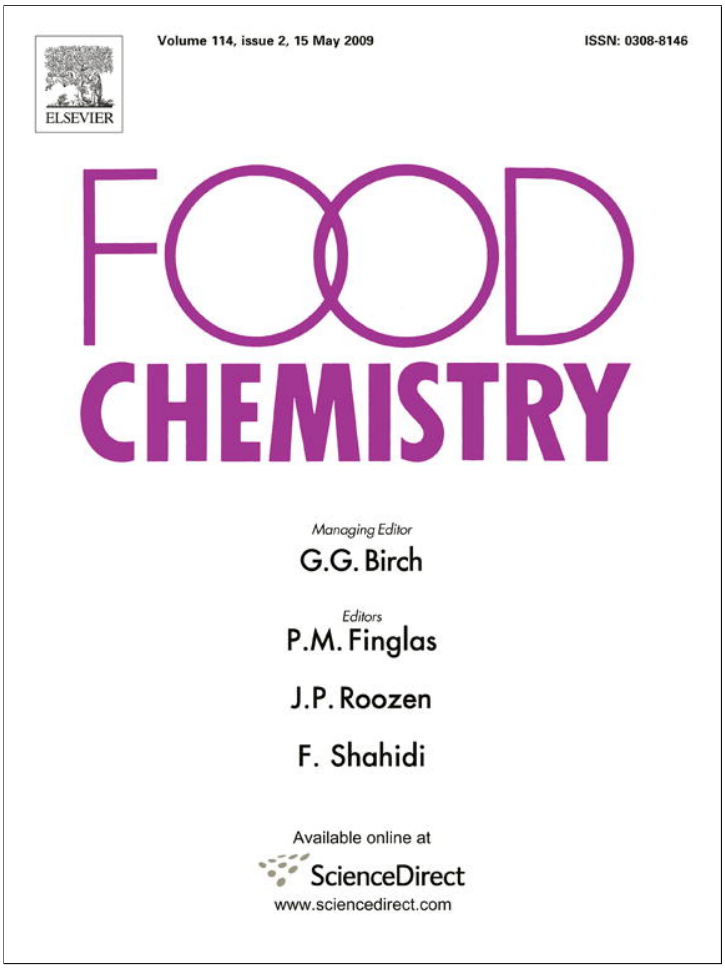

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Analytical Methods

Optimization of SC–CO_2 extraction of zerumbone from *Zingiber zerumbet* (L) Smith

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ABSTRACT

Response surface methodology (RSM) was applied to optimize the variables affecting the Supercritical carbon dioxide (SC–CO_2) extraction of non-polar compounds from *Zingiber zerumbet* using the Box-Behnken design (BBD). Dependent variables were the percentage of the chemical components in the ginger *vis a vis* α-caryophyllene (y_1), camphene (y_2), and zerumbone (2,6,10-cycloundecatrien-1-one, 2,6,9,9-tetramethyl-) (y_3). Pressure was the most significant parameter affecting the amount of each compound extracted. When temperature was kept constant and pressure was increased, all of the dependent variables increased concomitantly. Since pressure and temperature are two of the major influential factors in the extraction using SC–CO_2, any combination of these two parameters could be selected to ascertain the optimum combination for a particular compound in the extract. Extraction at 30 °C and 55 MPa with total amount of 30 g of CO_2 used was found to maximize all the responses.

1. Introduction

Zingiber zerumbet well known as lempoyang, is a wild ginger belonging to the Zingiberaceae family, and has been used as an ingredient in some traditional medicines. It is used in local traditional medicine as a cure for swelling, sores and loss of appetite. The juice of the boiled rhizomes has also been used as a medicine for worm infestation in children. The volatile oils of the rhizomes have been shown to contain zerumbone, humulene and camphene (Nhareetsomchit & Nurshukriyah, 2003).

Zerumbone (2,6,10-cycloundecatrien-1-one, 2,6,9,9-tetramethyl-, (*E*,*E*,*E*)-) is a monocyclic sesquiterpene containing a cross-conjugated dienone system found as the main component of the essential oil of *Z. zerumbet* (Kitayama et al., 1999; Kitayama et al., 2002). Much of its chemistry remains to be explored in order to exploit the ready availability of this substance as a versatile starting material for conversion to other useful compounds (Kitayama et al., 2002). Authors have also reported that the achiral sesquiterpene zerumbone, which is readily available from wild ginger, has unique functionality and reactivity which makes it a potential starting material for conversion to useful compounds such as paclitaxel, provided that it can easily be transformed to chiral derivatives.

