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Abstract: Response surface methodology was employed to investigate the effects of operating conditions and predict the optimal conditions for supercritical carbon dioxide extraction of essential oil from *Amomum krevanh Pierre*. The factors investigated were operating temperature (33–67°C), the operating pressure (91–259 bar), and the extraction time (20–70 min). The main effect of the operating pressure and the interaction effect between the operating temperature and the extraction time were found to be significant factors. From the response surface model, an optimal condition for essential oil content within the range of experimental study was found to be at 33°C, 175 bar, and 70 min, which gave the oil yield of 17.3 mg/g dry wt. The essential oil yield obtained at all conditions were higher than that obtained by organic solvent extraction (9.74 mg/g dry wt.) while the composition of the extract was similar, which were 1,8-cineole (70.87%), β-pinene (8.89%), and limonene (4.81%).

Keywords: Cardamom, 1,8-cineole, extraction, optimization, orthogonal design

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INTRODUCTION

Amomum krevanh Pierre, an herb belonging to the Zingiberaceae family, is one of the most commonly used spices in South East Asia. In Thailand, it is commonly known as *Krevanh*. The oil from the seeds of amomum contains 1,8-cineole as a major component, and is widely used as flavor and fragrance in foods such as baked goods, meat, and coffee. Medically, such oil is used for flatulent indigestion, as a carminative, and to stimulate the appetite (1).

Generally, essential oils are isolated from plants by solvent extraction, steam distillation, or hydrodistillation. In extractions of essential oils with organic solvents, evaporation of the solvent is typically required and the process conditions employed for evaporation could cause product degradation. Alternative to organic solvents, steam distillation and hydrodistillation are used, however, the methods have some disadvantages such as requiring a long extraction time, and the processes lead to thermal degradation (2–4). In recent years, supercritical fluid technology has gained increasing popularity for the applications in food processing industry, and extraction with supercritical fluid has become an interesting alternative to the conventional extraction procedures. The physicochemical properties of supercritical fluids such as high diffusivity and low viscosity allow them to better diffuse through the natural solid matrix, and thus better extract the natural compounds than the conventional liquid solvents. The most frequently employed supercritical solvent in food and natural products processing is supercritical carbon dioxide (SC-CO_2) due to its low toxicity, low cost, and low critical temperature (31.1°C) and pressure (73.8 bar) (5).

Several studies have been carried out on essential oil extraction with SC-CO_2 to determine the effect of the operating conditions on the process (6–8), including the study on extraction of *Elettaria cardamomum L.*, a similar plant belonging to the same family as *Amomum krevanh Pierre* (9–10). In most of the previous studies, the information regarding the process conditions is obtained by conducting one-variable-at-a-time experiments. In such cases, no interaction was assumed between process variables. This assumption indeed might lead to biased results, and thus the true optimality would not be reached. Statistical experimental design has been demonstrated to be a powerful tool for determining the factors effects and their interactions, which allows the process optimization to be conducted effectively (11–13).

The aim of the present work is therefore to employ the central composite orthogonal rotatable experimental design and statistical analysis for the investigation of the effects of the operating pressure, the temperature, and the extraction time for the SC-CO_2 extraction of essential oil

from *Amomum krevanh Pierre*. The essential oil obtained by solvent extraction was used for comparison.

MATERIALS AND METHODS

Plant Material and Chemicals

The seeds of *Amomum krevanh Pierre* used in this experiment were obtained from a local market in Thailand. They were ground in a coffee grinder to fine powder whose average particle size was approximately 300 μm, and the ground samples were stored in a dry place until use. High purity carbon dioxide used for extraction was purchased from Thonburiwattana Co. (Bangkok, Thailand). Hexane (purity >99.5%) was supplied by Sigma-Aldrich. 1,8-cineole, *β*-pinene (7.91%), and limonene standards used for identification of chromatographic peak were purchased from Fluka. Nitrogen was purchased from Thai industrial gas Co. (Bangkok, Thailand).

