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OPTIMIZATION OF *CURCUMA LONGA* L. RHIZOME SUPERCRITICAL CARBON DIOXIDE EXTRACTION ($SC\text{-}CO_2$) BY RESPONSE SURFACE METHODOLOGY (RSM)

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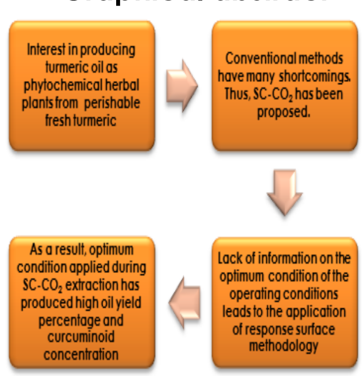
Graphical abstract



Abstract

Fast, selective and reliable extraction technique namely Supercritical Carbon Dioxide Extraction ($SC\text{-}CO_2$) has been proposed in this study. Experiments were carried out to determine the optimum conditions for pressure and temperature of $SC\text{-}CO_2$ extraction for *Curcuma longa* L. using response surface methodology (RSM) based on central composite design (CCD) with the aim to obtain the highest percentage of yield and curcuminoid concentration. At a pressure of 4807 psi and temperature of 90°C, extraction of turmeric rhizome using $SC\text{-}CO_2$ extraction resulted in oil yield of 7.54% (dry weight basis) and curcuminoid concentration of 0.023 mg 100^{-1}g of dry turmeric rhizome. From the study, it can be concluded that the percentage yield and curcuminoid concentration was significantly influenced by pressure and temperature.

Keywords: Supercritical carbon dioxide extraction ($SC\text{-}CO_2$), response surface methodology (rsm), oil yield, curcuminoid concentration

1.0 INTRODUCTION

Turmeric (Curcuma longa L.) is one of herbaceous plant in the plant kingdom. All parts of the plant from the leaves to the rhizomes are useful and have various applications. Some literatures reported that the rhizomes are widely used as a spice and a common food and beverage additive [1]. Interestingly, turmeric plant contains invaluable natural compounds which are currently drawing interest to be formulated into therapeutic product.

Turmeric consists of light fraction of volatile compounds which is known to be the character impact compounds of turmeric, contributing to the camphory note, and have been reported to be responsible for the top note of dry turmeric [2]. It also consists of heavy fraction of non-volatile compounds which acts as colouring agent and was found to be a rich source of phenolic compounds namely, curcumin, demethoxycurcumin and bisdemethoxycurcumin [3].

Invaluable extracts of turmeric usually being extracted using many extraction techniques from conventional such as hydrodistillation and steam distillation to modern techniques such as SC-CO2 extraction. Selection of proper extraction technique will result in extracting highest yield of extract and simultaneously, enable to extract targeted compounds in plants that eventually enhance the quality of the end product. Conventional extraction methods has many shortcomings such as longer extraction time, employs costly and toxic organic solvent, low extraction selectivity [4] and require a post-extraction process [5] to remove solvent from the extract. Therefore, in this study, SC-CO2 extraction has been proposed.

SC-CO2 extraction has emerged as a superior alternative technique for the extraction of bioactive species from natural products due to shorter extraction time, production of clean extract, environmental benignity, higher selectivity and does not utilise toxic organic solvents [6][7][8]. Different operating parameter of SC-CO2 will give different effect to the extraction product. Hence, the choosing of suitable operating parameter is crucial in order to obtain desirable extract. In this study, the relationship between pressure and temperature of SC-CO2 was optimised using RSM. RSM is a reliable statistical methods used for designing experiments and finding the optimum conditions of variables to achieve desirable targeted responses [9]. Besides, it is also helpful in reducing the number of experimental trials required by optimizing the extraction process [10] and simultaneously evaluate the variations of all the factors and responses and also distinguishing the relationship among them [11].

Literature pertaining optimisation of SC-CO2 extraction particularly for oil yield and curcuminoids of Curcuma longa L. is still scarce especially in Malaysia. Hence, the aim of the present study was to elucidate the optimum conditions of pressure and temperature for the SC-CO2 extraction of Curcuma longa L. rhizome

2.0 EXPERIMENTAL

2.1 Materials

All the chemicals used were of analytical grade and purchased from Merck Sdn. Bhd. (Selangor, Malaysia).

