



Original Research Article

**Response Surface Methodology Applied to the Supercritical Carbon Dioxide
Extraction of *Zingiber officinale* Oleoresin**

Shahin Ahmadi^{1*}, Sedighe Pardis kian², Maryam Moghaddas²

^{1*} Department of Pharmaceutical Chemistry, Tehran medical sciences, Islamic Azad University, Tehran, Iran.

² Department of Chemistry, Safadasht Branch, Islamic Azad University, Tehran, Iran.

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ABSTRACT

This study explores the extraction of oleoresin from the rhizome of ginger (*Zingiber officinale*) using supercritical carbon dioxide (SC-CO₂) extraction and Soxhlet extraction techniques. Key operational parameters for SC-CO₂ extraction, including pressure (10–20 MPa), temperature (35–45 °C), and flow rate (10–16 g min⁻¹), were optimized to evaluate their influence on extraction yield, radical scavenging activity, and total phenolic content. A Box–Behnken design was employed for experimental design and analysis. Regression analysis confirmed that the experimental data conformed well to both linear and second-order polynomial models. The SC-CO₂ method achieved a maximum oleoresin yield of 6.47 ± 0.07%, significantly higher than the 3.19 ± 0.22% obtained using Soxhlet extraction. The antioxidant potential of the extracts, determined through 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity, revealed values of 50.70 ± 0.52% for SC-CO₂ extraction and 88.50 ± 0.18% for Soxhlet extraction. The total phenolic content, quantified via the Folin–Ciocalteu method, was 103.24 ± 1.58% for SC-CO₂ extracts under optimal conditions, compared to 31.10 ± 0.28% for Soxhlet extracts.

Keywords: *Zingiber officinale*, Oleoresin, Supercritical carbon dioxide, Response surface optimization, Antioxidant activity, Total phenolic content

*Corresponding author email address: ahmadi.chemometrics@gmail.com

Introduction

In complex matrices such as biological, environmental, and food samples, analyte concentrations often fall below the detection limits of many analytical instruments. As a result, sample pretreatment is an essential step in analytical workflows prior to analysis. To date, a variety of extraction and pretreatment techniques have been developed and applied, including Soxhlet extraction, microwave-assisted extraction (MAE), and supercritical fluid extraction (SFE), for isolating target compounds from solid samples. SFE offers several advantages, such as significantly reduced extraction times and the use of smaller quantities of extraction solvents. Supercritical fluids are characterized by their low viscosity, high diffusivity, and ability to dissolve a wide range of substances [1]. Among various supercritical solvents (e.g., ethane, propane, argon, and carbon dioxide), CO₂ is the most widely used due to its notable benefits, including non-toxicity and non-flammability.

Ginger (*Zingiber officinale*) is a plant whose rhizome is extensively used as a spice and flavoring agent in food. Extracts from the rhizome of *Zingiber officinale* are rich in polyphenolic compounds known for their anti-inflammatory properties [2]. Additionally, the plant contains various antioxidants, including ascorbic acid, alkaloids, and beta-carotene, all of which play critical roles in promoting human health [3-8]. The essential oil and oleoresin of ginger are utilized not only in the food industry but also in pharmaceutical applications [9, 10]. Several methods, such as Soxhlet extraction and steam distillation, have been employed for isolating oleoresin from ginger. However, these conventional techniques often require large quantities of organic solvents, elevated temperatures, and extended extraction times. Supercritical carbon dioxide extraction has emerged as a promising alternative, offering the advantage of isolating

natural products at room temperature while minimizing the degradation of sensitive antioxidants [10].

The scientific literature documents various methods for extracting ginger oleoresin, including traditional techniques such as Soxhlet extraction and cold percolation, which rely on the use of organic solvents and elevated temperatures. However, these conventional methods have notable drawbacks, including prolonged extraction times, high solvent consumption, and the potential degradation or alteration of key oleoresin components, such as antioxidant activity and total phenolic content [9].

In recent years, growing interest has focused on innovative extraction techniques that require shorter extraction times and minimal solvent use, both to reduce environmental impact and lower costs. Emerging methods, such as enzyme-assisted three-phase partitioning [10], microwave-assisted extraction (MAE) [11], ultrasound-assisted supercritical extraction [12, 13], and supercritical fluid extraction (SFE) [9, 14], have been increasingly employed for isolating ginger constituents.

The Box–Behnken design (BBD), a type of response surface methodology (RSM), has proven to be an effective tool for optimizing analytical procedures. As a second-order multivariate approach, BBD employs a three-level partial factorial design that determines the required number of experiments using the formula $N = 2k(k-1) + C_0$, where k represents the number of factors and C_0 denotes the number of central points [15, 16].

This study aims to compare the extraction yield (EY), free radical scavenging activity (RSA), and total phenolic content (TPC) of ginger (*Zingiber officinale*) oleoresin obtained through two methods: supercritical carbon dioxide (SC-CO₂) extraction and Soxhlet extraction. The antioxidant activity of the oleoresin was assessed using N-vanillylnonanamide and the 2,2-

diphenyl-1-picrylhydrazyl (DPPH) method, while the TPC of each extract was measured using the Folin–Ciocalteu procedure.

BBD was utilized to guide the experimental optimization of the extraction process. Linear and quadratic models were developed to explore the relationships between EY, RSA, and TPC, and key extraction variables such as temperature, pressure, and CO₂ flow rate. The model's accuracy and reliability were assessed using analysis of variance (ANOVA).