SC-CO_2 Extraction

SC-CO_2 extraction was carried out using SFX-220 (the maximum extraction temperature of 150°C and the maximum pressure of 680 bar, Isco Inc., Lincoln, NE USA.), which consisted of an extractor with a maximum capacity of 10 ml, a controller, a restrictor temperature controller, and a syringe pump. The schematic diagram of the apparatus is shown in Fig. 1. For each experimental run of essential oil extraction, 3.0 g ground seeds of *Amomum krevanh Pierre* was mixed with silica sand and charged into the extraction chamber. The extraction was carried out at a desired temperature and pressure for a specified extraction period with the static time of 2 min. The extract was trapped in a tube containing *n*-hexane. After extraction, *n*-hexane was removed with nitrogen stream at room temperature and stored at 5°C until analysis.

Solvent Extraction

The cardamom seed powder (3.0 g) was extracted with *n*-hexane (30 ml) under sonication for 30 min in an ultrasonic bath (275DAE, Crest Ultrasonics, USA), after which the sample were macerated at 25°C for 24 h. The extraction flask was partially immersed into the ultrasonic bath, which contains 2.21 of water. The bottom of the flask was about 5 cm

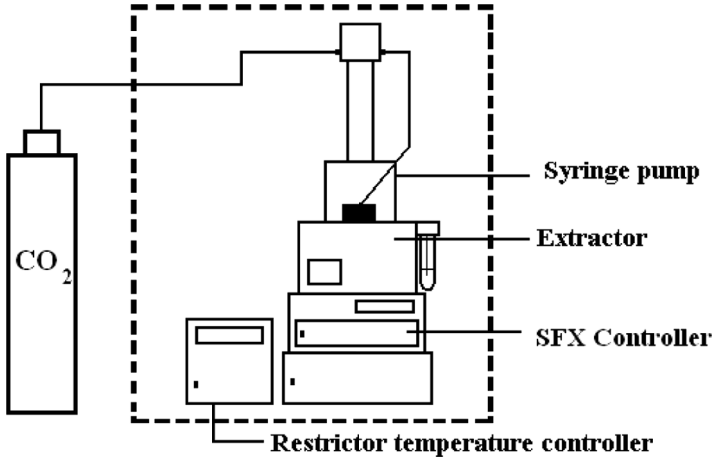

Figure 1. Schematic diagram of supercritical fluid extraction apparatus.

above the bottom of the bath. The solvent surface in the flask was kept at the level of the water in the ultraasonic bath. The extract was then filtered through a 0.45 μm nylon membrane filter (Millipore, USA) and the filtered extract was concentrated by removing the solvent under nitrogen stream. The concentrated sample was then stored at 5°C until analysis.

GC Analysis

GC analyses were performed using a Shimadzu GC-2010 gas chromatography whose system was equipped with a DB-WAX capillary column (30 m × 0.25 mm i.d., film thickness 0.25 μm). The SFE samples (1 μl) were injected using the split mode with a split ratio of 1/30. Oven temperature was programmed to increase from 80°C to 130°C at a rate of 5°C/min. The injector and detector temperatures were held at 230 and 250°C, respectively.

Experimental Design and Statistical Analysis

In this study, the experimental design was used to evaluate the main and interaction effects of the factors: the temperature (X_1), the pressure (X_2), and the extraction time (X_3) on essential oil yield obtained with SC-CO_2 process. Seventeen experiments were performed: eight factorial points for the three factors each at five levels, six axial points, and three

Table 1. Factors and levels tested for the designed experiment

	Levels				
Variables	−1.68	−1	0	+1	+1.68
X_1:Temperature (°C)	33	40	50	60	67
X_2: Pressure (bar)	91	125	175	225	259
X_3: Extraction time (min)	20	30	45	60	70

repeatability experiments for the measurements at the center of the experimental domain. The ranges and the levels of the factors investigated in this study are summarized in Table 1.