Response surface methodology (RSM) is an optimization approach commonly used in industrial process control and engineering where the goal is to find levels of input variables that optimize a particular response (Dhungana, Eskridge, Weiss, & Baenziger, 2006). RSM is a technique consisting of: (a) designing of experiments to provide adequate and reliance measurements of the response, (b) developing a mathematical model having the best fit to the data obtained from the experimental design and (c) determining the optimal value of the independent variables that produce maximum or minimum value of the response. Lee, Yusof, Hamid, and Baharin (2006) used RSM in the determination of optimum extraction temperature and time to produce an acceptable banana juice extract.

Supercritical fluid extraction (SFE) is known to be a fast and efficient method for the extraction of non-polar compounds from plant matrices. Carbon dioxide is the most widely used solvent for extraction of natural products for foods and medicines, under mild conditions. It is an inert, inexpensive, odorless, tasteless and environment-friendly solvent. Furthermore, there is no solvent residue in the extract, since it is a gas under ambient conditions (Mukhopadhyay, 2000; Taylor, 1996).

Therefore, using RSM in supercritical carbon dioxide (SC–CO_2) could be an excellent method to optimize the extraction and, by manipulating the combined configuration of temperature and pressure, the type of compound to be extracted can also be selected from *Z. zerumbet* extract. Thus, the objective of this study is to employ RSM to assess the effect of different combinations of

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temperature, pressure and carbon dioxide consumption on the amount of different extractable compounds from *Z. zerumbet*.

2. Experimental

2.1. Materials

Z. zerumbet plants with rhizomes were collected from a secondary forest at Kubang Kerian, Kelantan, Malaysia and were identified by the Department of Botany, School of Biological Science, Universiti Sains Malaysia, Penang, Malaysia. One kilogram of rhizomes of *Z. zerumbet* was prepared for the extraction. The rhizomes were washed with distilled water, chopped into small pieces of about 0.5–1.0 cm, dried in an oven (40 °C) for about 1–2 days and then thoroughly ground with a dry mixer (Waring, Laboratory, USA) at low grinding speed for about 30 s. They were packed 10 g each into zipped plastic bags and stored at −20 °C until ready for supercritical fluid extraction. Commercial liquefied carbon dioxide (purity, 99.9%) and helium (as a GC analysis carrier gas with the purity of 99.9%) were purchased from Malaysian Oxygen Ltd., Penang. *n*-Hexane, AR Grade obtained from R & M marketing UK.

2.2. Supercritical CO_2 extraction

Supercritical CO_2 was carried out using SFX 220 extraction system (ISCO, Lincoln, NE, USA) comprising of a carbon dioxide cylinder, chiller (BL-730, Yih Der, Taiwan), CO_2 syringe pump (ISCO, Model 100DX), modifier syringe pump (ISCO, Model 100 DX), extraction chamber (ISCO, SFX 220), extraction cartridge, controller (ISCO, SFX 200), and restrictor temperature controller (ISCO).

Approximately 0.4 g of ground *Z. zerumbet* powder was placed in the 2 ml sample cartridge with cotton wool filling up the top and bottom of the cartridge. The sample cartridge was placed in the ISCO extraction chamber and allowed to equilibrate to the preset extraction temperature of 40 °C. CO_2 was then charged from the CO_2 tank into the high pressure syringe pump, which was cooled by a continuous flow of ethylene glycol–water solution (ratio 2:3). The temperature of the chiller was maintained at 0 °C. The extracting pressure and temperature were automatically controlled and maintained throughout the system. The high pressure pump compressed the CO_2 to the desired pressure. When both the desired pressure and temperature were reached, the extraction began by opening the valve between the pump and the sample cartridge to allow the CO_2 to flow through the sample. The product gas, CO_2 plus extract were collected in the collecting tube, which was wrapped with aluminum foil to prevent photo degradation of the extract. This product gas was cooled with the circulating ethylene glycol–water inside a modified conical flask with collection tube. The extraction was designed to use only 30, 40 and 50 g of CO_2 for each designated extraction run. The mass of CO_2 used in the SC–CO_2 extraction was based on the calculation produced by ISCO pressure–temperature–density (SF-Solver Software. Lincoln, NE, USA).

2.3. Experimental design

An experiment was carried out to study the effect of three factors (variables) namely temperature, pressure and mass of CO_2 on the extraction of chemical compounds from *Z. zerumbet*. Three responses in the form of different components of the extract were evaluated:

(i) α-caryophyllene (y_1),
(ii) camphene (y_2), and
(iii) zerumbone (2,6,10-cycloundecatrien-1-one, 2,6,9,9-tetramethyl-) (y_3).