Materials and Methods

Plant Materials

Fresh ginger rhizomes (*Zingiber officinale*) of Indian origin were procured from a local market in Tehran. The rhizomes were thoroughly washed, peeled, and sliced into thin pieces measuring 1–5 mm in thickness. The prepared slices were dehydrated in a hot air oven at 35±5 °C for two days until a final moisture content of approximately 8% was achieved. The dried ginger was stored in paper bags at 4 °C until further use.

Prior to experimentation, the dried ginger was ground into fine particles using a ball mill and sieved with I.S. Standard test sieves. Based on preliminary findings, a particle size of 250 µm was selected for the experiments. Smaller particle sizes were avoided as they increased packing density within the extraction column, which impeded solvent mass transfer and subsequently reduced the extraction yield (EY).

Reagents

Carbon dioxide (99.99% purity) was supplied in a cylinder equipped with an eductor tube by Sabalan Co. (Tehran, Iran). HPLC-grade acetonitrile and methanol, along with hydrochloric acid, acetic acid, ammonium chloride, gallic acid, silver nitrate, and Folin–Ciocalteu phenol reagent (analytical grade), were obtained from Merck (Darmstadt, Germany). DPPH was sourced from

Sigma–Aldrich (Milwaukee, WI, USA), and butylated hydroxytoluene (BHT) was procured from Merck (India). Deionized water used in all procedures was generated using a Milli-Q purification system.

Supercritical Fluid Extraction (SFE)

A continuous SC–CO₂ extraction system was utilized for oleoresin extraction. Liquid CO₂ was pressurized into a vertical surge tank using an air-driven, oil-free reciprocating pump (Haskel pump, MS-110, USA) to achieve the desired pressure. The pressure within the extraction and separation vessels was regulated using a pressure controller, while a heat-exchange system maintained the required temperature. Dial pressure gauges were strategically installed at various points to monitor pressure, and a manual pressure regulator at the separator was used to release CO₂.

In each extraction, 30 g of dried ginger powder was loaded into the extraction vessel, interspersed with glass beads. The inclusion of glass beads reduced packing density and enhanced solvent diffusion, thereby improving mass transfer. Each extraction was conducted over a duration of 30 minutes. The yield of the extracted oleoresin was determined gravimetrically on a weight-to-weight (w/w) basis using an analytical balance. During the experiments, pressure, temperature, and solvent flow rate were manipulated as independent variables.

Soxhlet Extraction

To compare the SC-CO₂ extraction method with traditional techniques, oleoresin was extracted using the continuous percolation method in a Soxhlet extractor. The procedure outlined by Said et al. [9] was adapted for this study. Approximately 30 g of dried ginger rhizome powder was placed in a cellulose thimble and positioned in a Soxhlet extractor (Vapodest 30, Gerhardt,

Germany). The extraction was carried out using acetone at 60 °C for 4 hours. After extraction, the solvent was evaporated using a vacuum oven (Ehret, Germany) at 35 °C until the extraction vessels reached a constant weight. The oleoresin was then allowed to cool before being weighed.

The extraction yield (EY) of the oleoresin was calculated using the following formula:

$$EY (\%) = \frac{W_2 - W_1}{W} \times 100 \quad (1)$$

where W_1 is the weight of the empty extraction vessel, W_2 is the weight of the extraction vessel containing oleoresin, and W is the weight of the dried ginger rhizome powder. Each extraction was performed in triplicate to reduce experimental error. The oleoresin was stored in amber-colored bottles and kept in a refrigerator. The oleoresin yield was found to be $3.19 \pm 0.22\%$.

Response Surface Methodology (RSM)

A three-level, three-factor Box-Behnken Design (BBD) [15] was employed for experimental design, optimization, and response function fitting in response surface methodology (RSM) using Design Expert Version 10.0.8 (Stat Ease, USA) [17-19]. Since extraction pressure (X_1), temperature (X_2), and CO₂ flow rate (X_3) significantly affect extraction efficiency, these parameters were chosen as critical variables to optimize, aiming to achieve the highest extraction yield (EY), free radical scavenging activity (RSA), and total phenolic content (TPC) as dependent variables (Y). Extraction experiments were conducted at varying pressures (10–20 MPa), temperatures (35–45 °C), and flow rates (10–16 g min⁻¹) to determine the optimal conditions. The ranges for the independent variables (X_1 , X_2 , and X_3) were selected based on previous studies [9, 14, 20].

A total of 17 experimental runs were conducted following the BBD design, with low, middle, and high levels of the coded values assigned as -1, 0, and +1, respectively.

Table 1. Coded and actual values of the independent variables in BBD matrix with the experimental and predicted values of extraction yield (%), radical scavenging activity and TPC of Zingiber officinale oleoresin.

test	Coded variables			Actual values			Extraction yield (%)		Radical scavenging activity (%)		Total phenolic content	
	X ₁	X ₂	X ₃	x ₁	x ₂	x ₃	Exp.	Prd.	Exp.	Prd.	Exp.	Prd.
1	0	0	0	15	40	13	5.01	5.08	86.46	86.73	78.29	77.42
2	0	1	-1	15	45	10	5.60	5.65	88.18	88.07	92.44	90.54
3	0	0	0	15	40	13	5.15	5.08	86.86	86.73	80.07	77.42
4	0	-1	-1	15	35	10	5.60	5.53	82.95	82.97	79.91	78.30
5	1	0	1	20	40	16	5.35	5.41	80.59	80.66	85.01	83.53
6	0	0	0	15	40	13	5.10	5.08	86.83	86.73	76.46	77.42
7	-1	-1	0	10	35	13	4.25	4.17	85.12	85.12	58.14	58.19
8	0	0	0	15	40	13	5.08	5.08	86.90	86.73	75.44	77.42
9	-1	1	0	10	45	13	4.32	4.29	86.20	86.32	69.52	70.43
10	0	0	0	15	40	13	5.00	5.08	86.60	86.73	77.20	77.42
11	-1	0	1	10	40	16	3.59	3.71	82.85	82.69	58.69	57.31
12	1	0	-1	20	40	10	6.41	6.44	82.43	82.48	95.56	97.53
13	1	1	0	20	45	13	6.08	5.99	85.25	85.25	96.28	96.65
14	0	-1	1	15	35	16	4.55	4.50	83.98	84.09	62.54	64.30
15	-1	0	-1	10	40	10	4.70	4.74	84.47	84.51	70.19	71.31
16	1	-1	0	20	35	13	5.81	5.86	82.24	82.12	84.54	84.41
17	0	1	1	15	45	16	4.70	4.62	83.34	83.32	75.86	76.54