The test factors were code according to the following equation:

$$x_i = \frac{X_i - X_i^*}{\Delta X_i} \tag{1}$$

where x_i is coded value of the ith factor, X_i the uncoded value of the ith factor, X_i^* the uncoded value of the factor at the center point, and ΔX_i is the step change value. More specially, coded values of the factors were calculated as follows:

$$\text{Temperature}; x_1 = \frac{X_1 - 50}{10} \tag{2}$$

$$\text{Pressure}; x_2 = \frac{X_2 - 175}{50} \tag{3}$$

$$\text{Extractiontime}; x_3 = \frac{X_3 - 45}{15} \tag{4}$$

The statistical analysis of variance (ANOVA) of the experimental results was employed to determine the main effect and the interaction of the factor effects using the SPSS program. The response surface equations were then proposed, from which the optimal conditions were determined.

RESULTS AND DISCUSSION

Statistical Analysis of Essential Oil Yields by SC-CO_2 Extraction

SC-CO_2 extractions were carried out at different conditions as indicated in Table 1. It is noted that the sample size of 300 μm was selected based on our preliminary experiment in which three particle sizes at nominal

Table 2. Experimental matrix and experimental results for the design of experiment

Run	Temperature	Pressure	Time	Essential oil yield (mg/g)
1	−1	−1	−1	14.08
2	1	−1	−1	12.25
3	−1	1	−1	11.30
4	1	1	−1	13.58
5	−1	−1	1	13.49
6	1	−1	1	10.97
7	−1	1	1	14.96
8	1	1	1	12.17
9	−1.68	0	0	14.03
10	1.68	0	0	15.00
11	0	−1.68	0	9.74
12	0	1.68	0	12.51
13	0	0	−1.68	11.04
14	0	0	1.68	14.62
15	0	0	0	13.91
16	0	0	0	13.77
17	0	0	0	12.92

average diameters of 215 μm, 300 μm, and 580 μm average were extracted at 40°C, 225 bar, for 30 min. The results showed that the sample with particle size of 300 μm gave the highest oil yield. Although the sample with a smaller particle size would usually give a larger surface area for mass transfer, in this study the extraction yields from the sample of the smallest particle size was lower, possibly because the volatile essential oil was more readily lost from the small size sample and too small particles may cause high pressure drop inside the extraction chamber, plugging of fine particles, as well as channeling, which lessens extraction efficiency.

In this study, the central composite orthogonal rotatable design experimental was used to evaluate the main effects and the interaction effects on the performance of SC-CO_2, measured in terms of the essential oil yield. The experimental matrix and the results for all 17 extraction experiments are shown in Table 2. From these results, the analysis of variance (ANOVA) was used to determine the factors that have important effects on the essential oil yields. The analysis results obtained using a statistical program SPSS 15.0 are shown in Table 3, which shows that for the confidence interval of 90% ($P < 0.1$), the factors that have significant effects on the essential oil yields were the operating pressure, and the interaction between the operating temperature and the extraction time. Each of these effects is described in more detail as follows.

Table 3. ANOVA table for essential oil yields

Source	Type III sum of squares	df	Mean square	F	Sig.
Corrected Model	37.028(a)	14	2.645	9.214	.102
Intercept	1675.982	1	1675.982	5838.980	.000
x1	4.569	3	1.523	5.306	.163
x2	10.989	3	3.663	12.762	.074
x3	7.020	3	2.340	8.153	.111
x1 * x2	1.842	1	1.842	6.418	.127
x1 * x3	4.129	1	4.129	14.383	.063
x2 * x3	2.121	1	2.121	7.389	.113
x1 * x2 * x3	2.384	1	2.384	8.305	.102
Error	.574	2	.287		
Total	2893.338	17			
Corrected Total	37.602	16			

R Squared = .985 (Adjusted R Squared = .878)

Main Effect of Operating Pressure on Essential Oil Yields

The main effect of the operating pressure on essential oil yields is shown in Fig. 2. The results show that the essential oil yields were higher with increasing operating pressure in the range of 91–175 bar. However, for the operating pressures in the range of 175–259 bar, the essential oil yields

