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Research paper

Optimisation of SC-CO_2 Extraction of *Curcuma zedoaria* by Response Surface Methodology

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Abstract

In this study, *Curcuma zedoaria* was extracted using supercritical fluid extraction system (SFE) to obtain valuable extract. Optimisation of extraction condition for high yield and curzerene concentration was carried out by employing the Response Surface Methodology (RSM) particularly, Central Composite Design (CCD). The objectives of this work are to investigate the effect of temperature (40^oC to 60^oC) and pressure (100 to 300 bar) on extraction yield and concentration of major chemical constituents and determine the optimum parameter for high oil yield and curzerene concentration using RSM. Chemical constituents of the oil were analysed using gas chromatography (GC) and Gas Chromatography Spectrometry (GCMS) and showed that curzerene as the key chemical constituent in the oil extract. The results demonstrated that the oil yield obtained were ranged from 0.6 w/w% to 1.8 w/w% and curzerene concentration in range of 1.6% to 4.1%. The optimum parameters predicted by RSM for the highest oil yield and curzerene concentration were found to be 138.65 bar and 40^oC, which produced the best oil yield and curzerene concentration predicted as 1.7 w/w % and 3.7% respectively. In conclusion, the pressure and temperature significantly influence the oil yield and oil concentration as $p \leq 0.05$ for both factor.

Keywords: *Curcuma zedoaria; Curzerene; Oil yield; RSM; SFE;*

1. Introduction

Curcuma zedoaria (Berg.) Rosc. (Zingiberaceae) oil can be obtained using either conventional or novel extraction method. Hydrodistillation is one of the conventional methods while supercritical fluid extraction (SFE) is a novel extraction method. The oil obtained using conventional method has been recognised as the most favourable for many decades among researchers. However, the conventional oil extraction methods require long processing time and consume relatively large amounts of organic solvent. Ouzzar et.al (1995) conducted an extraction of natural plant using conventional method almost 5 hours and concluded that some solvent residue remained in the products at the end of the processes. Furthermore, the elevated temperature set during the hydrodistillation process degraded the extracts quality as the temperature triggered chemical modifications to the oil component and this led to the loss of valuable volatile components.

In contrast, SFE has been recognized as safe and fast technique to produce high-quality oil with solvent-free products. In SFE technique, carbon dioxide (CO_2) is the most popular and common solvent used due to its environmentally friendly properties such as non-toxic, non-flammable, odourless and inert. SFE is an efficient extraction process compared to conventional extraction method because of its high rate of extraction and the solvating power. Shih et.al (2015) conducted SFE of *C. zedoaria* rhizome within two hours and found that the extracted yield was slightly higher compared to the hydrodistillation. The oil yields acquired by SFE were 0.82% and 0.99% while for hydrodistillation, it was 0.63%. Higher throughput of SFE is often defined by the solubility maxima of solutes in a critical fluid (Ozkal, 2004) and thus contributes to the higher yield. The solubility of the solute in a supercritical fluid depends on several factors such as solvent density, solute volatility (vapour pressure) as well as polarity. The increase in solvent density depends on temperature and pressure and it also increases the solubility. Meanwhile, higher solute volatility means high vapour pressure which makes it easier to remove the solute. The increase in vapour pressure leads to an increase in solubility.

For this case, supercritical fluid extraction is used as a technique for *C. zedoaria* extraction. In SFE, the operating parameters were expected to give different effects on the extraction yield and oil component properties. Hence, optimum parameters for the SFE application are essential. The optimum parameter can be determined using Response Surface Methodology (RSM) method. The RSM is an empirical statistical design of experiment method that aims to find the optimum parameters and achieve the best response. RSM also helps to reduce the number of the required experimental trials and reveal the relationship between independent variables towards the responses using multiple regression. In this work, the parameters used including pressure and temperature meanwhile the responses are the extracts oil (oil yield) and curzerene concentration.

The objectives of this study are to investigate the effect of pressure and temperature on the oil yield and curzerene concentration and to determine the optimum parameter of pressure and temperature to obtain high oil yield and curzerene concentration using RSM. The ranges of temperature and pressure used were between 100 bar to 300 bar, and 40^oC to 60^oC respectively. Besides, the major chemical constituents in SC-CO_2 *C. zedoaria* extracts was identified via GC and GCMS.

