Response Surface Optimization of *Curcuma zedoaria* Oil yield by SC-CO_2 Extraction

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***Abstract*- With the increasing of public interest in natural or herbal products, the Supercritical Fluid Extraction (SFE) of *Curcuma zedoaria* (Berg.) Rosc. (Zingiberaceae) was study. *Curcuma zedoaria* was selected to be extracted due to its great potential in pharmaceutical. SFE technique was safe and time effective in extraction process. Although SFE was viable alternative, there was major drawback on this application which was the high capital and operating cost due to requirement of extreme pressure on the process. Besides, the operating parameter in SFE also will be give different effect on the extraction yields as well as oil component properties. Therefore, it was crucial to determine the optimum parameter used in SFE. The parameters used in SFE were pressure and temperature. The optimum parameters were determined by employing the Response Surface Methodology (RSM) based on the Central Composite Design (CCD). The *Curcuma zedoaria* was extracted by SFE with the pressure and temperature range between 100 to 300 bar and 40°C to 60°C respectively. The extracts or oil yield was collected, weighed and analyzed by using gas chromatography mass spectrometer (GCMS) and gas chromatography flame ionization detector (GCFID). Based on the results, most of the extraction of SFE gave oil yield ranged from 0.6% to 1.8%. For the analysis, curzerene concentration was found high at SFE 11 and low at SFE 8. Hence, the optimum parameter of Supercritical Carbon Dioxide (SC-CO_2) of *Curcuma zedoaria* at best oil yield and curzerene concentration obtained was 138.65 bar and 40°C. This optimum parameter gives the best predicted oil yield and curzerene concentration at 1.664 % and 3.696% respectively. In conclusion, the pressure and temperature significantly influence the oil yield as well as concentration of oil compounds.**

***Keywords* : SC-CO_2, SFE, Curcuma zedoaria, RSM, Oil yield, Curzerene**

I. INTRODUCTION

Curcuma zedoaria (Berg.) Rosc. (Zingiberaceae) oil can be obtained by using extraction method either conventional or novel method. For example, hydrodistillation is one of the conventional methods while Supercritical Fluid Extraction (SFE) is a novel extraction method. The oil obtained by using conventional method has been recognized as the most favorable for many decades among researchers. Conventional methods of extraction require much time for the process and also require relatively large amounts of solvent. Ouzzar et.al (1995) did an extraction of natural plant by using conventional method. He consumed almost 5 hour for the extraction and concluded that there is a solvent residue left behind in the products. In fact, the elevated temperature used in hydrodistillation can degrade the quality of extracts as the temperature can cause chemical modifications to the oil component and loss of valuable volatile components.

In contrast, SFE is a safe and fast technique that has potential to give high quality oil with solvent free products. SFE is an efficient extraction process compared to conventional extraction method because of its high rate of extraction, environmental friendly and the solvating power. Shih et.al (2015) did SFE of *Curcuma zedoaria* rhizome and consumed 1 and 2 hours for extraction time. He found that the extract oil is slightly higher compared to the hydrodistillation. The extract oil acquired by SFE were 0.82% and 0.99% while for hydrodistillation is 0.63%. This is because the solvent used in SFE is a carbon dioxide (CO_2). Carbon dioxide (CO_2) is a fluid that has good solvent properties such as high solvent power which in turn gives high diffusivity just like the gas. In addition, CO_2 is non-toxic, non-flammable, odorless, tasteless and low cost.

For this case, Supercritical Fluid Extraction is used as a technique for *Curcuma zedoaria* extraction. In SFE, the operating parameters were expected to give different effect on the extraction yield as well as oil component properties. Hence, it is essential to choose the suitable optimum parameter used in SFE application. The optimum parameter has been determined by using Response Surface Methodology (RSM) method. RSM is an empirical statistical method that aims to design the experiment and find the optimum parameters at best response. RSM also help to reduce the number of experimental trials required and reveal the relationship between independent variables towards on the responses using multiple regression. The parameters used are pressure and temperature while the responses are extracts oil (oil yield) and curzerene concentration.