The coded variables X₁, X₂, and X₃ were calculated from their respective real values x₁, x₂, and x₃ using the equations $X_1 = (x_1 - 15)/5$, $X_2 = (x_2 - 40)/5$ and $X_3 = (x_3 - 13)/3$, and X₁, X₂, and X₃ corresponded to the independent variables including extraction pressure (MPa), temperature (°C) and CO₂ flow rate (g min⁻¹), respectively.

The coded and actual values for the independent variables, along with the results for the four responses in the BBD experimental design matrix, are presented in Table 1.

The BBD optimization process typically involves executing the designed experiments, estimating the coefficients in the mathematical model, predicting the responses, and validating the model's adequacy. In this study, BBD experimental design, statistical ANOVA, regression model analysis, and the generation of three-dimensional response surface plots were all performed using statistical software.

In the BBD design, the independent variable x_i was coded as X_i , a dimensionless term, using the following equation:

$$X_i = (x_i - x_0) / \Delta x_i \quad (2)$$

The response variable (Y) was fitted to a second-order polynomial model, expressed as:

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

where Y is the predicted response, β_0 is the intercept, β_i represents the linear effect of the independent variable X_i , β_{ii} is the quadratic effect of X_i , and β_{ij} represents the interaction effect between X_i and X_j . Additionally, k denotes the number of variables.

All extraction tests were performed in triplicate and randomized. The coefficients of the mathematical model were determined, and the optimal conditions for extraction pressure (X_1), temperature (X_2), and CO₂ flow rate (X_3) were identified through BBD analysis. The optimal values for the variables were determined using Design Expert Version 10.0.8 (Stat Ease, USA).

DPPH Free Radical Scavenging Activity Assay

The antioxidant activities of the supercritical fluid extracts were evaluated using the DPPH free radical scavenging activity (RSA) assay. The method followed was based on that of Shortle et al. [21], with slight modifications. In brief, 1 mL of a 0.3 mM DPPH methanol solution was added

to 2.5 mL of the sample and incubated at room temperature for 30 minutes. After incubation, the absorbance was measured at 517 nm using a spectrophotometer (Cary 100 UV-Vis, Agilent Technologies, USA).

Blank samples consisted of 1 mL of ethanol and 2.5 mL of varying concentrations of ginger extract, while the control sample contained 1 mL of 0.3 mM DPPH and 2.5 mL of ethanol. A methanolic solution of BHT (0.01%, w/v), a known antioxidant, was used as a positive control. The RSA of the samples was calculated using the following equation:

$$\text{RSA (\%)} = \frac{A_{\text{control, 517}} - A_{\text{sample, 517}}}{A_{\text{control, 517}}} \times 100 \quad (4)$$

where $A_{\text{control, 517}}$ and $A_{\text{sample, 517}}$ are the absorbance of the control and the sample in 517 nm, respectively

Determination of Total Phenolic Content

The total phenolic content (TPC) of each extract was measured in triplicate using the Folin–Ciocalteu method, as described by Sun, Powers, and Tang [21], with minor modifications. In brief, the Folin–Ciocalteu reagent was diluted 10-fold with deionized water. A 0.1 mL aliquot of 70% methanolic ginger oleoresin was mixed with 0.75 mL of the diluted reagent and incubated at room temperature for 10 minutes. Then, 0.75 mL of a 2% sodium carbonate (w/v) solution was added. The mixture was allowed to stand in the dark for 45 minutes before absorbance was measured at 765 nm using a UV-Visible spectrophotometer (Pharmacia LKB-novaspec II, UK). The blank contained deionized water instead of the sample extract.

TPC values were calculated using a calibration curve created with a series of gallic acid standards (10, 20, 40, 60, 80, and 100 mg/L). The results are expressed as milligrams of gallic acid equivalents per gram of oleoresin.

Results and Discussion

Response Surface Analysis

The Box-Behnken Design (BBD) with three factors—pressure, temperature, and CO₂ flow rate—was employed to assess the impact of these factors on extraction yield (EY, Y₁), radical scavenging activity (RSA, Y₂), and total phenolic content (TPC, Y₃) (Table 1). Key criteria, including the coefficient of determination (R²), adjusted R² (Adj R²), predicted R² (Prd R²), lack of fit, and p-value, were considered in the ANOVA to determine the suitability of the models.

Optimization of the Experimental Conditions for Extraction Yield

The experimental conditions and extraction yields (EY, %) based on the Box-Behnken Design (BBD) method are summarized in Table 1. The experimental data for the percentage yield of oleoresin were fitted using a linear regression model, as shown in Equation 5:

$$EY (\%) = +5.08 + 0.85X_1 + 0.061X_2 - 0.52X_3 \quad (5)$$

As shown in Table 2, the analysis of variance (ANOVA) for the model indicated that the predicted model adequately represented the experimental values. The model's F-value of 467.50 suggested its statistical significance, and a p-value of <0.0001 further confirmed the suitability of the model for the current experiment. Additionally, the p-value for lack of fit was 0.3414, indicating that the lack of fit was insignificant compared to the pure errors, further validating the model's suitability.

