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# Process optimization for the supercritical carbon dioxide (SC-$CO_2$) extraction of wheat germ oil with respect to yield, and phosphorous and tocol contents using a Box Behnken design

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**SUMMARY:** The supercritical carbon dioxide (SC-$CO_2$) extraction technique has emerged as one of the best possible alternatives to organic solvent (hexane) extraction. However, very limited information is available on process optimization for this extraction technique and the lack of available engineering data is causing the slow growth of this technique. In the present investigation, SC-$CO_2$ extraction was carried out to extract the oil from wheat germ under various operating conditions and the oil samples were characterized for properties such as phosphorous and tocol contents (vitamin E). A three-level Box Behnken design from response surface methodology was applied to optimize the SC-$CO_2$ extraction parameters such as pressure, temperature and $CO_2$ flow rate with an objective to obtain high oil yield, rich tocol contents and low phosphorous content. The process parameters were maintained between 30 to 50 MPa, 40 to 60 °C and a flow rate of 10 to 30 g·min$^{-1}$ in a Box Behnken design matrix. Three different second order polynomial models were obtained for oil yield, phosphorous content and tocol contents with high $R^2$ values. The optimum conditions were found to be 50 M Pa, 60 °C and 30 g·min$^{-1}$ where the predicted oil yield, phosphorous content and tocol contents were found to be 8.87%, 31.86 mg·Kg$^{-1}$ and 2059.92 mg·Kg$^{-1}$ respectively. Under the optimum conditions, the experimental oil yield, phosphorous content and tocol contents obtained were found to be very close to the values predicted by the model.

***KEYWORDS:*** *Box Behnken design; Oil yield; Phosphorous content; Supercritical carbon dioxide extraction; Tocol contents; Wheat germ*

**RESUMEN:** ***Optimización mediante el diseño Box Behnken del proceso de extracción con dióxido de carbono supercrítico (SC-$CO_2$) de aceite de germen de trigo en relación al rendimiento, contenido de fósforo y tocoles.*** La técnica de extracción mediante dióxido de carbono supercrítico (SC-$CO_2$) ha surgido como una de las mejores alternativas posibles a la extracción con solventes orgánicos (hexano). Sin embargo, se dispone de información muy limitada sobre la optimización del proceso y la falta de disponibilidad de datos de ingeniería es la causa del lento crecimiento de esta técnica. En la presente investigación, la extracción con SC-$CO_2$ se llevó a cabo para obtener aceite de germen de trigo en diversas condiciones operacionales. Los aceites se caracterizaron mediante sus contenidos en fósforo y tocoles (vitamina E). Se aplicó el diseño Box Behnken de tres niveles a partir de la metodología de superficie de respuesta para optimizar los parámetros de la extracción, presión, temperatura y flujo de $CO_2$ para obtener un alto rendimiento de aceite, alto contenido de tocoles y bajo contenido de fósforo. Los parámetros del proceso se mantuvieron entre 30 - 50 MPa, de 40 a 60 °C y de 10 a 30 g·min$^{-1}$ de caudal de $CO_2$ en la matriz de diseño Box Behnken. Se obtuvieron tres modelos polinomiales de segundo orden diferentes para rendimiento de aceite, contenido de fósforo y contenido de tocoles, con altos valores de $R^2$. Las condiciones óptimas fueron: 50 M Pa, 60 °C y 30 g·min$^{-1}$ donde el rendimiento de aceite, el contenido de fósforo y el contenido de tocoles previstos fueron 8.87%, 31,86 mg·Kg$^{-1}$ y 2059,92 mg·Kg$^{-1}$ respectivamente.

Bajo las condiciones óptimas, el rendimiento de aceite, el contenido de fósforo y el contenido de tocoles presentaron valores muy cercanos a los predichos por el modelo.

***PALABRAS CLAVE:*** *Contenido de fósforo; Contenido de Tocols; Diseño Box-Behnken; Extracción supercrítica con dióxido de carbono; Germen de trigo; Producción de aceite*