Therefore, the objectives in this study is to determine the optimum parameter at best oil yield and curzerene concentration by using RSM. Besides, the major chemical compound in SC-CO_2 *Curcuma zedoaria* extracts is identified by gas chromatography mass spectrometer (GCMS) and gas chromatography flame ionization detector (GCFID).

II. METHODOLOGY

A. Materials

Dried *Curcuma zedoaria* was purchased from Kuala Krau, Kedah. The rhizomes were washed, oven dried and pulverized until its diameter size is range within 1 to 3mm. The moisture content of dried rhizome was 6.28%. All chemicals and solvents used were of analytical grade. Carbon dioxide, CO_2 with 99.9 % purity, dicholoromethane, DCM with 99.9% purity and helium with 99% purity were used in this experiment.

B. Experimental apparatus and procedure

Supercritical CO_2 (SC-CO_2) extraction of Curcuma zedoaria was performed by using a laboratory scale supercritical-fluid extraction system (SFE 500MR, Thar Technology). The range of pressure and temperature used were 100 – 300 bar and 40°C – 60°C respectively. The experimental procedure was begun by placing a 200g of grinded dried *Curcuma zedoaria* in the extraction vessel as shown in Figure 1.1. After the recirculating chiller reached to 3°C, the CO_2 that was supplied from the gas cylinder was continuously fed into the extractor with fixed flow rate of 35g/min. The gas CO_2 was cooled by cooling heat exchanger and make it liquefied and denser CO_2. The liquefied CO_2 then, was pressurized by CO_2 pumping to the desired pressure and heated to the specific temperature with the purpose to reach the supercritical state. The extraction process was carried out 30 minutes for static and 1 hour dynamic extraction time. The pressure within the extraction vessel was regulated by automated back pressure regulator (ABPR) while manual back pressure regulator (MBPR) maintained the pressure in the collector constant. After the extraction process, the pressure in extraction vessel was depressurized by ABPR and extracted oil yield was collected through drain valve. The *Curcuma zedoaria* extract oil yield were calculated using equation 1.

$$\text{Yield } (\%) = \frac{M_{extract}(g)}{M_{feed}(g)} \times 100 \quad (1)$$

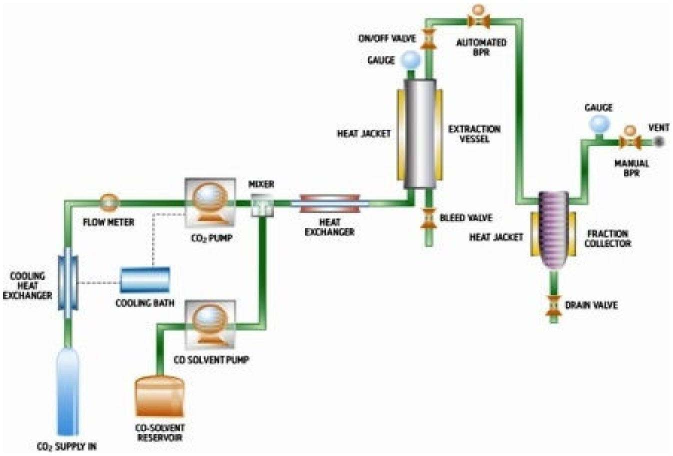

Figure 1.1: The process flow diagram of supercritical fluid extraction.

C. *Experimental design*

The SC-CO_2 extraction parameters were optimized by employing the Response Surface Response (RSM). In RSM, the relationship between independent variables (pressure and temperature) towards on the responses oil yield was examined. 13 experiments included 4 factorial points, 4 axial points and 5 central points were assigned based on the Central Composite Design (CCD). By using Expert Design version 7.0 sofware, the contrast coefficients among the obtained experimental data were well tuned and statistically analyzed by Analysis of Variance (Manickam et.al, 2012). The mathematical models for each responses were predicted by using multiple regression model and were fitted into second order polynomial equation 2.

$$Y = \beta_o + \sum \beta_i X_i + \sum \beta_{ii} X_i^{\,2} + \sum\sum \beta_{ij} X_i X_j \quad (2)$$

Where Y is the response variable, β_o is a constant, and β_i, β_{ii}, and β_{ij} represents the linear, quadratic and interactive coefficients respectively. X_i and X_j are the independent variables. The coefficient of determination, R^2 were determined and the F-test of significance of the equation parameters for each response variable was analyzed. According to Paulucci et.al, only the factors with significance higher than or equal to 5% ($p \leq 0.05$) were considered.