The goodness-of-fit for the model was evaluated using R², adjusted R² (Adj R²), and predicted R² (Prd R²) values. The R², Adj R², and Prd R² values for the predicted model were 0.9908, 0.9887, and 0.9827, respectively, indicating that more than 98% of the variability in the response could be explained by the model. Overall, the statistical analysis demonstrated that the experimental

values were well-aligned with the predicted ones, confirming the accuracy and reliability of the linear model for optimization.

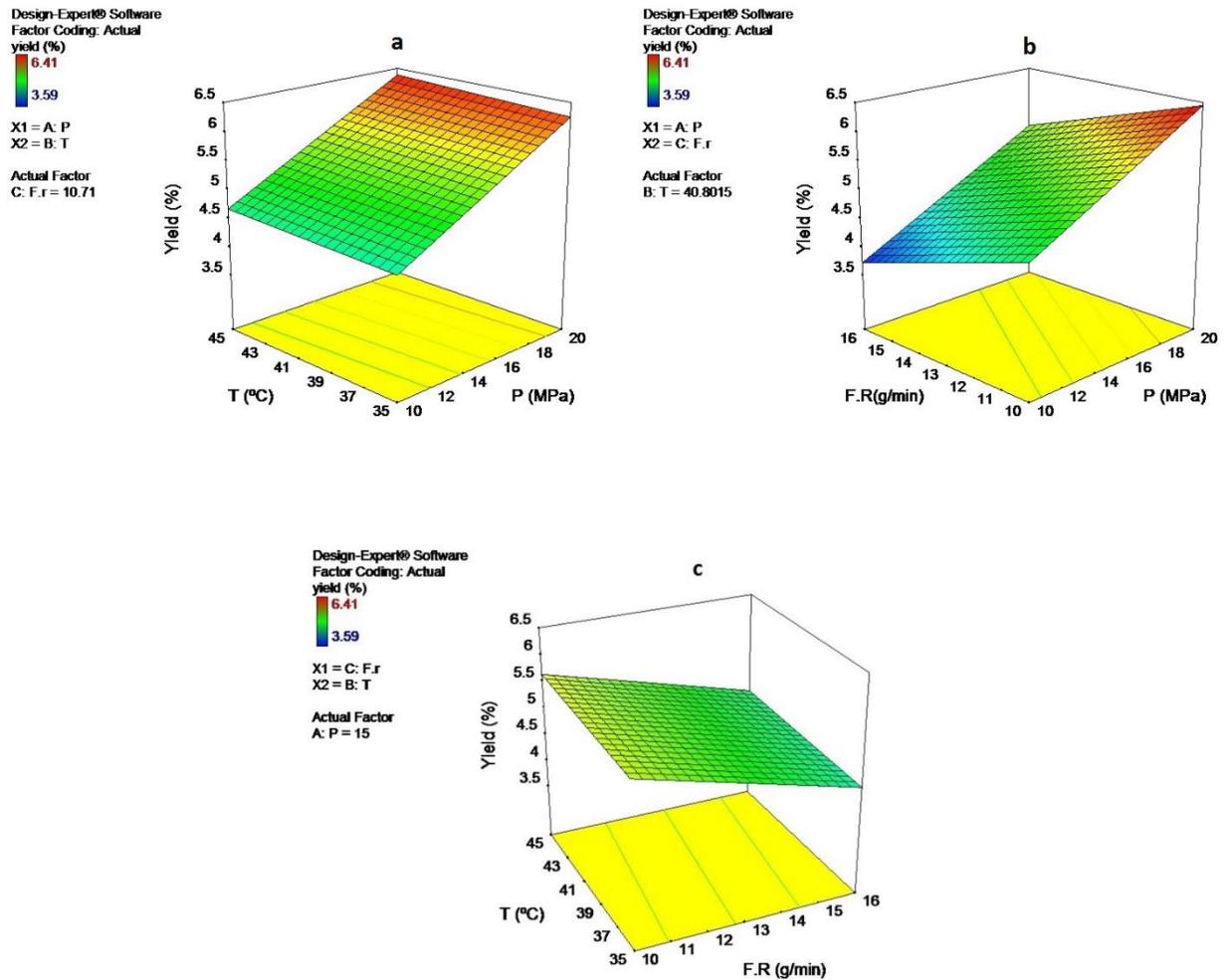


Figure 1. Response surface plots for extraction yield (EY, %) as a function of: (a) temperature and pressure; (b) CO₂ flow rate and pressure; (c) temperature and CO₂ flow rate.

The model's significant parameters included pressure (X_1), temperature (X_2), and CO₂ flow rate (X_3). The three-dimensional surface responses for the independent parameters are shown in Fig. 1, illustrating the relationships between EY and these factors. Fig. 1a shows the 3D plot for EY versus pressure and temperature at a fixed CO₂ flow rate. As indicated in Equation 5, pressure

(X_1) had the most significant positive effect on EY, as reflected by its highest positive coefficient. It is important to note that a positive sign in the linear model indicates that an increase in the parameter leads to an increase in the response, whereas a negative coefficient indicates a decrease in the response with an increase in the factor. As seen in Figs. 1a-c, EY increased as pressure was raised from 10 to 20 MPa, likely due to the increase in CO_2 density, which in turn enhanced the solubility of oleoresin in SC- CO_2 .

