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Optimization of Supercritical CO_2 Extraction of Safflower Seed Oil Using Response Surface Methodology

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The present study examined extraction of safflower seed oil by using supercritical carbon dioxide. Response surface methodology was used to evaluate the effects of the process parameters, namely pressure, temperature and extraction time on the yield of safflower seed oil. The quadratic terms of time ($p < 0.01$), temperature ($p < 0.001$) and pressure ($p < 0.001$), interaction between time and pressure ($p < 0.001$), as well as time and pressure ($p < 0.001$) showed significant effect on the safflower oil yield in the present model. Optimum yield of safflower seed oil was predicted as 26.47 % at pressure of 450 bars, temperature 68.49 °C and extraction time of 30 min. Colour, acid value, saponification value and induction time of safflower seed oil extracted using supercritical CO_2 were compared with that obtained by solvent extraction. Minor differences were found in all the studied parameters of the oils extracted by the two methods. Acid value of oil extracted using supercritical CO_2 was approximately five times lower than the value obtained for the oil from solvent extraction. However, a slightly shorter induction period was recorded for the supercritical CO_2 extracted safflower seed oil as compared to the solvent extracted oil at all the studied temperatures *i.e.*, 120, 140, 160 and 180 °C. Minor differences were observed in the fatty acid composition of the oils extracted using two methods.

Keywords: Acid value, Fatty acid composition, Response surface methodology, Supercritical CO_2 extraction, Safflower oil.

INTRODUCTION

Safflower (*Carthamus tinctorius* L.) a member of composite family, is one of the important oilseed crops in India. It has been grown traditionally for its flowers as a source of dye for colouring food and fabrics. But nowadays it is grown for edible oil, animal meal, bird feed, medicinal value, *etc.* [1]. Safflower seed contains 22-34 % oil [2] and seed oil is rich in the linoleic acid (70-87 %) and tocopherols [3]. Safflower is cultivated in India, China, USA, Mexico, Turkey and Kazakhstan [4]. India is the largest producer of safflower (approximately 2 lakh tones) in the world with highest acreage of 4.3 lakh hectares [5]. The crop has gained importance because of its adaptability to different environmental conditions and due to the quality of its seed oil [6].

Recently, attention has been given to safflower seed oil as an excellent health care product, because it has been reported to be effective for the treatment of hyperlipemia, coronary heart disease and it has been reported to enhance the micro-circulation [7]. The oil is good for the skin and the intestinal mucosa and also known to find applications in the prevention as well as treatment of rheumatic disorders [8].

Commercially oil is extracted using hexane or petroleum ether but because of consumer awareness and restrictions imposed by certain governments on use of organic solvents the industries are looking for some other green technologies for oilseed processing. Supercritical fluid extraction technology (SCFE) is gaining importance as a green technology. As compared to conventional solvent extraction and mechanical pressing of oils, supercritical extraction using CO_2 as a solvent can become potential alternative for extracting oils and other materials because of easy availability, no toxicity and low-cost price of CO_2. Many plant seed oils have been extracted using supercritical CO_2 extraction.

The efficiency of supercritical CO_2 extraction can be affected by many factors such as temperature, pressure, particle size and moisture content of feed material, extraction time, CO_2 flow rate and solvent and solid ratio. When multiple variables may influence the output, the response surface methodology (RSM) could be used as an effective technique for optimizing the process [9]. The advantage of response surface methodology over the one-factor-one time approach is that it allows the evaluation of the effect of multiple variables and their inter-actions on the output variables with reduced number of trials [9,10]. Response surface methodology (RSM) has been used to optimize food processing operations including supercritical CO_2 extraction of oils from cottonseed [11], canola, rapeseed, soybean, sunflower, jojoba, sesame, *etc.* [12-14]. Keeping in view the potential of SCFE technology and importance of safflower crop, the present study was designed to optimize the conditions, including temperature, pressure and extraction time, for supercritical CO_2 extraction of safflower seed oil using the response surface methodology, while considering the yield of oil as the response variable. The chemical analysis of the oil extracted using solvent as well as supercritical fluid extraction was also compared.