D. *GCMS and GCFID analysis*

The chemical analysis composition of extracts was analyzed by gas chromatography (GC) equipped with a DB Wax column (30 m x 0.25 mm i.d, film thickness 0.25 µm) .The detectors used was Mass Spectrometer Detector (MSD) and Flame Ionization Detector (FID). The carrier gas was used helium at pressure 210kPa and the temperature of injector as well as detector were set to 240°C. The oven's temperature was set at 60 °C for 1 minute, then programmed for heating from 60 to 240 °C at rate of 5 °C/min and 240 °C for 1 minute. 1 µl of sample was injected and the split ratio was 1:20.

III. RESULTS AND DISCUSSION

A. *SFE of Curcuma zedoaria rhizome*

The result of oil yields of SC-CO_2 of *Curcuma zedoaria* were tabulated in Table 4.1. Most of the extraction of SFE gives oil yield ranged from 0.6% to 1.8% according to its parameter. This obtained yield range of oil yield is within the expected range pf less than 5%. A previous study on Curcuma showed that yield of essential oil obtained from both fresh and dried rhizomes of turmeric ranged from 0.7% to 1.1% (Hong et.al, 2014). Awasthi et.al claimed that the oils obtained by conventional hydro distillation of the rhizomes and leaves of *C. longa* were 0.36% and 0.53% respectively. Thus, it can be concluded that the oil yield percentage obtained was some-what similar in trend of related studies even though different parameter and extraction method used. In addition, a recent Chinese report showed that the zedoary oil can be formulated as submicron emulsion. Based on the report, 2.0g of zedoary oil was required in the formula and this value proved that the oil yield obtained from the experiment able to meet the pharmaceutical requirement (Zhihui et.al, 2014).

Table 1.1: The weight of oil from SFE of *Curcuma zedoaria* dried rhizome according to the following parameter.

Run	Factors		Wt of oil yield (g)	Oil yield (%)
	A= Pressure (bar)	B= Temperature (°C)		
SFE 1	100	50	1.54	0.769
SFE 2	200	50	3.22	1.612
SFE 3	100	60	1.22	0.609
SFE 4	200	50	3.14	1.57
SFE 5	300	50	3.18	1.589
SFE 6	300	40	2.83	1.414
SFE 7	300	60	3.21	1.604
SFE 8	100	40	3.31	1.654
SFE 9	200	50	3.18	1.593
SFE 10	200	50	3.18	1.592
SFE 11	200	40	3	1.499
SFE 12	200	50	3.24	1.62
SFE 13	200	60	3.74	1.869

B. *Chemical investigation*

Based on the GCMS and GCFID analysis, if found that more than 30 compounds were identified in extracted oil. The oil mainly contains of sequiterpene and mono sesquiterpene hydrocarbons (terpenes). This behavior is similar with the finding of Shih et.al (2015) that the essential oil of *Curcuma zedoaria* contained high ratio of terpenes compounds. Among 30 compounds, there were two interesting major compound identified which are curzerene and curzerenone. Each compound gives a different concentration percentage according to the parameter and was summarized in Table 1.2. SFE 11 (temperature 40°C, pressure 200 bar) had the highest percentage concentration of curzerenone (6.87%) while the lowest percentage concentration was SFE 8 (temperature 40°C, pressure 100 bar) with 5.05%. Even though SFE 8 had the lowest percentage of curzerenone, it stills was the highest percentage of curzerene which is 4.07%. SFE 3(temperature 60°C, pressure 100 bar) with 1.57% of curzerene was the lowest percentage obtained compare to the other SFE parameters.