2.2 Method

2.2.1 Preparation of Dried Turmeric Powder

Fresh turmeric rhizomes (*Curcuma longa* L.) were bought from local market in Shah Alam, Selangor. The rhizomes were cleaned with water and sliced about 3 mm thick and air dried at room temperature until the moisture content reached 10 to 15% as reported by Zaibunnisa *et al*. [2]. Samples were then ground and sieved to 2 mm particle size and were put into vacuum packed and stored at -20°C until needed for further analysis.

2.2.2 Optimisation of Turmeric Oil Using Central Composite Design (CCD)

Extractions were carried out using supercritical carbon dioxide (SC-CO_2) laboratory scale extraction system (Taiwan Supercritical Technology, Taiwan). Samples (20 g) of the turmeric rhizomes were accurately weighed and placed into 60 ml vessel and fixed into extraction oven. Incoming and outgoing tubes were attached at the bottom and upper parts of the vessel, respectively. Turmeric oil was extracted with supercritical carbon dioxide in a continuous-flow extractor. Carbon dioxide gas was passed through a cooling bath and then compressed and heated up to the selected supercritical condition. The temperature was measured using a thermocouple device. The operating conditions for pressure and temperature were as suggested by the central composite design. The extract was collected in a glass tube for further analysis.

2.2.3 Experimental Design Approach

To provide sufficient information regarding the interior of the experiment region and also allowing for the evaluation of the curvature, 14 experiments were assigned which included 4 axial points, 4 factorial points and 6 central points based on central composite design (CCD). The experiments were conducted within two blocks as the extraction process could not be able to be conducted in a day. In this study, CCD was used to study the main and combined effects of SFE extraction conditions on the quality and quantity of turmeric rhizome oil. The experimental design was generated using Design-Expert version 6.0.4 (Stat Ease Software). Each of the variables had levels set at five coded levels: -α, -1, 0, +1 and +α. For SC-CO_2 extraction of *Curcuma longa* L., pressure (A) and temperature (B) were the independent variables selected for optimizingthe oil yield and curcuminoid concentration. The levels of variables chosen for CCD are shown in Table 1. In order to correlate the response variable to the independent variable, the response variables (oil yield and curcuminoid concentration) were fitted into a second-order model and the general equation of the second-order polynomial is given in Equation (1).

$$Y = \beta_0 + \beta_1 A + \beta_2 B + \beta_{12} AB + \beta_{11} A^2 + \beta_{22} B^2 + \varepsilon \quad (1)$$

In this equation, Y is the response (dependent variables), β_0 isthe constant coefficient, $\beta_1 A$ and $\beta_2 B$ are the linear coefficients, $\beta_{11} A^2$ and $\beta_{22} B^2$ are the quadratic coefficients, $\beta_{12} AB$ is the interaction effect, A and B are the factors and ε is the error.

Table 1 CCD used for *Curcuma longa* L. oil extraction

Level	-a	-1	0	+1	+a
Pressure (psi)	1000	1586	3000	4414	5000
Temperature (°C)	30	40	65	90	100

Percentage yield (%)

All extracts obtained from extraction were weighed and calculated using the formula used by Zaibunnisa *et al.* [2], as shown below:

$$\text{Oil yield (dry weight basis, \%)} = \frac{\text{Weight of extract}}{\text{Weight of dried sample (g)}} \times 100 \text{ (g)}$$

Curcuminoid content

The curcuminoid content in the turmeric oil was determined by spectrophotometric method according to Haiyee *et al*. [12]. Turmeric oil (0.002 g) was dissolved with 10 ml of 95% ethanol. The absorbance of the extract was measured at 425 nm using 95% ethanol as blank. Curcuminoid concentration in the sample was quantified using standard calibration curve of pure curcumin

2.2.4 Data Analysis

The experimental design and statistical analysis were performed using response surface methodology (RSM) with Design Expert Version 6.0.4, (StatEase, 2001) software.

3.0 RESULTS AND DISCUSSION

From the study, the relationship between two independent factors (pressure and temperature) and the responses (oil yield and curcuminoid concentration) was observed. Experiments were run in random order to minimize the effects of unexplained variability in the actual responses due to extraneous factors while the center point was repeated six times to calculate the repeatability of the method [9].