Table 2. RSM model adequacy and ANOVA for model of percentage yield of oleoresin

Source	Std. Dev.	R-Square	Adjusted R-Square	Predicted R-Square	p-Value	PRESS
Linear	0.075	0.9908	0.9887	0.9827	< 0.0001	0.14
2FI	0.076	0.9929	0.9886	0.9714	0.4537	0.23
Quadratic	0.065	0.9963	0.9916	0.9701	0.1732	0.24

Source	Sum of squares	df	Mean-square	F-value	p-value	Significance
Model	7.91	3	2.64	467.50	< 0.0001	Significant
X_1	5.76	1	5.76	1021.21	< 0.0001	Significant
X_2	0.030	1	0.030	5.32	0.0382	Significant
X_3	2.12	1	2.12	375.98	< 0.0001	Significant
Residual	0.073	13	0.005643			
Lack of fit	0.057	9	0.006387	1.61	0.3414	Not significant
Pure error	0.016	4	0.003970			
Core total	7.99	16				

The 3D plots also reveal that the highest yield was obtained at the highest temperature. This is likely because increased temperature raises the vapor pressure and volatility of the oleoresin in SC-CO₂, while also improving the diffusion coefficient. However, as shown in Table 2, the effect of temperature on EY was less significant than other parameters (F-value = 5.32). CO₂ flow rate had a negative linear effect on the extraction yield.

The optimal conditions for achieving the highest EY were determined to be a pressure of 20 MPa, a temperature of 45 °C, and a CO₂ flow rate of 12 g min⁻¹. Under these conditions, the maximum EY was predicted to be 6.47 ± 0.07%. Experimental validation of the model yielded an average EY of 6.45 ± 0.08%, which closely aligned with the predicted value.

Optimization of the Experimental Conditions for Percentage Free Radical Scavenging Activity (RSA)

The experimental conditions and RSA values, determined by the Box-Behnken Design (BBD) method, are provided in Table 1. A second-order polynomial regression, incorporating interaction terms, was used to fit the experimental data. The regression equation, based on the coded values, is given by:

$$RSA (\%) = 86.73 - 1.02X_1 + 1.08X_2 - 0.91X_3 + 0.48X_1X_2 - 1.47X_1X_3 - 2.03X_1^2 - 2.12X_3^2$$

(6)

The insignificant factors were excluded from the final equation. According to the ANOVA in Table 3, the predicted model effectively represented the experimental data. The model's F-value of 403.73 indicated that the model was statistically significant, and a p-value of <0.0001 further confirmed its suitability for the study. Additionally, the p-value for the lack of fit was 0.7791, which suggested that the model fit the data well.

The goodness of fit was assessed through R^2 , adjusted R^2 (Adj R^2), and predicted R^2 (Prd R^2). The R^2 and Adj R^2 values for the predicted model were 0.9968 and 0.9944, respectively, while the Prd R^2 was 0.9884, indicating that more than 98% of the variability in the response could be explained by the model. The predicted residual sum of squares (PRESS = 0.84) also showed that the model fit each data point well, with a small value of PRESS signifying better model accuracy, the statistical analysis indicated that the experimental data aligned closely with the predicted values, confirming the robustness and reliability of the polynomial model for further optimization.

The significant terms identified in the model included pressure (X_1), temperature (X_2), CO_2 flow rate (X_3), quadratic terms for pressure (X_1^2) and CO_2 flow rate (X_3^2), as well as interaction terms between pressure and temperature (X_1X_2) and pressure and CO_2 flow rate (X_1X_3). The three-dimensional surface response plots for the independent parameters are shown in Fig. 2, which illustrate the relationships between RSA (%) and these factors.

The 3D surface plot of RSA (%) versus pressure and temperature at a fixed CO_2 flow rate of 13 g min^{-1} is presented in Fig. 2a. Figures 2a-c depict the interactions between temperature and pressure, CO_2 flow rate and pressure, and temperature and CO_2 flow rate, respectively. All plots were generated by holding one variable at its coded zero level while varying the other two within their experimental ranges.

The optimized process conditions were found to be: pressure = 14.5 MPa, temperature = $45.0 \text{ }^\circ\text{C}$, and CO_2 flow rate = 11.0 g min^{-1} . These conditions were expected to yield the highest RSA (%), which was predicted to be $88.50 \pm 0.18\%$ based on the response surface model. Experimental validation of the optimization model resulted in an average RSA (%) of $87.50 \pm 0.21\%$, which

was in close agreement with the predicted value. This confirms the effectiveness of the model in predicting and optimizing antioxidant activity.

Table 3. RSM model adequacy and ANOVA for model of percentage radical scavenging activity

Source	Std. Dev.	R-Square	Adjusted R-Square	Predicted R-Square	p-Value	PRESS
Linear	1.92	0.3355	0.1822	-0.1364	0.1383	82.35
2FI	1.96	0.4677	0.1483	-0.6767	0.5082	121.51
Quadratic	0.18	0.9970	0.9931	0.9803	< 0.0001	1.43
¹ Quadratic2	0.16	0.9968	0.9944	0.9884	< 0.0001	0.84
Source	Sum of squares	df	Mean-square	F-value	p-value	Significance
Model	72.25	9	8.03	256.82	< 0.0001	Significant
X ₁	8.28	1	8.28	265.05	< 0.0001	Significant
X ₂	9.41	1	9.41	301.17	< 0.0001	Significant
X ₃	6.62	1	6.62	211.66	< 0.0001	Significant
X ₁ X ₂	0.93	1	0.93	29.82	0.0009	Significant
X ₁ X ₃	0.011	1	0.011	0.36	0.5677	Insignificant
X ₂ X ₃	8.63	1	8.63	276.25	< 0.0001	Significant
X ₁ X ₁	17.27	1	17.27	552.52	< 0.0001	Significant
X ₂ X ₂	0.000004211	1	0.000004211	0.0001347	0.9911	Insignificant
X ₃ X ₃	18.86	1	18.86	603.29	< 0.0001	Significant
Residual	0.22	7	0.031	256.82	< 0.0001	Significant
Lack of fit	0.075	3	0.025			
Pure error	0.14	4	0.036	0.70	0.6010	Not significant
Core total	72.47	16				