EXPERIMENTAL

Procurement of safflower seed: Seeds of safflower variety PBNS 12 were procured from Directorate of Oilseed Research, Hyderabad, India. The seeds were ground and oil was extracted using solvent as well as supercritical technology.

Solvent extraction: For solvent extraction, 10 g of ground seed was weighed and placed in a Soxhlet apparatus. Crushed material was then continuously extracted for 8 h using *n*-hexane as solvent. After extraction, the solvent was evaporated. The extracted oil was stored at 20 °C till further biochemical analysis.

Supercritical CO_2 extraction: Speed SFE model 7071 instrument (Applied Separations, Allentown, PA, USA), equipped with a pressure unit (Model 7071) and a thick-walled stainless steel cylindrical extractor vessel was used for oil extraction. Carbon dioxide was cooled by a chiller before pressurizing and pumping into the system. Seed sample of 20 g was crushed, weighed and filled in the extractor. The flow rate of the CO_2 at the exit of the system was controlled and kept constant at 3 L/min during the experiments. The pressures, temperatures and extraction time were varied to determine the effects of these parameters on the extraction yield according to the experimental design in present study. All samples were collected in sample vials and the extracted oil was stored at 20 °C till further biochemical analysis.

Experimental design: A 3^3 factorial experimental design was used to evaluate the effects of three independent variables: X_1 extraction time (t, 30-75 min), X_2 temperature (T, 50-80 °C), X_3 Pressure (300-450 bars) on the seed oil extraction yield (Y_1, %). Table-1 represents coded and uncoded levels of the independent variables used in the experimental design. Coded value 0 stands for centre point of the variables and repeated for experimental error. Factorial points are coded as ± 1. Five replicates at the center of the design were used to allow for the estimation of a pure error sum of squares. Differences between variables were tested for significance using the one-way ANOVA analysis procedure. The statistical software package 'Design Expert 8.0' (Version 8.0.2, Stat-Ease, Minneapolis, MN) was used for regression analysis of the data and estimation of the regression equation coefficients. A multiple regression analysis was applied to obtain an empirical model that relates the response measured to the independent variables. A second-order polynomial equation was used to express the yield (Y) as a function of the independent variables,

$$Y = \beta_0 + \beta_1X_1 + \beta_2X_2 + \beta_3X_3 + \beta_{11}X_1^2 + \beta_{22}X_2^2 + \beta_{33}X_3^2 + \beta_{12}X_1X_2 + \beta_{13}X_1X_3 + \beta_{23}X_2X_3$$

TABLE-1
UNCODED AND CODED LEVELS OF INDEPENDENT VARIABLES USED IN THE RESPONSE SURFACE METHODOLOGY DESIGN

Independent variables	Symbol	Level		
		Low (–1)	Middle (0)	High (+1)
Extraction time (min)	X_1	30	52.5	75
Temperature (°C)	X_2	50	65	80
Pressure (bars)	X_3	300	375	450

where, Y represents the response variable and X_1, X_2, X_3 are the coded values of independent variables. β_0 is a constant, β_1, β_2, β_3 are the linear coefficients, β_{11}, β_{22}, β_{33} are the quadratic coefficients, β_{12}, β_{13}, β_{23} are the interaction coefficients.

Physico-chemical characteristics: Colour of oil was measured using tintometer. Peroxide value (PV) expressed as meq O_2/kg oil was measured according to the American Oil Chemists' Society (AOCS) official method [15]. Free fatty acid content of the seed oil (expressed as wt % oleic acid) was measured according to International Union of Pure and Applied Chemistry standard method 2.201 [16].

Oxidative stability index: Oxidative stability of the oil was determined at different temperatures with the Rancimat (Metrohm AG) apparatus, according to the AOCS official method [17].