The final equation in terms of actual value is given in equations (2) and (3). In the equations, oil yield and curcuminoid concentration are the responses while A and B represents pressure and temperature, respectively. Positive sign at the front of the terms indicates synergistic effect, while negative signs indicates antagonistic effect. Analysis ofvariance (ANOVA) was performed in order to evaluate the goodness of fit of the model.

$$\text{Yield (\%)} = 4.83 + 3.06\times10^{-3}A - 0.16B - 9.81\times10^{-7}A^2 + 1.31\times10^{-3}B^2 + 4.11\times10^{-5}AB + 7.60\times10^{-9}A^2B - 4.78\times10^{-7}AB^2 \quad (2)$$

$$[\text{Curcuminoid}] = -1.82\times10^{-3} + 6.19\times10^{-6}A - 7.09\times10^{-5}B - 1.55\times10^{-9}A^2 + 2.46\times10^{-6}B^2 - 1.04\times10^{-7}AB + 4.94\times10^{-11}A^2B - 1.06\times10^{-9}AB^2 \quad (3$$

According to Zaidi *et al*. [9], regression analysis is the common approach used to fit the empirical model with the collected response variable data. Table 2 represents the CCD matrix experimental and predicted response of oil yield and curcuminoid concentration. Statistical results suggest that the quadratic model is the most suitable model for both oil yield and curcuminoid concentration.

From Table 3, statistical results suggest that the coefficient of determination (R^2) value for all response variables were higher than 0.75, which indicates that the regression model explained the response well. The R^2 values for oil yield and curcuminoid concentration were 0.9948 and 0.9983, respectively, indicating a good fit. For the oil yield, the R^2 indicates that the sample variation of 99.48% for oil yield is attributed to the independent variables and only 0.52% of the total variations could not be explained by the model. For curcuminoid concentration, the R^2 indicates that only 0.17% of the total variations could not be explained by the model.

The lack-of-fit tests were insignificant for both oil yield and curcuminoid concentration which also showed a good fit between the experimental data and the mode

Table 2 Experimental determined and predicted values of total oil yield and curcuminoids concentration of *Curcuma longa* L

Run	Factors		Oil yield (%)		Curcuminoid concentration (mg 100g $^{-1}$) x 10^{-3}	
	A (psi)	B (°C)	Experimental	Predicted	Experimental	Predicted
1*	3000	65	6.81	6.72	3.613	3.934
2*	3000	65	6.80	6.72	3.583	3.934
3	1586	90	4.81	4.79	0.564	0.522
4	1586	40	5.15	5.14	1.022	0.980
5*	3000	65	6.50	6.72	4.436	3.934
6	4414	40	4.85	4.83	9.285	9.243
7	4414	90	7.94	7.93	17.00	16.00
8	1000	65	2.79	2.81	0.500	0.542
9	3000	100	6.61	6.63	1.814	1.856
10	5000	65	5.96	5.97	20.00	20.00
11*	3000	65	6.49	6.34	4.223	3.781
12*	3000	65	6.22	6.34	3.491	3.781
13	3000	30	5.73	5.75	3.918	3.960
14*	3000	65	6.37	6.34	3.796	3.781

*replication of the center point, A: Pressure (psi); B: Temperature (°C)

Table 3 ANOVA for response surface for curcuminoid concentration and oil yield employing CCD

Response	Transform	Lack of fit	R^2
Oil yield (%)	None	Not significant	0.9948
Curcuminoid concentration (mg 100^{-1}g)	None	Not significant	0.9983

Table 4 shows the predicted values for oil yield calculated using the regression model and compared with experimental values obtained from the experiment. The quadratic term of pressure (A^2) with an *F*-value of 349.233 is more significant than the temperature (B^2) with an *F*-value of 2.13. It was also observed that pressure (A) has a significantly large effect on the oil yield due to high *F*-value of 249.98. The coefficients for all independent variables (pressure and temperature) were in positive values which showed that as the variables increased, the percentage of oil yield also increased. However, the quadratic term of temperature (B^2) has no significant effect on the oil yield due to the prob>*F* value of 0.2040 is greater than 0.1000.