2. Methodology

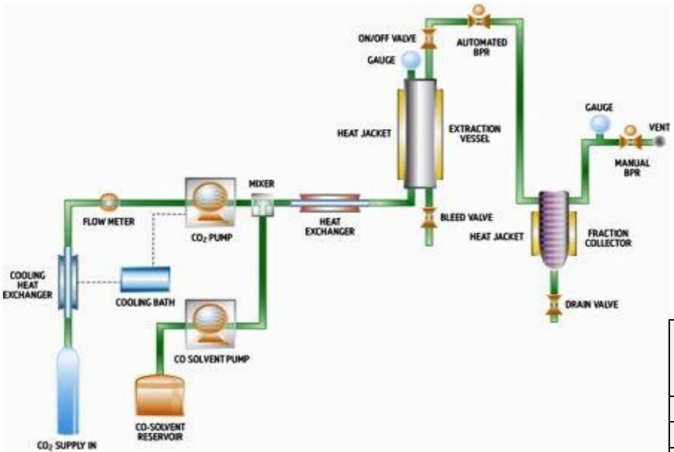
2.1. Materials

Dried *C. zedoaria* was purchased from Kuala Krau, Pahang. The rhizomes were washed, oven dried and pulverised until their diameter size was within 1 to 3 mm. The moisture content of dried rhizome was 6.28% on a dry basis. All chemicals and solvents used were of analytical grade. Carbon dioxide (CO_2) with 99.9 % purity, dichloromethane (DCM) with 99.9% purity and helium with 99% purity were used in this experiment.

2.2. Experimental Apparatus and Procedure

Supercritical CO_2 (SC-CO_2) extraction of *C. zedoaria* was performed using a laboratory scale supercritical-fluid extraction system (SFE 500MR, Thar Technology). The ranges of pressure and temperature used were 100 – 300 bar and 40°C – 60°C respectively. About 200g ground dried *C. zedoaria* was placed in the 1000 ml extraction vessel. After the recirculating chiller reached 3°C, CO_2 was supplied continuously from the gas cylinder into the extractor at a fixed flow rate of 35g/min. The gas CO_2 was cooled using a cooling heat exchanger and liquefied into denser CO_2. The liquefied CO_2 was then pressurised by pumping the CO_2 to the desired pressure and heated to a specific temperature with the purpose of reaching the supercritical state. The extraction process was carried out in 30 minutes for static and 1 hour for dynamic extraction time. The pressure within the extraction vessel was regulated by an 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 depressurised by ABPR and the extracted oil yield was collected through the drain valve. The *C. zedoaria* extract oil yield was calculated using Eq. (1).

$$\text{Yield (\%)} = [M_{extract}\,(g) / M_{feed}\,(g)] \times 100 \qquad (1)$$



2.3. Experimental Design

The SC-CO_2 extraction parameters were optimised by employing the Response Surface Response (RSM). In the RSM, the relationship between independent variables (pressure and temperature) towards the responses oil yield was investigated. 13 experiments including 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 software, the contrasting coefficients among the obtained experimental data were well tuned and statistically analysed using Analysis of Variance (Manickam et.al, 2012). The mathematical models for each response were predicted by using multiple regression models and were fitted into second order polynomial Eq. (2).

$$Y=\beta_o + \Sigma\beta_i X_i + \beta_{ii} X_i^2 + \Sigma\Sigma\beta_{ij} X_i X_j \qquad (2)$$

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 was determined and the F-test of significance of the equation parameters for each response variable was analysed. According to Paulucci et.al, only the factors with significance higher than or equal to 5% ($p \leq 0.05$) were considered.

2.4. GC and GCMS Analysis

The chemical analysis composition of extracts was analysed via gas chromatography (GC) equipped with a DB Wax column (30 m x 0.5 mm i.d, film thickness 0.25 μm). The detectors used were Mass Spectrometer Detector (MSD) and Flame Ionisation Detector (FID). The carrier gas used was helium at pressure 210kPa and the temperature of the injector as well as the detector were set to 240°C. The oven's temperature was initially set at 60 °C for 1 minute and then the heating process was programmed from 60 to 240°C at the rate of 5 °C/min and finally remained at 240 °C for another minute. 1 μl of the sample was injected and the split ratio was 1:20.

3. Result and Discussion

3.1. SFE of *Curcuma Zedoaria* Rhizome

The results of the oil yields of SC-CO_2 of *C. zedoaria* were presented in Table 1.1. Results showed that extraction yield of the oil were from 0.6 w/w% to 1.8 w/w% for pressure ranging from 100 to 300 bar and 40°C to 60°C. These results agree with the assertion that most extraction of medicinal plants will yield less than 5%. A previous study on Curcuma showed that the 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 hydrodistillation 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 somewhat similar to the trend of related studies even though different parameters and extraction method were used. In addition, a recent Chinese report showed that the zedoary oil can be formulated as a submicron emulsion. According to the report, a 2.0 g of zedoary oil was required in the formula and this value proved that the oil yield obtained from the experiment was 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 (w/w%)
	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