Response surface methodology (RSM) was employed to study the effect of the three factors on the three responses mentioned above and identify the combination that will optimize the extraction. Box-Behnken Design (BBD) was used to perform this experiment; the design is a three-level design for fitting a second-order response surface, and it is a rotatable design. BBD is very efficient in terms of the number of required run (Myers & Montgomery, 2002).

Seventeen runs were required to cover all possible combinations of factors levels. The coded and uncoded independent variables used in RSM design are shown in Table 1. The study was carried out according to Box-Behnken design and the experimental points used according to this design are shown in Table 2. Experimental data were analysed by RSM to fit the following second-order polynomial model

$$y = \beta_0 + \sum_{i=1}^{3} \beta_i + \sum_{i=1}^{3} \beta_{ii} x_i^2 + \sum_{i<j} \sum \beta_{ij} x_i x_j \quad (1)$$

where β_0, β_i, β_{ii} and β_{ij} are regression coefficients, and x_i are the coded variables.

2.4. Gas chromatography-mass spectrometry

The collected extracts of SC–CO_2 were placed in 4 ml glass vials (Supelco, USA) for identification and composition analysis. The extracts were diluted to 1 ml with hexane and 1 μl of the solution was injected into GC–MS without derivatization. The identification of the compounds was done by using a GC model HP 6890 and HP 5973 mass selective detector (Agilent, Wilmington, USA) and Chemstation data system. Electron impact-mass of the extracted component was performed at electron energy of 70 eV with a source temperature of 250 °C and a scan range of 40–650 amu at a rate of 0.81 scan per second. The column used was a 30 m × 0.25 mm × 0.25 μm (HP-5MS, Agilent 19091S-433, Wilmington, USA) 5%-phenyl-methylpolysiloxane capillary column.

Table 1
Treatment levels and code values for each of the independent variable

Symbol	Independent variables	Coded level		
		−1	0	+1
Factor levels:				
x_1	Temperature (°C)	30.0	45.0	60.0
x_2	Pressure (MPa)	30.0	42.5	55.0
x_3	CO_2 used (g)	30.0	40.0	50.0

Table 2
BBD for the three factors in the experiment

X_1	X_2	X_3	y_1	y_2	y_3
−1	−1	0	6.4	5	51.7
1	−1	0	6.7	4.2	52.9
−1	1	0	8.9	6	50.5
1	1	0	7.6	5.1	49.6
−1	0	−1	8.9	6.5	54.9
1	0	−1	9.4	5.5	54
−1	0	1	6.3	5.7	53.3
1	0	1	9.9	5.8	54.9
0	−1	−1	7.9	5.9	53.5
0	1	−1	9.8	7.8	60.1
0	−1	1	6	5.1	54.1
0	1	1	9.6	7	54.4
0	0	0	7.4	5.3	49.7
0	0	0	7.8	5.6	52.6
0	0	0	7	5.1	51.4
0	0	0	7.2	5.2	50.1
0	0	0	7	5.4	54.4

The oven temperature was set constant at 70 °C for 2 min and programmed to rise up to 230 °C at a rate of 7 °C/min and then remained at that temperature for 6 min. Helium at a flow rate of 1 ml/min was used as a carrier gas. The splitless injector was kept at 250 °C. The injector and MS detector temperature was 250 and 280 °C, respectively. The percentage composition of the oil was computed electronically from the GC peak areas without the use of an internal standard or detector response factors (Sirat, Lim, Saat, Leh, & Leng, 2000).

3. Results and discussion

Most of the SFE studies used in the extraction of secondary metabolites involved the yield of extractable components. In some cases, temperature acted as the influencing factor since solvents can penetrate regardless of the physical property of the sample matrix as affected by temperature (Xu & Gidber, 2000). The RSM used in this study is meant to construct and explore an approximate functional relationship between variables such as pressure, temperature and mass of CO_2 used and yield of chemical compounds. The range of pressure tested was between 30 and 55 MPa and the temperature varied from between 30 and 60 °C (Table 1). The amount of CO_2 used was 30, 40 and 50 g in the study on α-caryophyllene extraction under different pressure and temperature combination regimes.

The target compounds of the extraction were bicyclic sesquiterpene α-caryophyllene (y_1), bicyclic monoterpene camphene (y_2) and sesquiterpene zerumbone (2,6,10-cycloundecatrien-1-one, 2,6,9,9-tetramethyl-) (y_3). To find the interactions between the parameters (pressure, temperature and CO_2 used) on the target compounds a statistical analysis was applied (Myers and Montgomery, 2002). Experimental data were obtained according to the response surface methodology (RSM) design of Table 2.