From the results, it can be concluded that the major compound found in oil *Curcuma zedoaria* were curzerene and curzerenone. Both compounds have different concentration as the pressure and temperature influence the concentration of compounds. The results of major compound in oil *Curcuma zedoaria* was in good agreement with those reported by Angel et.al (2014) in which cuzerene (8%) and epicurzerenone(19%) were the major compounds in essential oil of *Curcuma zedoaria* from Kerala, India. In addition, Richard, Kirti, and Annie (2009) conveyed that curzerenone (22.3%), 1,8cineole (15.9%) and germacrone (9%) as the major compound of the essential oil whereas Mau et al (2003) had identified curzerene (10.36%), epicurzerenone (24.08%), isocurcumenol (2.98%), 5-isopropylidene-3,7,-dimethyl-1 (5H)-azulnone (4.3%) and curdione(7%).

Table 1.2: Major compound of oil extracted of *Curcuma zedoaria*

Run	Total identified compounds	Major Compounds				
		Curzerenone		Curzerene		Identification
		Concentration (%)	KI	Concentration (%)	KI	
SFE 1	33	5.356	2100	3.158	1851	MS, RI
SFE 2	34	5.0865	2100	2.75	1852	MS, RI
SFE 3	33	6.450	2100	1.579	1851	MS, RI
SFE 4	34	5.134	2100	2.89	1852	MS, RI
SFE 5	34	6.361	2100	2.06	1852	MS, RI
SFE 6	34	5.516	2100	2.199	1852	MS, RI
SFE 7	34	6.257	2100	2.326	1851	MS, RI
SFE 8	34	5.053	2100	4.073	1854	MS, RI
SFE 9	34	5.097	2100	2.92	1852	MS, RI
SFE 10	34	5.098	2100	2.913	1852	MS, RI
SFE 11	39	6.877	2100	2.818	1852	MS, RI
SFE 12	34	5.147	2100	2.97	1852	MS, RI
SFE 13	35	5.356	2100	2.196	1852	MS, RI

C. *Response Surface Methodology (RSM)*

The Response Surface Methodology (RSM) was used to determine the optimum variables at best responses. In this case, oil yield and curzerene concentration are the responses while pressure and temperature are two independent variables. The relationship between two independent variables and response have been analyzed by RSM so that the predicted of both response can be obtained. Table 1.3 showed the Central Composite Design (CCD) matrix of experimental and predicted of oil yield and curzerene concentration based on the independent variables (pressure and temperature). A quadratic model is suggested since CCD can fit a full quadratic model.

Table 1.3: The independent variables for extraction of *Curcuma zedoaria* along with experimental and predicted values of oil yield and curzerene concentration

Run	Oil yield %		Curzerene concentration	
	Experimental	Predicted	Experimental	Predicted
SFE 1	0.769	0.935	3.158	3.089
SFE 2	1.612	1.589	2.75	2.845
SFE 3	0.609	0.658	1.579	1.707
SFE 4	1.57	1.589	2.89	2.845
SFE 5	1.589	1.460	2.06	2.347
SFE 6	1.414	1.345	2.199	1.962
SFE 7	1.604	1.801	2.326	2.276
SFE 8	1.654	1.437	4.073	4.014
SFE 9	1.593	1.589	2.92	2.845
SFE 10	1.592	1.589	2.913	2.845
SFE 11	1.499	1.783	2.818	3.114
SFE 12	1.62	1.589	2.97	2.845
SFE 13	1.869	1.62211	2.196	2.118

The Analysis of Variance (ANOVA) was performed so that the goodness of the fit can be evaluate. The ANOVA of the model predict the values for oil yield and curzerene concentration calculated using regression model and compared with experimental values obtained from the experiment. Figure 1.2 and 1.3 showed the correlation between experimental and predicted values of oil yield and curzerene concentration. Both response variables were fitted into a second order polynomial equation presented in Eq (3) and (4) respectively. Based on the figure 1.2 and 1.3, the experimental responses are closer to the predicted responses as most of the point scatter around the 45 degree line.