Fatty acid analysis by gas liquid chromatography (GLC): Methyl esters of oil samples were prepared by transesterification according to the method [18] with slight modifications. 1 mL of the methyl ester sample was injected into SP 2300 + 2310 SS column. A Nucon model 5765 gas chromatograph equipped with flame ionization detector (FID) was used. The oven, injector and detector temperature were 240, 230 and 250 °C, respectively. The carrier gas was nitrogen at flow rate of 40-50 mL/min. Peaks of fatty acid methyl esters were identified by comparing their retention time with that of the known standards run under similar separation conditions. Individual fatty acids were expressed as % of the total fatty acids.

RESULTS AND DISCUSSION

Fitting the model: Response surface methodology was used to optimize the conditions for extraction of oil from the safflower seed sample by using supercritical fluid extraction technology. In order to study the effects of three independent variables *viz.* X_1 extraction time (t, 30-75 min), X_2 temperature (T, 50-80 °C) and X_3 pressure (P, 300-450 bars) on extraction yield of safflower seed oil, response surface methodology was applied. Table-2 represents the coefficients R^2, adjusted R^2, standard deviation (SD), mean and CV %. The results revealed that seed oil yield (Y_1, %) was in the range of 10.45-26.96 % under experimental conditions. The highest yield of 26.96 % was observed at temperature, pressure and extraction time of 65 °C, 450 bars and 75 min, respectively. The lowest yield of 10.45 % was found at the conditions temperature, pressure and extraction time of 65 °C, 300 bars and 30 min, respectively (Table-2). Analysis of variance (ANOVA) showed that the resultant second order polynomial model adequately represented the experimental data with the coefficient of

TABLE-2
EXPERIMENTAL CONDITIONS AND EXTRACTION YIELD OF SUPERCRITICAL FLUID EXTRACTED SAFFLOWER SEED OIL

Standard	Run	Extraction time (X_1, min)	Temp. (X_2, °C)	Pressure (X_3, bars)	Yield (Y_1, %)
13	1	52.5	80	300	15.39
4	2	30.0	65	450	26.14
14	3	30.0	50	375	15.30
17	4	52.5	65	375	26.46
11	5	30.0	65	300	10.45
7	6	75.0	80	375	26.13
8	7	52.5	65	375	26.46
5	8	52.5	65	375	26.46
9	9	52.5	50	450	26.54
16	10	75.0	65	450	26.96
3	11	52.5	80	450	26.82
2	12	75.0	50	375	26.31
12	13	52.5	50	300	15.35
6	14	75.0	65	300	24.80
10	15	30.0	80	375	18.62
1	16	52.5	65	375	26.46
15	17	52.5	65	375	26.46
Std. Dev.	–	–	–	–	0.87
Mean	–	–	–	–	23.01
C.V. %	–	–	–	–	3.76
R^2	–	–	–	–	0.9893
Adj R^2	–	–	–	–	0.9755

multiple determinations (R^2) and adjusted R^2 for the oil yield, being 0.9893 and 0.9755, respectively.

The results of analysis of variance (ANOVA) were used to evaluate the significance of the coefficients of the model and are represented in Table-3. Significant effects of the variables as linear, quadratic, or an interaction coefficient on the response was checked. As discussed by Quanhong and Caili [19], a large regression coefficient and a small p-value, for any of the terms in the model indicates a significant effect on the given response variable. The quadratic terms of time ($p < 0.01$), temperature ($p < 0.001$) and pressure ($p < 0.001$), interaction between time and pressure ($p < 0.001$), as well as time and pressure ($p < 0.001$) showed significant effect on the safflower oil yield in the present model.

TABLE-3
REGRESSION COEFFICIENTS OF THE FITTED QUADRATIC EQUATION AND STANDARD ERRORS FOR THE YIELD OF OIL (%, Y_1)

Regression coefficient	Yield of oil (%, Y_1)	
	Regression coefficient	Standard error
β_0	26.46**	0.39
Linear		
β_1	+4.21**	0.31
β_2	+0.43	0.31
β_3	+5.06**	0.31
Quadratic		
β_{11}	-1.90*	0.42
β_{22}	-2.97**	0.42
β_{33}	-2.47**	0.42
Interaction		
β_{12}	-0.88	0.43
β_{13}	-3.38**	0.43
β_{23}	-0.060	0.43

*$p < 0.01$; ** $p < 0.001$; β_0 is a constant, β_{ii} and β_{ij} are the linear, quadratic and interactive coefficients of the second order polynomial equation, respectively. Where 1, 2 and 3 corresponds to extraction time, temperature and pressure, respectively.