Table 4 ANOVA for the regression model and respective model term for oil yield

Source	Sum of squares	Degree of Freedom	Mean square	*F*-Value	Prob>*F*	Remarks
Model	19.27	7	2.75	137.15	<0.0001	Significant
A	5.02	1	5.02	249.98	<0.0001	Significant
B	0.39	1	0.39	19.41	0.0070	Significant
A^2	7.01	1	7.01	349.23	<0.0001	Significant
B^2	0.043	1	0.043	2.13	0.2040	Not significant
AB	2.96	1	2.96	147.60	<0.0001	Significant
A^2B	0.28	1	0.28	14.11	0.0132	Significant
AB^2	0.34	1	0.34	17.07	0.0091	Significant
Residual	0.10	5	0.020			
Lack of fit	0.0044	1	0.0044	0.18	0.6909	Not significant
Pure error	0.096	4	0.024			

The ANOVA for the regression model and respective model term for curcuminoid concentration is shown in Table 5.The quadratic term of pressure (A^2) with an *F*-value of 524.97 has a large effect on the curcuminoid concentration due to the high *F*-value. Moreover, the coefficients for all independent variables (pressure and temperature) were in positive values which showed that as the variables increased, the curcuminoid concentration also increased. Nevertheless, temperature only shows slight significant effect due to low *F*-value which is 14.33.

Table 5 ANOVA for the regression model and respective model term for curcuminoid concentration

Source	Sum of squares (x10^{-4})	Degree of Freedom	Mean square (x10^{-4})	*F*-Value	Prob>*F*	Remarks
Model	4.584	7	0.650	420.70	<0.0001	Significant
A	1.946	1	1.946	1260.4	<0.0001	Significant
B	0.022	1	0.022	14.33	0.0128	Significant
A^2	0.811	1	0.811	524.97	<0.0001	Significant
B^2	0.014	1	0.014	9.10	0.0295	Significant
AB	0.149	1	0.149	96.31	0.0002	Significant
A^2B	0.119	1	0.119	77.30	0.0003	Significant
AB^2	0.017	1	0.017	10.86	0.0216	Significant
Residual	0.0077	5	0.0015			
Lack of fit	0.0003	1	0.0003	0.18	0.6938	Not significant
Pure error	0.0074	4	0.0018			

Figures 1 and 2 showed the correlation between experimental values and predicted values of oil yield and curcuminoid concentration, respectively. The predicted versus actual response for oil yield and curcuminoid concentration revealed that most of the points generally fall along the straight line implying that the actual response approximately fulfill the prediction.

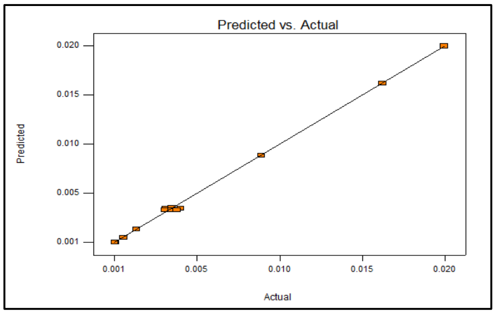


Figure 1 Relationship between predicted and experimental values of oil yield using equation

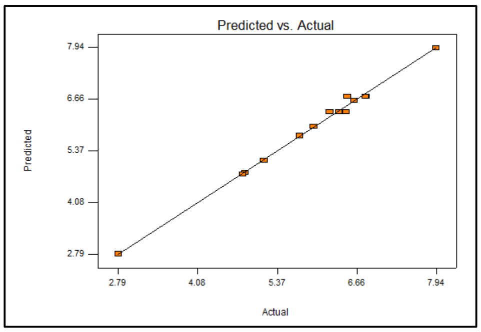


Figure 2 Relationship between predicted and experimental values of curcuminoid concentration using equation (3)

Figure 3 shows the response surface for the interaction between temperature and pressure of SC-CO_2 during extraction of turmeric oil. From the 3D plot, oil yield increased when temperature was increased up to the optimum point at each pressure. It is clearly shows that the interaction between low temperatures (30°C) with high pressure (5000 psi) will produce low oil yield. Increasing the pressure at each temperature showed an increase in oil yield. Similarly, the combination of low pressure with high temperature will produce low oil yield.

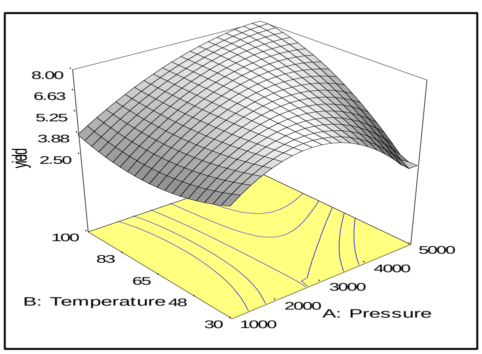


Figure 3 3D-Surface plot of percentage yield of turmeric oil as a function of pressure and temperature

Highpercentage of oil yield is desirable in any extraction processes. SC-CO_2 with optimised conditions is capable to produce high percentage of oil yield, save time and cost of extraction. Traditional methods such as hydrodistillation only produce small percentage of oil yield which is 2.1% [3]. While Soxhlet extraction with co-solvent produce high percentage of oil yield. However, in this study, the usage of organic or inorganic solvent was avoided.