3.2. Chemical Investigation

Based on the GC and GCMS analysis, results revealed that more than 30 constituents were identified in the extracted oil. The oil mainly contained sesquiterpene and mono sesquiterpene hydrocarbons (terpenes). This behaviour is coincided to the finding of Shih et.al (2015) that the essential oil of *C. zedoaria* extracted by SFE contained a high ratio of terpenes constituents. Among 30 constituents, curzerene was found as the desired major constituent. SFE 8 (temperature 40^{o}C, pressure 100 bar) gave the highest percentage concentration of curzerene (4.07%) while the lowest percentage concentration was from SFE 3 (temperature 60^{o}C, pressure 100 bar) with 1.58%. The results of the major constituents in oil *C. zedoaria* agree to those reported by Angel et.al (2014) in which cuzerene (8%) and epicurzerenone (19%) were the major compounds in the essential oil of *C. zedoaria* from Kerala, India. In addition, Richard, Kirti, and Annie (2009) conveyed that curzerenone (22.3%), 1,8 cineole (15.9%) and germacrone (9%) as the major compounds 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 constituents	Curzerene Concentration (%)	Curzerene KI	Identification
SFE 1	33	3.158	1851	MS, RI
SFE 2	34	2.75	1852	MS, RI
SFE 3	33	1.579	1851	MS, RI
SFE 4	34	2.89	1852	MS, RI
SFE 5	34	2.06	1852	MS, RI
SFE 6	34	2.199	1852	MS, RI
SFE 7	34	2.326	1851	MS, RI
SFE 8	34	4.073	1854	MS, RI
SFE 9	34	2.92	1852	MS, RI
SFE 10	34	2.913	1852	MS, RI
SFE 11	39	2.818	1852	MS, RI
SFE 12	34	2.97	1852	MS, RI
SFE 13	35	2.196	1852	MS, RI

3.3. Response Surface Methodology

The Response Surface Methodology (RSM) was used to determine the optimum variables with the best responses. In this case, the oil yield and curzerene concentration were the responses while pressure and temperature were two independent variables. The relationship between two independent variables and response have been analysed by RSM to obtain the predicted of both responses. Table 1.3 shows 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 was 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 evaluated. The ANOVA predicted the values for oil yield and curzerene concentration calculated using regression model and compared with experimental values obtained from the experiment. Figure 1.1 and 1.2 show 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 Figures 1.1 and 1.2, the experimental responses were closer to the predicted responses as most of the point concentrated around the 45-degree line.

Oil yield % =
$$0.91-0.07A+0.069B+0.11AB-0.27A^2-0.19B^2 \quad (3)$$

Curzerene concentration% =
$$2.84-0.37A-0.5B+0.66AB-0.13A^2-0.23B^2 \quad (4)$$

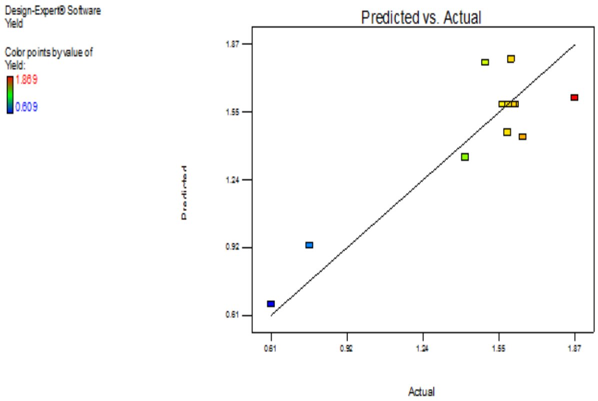


Fig. 1.1: Relationship between experimental and predicted values of oil yield

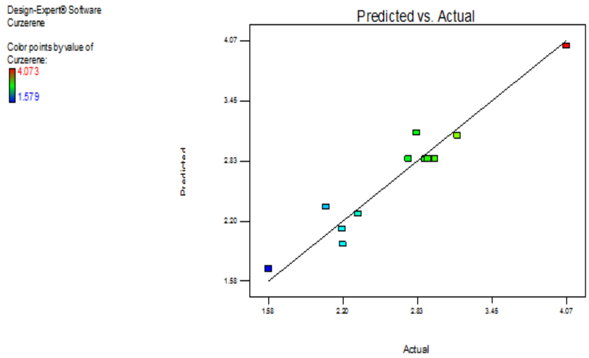


Fig.1.2: Relationship between experimental and predicted value of the curzerene concentration

According to Azmir et.al (2014), the coefficient of determination (R^2) of the model of 0.99 indicated a similarity between the experimental and predicted response variables. Meanwhile, Siti et.al (2015) stated that the R^2 value for all response variables that were higher than 0.75 implying that the regression model capable of presenting the response well. The R^2 value of oil yield and curzerene concentration were 0.8180 and 0.9357 respectively. Therefore, it can be concluded that the regression model can explained the response well with a good fit.