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## 1. INTRODUCTION

Extraction is a primary unit operation for the processing of vegetable oils and fats. The selected extraction technique should give the maximum possible amount of oil with desired quality. Generally, oils and fats are extracted using hexane, a solvent obtained from a petroleum source. It has been identified and enlisted as a hazardous air pollutant by the US EPA in the Clean Air Amendments of 1990. Hexane may react with other pollutants and create health hazards (Wan *et al.*, 1995; Hanmoungjai *et al.*, 2000). Traces of hexane present in the extracted oil reduce the food and nutritional value of the oil. All these factors raised interest for finding alternatives to hexane. Carbon dioxide exhibits supercritical fluid properties above 31 °C and 7.397 MPa and is chemically stable, non-toxic and non-flammable. The other main advantages of the SC-$CO_2$ are its higher solubility, higher efficiency of extraction and better selectivity. SC-$CO_2$ extraction shows supercritical properties at near to ambient temperature and it helps in processing heat-sensitive products. Because of all these reasons, in recent years, SC-$CO_2$ extraction has gained huge importance as a green solvent. This technique was used to extract oil from various oil bearing materials (Avila *et al.* 2017; Bozan and Temelli, 2002; Haloui *et al.*, 2017; Han *et al.*, 2009; Roy *et al.*, 2006). However, the process is not properly understood as studies on process optimization are yet to be done.

Wheat germ is a by-product from the processing of wheat grain. It is the reproductive part of the grain and contains large amounts of vitamins. It contains around 8-14% oil (Sonntag, 1979; Dunford and Zhang, 2003). Wheat germ oil contains up to 2500 mg·$Kg^{-1}$ of tocopherols and tocotrienols, inclusively known as tocols (vitamin E). The presence of large quantities of tocols gives antioxidant properties (Tracy *et al.*, 1944; Saleh *et al.*, 2010). This oil was reported to have anti-cancer and anti-inflammatory properties (Zalatnai *et al.*, 2001; Reddy *et al.*, 2000; Janthachotikun *et al.*, 2015). One very significant observation was noted that, although conventional hexane extracted oil contains considerable amounts of phospholipids (> 600 mg·$Kg^{-1}$ of phosphorous content) (Taniguchi *et al.*, 1985). The SC-$CO_2$ extracted oil had a significantly lower amount of phospholipids (< 50 mg·$Kg^{-1}$ of phosphorous content). The presence of a higher amount of phospholipids leads to a unit operation called de-gumming where water/phosphoric acid etc. are used to reduce the phosphorous content for further refining. This will result in oil loss and a reduction in tocols. Moreover, this process generates considerable amounts of effluents that need to be treated. SC-$CO_2$ extraction does not require this step of refining if the phosphorous content is kept low. It was, therefore, decided to optimize the SC-$CO_2$ extraction of wheat germ oil yield with respect of phosphorous content and tocol contents.

The literature reports the application of a Box Behnken design to optimize the supercritical parameters of oil yield (Zahedi and Azarpour, 2011; Tao *et al.*, 2014; Aladic *et al.*, 2016). This design was preferred because relatively few experimental combinations of variables are adequate to estimate complex response functions. Some researchers carried out studies on the supercritical fluid extraction of wheat germ (Zacchi *et al.*, 2006; Gómez and Ossa, 2000) and the optimization of process conditions either for oil yield (Shao *et al.*, 2008; Jiang and Niu., 2011) or for tocol contents (Ge *et al.*, 2002) using response surface methodology. These data only give limited information and do not give any input on phosphorous content. Because phosphorous content is one of the major aspects of processing and it has direct impact on the ultimate refined oil yield and quality, the Box Behnken design was adopted for the optimization of SC-$CO_2$ extraction for phosphorous content also. Accordingly, the effects of process parameters such as pressure, temperature and $CO_2$ flow rate on yield of oil, phosphorous content and tocol contents of the extracted oils were studied and the optimized conditions were predicted using the Box Behnken design. At the optimized conditions, experiments were carried out and the results obtained were compared with the predictions by the design.

## 2. MATERIALS AND METHODS

### 2.1. Materials

Wheat germ was supplied by a local wheat processing industry, Andhra Pradesh, India. The raw wheat germ was pretreated by heating in an oven at 105 °C for 30 to 45 min to remove the possible free moisture and to inactivate the enzymes. Then the sample was kept in tightly sealed plastic bags for further extraction. All the chemicals and solvents used in this study were purchased from SD Fine Chemical Ltd. (Mumbai, India) and were of laboratory reagent grade.

### 2.2. Supercritical carbon dioxide (SC-$CO_2$) extraction

The oil in the wheat germ was extracted using a SC-$CO_2$ extraction unit with 500 $cm^3$ capacity supplied by the Waters Corporation, Milford, USA. The unit contains a chiller, $CO_2$ pump, co-solvent pump, heat exchanger, extraction vessel, automatic back pressure regulator, fraction collector and other accessories. The system is designed to withstand pressures and temperatures up to 60 M Pa and 80 °C, respectively. The system is controlled by a programmable logic controller (PLC). Initially, 150 g of wheat germ sample were taken in a 0.45 µm cotton filter bag and inserted into the extraction vessel and the chiller temperature was brought down to 5 °C. The $CO_2$ filled cylinder was connected to the unit and $CO_2$ was allowed to flow through the system. The $CO_2$ gas was pre-liquefied by passing through a shell side heat exchanger and then pumped into the extraction vessel where the temperature and pressure were kept at the desired conditions. The supercritical state of $CO_2$ was achieved at this stage. The oil extraction was carried out and the extract was collected in a sample bottle from the collection vessel and the collected oil was dried and weighed as yield. The sample bottle was tightly sealed and kept in a refrigerator for further analysis.