Data were analysed by multiple regressions to fit the quadratic equations to the dependent variables. Statistical analysis was performed using RSM software, design-expert 6-0-10. A mathematical model was established to describe the behavior of each response.

A second-order polynomial model was applied to express the responses as a function of the chosen variables.

$$y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \beta_{11} x_1^2 + \beta_{12} x_1 x_2 + \beta_{22} x_2^2 + \beta_{13} x_3 + \beta_{23} x_2 x_3 + \beta_{33} x_3^2 \quad (2)$$

Multiple regression analysis was performed to determine the regression coefficients of the model. The estimated coefficients (β_i) of second-order response models generated from the statistical analysis for all responses are shown in Table 3. Measured fit of the model to the data ($r2$) for all responses were very high for all of the parameters. The p-value for all responses were <0.01. Accordingly, it could be taken to mean that at 99% confidence level, the models were significant for the responses.

Table 3
Estimated coefficients (β_i) of second-order response models for the responses

	(y_1)	(y_2)	(y_3)
Intercept	7.04	5.34	51.52
x_1	0.34	0.11	−0.30
x_2	1.19	0.81	0.16
x_3	−0.42	−0.42	−1.16
x_1 x_1	0.14	−0.083	−1.3
x_2 x_2	0.34	0.12	0.22
x_3 x_3	0.27	0.84	4.58
x_1 x_2	−0.38	−0.18	1.15
x_1 x_3	0.5	0.05	1.15
x_2 x_3	−0.1	−0.25	−0.18
r^2	0.984	0.993	0.979

Figs. 1–3 show the effect of temperature and pressure on the extraction of the three compounds which α-caryophyllene (y_1), camphene (y_2), and zerumbone (2,6,10-cycloundecatrien-1-one, 2,6,9,9-tetramethyl-) (y_3), respectively.

The predicted data fit very well with the experimental data within the range investigated. Basically interactions between pressure and temperature will reduce the yield and the interaction between temperature and mass of CO_2 used is positive. There is a positive interaction between the temperature and mass of CO_2 used while the interaction between the pressure and temperature as well as pressure and mass of CO_2 used could lead to a reduced caryophyllene yield.

Fig. 1 illustrates the effect of different combinations of pressure and temperature on the amount of α-caryophyllene extracted using 30 g CO_2. It is apparent that maximum α-caryophyllene can be obtained by setting the temperature between 30 and

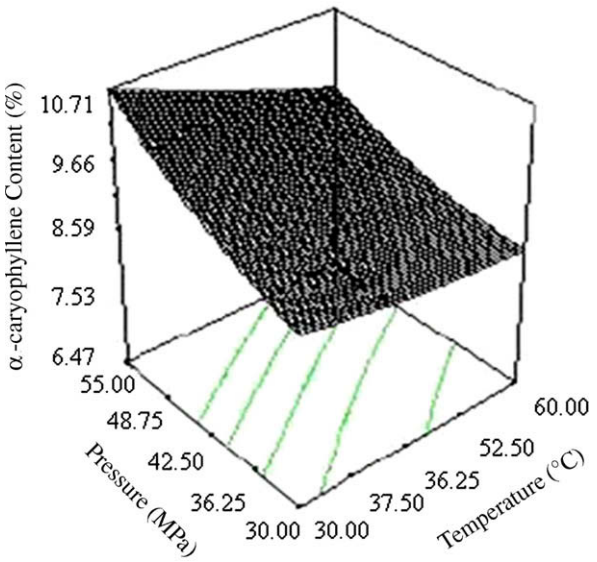

Fig. 1. Three-dimensional response surface of α-caryophellene over pressure ranges of 30–55 MPa at 30–60 °C.

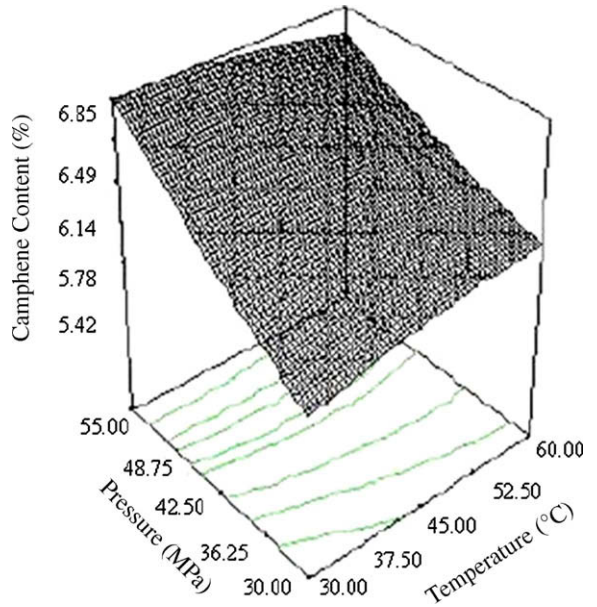

Fig. 2. Three-dimensional response surface for camphene at 30 g CO_2 used.