¹ Quadratic after removing of insignificant terms

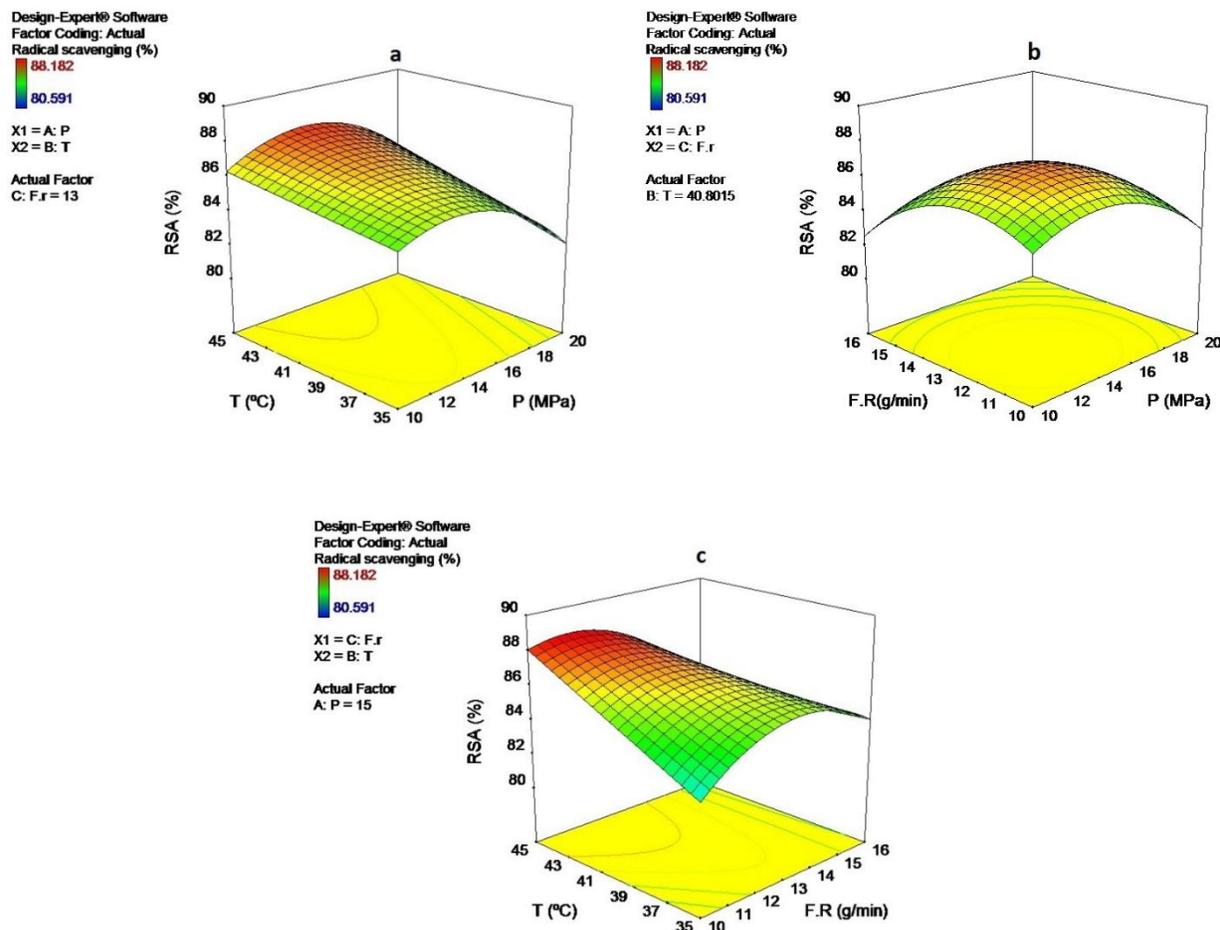


Figure 2. Response surface plots for radical scavenging activity (RSA, %) as a function of: (a) temperature and pressure; (b) CO₂ flow rate and pressure; (c) temperature and CO₂ flow rate.

Optimization of the Experimental Conditions for Total Phenolic Content (TPC)

The experimental conditions and TPC values, determined using the Box-Behnken Design (BBD) method, are provided in Table 1. The TPC ranged from 58.14 to 96.28 mg/g. Regression analysis was performed to relate TPC to the test variables, resulting in the following linear equation:

$$\text{TPC} = 77.42 + 13.11X_1 + 6.12X_2 - 0.700X_3 \quad (7)$$

ANOVA results for the regression model showed that the linear model was statistically significant, as indicated by the high model F-value ($F = 274.22$) and the low p-value ($p < 0.0001$). The significance of each coefficient was evaluated using these values, with higher F-values and lower p-values signifying greater significance. The model's goodness of fit was further confirmed by the high values of the determination coefficient ($R^2 = 0.9844$), adjusted determination coefficient (Adj. $R^2 = 0.9809$), and predicted coefficient (Pred. $R^2 = 0.9733$), which indicated a strong correlation between the experimental and predicted TPC values. The coefficient of variation (CV) was 2.05%, demonstrating a high level of precision and reliability of the experimental results. Additionally, the p-value for the lack of fit ($p = 19.98$) confirmed that the lack of fit was statistically insignificant, further validating the model.