### 2.3. Solvent (hexane) extraction

The oil was also extracted from wheat germ in a soxhlet apparatus with hexane and the extraction procedure was continued up to 8 h to extract the maximum amount of oil. The oil content was determined as a percentage of the extracted oil of the sample weight ($w{\cdot}w^{-1}$).

### 2.4. Experimental design

The Box Behnken design from response surface methodology (RSM) was used to perform the SC-$CO_2$ extraction. Three factors were included in the model: Pressure ($X_1$); Temperature ($X_2$); $CO_2$ flow rate ($X_3$). The coded and uncoded levels of both independent variables and experimental design are provided in Table 1 and low, middle and high levels are coded as -1, 0 and +1, respectively. With the center point assigned as '5', 17 runs in total were obtained using the Box Behnken design.

The responses assessed using the Box Behnken design were oil yield ($Y_1$), phosphorous content ($Y_2$) and tocol contents ($Y_3$). These contents were estimated after each experimental run. The SC-$CO_2$ extraction time was fixed at 3 hrs, which was based on a few initial experimental trials. The responses (Y) investigated were expressed in second order polynomial equation (Eq. 1) as a function of independent coded variables ($X_1$, $X_2$,…., $X_n$) affecting the responses. The regression coefficients for intercept, linear, quadratic, are $\beta_0$, $\beta_i$ (i = 1, 2,.., k), $\beta_{ii}$ (i = 1, 2, …, k), and $\beta_{ij}$ (i = 1, 2, …., k, j = 1, 2, …., k), where k is the number of variables.

$$Y=\beta_0+\sum_{i=1}^{k}\beta_i X_i+\sum_{i=1}^{k}\beta_{ii}X_i^2+\sum\sum_{i<j}\beta_{ij}X_iX_j \quad \text{(Eq. 1)}$$

A statistical analysis was performed using RSM software Design Expert® v.10. The responses were analyzed using analysis of variance (ANOVA). The efficiency of model was ascertained by determined coefficient ($R^2$) and its implication was found by an F-test.

### 2.5. High performance liquid chromatography analysis of tocol contents

The tocols present in the wheat germ oil were analyzed according to the AOCS prescribed analytical method (AOCS 1994 Ce 8–89) using high performance liquid chromatography (HPLC). Commercially available α-Tocopherol (> 96% purity), β-Tocopherol (> 90% purity), γ-Tocopherol (> 96% purity) and δ-Tocopherol (> 90% purity), Sigma Aldrich, USA were used as standards. An Agilent 1100 series HPLC unit equipped with a fluorescence detector was used. The normal phase silica column (LiChroshper Si-60; 250 mm × 4.0 mm × 5 µm) (Merck Millipore, UK) was

TABLE 1. Codes and uncoded levels of independent variables for SC-$CO_2$ extraction.

| Independent Variables | Symbol | Coded Levels | | |
|---|---|---|---|---|
| | | Low (-1) | Middle (0) | High (+1) |
| Pressure (M Pa) | $X_1$ | 30 | 40 | 50 |
| Temperature (°C) | $X_2$ | 40 | 50 | 60 |
| $CO_2$ flow rate ($g{\cdot}min^{-1}$) | $X_3$ | 10 | 20 | 30 |

used. The excitation and emission wave lengths of the detector were maintained at 292 and 330 nm, respectively. The isocratic mobile phase containing hexane and isopropyl alcohol (99:1, vol·vol$^{-1}$) was used at a flow rate of 1.0 cm$^3$·min$^{-1}$. The total tocol (vitamin E) content was measured in parts per million (mg·Kg$^{-1}$).

### 2.6. Analysis of phosphorous content

The phosphorous content in the samples was estimated using the International Union for Pure and Applied Chemistry (IUPAC) method (Pacquot and Hautfenne, 1987). A sample (2-10 g) was taken in a 50-100 cm$^3$ silica crucible along with magnesium oxide (0.1 g) burnt in a fume hood. The crucible was put in a muffle furnace at 800 °C for 1 hr to obtain the ash. This ash was then dissolved in exactly 5 cm$^3$ of an aqueous nitric acid (6 N) solution. 20 cm$^3$ of 1:1 aqueous ammonium molybdate and ammonium vanadate solutions were added to the sample. A blank solution was also prepared under the same conditions. The absorbance was recorded at 460 nm using UV-spectrometer (Perkin Elmer, Lambda 35) for both sample and blank and the corresponding concentrations were taken. Phosphorous content was calculated accordingly as per the method.