Table 1.4 shows 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 is shown in Table 1.5. The linear and square effects of pressure give both significant and non-significant effect respectively. For the temperature effects, both linear and quadratic term also expressed significant and non-significant. The interaction between pressure and temperature displayed a significant effect 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 1.3 and 1.4 illustrate the interaction between independent variables and responses represented in 3-Dimensional response surface. Based on Figure 1.3, the oil yield increased as the pressure increased at a constant temperature. However, this only happened when it is increased up to the 200 bar for temperature ranges. Note that, above 200 bar, there was a fluctuation in oil yield percentage even though the pressure was high. Meanwhile, at constant pressure, the oil yield increased as the temperature increased. It is obviously shown that high temperature (60^{o}C) with low pressure (100 bar) did not exhibit a good oil yield and is in contrast with the combination of low temperature (40^{o}C) and high pressure (300 b). According to Figure 1.4, the temperature had the important influence on the curzerene concentration. It is showed that the curzerene concentration decreased with the increase of temperature at a constant pressure. The use of high temperature may lead to the degradation of extracts' quality especially heat sensitive materials.

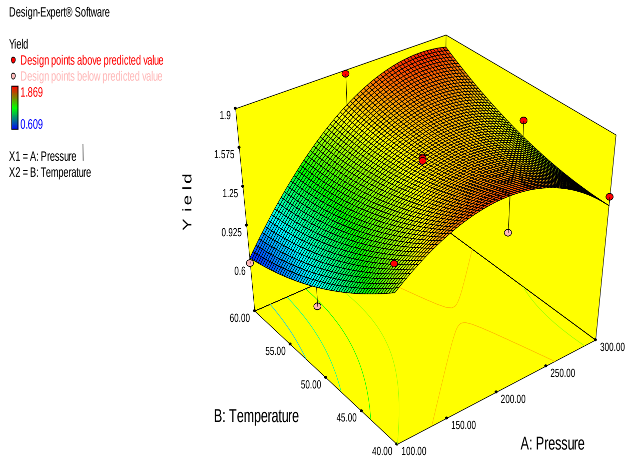


Fig. 1.3: Response surface for oil yield percentage as a function of temperature and pressure

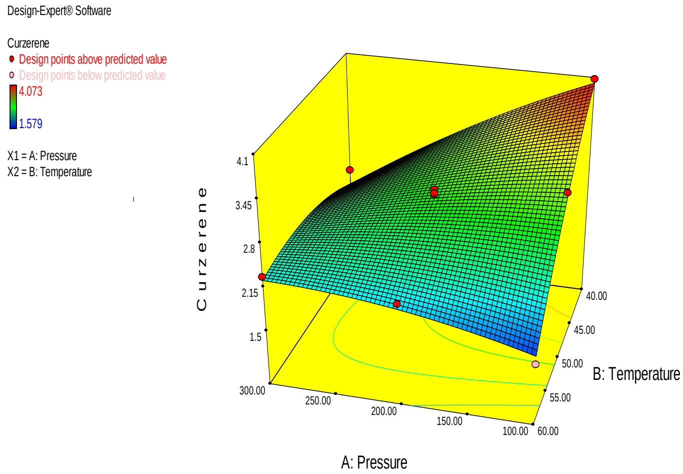


Fig. 1.4: Response surface for curzerene concentration percentage as a function of temperature and pressure

The optimum pressure and temperature were determined based on the high desirability of response. In this study, it is desirable to obtain high oil yield and curzerene concentration within the ranges of pressure and temperature that are cost-effective. In economic perspective, low pressure and temperature consumption reduces the overall operating cost and capital cost. The optimum pressure and temperature in producing highest oil yield and curzerene concentration were 138.65 bar and 40^{O}C respectively. The predicted oil yield percentage and curzerene concentration were 1.664 w/w % and 3.696%. Technically, these optimum values complied with the values obtained from experiment (100 bar and 40^{O}C) which are 1.654 w/w% and 4.073%. The pressure and temperature significantly affected the oil yield and the concentration of oil compounds with $p \leq 0.05$ for both factors. Particularly, the oil yield and curzerene concentration increased as the pressure and temperature increased.

4. Conclusion

In summary, the optimisation of SFE of *C. zedoaria* parameters using response surface methodology (RSM) was accomplished in this study. According to the analysis:

1) Curzerene is the major constituent identified in the extracts. A kovats index (KI) has been calculated to confirm the presence of constituent. The highest percentage of curzerene was 4.07%. This major constituent is valuable and unique for bioactive compound in pharmaceutical applications.
2) Supercritical extraction of *C. zedoaria* at 138.65 bar and 40^{o}C has been determined by RSM as the optimum conditions. These optimum parameters demonstrated as the best predicted oil yield and curzerene concentration at 1.664 w/w% and 3.696%, respectively.

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