Response surface analysis: The best way to visualize the effect of the independent variables on the dependent ones is to draw surface response plots of the model. Within the experimental range two variables were varied and third was kept constant at the central point. The result showed that in present study, significant model was obtained for the dependent variable Y_1. However, multiple regression coefficients were determined by the least-squares technique in order to predict quadratic polynomial model for the tested response variable and the regression equation were obtained as shown below:

$$\text{Yield } (Y_1) = +26.46 + 4.21*t + 0.43*T + 5.06*P - 0.88*t*T - 3.38*t*P + 0.06*T*P - 1.90*t^2 - 2.97*T^2 - 2.47*P^2$$

The regression model obtained for the yield of extracted safflower seed oil, showed the F-test, with a high significant fit ($P < 0.001$). The lack of fit was insignificant and hence confirmed the adequacy of the quadratic model. Fig. 1a is a response surface plot showing the effect of pressure and temperature on the oil yield at a fixed time. Supercritical fluid extraction recovery and selectivity depend greatly on the equilibrium between fluid density and solute vapour pressure. Generally, an increase in pressure would increase the fluid density, consequently increasing the solubility of oil and yield. In present results, pressure showed a positive linear effect on the oil yield. At very low pressure, the yield of the extracted oil was less and it increased significantly with an increase in extraction pressure (Fig. 1a). Similar results have been reported by other workers [20-22]. This could be due to the improved oil solubility because of the increased solvent density at high pressure [23]. In present results at high-pressure levels, however, the negative and significant quadratic effect of pressure and temperature on the oil yield was observed. High negative quadratic effect of pressure could be due to the increased repulsive solute-solvent interactions resulted from the highly compressed CO_2 at high-pressure levels [24].

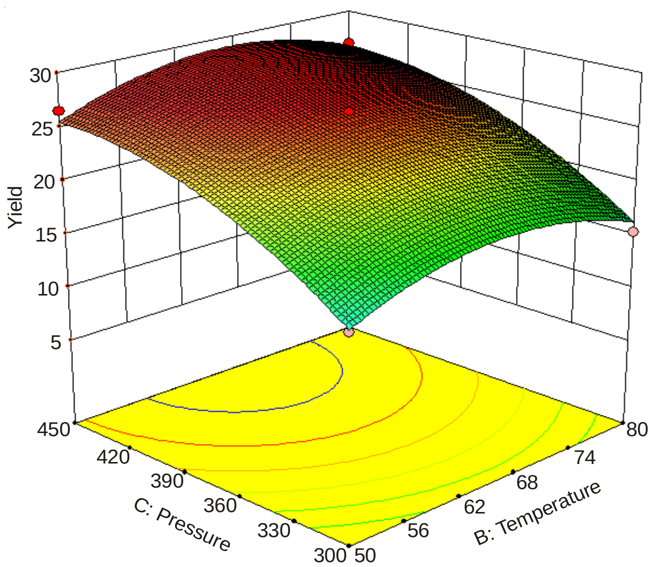

Fig. 1a. Surface plot of the oil yield (Y_1) as a function of pressure and temperature

Fig. 1b is a response surface plot showing the effect of temperature and time on the oil yield. At a particular extraction time, yield increased linearly with increase in temperature. An increase in temperature would reduce the fluid density, which decreases the solubility. An increase in temperature would also increase the solute vapour pressure, which would increase the solubility. However, in the present study yield was not affected significantly by the temperature. Interaction effect of time and temperature was not significant. Quadratic effect of temperature was negative and significant.