Oil yield % =

$$0.91-0.07A+0.069B+0.11AB-0.27A^2-0.19B^2 \qquad (3)$$

Curzerene concentration% =

$$2.84-0.37A-0.5B+0.66AB-0.13A^2-0.23B^2 \qquad (4)$$

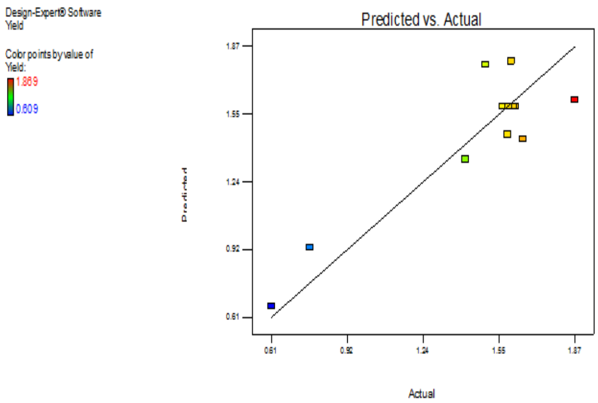

Figure 1.2: Relationship between experimental and predicted values of oil yield

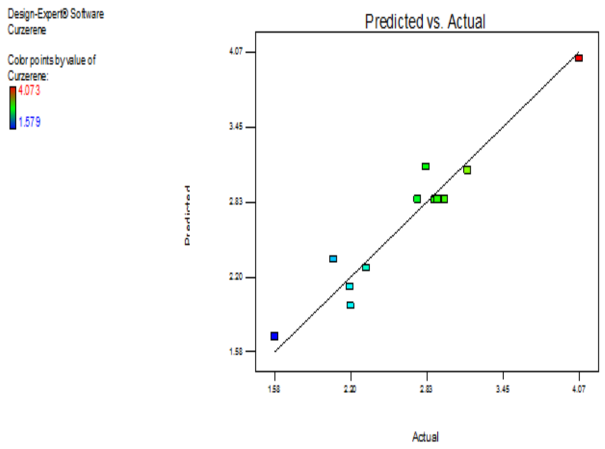

Figure1.3: Relationship between experimental and predicted value of curzerene concentration

According to Azmir et.al, the coefficient of determination (R^2) of the model of 0.99 indicates a similarity between the experimental and predicted of both response variables. Meanwhile, Siti Hafsah et.al stated that the R^2 value for all response variables that were higher than 0.75 shows a regression model explained the response well. The R^2 value of oil yield and curzerene concentration were 0.8180 and 0.9357 respectively. Thus, it can be concluded that the regression model can explained the response well and a good fit.

Based on Table 1.4, it showed that the quadratic term of pressure (A^2) and linear pressure (A) effects were highly significant ($p \leq 0.05$). However, linear temperature (B) and quadratic term of temperature (B^2) effects give ($p \geq 0.05$) which is non-significant. The interaction between temperature (B) and pressure (A) was significant ($p \leq 0.05$) within the experimental ranges. The ANOVA regression model for curzerene concentration was shown in table 1.5. The linear and square effects of pressure gives both significant and non significant respectively. For the temperature effects, both linear and quadratic term expressed a significant and non-significant too. The interaction between pressure and temperature displayed a significant effects within the experimental ranges.

Table 1.4: The ANOVA for regression model and respective model term for oil yield

Source	P < F	Remarks
Model	0.0159	Significant
A-Pressure	0.0149	Significant
B-Temperature	0.3558	Not significant
AB	0.0177	Significant
A^2	0.0140	Significant
B^2	0.3798	Not significant

Table 1.5: The ANOVA for regression model and respective model term for Curzerene concentration

Source	P < F	Remarks
Model	0.0005	Significant
A-Pressure	0.0031	Significant
B-Temperature	0.0006	Significant
AB	0.0004	Significant
A^2	0.3409	Not significant
B^2	0.1076	Not significant

Figure 1.4 and 1.5 illustrates the interaction between independent variables and response represented in three dimensional response surface. Based on the figure 1.4, the oil yield is increases as the pressure increase at constant temperature. However, it only increasing up to the 200 bar for all range temperature. Above 200 bar, there is fluctuation in oil yield percentage even though the pressure is high. Meanwhile at constant pressure, the oil yield is increasing as the temperature increased. It is obviously shown that high temperature (60°C) with low pressure (100 b) did not exhibit a good oil yield and is on contrast with the combination of low temperature (40°C) and high pressure (300 b). Based on figure 1.5, temperature had the important influence on the curzerene concentration. It shown that curzerene concentration decreased with an increase in temperature at each pressure. Use of high temperature may lead to the degradation of extracts quality especially heat sensitive materials.