## 3. RESULTS AND DISCUSSION

### 3.1. Response surface analysis

The SC-$CO_2$ extraction of wheat germ was carried out under 17 experimental conditions which were obtained from the Box Behnken design. All experiments were carried out in triplicate at a fixed extraction time of 3 h by varying pressure (30-50 M Pa), temperature (40-60 °C) and $CO_2$ flow rate (10-30 g·min$^{-1}$). The oils obtained for each experiment were analyzed for phosphorous content and tocol contents. The Box Behnken experimental design and observed responses are shown in Table 2. The surface plots of the yield, phosphorus content and tocol contents as a function of the various independent variables such as pressure, temperature and $CO_2$ flow rate are shown in Figures 1-3. Keeping the third variable constant, the response surface plots were obtained by varying two independent variables. The significance of linear, quadratic and interaction coefficients were studied at the *p* values of less than 0.0001, 0.005 and 0.05. The oil yield, phosphorous content and tocol content values varied in the range of 3.92 to 9.8%, 26 to 48.4 mg·Kg$^{-1}$ and 1384 to 2286.3 mg·Kg$^{-1}$, respectively, in the Box Behnken design matrix. It was found that the effect of pressure was more eminent on the extraction

TABLE 2. Response surface analysis and observed responses

| | Factor 1 | Factor 2 | Factor 3 | Response 1 | Response 2 | Response 3 |
|---|---|---|---|---|---|---|
| Run | Pressure (M Pa) | Temperature (°C) | $CO_2$ flow rate (g · min$^{-1}$) | Oil Yield* (%) | Phosphorous content* (mg · Kg$^{-1}$) | Tocols content* (mg · Kg$^{-1}$) |
| 1 | 30 | 60 | 20 | 4.80 | 29.39 | 2050.00 |
| 2 | 50 | 50 | 10 | 7.10 | 31.00 | 1680.00 |
| 3 | 30 | 40 | 20 | 5.00 | 36.00 | 1680.00 |
| 4 | 40 | 60 | 30 | 8.00 | 44.53 | 2286.30 |
| 5 | 30 | 50 | 10 | 3.92 | 37.00 | 1920.60 |
| 6 | 40 | 50 | 20 | 8.25 | 48.30 | 1920.00 |
| 7 | 40 | 40 | 30 | 9.33 | 48.24 | 1694.30 |
| 8 | 40 | 40 | 10 | 6.80 | 42.00 | 1650.00 |
| 9 | 40 | 50 | 20 | 8.30 | 48.40 | 1924.00 |
| 10 | 30 | 50 | 30 | 5.50 | 41.13 | 2050.40 |
| 11 | 40 | 50 | 20 | 8.02 | 47.50 | 1927.00 |
| 12 | 50 | 60 | 20 | 8.20 | 26.00 | 1850.00 |
| 13 | 40 | 60 | 10 | 6.02 | 39.00 | 1960.00 |
| 14 | 50 | 50 | 30 | 9.80 | 39.00 | 1820.40 |
| 15 | 50 | 40 | 20 | 9.60 | 27.00 | 1384.00 |
| 16 | 40 | 50 | 20 | 8.03 | 48.10 | 1939.00 |
| 17 | 40 | 50 | 20 | 7.90 | 47.70 | 1960.00 |

*All experiments were carried out in triplicate and the results expressed as mean.