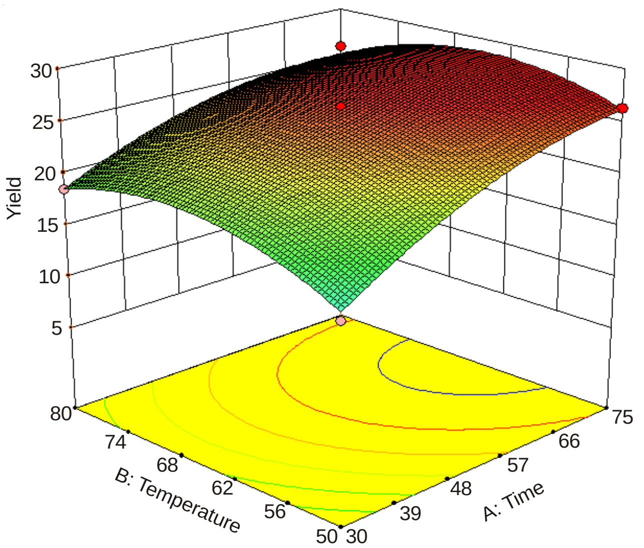

Fig. 1b. Surface plot of the oil yield (Y_1) as a function of temperature and extraction time

Fig. 1c represents response surface plot showing the effect of pressure and time on the yield (%) of oil. It could be seen that safflower oil yield increased with increase in extraction time at a particular pressure. Extraction time showed positive and significant effect on yield in linear term, however, quadratic term of extraction time showed negative but significant effect. Similar effects were observed by Xu *et al.* [20] during the extraction of sea buckthorn oil. Interactive effect of time and pressure was also significant but negative.

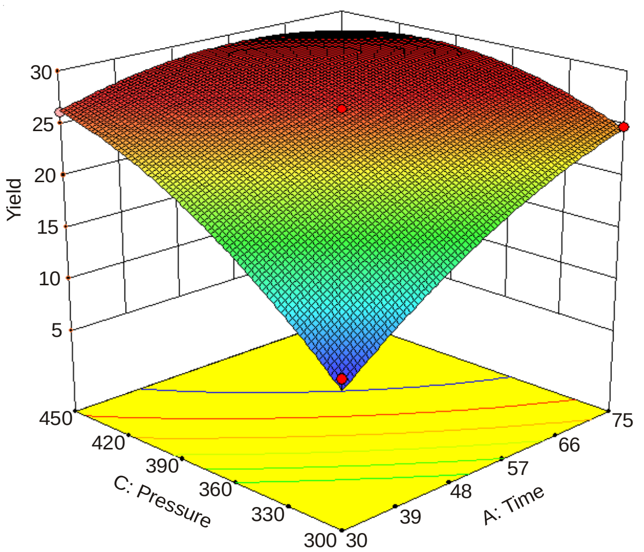

Fig. 1c. Surface plot of the oil yield (Y_1) as a function of pressure and extraction time

Optimization of extraction conditions: Response optimization is conducted to predict the optimum levels of independent variables leading to the desired response goal [25]. In order to check the optimized conditions, a numerical optimization was employed. Time was kept minimum, temperature and pressure were within the range while yield was kept at maximum. The numerical optimization results showed that the overall optimum area was predicted to be obtained by the supercritical CO_2 extraction at the combined level of 450 bars, 68.49 °C and 30 min for pressure, temperature and extraction time, respectively with desirability of 0.985 % by response surface plots and response optimizer. The corresponding predicted response values based on the final reduced model for yield were 26.47 %.

Physico-chemical characteristics of safflower seed oil: Physico-chemical characteristics of hexane extracted and SCFE oil was compared (Table-4). Acid value of supercritical CO_2 extracted oil was low (0.73) as compared to the hexane extracted oil (1.76) which is a significant indicator of quality of oil. According to Demian [26] acid values are used to measure the extent to which glycerides in the oil has been decomposed by lipase, heat and light. The results further revealed that colour reading, saponification value and peroxide value (meqv/kg) for SCFE oil were 14.6, 185.5 and 8.04, respectively and the values were slightly lower than the values obtained for hexane extracted oil 16.3, 187.8 and 9.63, respectively. The results showed supercritical CO_2 extracted oil was better than hexane extracted oil in colour as well as other parameters.