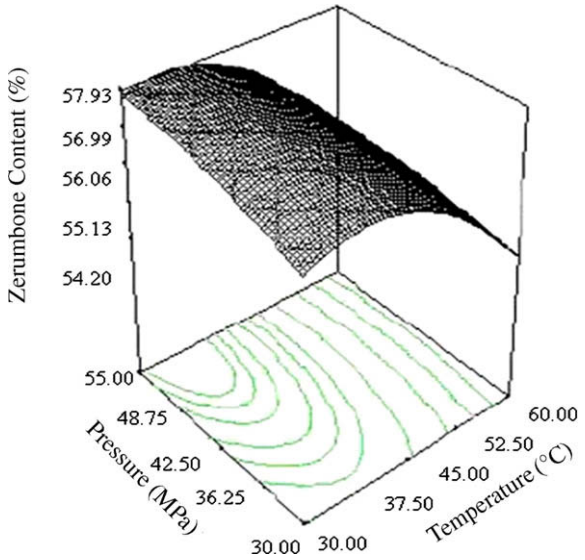

Fig. 3. Three-dimensional response surface Zerumbone, (2,6,10-Cycloundecatrien-1-one, 2,6,9,9-tetramethyl-) at 30 g CO_2 used.

45 °C, with pressure greater than 42.5 MPa and CO_2 consumption of only 30 g. At higher temperatures of greater than 45 °C and pressure less than 42.5 MPa the amount of α-caryophyllene is the lowest. Simultaneous application of high pressure and temperature did not achieve as high a yield as with low temperature and high pressure. Pressure greater than 50 MPa and lower temperature of between 30 and 37 °C gave good yields of α-caryophyllene. The effect of increasing pressure on the yield of α-caryophyllene was more noticeable than that of temperature. The increase in yield at higher pressure could be due to structural change in the matrix that facilitates the diffusion out of the solute and contact with the supercritical CO_2.

With greater CO_2 usage, the low temperature and high pressure interaction did not give as high yield as when lower amount of CO_2 was used. Low pressure and temperature combination gave the lowest yield while high pressure (>50 MPa) and low temperature (30–40 °C) seemed to benefit the extraction. However, the effect of temperature is not as great as that of pressure.

The amount of camphene extracted is dependent on the pressure rather than temperature as observed in Fig. 2. Increasing the pressure will lead to the simultaneous increase in yield of camphene. However, increasing the temperature at constant pressure did not have such a great effect on the yield. The highest amount of chemical component obtained was zerumbone (2,6,10-cycloundecatrien-1-one, 2,6,9,9-tetramethyl-) (Fig. 3) and this was optimally produced at about temperature 40 °C and pressure 55 MPa. Higher temperature will bring down the amount extracted.

The maximum content of the compounds could be obtained from low temperature of around 30 °C and high pressure of 55 MPa. The yields of these compounds are not positively associated with extraction temperature. Nonetheless the positive effect of temperature has been explained in terms of its ability to alter the physical properties of the sample matrix making it easier for the CO_2 to penetrate. At the same time, at higher temperature and constant pressure, the density of supercritical CO_2 decreased thus making it easier to be in contact with the compounds to be extracted. The long extraction period (24 h) carried out may not be feasible because of the high consumption of supercritical CO_2.

Our intention was to find the best settings for temperature, pressure and mass of CO_2, that produce maximum responses, meaning finding a compromised solution among all components (Table 1). We found that the optimum compromising processing extraction parameters are temperature 30 °C, pressure 55 MPa and 30 g of CO_2. The quality of the model was verified by good agreement between experimental and predicted responses.

4. Conclusions

The extraction rate of non-polar compounds in SC–CO_2 was measured as a function of pressure, temperature and mass of CO_2 used at constant extraction time. Results showed that optimized extraction responses using SC–CO_2 based on conditions studied indicate more sensitive to pressure than temperature. These trends could be observed in the extraction process for all responses. By applying a Box-Behnken design, the effects of three SC–CO_2 parameters on the compound recovery were measured. The optimization of this extraction process could be utilized to extract key components known for specific pharmaceutical functions.

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