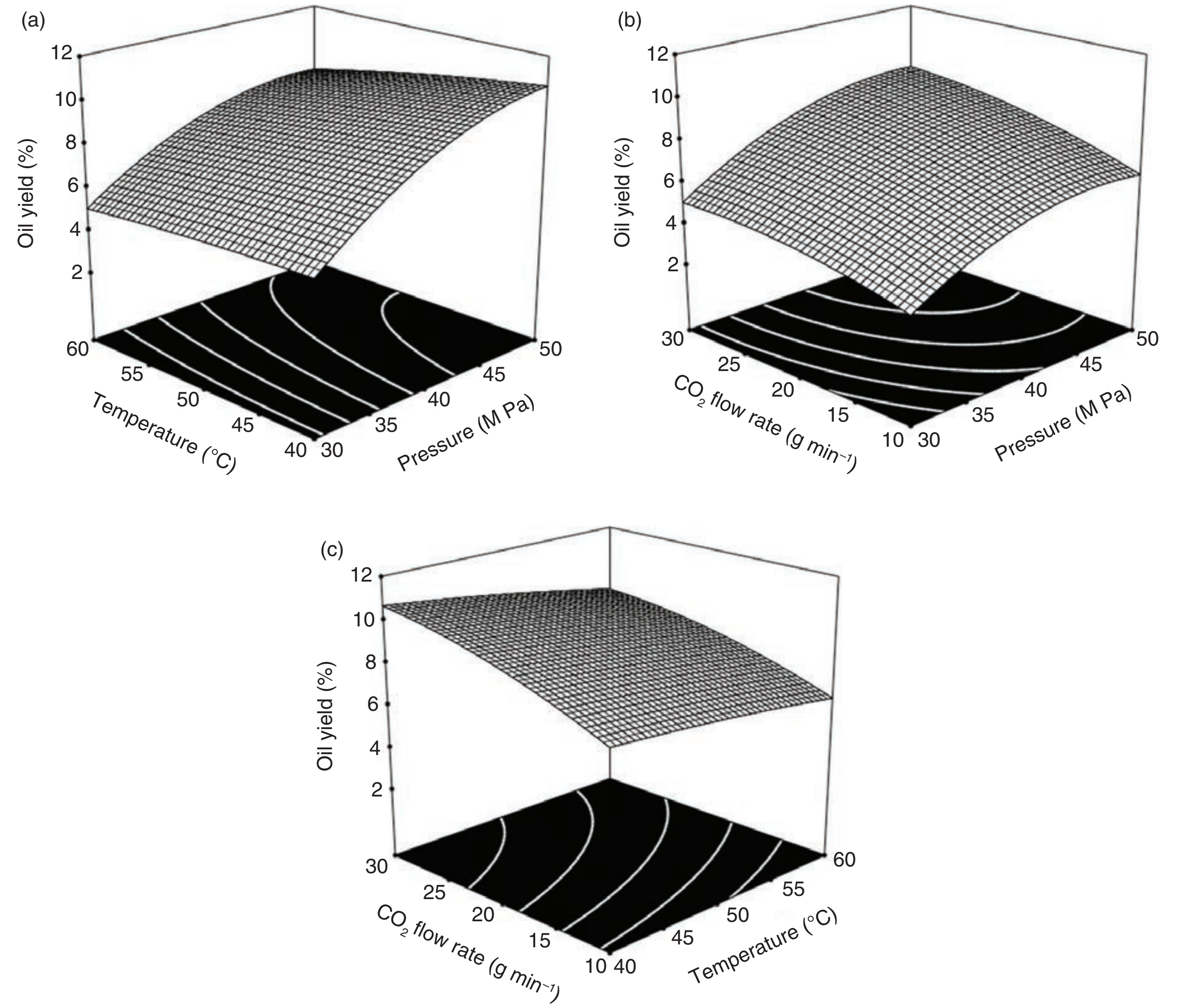


**FIGURE 1. Response surface plots showing the effects of the investigated variables on oil yield (a). $CO_2$ flow rate constant at 20 g·min$^{-1}$ (b). Temperature constant at 50 °C (c). Pressure constant at 40 MPa.** The figures resulted from the mean of the three replicates of Response 1 (oil yield).

yield compared to other parameters. As expected, the oil yield increased with increasing pressure as increase in pressure leads to an increase in the density of SC-$CO_2$ thereby increasing the solubility of the oil in SC-$CO_2$ (Ghoreishi *et al.*, 2016). As evident in Table 2, the high pressure of 50 M Pa yielded more oil compared to the low pressure of 30 MPa. On the other hand, the rise in temperature decreases the density of SC-$CO_2$ and solubility decreases, due to which the oil yield decreased with the increase in temperature (Zacchi *et al.*, 2006; Kamali *et al.*, 2015). Therefore, the maximum extraction oil yield was observed at the low temperature of 40 °C. The increase in $CO_2$ flow rate resulted in a slight increment in oil yield. The effects of processing parameters on the yield are shown in the response surface plots in Figure 1.

The phosphorous content initially increased with the increase in pressure and temperature at a constant $CO_2$ flow rate and then was decreased gradually. A small increment in phosphorous content was also observed with increasing $CO_2$ flow rate. Figure 2 shows the effects of process parameters on phosphorus content in the SC-$CO_2$ extracted oils. The tocols contents in the oil decreased with an increase in pressure and increased when the temperature was raised from 40 to 60 °C. A small increment in tocol contents was observed with increasing $CO_2$ flow rate. Figure 3 shows the dependence of tocol contents on the pressure, temperature and $CO_2$ flow rate.