TABLE-4
COMPARISON OF PHYSICO-CHEMICAL PROPERTIES OF HEXANE AND SCFE EXTRACTED SAFFLOWER SEED OIL

Parameters	Hexane extracted	SCFE extracted oil (P: 375 bars, T: 65 °C, Extraction time: 52.5 min)
Oil (%)	24.36 ± 0.150	26.46 ± 0.200
Colour reading	16.30 ± 0.490	14.60 ± 0.210
Saponification value	187.80 ± 0.260	185.5 ± 0.390
Peroxide value (meq/kg)	9.63 ± 0.140	8.04 ± 0.130
Acid value (%)	1.79 ± 0.020	0.43 ± 0.020
Induction time (h) at 120 °C	0.49 ± 0.005	0.40 ± 0.005
at 140 °C	0.20 ± 0.010	0.05 ± 0.010
at 160 °C	0.16 ± 0.005	0.03 ± 0.001
at 180 °C	0.03 ± 0.001	0.02 ± 0.001
Fatty acid (g/100 g)		
Palmitic acid	5.67 ± 0.500	6.60 ± 0.100
Stearic acid	1.60 ± 0.660	1.97 ± 0.210
Oleic acid	11.47 ± 0.650	15.03 ± 0.150
Linoleic acid	80.90 ± 1.300	76.20 ± 0.200
MUFA	11.47 ± 0.650	15.03 ± 0.150
PUFA	80.90 ± 1.300	76.20 ± 0.200

MUFA = Monounsaturated fatty acid
PUFA = Polyunsaturated fatty acid

Oxidative stability: Lipid oxidation is the main process leading to the deterioration of edible oils [27]. It produces rancid odors, unpleasant flavours, discolouration and decreased nutritional quality and safety. Induction period was recorded for the supercritical CO_2 extracted and solvent extracted safflower seed oil (Table-4). A slightly shorter induction period was recorded for the supercritical CO_2 extracted safflower seed oil as compared to the solvent extracted oil at different temperatures *i.e.* 120, 140, 160 and 180 °C. Mean value for induction period at 120 °C for Soxhlet extracted oil was 0.49 h and supercritical CO_2 extracted safflower seed oil was 0.40 h. Similar trend was observed at other temperatures also. Other workers have also reported about slightly lower oxidative stability of the supercritical CO_2 extracted oil [28,29]. According to different workers introduction of oxygen into the apparatus during the extraction, presence of oxygen in commercial CO_2 [28] contamination of oil by impurities present on the surface of the extractor circuit (*e.g.,* metals and water) and in the gas phase (*e.g.,* oxygen and vapour [30] could be the reasons for this instability. Removal of co-extracted water could help to increase oil stability.

Fatty acid composition: Fatty acid composition of the seed oil using SCFE and solvent extraction is given in Table-4. The results of GC revealed that no major differences were observed in the fatty acid composition of the oils extracted by the two methods. Major fatty acid was found to be linoleic acid and hence oil is good source of omega-6 linoleic acid. Similar results showing no marked changes in seed oil composition has also been reported by other workers [31] for pomegranate seed oil.

Conclusion

Response surface methodology was applied for supercritical CO_2 extraction optimization of safflower oil. The high regression coefficients of the second-order polynomial showed that the model was well fitted to the experimental data. The quadratic terms of time ($p < 0.01$), temperature ($p < 0.001$) and pressure ($p < 0.001$), interaction between time and pressure ($p < 0.001$), as well as time and pressure ($p < 0.001$) showed significant effect on the safflower oil yield in the present model. The ANOVA implied that extraction pressure was the most significant factor affecting the yield in supercritical CO_2 extraction. The optimum extraction conditions were extraction pressure of 450 bars and temperature of 68.49 °C with extraction time of 30 min which predicted the yield of 26.47 %. Seed oil extracted by supercritical CO_2 was better than solvent extracted oil though significant differences were not observed in the fatty acid composition. However, induction period of supercritical CO_2 extracted oil was found slightly shorter than hexane extracted oil.

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