The regression coefficients indicated that the significant parameters influencing TPC were pressure (X_1), temperature (X_2), and CO_2 flow rate (X_3). The three-dimensional response surfaces for the independent variables are shown in Fig. 3, illustrating the relationships between TPC and the independent factors.

According to the literature, increasing pressure leads to higher density, which in turn enhances the solvating power of SC-CO_2 [23, 24]. Additionally, raising the temperature from 35 to 45 °C improves the diffusion coefficient, thereby increasing the extraction of phenolic compounds.

However, TPC decreased as the CO_2 flow rate increased from 10 to 16 g min^{-1} . A lower CO_2 flow rate improves the solvent-to-feed ratio, thereby enhancing extraction. As seen in Figures 3b and 3c, the CO_2 flow rate had a small negative effect on TPC within the current experimental range, while pressure and temperature had a more significant positive influence.

The optimized process conditions for maximum TPC were determined to be a pressure of 20 MPa, a temperature of 45 °C, and a CO_2 flow rate of 10 g min^{-1} . These conditions were

predicted to yield a maximum TPC of 103.24 ± 1.58 mg/g according to the RSM model. Experimental validation of the optimized conditions resulted in an average TPC of 102.92 ± 1.32 mg/g, which closely matched the predicted value, confirming the model's accuracy and its ability to effectively predict and optimize antioxidant activity.

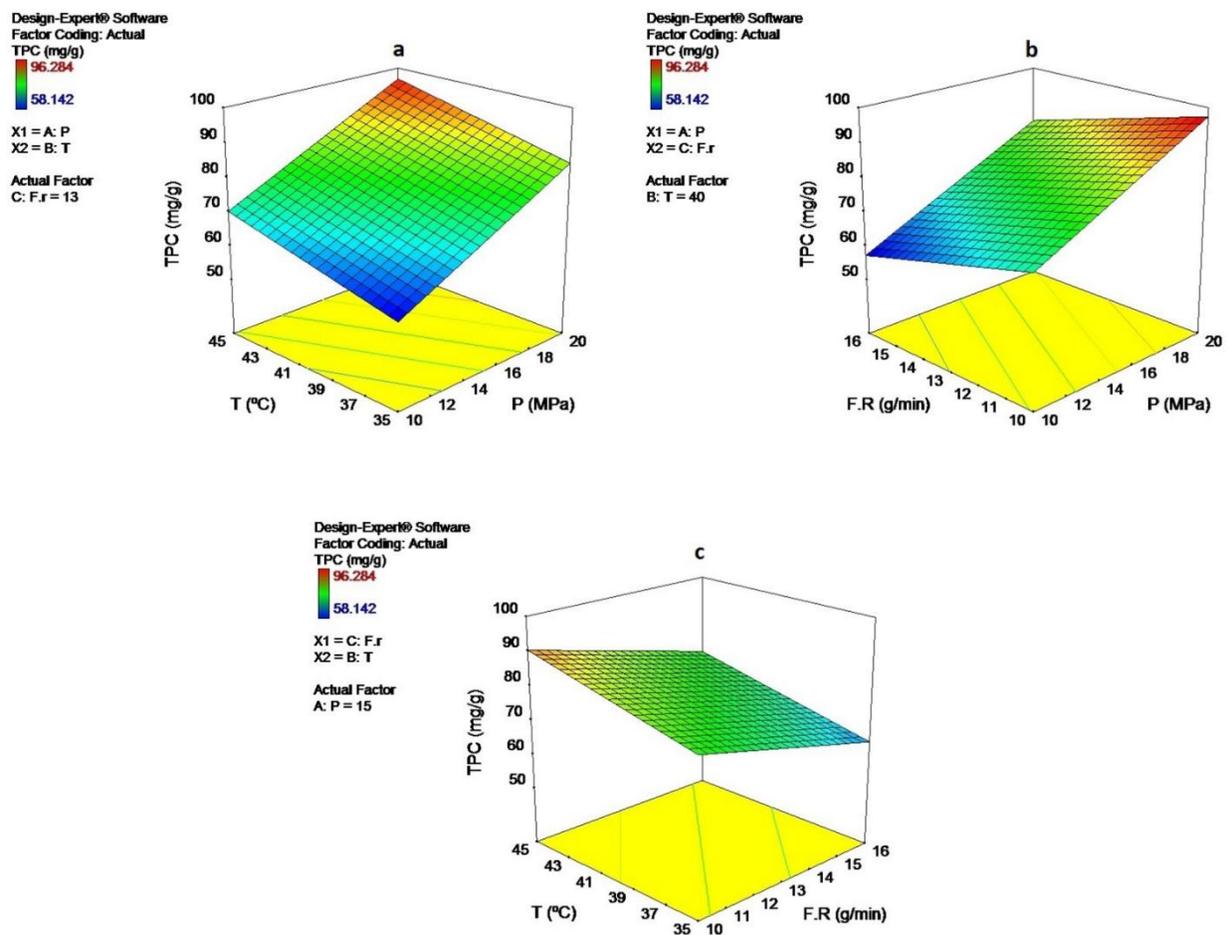


Figure 3. Response surface plots for total phenolic content (TPC) as a function of: (a) temperature and pressure; (b) CO₂ flow rate and pressure; (c) temperature and CO₂ flow rate.