The coefficients of the second order polynomial equations derived from the models are shown in Table 3. The linear coefficients of $X_1$, $X_2$ and $X_3$,

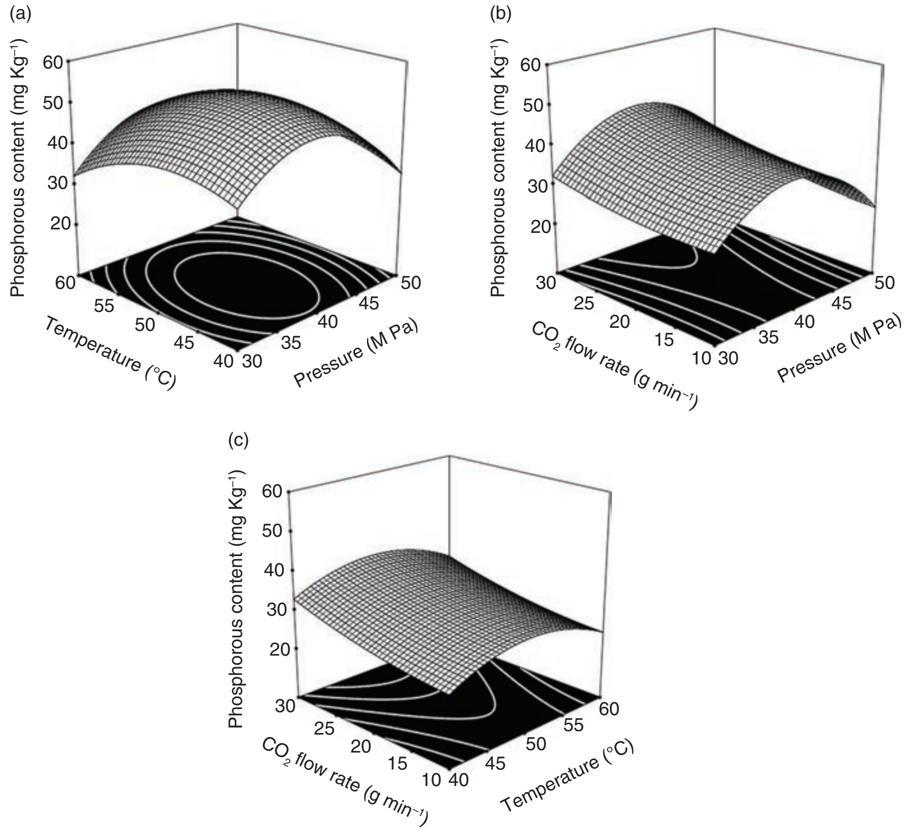


**FIGURE 2. Response surface plots showing the effects of the investigated variables on the extraction of phosphorous content (a). $CO_2$ flow rate constant at 20 g·min$^{-1}$ (b). Temperature constant at 50 °C (c). Pressure constant at 40 M Pa** The figures produced resulted from the mean of the three replicates of Responses 2 (phosphorous content).

and the quadratic coefficient of $X_1^2$ for oil yield, the linear coefficients of $X_1$ and $X_3$ and the quadratic coefficients of $X_1^2$ and $X_2^2$ for phosphorus content, the linear coefficients of $X_1$, $X_2$ and $X_3$ and the quadratic coefficients of $X_1^2$ and $X_2^2$ for tocol content are significant at $p < 0.0001$. Similarly, the quadratic coefficient of $X_3^2$ for oil yield, linear coefficient of $X_2$, the quadratic coefficient of $X_3^2$ and the interaction coefficient of $X_1X_2$ for phosphorus content, the quadratic coefficient of $X_3^2$ and the interaction coefficient of $X_2X_3$ for tocol contents are significant at $p < 0.005$. The interaction coefficients of $X_1X_2$ and $X_1X_3$ for oil yield, and the interaction coefficient of $X_1X_3$ for phosphorus content are significant at $p < 0.05$, whereas the other coefficients are insignificant.

R-square was estimated to study the sustainability of the second-order polynomial equations and models (Daneshvand *et al.*, 2012; Banik and Pandey, 2008). For a good fit of a model, the R-square value should be at least 0.80 (Joglekar and May, 1987). The R-square values for the oil yield, phosphorous content and tocol contents were found to be 0.9961, 0.9970 and 0.9956, demonstrating a good correlation between the experimental data to the predicted data by the three models. Variations of 0.39%, 0.3% and 0.44% were observed for the responses predicted by the models for oil yield, phosphorous content and tocol contents, respectively, but they cannot be explained by the model. The predicted R-squared values (0.9722, 0.9609 and 0.9519 for the oil yield, phosphorous content and tocol contents) are in good agreement with the adjusted R-square values (0.9912, 0.9932 and 0.9899 for the oil yield, phosphorous content and tocol content) and the values were within very narrow differences (less than 0.2) for each other. Table 4 shows the F value with the corresponding *p* value of models, residual and lack of fit for oil yield, phosphorous content and tocol contents. The *p* values for lack of fitness were found to be more than 0.05 for all three responses and that indicates that lack of fit was insignificant and that the three models fit the data well.