From the 3D plot of Figure 4, it is clearly shown that the curcuminoid concentration was the lowest at low temperature and increased when temperature was increased at each point of pressure.

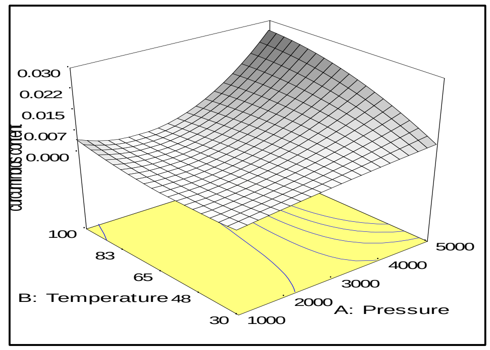


Figure 4 3D-Surface plot of curcuminoid concentration of turmeric oil as a function of pressure and temperature

Different extraction method requires different extraction conditions. This was also highlighted by Paulucci *et al*. [12], which reported that the quality, cost and the efficacy of the standardized phytopharmaceutical intermediate product were significantly determined by the method and processing conditions applied for the extraction of chemical markers from herbal raw materials. For instance, Paulucci *et al*. [13] found out that the optimum temperature for curcumin concentration was 80°C with 12 hours of extraction and 30 rpm agitation speed using maceration method and the curcumin yields ranging from 2.1 to 62.6%. Sogi *et al*. [14] reported that solvent extraction method at the temperature of 60°C and co-solvent assist their extraction and yields the curcumin ranging from 4.5 to 12.9%. Chen *et al*. [15] use the temperature of 60°C, 350 bar and 5 hours extraction time to extract *Jatropha* triglycerides using SC-CO_2 method andthey claimed that the extraction efficiency of SC-CO_2 was declined as temperature decreased. While Nur Ain *et al*. [16] use high temperature of 167°C, a pressure of 1203 psi and a static time of 20.43 minutes for the extraction of lemongrass oleoresin using pressurised liquid extraction (PLE) method.

From these previous reports, the use of high temperature plays a role in increasing the extraction efficiency. Ching *et al*. [17] added that the lowest the temperature used for curcumin extraction, the longest time needed. Thus, it is understandable that, in order to increase the efficiency of SC-CO_2 extraction and shorten the extraction time, high temperature is required. Nevertheless, it should be noted that the use of more than 100°C of extraction temperature might cause thermal degradation to the compounds in the extract and also inappropriate for long term usage of SC-CO_2, technically and economically.

Pressure and temperature were the main factors that significantly determine the successful of the extraction of valuable compounds from plant material. This is in accordance with the report by Azmir *et al*. [4] which stated that changing the temperature and pressure of SC-CO_2 can tune the solvation power of supercritical fluid and thus increase the selectivity of targeted compounds to be extracted out. According to Braga *et al*. [3], the solubility of carbon dioxide as extracting solvent was found to be dependent on pressure and temperature used, and some other factor. Application of higher extraction pressure has increased the solvent power and the solubility of turmeric oil to the supercritical fluid and thus increased the percentage of oil yield during extraction. Gopalan *et al*. [1] reported that the increasing of pressure during extraction can result in changing of the solubility of the oil.

For SC-CO_2, pressure becomes the most crucial factor in order to maintain the supercritical carbon dioxide condition in its supercritical form. Although increased in pressure and temperature will produce more yield and curcuminoid concentration, it is not advisable to increase the pressure and temperature for more than 5000 psi and temperature of more than 100°C as it is not economical in term of the usage of more liquid carbon dioxide and also not so compatible with SC-CO_2 instrument.

4.0 CONCLUSION

The optimum point was determined based on the highest desirability to the responses. Based on the result, optimum conditions for the extraction of turmeric rhizome (*Curcuma longa* L.) using SC-CO_2 were 4807 psi and 90°C for 90 minutes. At the optimum condition, extraction of turmeric resulted in oil yield of 7.54% and curcuminoid concentration of 0.023 mg 100^{-1} g of dry rhizome. The percentage of oil yield and curcuminoid concentration was significantly influenced by pressure and temperature. Increased the pressure and temperature resulted in increased in oil yield and curcuminoid concentration.

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