures ranging from 52.3 $^oC$ to 57.0 $^oC$, and the $CO_2$ flow rate ranging from 27.8 L/h to 31.2 L/h and the yield of flaxseed oil might be beyond 29% at a probability of 95% in these ranges.

### 3.5 Verification of the model

The suitability of the model equation for predicting the optimum response values was tested using the recommended optimal conditions. The set of optimal conditions, determined using the RSM optimization approach, were tested experimentally in order to the model equation. The experimental value (29.96%) was found to be close to the predicted one.

## 4. CONCLUSION

A quadratic polynomial model for predicting the values of yield of flaxseed oil was determined according to the RSM design. Three independent variables involved in the model were extraction pressure, extraction temperature and $CO_2$ flow rate. The results were:

(1) The $F$-test and $p$ value indicated that the extraction pressure ($X_1$), $CO_2$ flow rate ($X_3$) and the quadratic term of extraction pressure ($X_1^2$) had the largest effect on the yield of flaxseed oil.

(2) Frequency analysis indicated that the yield of flaxseed oil would be beyond 29% at a probability of 95% in the range of extraction pressure: 38.6-42.3 MPa, extraction temperature: 52.3-57.0 $^oC$, $CO_2$ flow rate: 27.8-31.2 L/h.

(3) The optimal extraction conditions were obtained at the extraction pressure of 41 MPa, extraction temperature of 56 $^oC$ and $CO_2$ flow rate of 31 L/h. In these conditions, the experimental yield of flaxseed oil was 29.96%, which was close to the predicted value (30.52%).

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