### 3.2. Optimization of SC-$CO_2$ extraction parameters

To attain the optimum conditions and to predict the yields, three second order polynomial equations for oil yield, phosphorous content and tocol contents were used. In the Box Behnken design, a desirability

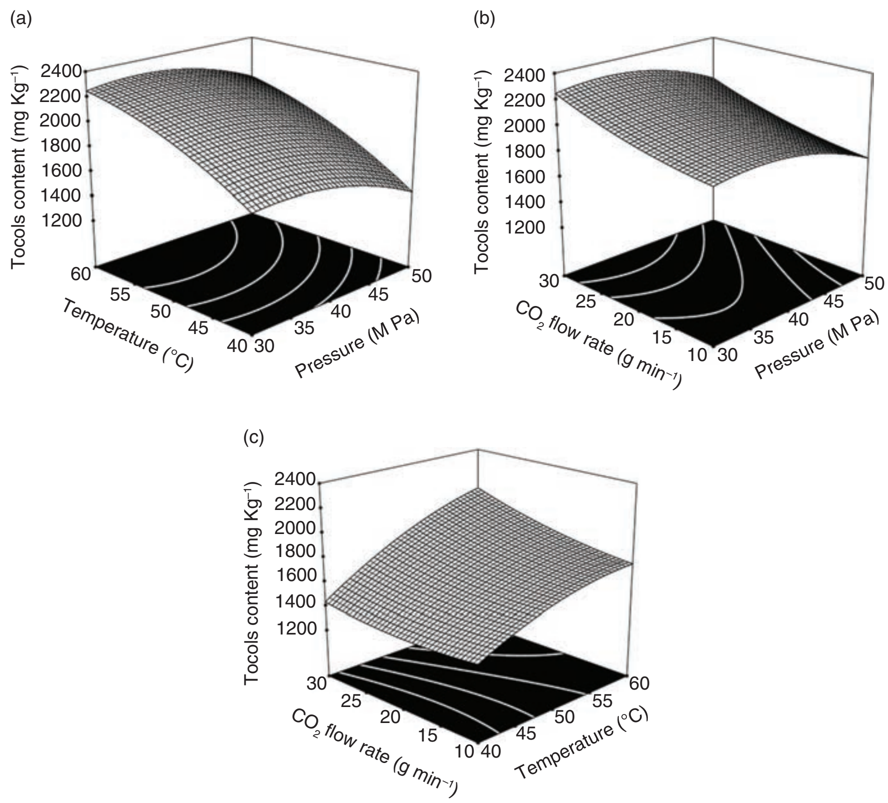


**FIGURE 3. Response surface plots showing the effects of the investigated variables on the extraction of tocol contents (a). $CO_2$ flow rate constant at 20 g·min$^{-1}$ (b). Temperature constant at 50 °C (c). Pressure constant at 40 MPa.** The figures produced resulted from the mean of the three replicates of Responses 3 (tocol contents).

TABLE 3. Estimated coefficients of the second order polynomial equations.

| Term | Coefficient | For Oil Yield | For Phosphorous content | For Tocols content |
|---|---|---|---|---|
| Intercept | $\beta_0$ | +8.10 | +48.00 | +1934.00 |
| $X_1$ | $\beta_1$ | + 1.94[a] | -2.56[a] | -120.82[a] |
| $X_2$ | $\beta_2$ | -0.46[a] | -1.79[b] | +217.25[a] |
| $X_3$ | $\beta_3$ | +1.10[a] | +2.99[a] | +80.10[a] |
| $X_1^2$ | $\beta_{11}$ | -1.08[a] | -12.41[a] | -111.40[a] |
| $X_2^2$ | $\beta_{22}$ | -0.12 | -6.00[a] | -81.60[a] |
| $X_3^2$ | $\beta_{33}$ | -0.44[b] | +1.44[b] | +45.25[b] |
| $X_1X_2$ | $\beta_{12}$ | -0.30[c] | +1.40[b] | +24.00 |
| $X_1X_3$ | $\beta_{13}$ | +0.28[c] | +0.97[c] | +2.65 |
| $X_2X_3$ | $\beta_{23}$ | -0.14 | -0.18 | +70.50[b] |