Optimization of Experimental Conditions for Total Phenolic Content

The experimental conditions and Total Phenolic Content (TPC) values, determined using the Box-Behnken Design (BBD) approach, are presented in Table 1. The TPC values ranged from 58.14 to 96.28 mg/g. A regression analysis was conducted on the experimental data, and the relationship between TPC and the test variables was modeled by the following linear equation:

$$\text{TPC} = 77.42 + 13.11X_1 + 6.12X_2 - 0.700X_3 \quad (7)$$

The results from the Analysis of Variance (ANOVA) for the regression model revealed that the linear model was statistically significant, as evidenced by the high model F-value ($F = 274.22$) and the low p-value ($p < 0.0001$). These values were used to assess the significance of each coefficient. A higher F-value and a lower p-value indicate greater significance for the corresponding coefficient. Additionally, the high values of the coefficient of determination ($R^2 = 0.9844$), the adjusted R^2 (adj. $R^2 = 0.9809$), and the predicted R^2 (Pred. $R^2 = 0.9733$) demonstrated a strong correlation between the experimental and predicted values of TPC. The coefficient of variation (CV), which reflects the precision of the model, was found to be 2.05%, suggesting a high degree of reliability and precision in the experimental results. The lack of fit for the model was found to be statistically insignificant ($p = 19.98$), further confirming the robustness of the model.

The regression coefficients indicated that the significant parameters influencing the linear model were pressure (X_1), temperature (X_2), and CO_2 flow rate (X_3). The three-dimensional surface plots of the independent parameters are presented in Fig. 3, which illustrate the relationships between TPC and these parameters.

Fig. 3a shows the effect of the interaction between pressure and temperature at a constant CO_2 flow rate. Both Figs. 3a and 3b indicate that an increase in pressure had a positive effect on TPC.

Previous studies have suggested that higher pressure increases the density of supercritical CO₂, enhancing its solvating power and, in turn, improving phenolic compound extraction [23, 24]. Moreover, TPC increased with temperature, from 35°C to 45°C, as elevated temperatures enhance the diffusion coefficient, facilitating the extraction of phenolic compounds. Conversely, TPC decreased with an increase in CO₂ flow rate from 10 to 16 g/min. A lower CO₂ flow rate improves the solvent-to-feed ratio, thus enhancing extraction efficiency. As observed in Figs. 3b and 3c, CO₂ flow rate had a minor negative effect on TPC within the examined range, when pressure and temperature were held constant.

The optimized process conditions for maximum TPC were determined to be 20 MPa pressure, 45°C temperature, and 10 g/min CO₂ flow rate. Under these conditions, the predicted TPC was 103.24 ± 1.58 mg/g, based on the Response Surface Methodology (RSM) model. The accuracy and validity of the optimization model were confirmed through experimentation, which yielded an average TPC of 102.92 ± 1.32 mg/g. This value closely aligned with the predicted value, demonstrating the effectiveness of the developed model in optimizing antioxidant activity.

Comparative Study of SC-CO₂ and Soxhlet Extraction Methods

In this study, the yield extraction, radical scavenging activity (RSA), and total phenolic content (TPC) of *Zingiber officinale* oleoresin were compared between the Soxhlet extraction and supercritical CO₂ (SC-CO₂) methods. Under optimal conditions, the oleoresin yield (EY) was found to be $3.19 \pm 0.22\%$ for the Soxhlet method and $6.47 \pm 0.07\%$ for the SC-CO₂ method. The RSA (%) under the same optimal conditions were $50.70 \pm 0.52\%$ and $88.50 \pm 0.18\%$ for the Soxhlet and SC-CO₂ methods, respectively. The TPC values under optimal conditions were 31.10 ± 0.28 mg/g for the Soxhlet method and 103.24 ± 1.58 mg/g for the SC-CO₂ method.

The characteristics of the oleoresin can be influenced by the extraction method used. The SC–CO₂ extraction technique is known to minimize the degradation of the oleoresin components compared to conventional methods such as Soxhlet extraction. Significant differences were observed between the yield extraction, RSA, and TPC of oleoresin obtained from the two methods. The results indicate that SC–CO₂ extraction is more effective than Soxhlet extraction, offering superior extraction yields, antioxidant activity, and phenolic content. Therefore, SC–CO₂ extraction can be considered the optimal technique for maximizing the yield, antioxidant properties, and TPC of *Zingiber officinale* oleoresin.

Conclusion

The present study aimed to investigate the extraction of oleoresin from *Zingiber officinale* and evaluate its extraction efficiency (EY), radical scavenging activity (RSA), and total phenolic content (TPC) using both supercritical CO₂ (SC–CO₂) and Soxhlet extraction methods. The effects of pressure (10–20 MPa), temperature (35–45 °C), and CO₂ flow rate (10–16 g/min) were assessed to optimize the SC–CO₂ extraction parameters for efficient yield, RSA, and TPC. Response Surface Methodology (RSM), utilizing a 3×3 Box-Behnken Design (BBD), was applied for this optimization. The results indicated that pressure, temperature, and CO₂ flow rate significantly influenced the yield, RSA, and TPC of the oleoresin. The optimal conditions for maximum EY were identified as a pressure of 20 MPa, a temperature of 45 °C, and a CO₂ flow rate of 12 g/min. For optimal RSA, the ideal conditions were found to be a pressure of 14.5 MPa, a temperature of 45.0 °C, and a CO₂ flow rate of 11.0 g/min. The best conditions for achieving the highest TPC were a pressure of 20 MPa, a temperature of 45.0 °C, and a CO₂ flow rate of 10.0 g/min. When comparing the results obtained from both extraction methods, the SC–CO₂ extraction method proved to be more efficient than the Soxhlet method. The findings of this

study highlight the superiority of SC-CO₂ in terms of extraction yield and antioxidant activity. Therefore, SC-CO₂ extraction can be considered the optimal method for maximizing both EY and RSA from *Zingiber officinale* oleoresin.

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