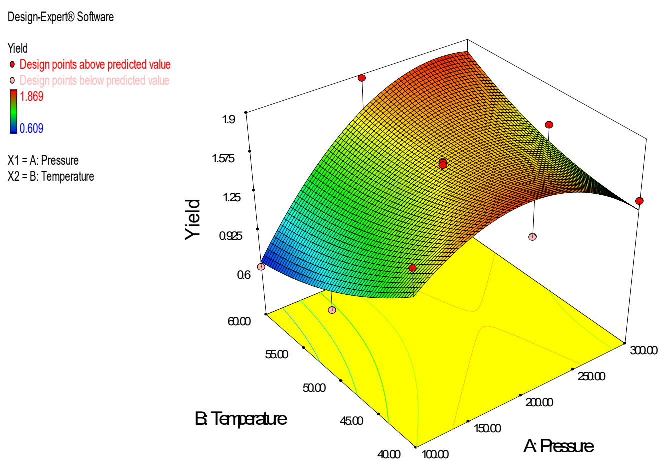

Figure 1.4: Response surface for oil yield percentage as a function of temperature and pressure

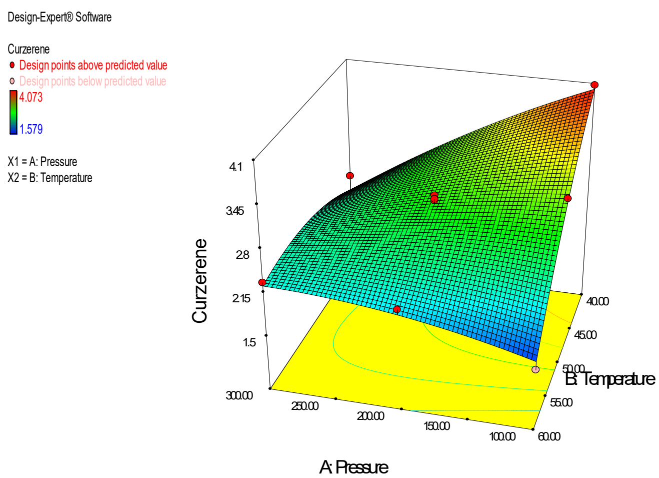

Figure 1.5: Response surface for curzerene concentration percentage as a function of temperature and pressure

The optimum pressure and temperature was determined based on high desirability of response. The optimum pressure and temperature in producing highest oil yield and curzerene concentration were 138.65 bar and 40^{O}C respectively. The predicted of oil yield percentage and curzerene concentration were 1.664 % and 3.696%. Logically, this optimum value is complying with the value that obtained from experiment (100 bar and 40^{O}C) which are 1.654% and 4.073%.The pressure and temperature were significantly control the oil yield as well as concentration of oil compounds. The oil yield and curzerene concentration were increased as the pressure and temperature increased.

IV. CONCLUSION

Summarizing the discussion, the optimization of SFE of *Curcuma zedoaria* parameters by response surface methodology (RSM) was accomplished in this study. According to the analysis:

1. There were a major compounds presents in SFE of *Curcuma zedoaria* which were curzerene and curzerenone. A kovats index (KI) has been calculated to confirm the present of particular compound. The highest percentage of curzerene and curzerenone compound found were 4.07% (SFE8) and 6.87% (SFE11) respectively. These major compounds are valuable and unique for bioactive compound in pharmaceutical application.
2. The optimum parameter of SFE of *Curcuma zedoaria* by RSM were obtained, which are 138.65 bar and 40^{o}C. This optimum parameter gives the best predicted oil yield and curzerene concentration at 1.664 % and 3.696% respectively

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