[a]significant at $p < 0.0001$, [b]significant at $p < 0.005$, [c]significant at $p < 0.05$; All the coefficients of second order polynomial equations were resulted from mean of three replicates of responses.

function was established which will simultaneously satisfy the requirements of all the responses. Using the desirability function, it is possible to get a point by numerical optimization that will maximize or minimize the function. By adjusting the weight or importance, the goal was set which was combined into one desirability function for multiple responses. The goal for extraction parameters in the SC-$CO_2$ extraction was set in range where the goal for oil yield and tocol contents were defined as maximum and phosphorous content was defined as minimum. The weight factor which illustrates the shape of the desirability function of the three responses was fixed as 1 and the "importance" value of 3 was chosen for all the goals in the study.

Table 4. Analysis of variance (ANOVA) of the modeled responses.

| Source | SS | DF | MS | F-value | *P*-value |
|---|---|---|---|---|---|
| **Oil Yield Recovery*** | | | | | |
| Model | 48.21 | 9 | 5.36 | 200.80 | < 0.0001 |
| Residual | 0.19 | 7 | 0.03 | | |
| Lack of fit | 0.07 | 3 | 0.02 | 0.85 | 0.5328 |
| Pure error | 0.11 | 4 | 0.03 | | |
| Total | 48.39 | 16 | | | |
| **Phosphorous Content Recovery**** | | | | | |
| Model | 996.88 | 9 | 110.76 | 259.67 | < 0.0001 |
| Residual | 2.99 | 7 | 0.43 | | |
| Lack of fit | 2.39 | 3 | 0.80 | 5.30 | 0.0704 |
| Pure error | 0.60 | 4 | 0.15 | | |
| Total | 999.86 | 16 | | | |
| **Tocols Content Recovery***** | | | | | |
| Model | 6.57E+005 | 9 | 73056.88 | 174.47 | <0.0001 |
| Residual | 2931.16 | 7 | 418.74 | | |
| Lack of fit | 1885.16 | 3 | 628.39 | 2.40 | 0.2081 |
| Pure error | 1046.00 | 4 | 261.50 | | |
| Total | 6.60E+005 | 16 | | | |

SS = Sum of Squares; DF = Degree of Freedom; MS = Mean Squares; *$R^2$- 0.9961, Adj. $R^2$- 0.9912, **$R^2$- 0.997, Adj. $R^2$- 0.9932, ***$R^2$- 0.9956, Adj. $R^2$- 0.9899; ANOVA of the modeled responses were resulted from the mean of the three replicates of responses.

The optimum extraction conditions for SC-$CO_2$ extraction obtained by the application of RSM were found to be pressure of 50 M Pa, extraction temperature of 60 °C, $CO_2$ flow rate of 30 g·min$^{-1}$. The optimum conditions resulted in the predicted oil yield of 8.87%, phosphorous content of 31.86 mg·Kg$^{-1}$ and tocol content of 2059.92 mg·Kg$^{-1}$ with a desirability factor of 0.775. To validate the sustainability of the models and the predicted values obtained, an experimental run was carried out under the optimal conditions and the results obtained were 8.68%, 42.5 mg·Kg$^{-1}$ and 2057.3 mg·Kg$^{-1}$ for oil yield, phosphorous content and tocol contents respectively. The experimental results were found to be within the range of predicted values, indicating that the optimization of SC-$CO_2$ extraction using the Box Behnken design was adequate to explain the process. Conventional solvent extraction using hexane as solvent resulted in 9.4% oil yield, 1056 mg·Kg$^{-1}$ phosphorous content and 2046.4 mg·Kg$^{-1}$ tocol content. Although the yield is shown to be a bit less, this may be due to the poor extractability of phospholipids by SC-$CO_2$.

## 4. CONCLUSIONS

In this present investigation, the Box Benhnken design from response surface methodology was used to find the optimum process conditions for the SC-$CO_2$ extraction of wheat germ oil with desirable characteristics. Keeping in mind the oil yield and quality of the oil with respect to phosphorus and tocol contents, the models obtained from the ANOVA analysis were with higher regression coefficients thus indicating that the data fit well. The optimum conditions obtained were 50 M Pa, 60 °C and 30 g·min$^{-1}$ where the predicted oil yield, phosphorus content and tocol contents were with high desirability factors. The experimental results obtained under the optimized conditions were found to be very close to those predicted by the model. The process conditions ensure that superior quality oil can be obtained from SC-$CO_2$ extraction with higher tocol contents and with lower phosphorous content. The present models can be used to design and scale up the SC-$CO_2$ extraction of high quality wheat germ oil.

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