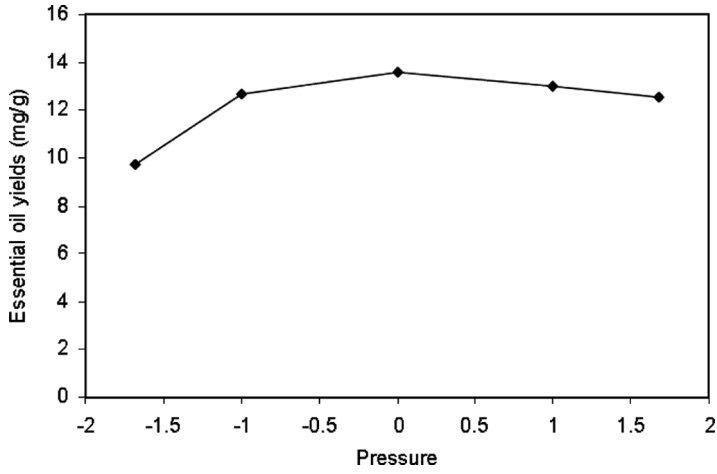

Figure 2. Main effect of operating pressure to essential oil yields.

decreased. The reason for this was that, in general, an increase in pressure causes an increase in the fluid density and this could have a double effect: an increase in the solvating power of the supercritical fluid and a reduced interaction between the fluid and the matrix as a consequence of a decrease in the diffusion coefficient at higher density (14).

Interaction Effects of Operating Temperature and Extraction Time on Essential Oil Yields

The statistical analysis of the experimental results showed that the interaction between the operating temperature and extraction time significantly affected the essential oil yields. Figure 3 shows the interaction effect between the operating temperature and the extraction time. It can be seen from this figure that for the extraction time of 30 min, the oil yield slightly increased as the operating temperature increased. However, as the time of extraction increased to 60 min, the oil yield decreased as the operating temperature increased. In typical extraction, longer extraction should generally result in higher yield as the time in which the sample and the solvents are in contact would increase. However, when extraction was carried out at higher temperature, the extraction was also subjected to loss of the volatile compound when the process took place at an extended time period, which resulted in the decrease in the yield as can be seen in the figure. Improved extract

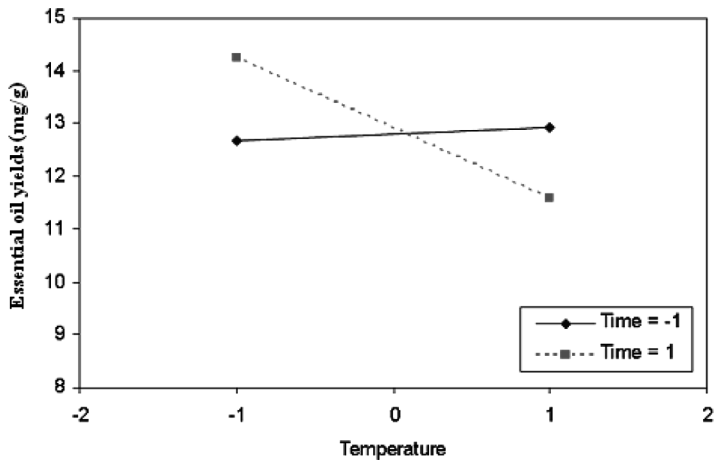

Figure 3. Interaction effect between temperature and extraction time for essential oil yields.

trapping procedure such as that using solid-phase sorbents could be devised (15–16).

Optimal Condition for SC-CO_2 Extraction of Essential Oil From *Amomum krevanh Pierre*

The relations between each factor and the extracts yields were modeled with a response surface using a second-order polynomial model. The analysis results obtained are summarized in Table 4. The response surface for essential oil yields is shown in Fig. 4 and the response surface equation obtained from the analysis was:

$$y = 13.355 - 0.237x_1 + 0.429x_2 + 0.469x_3 + 0.810x_1^2 - 1.208x_2^2 - 0.192x_3^2 + 0.480x_1x_2 - 0.718x_1x_3 + 0.515x_2x_3 \quad (5)$$

where y is the essential oil yields (mg/g dry wt.), x_1, x_2, and x_3 are coded variables for the operating temperature, the operating pressure, and the extraction time, respectively. The significance of each coefficient was determined by the P-values, that is, if the values of P are less than 0.1, this indicated that the model terms were significant. In this case, the quadratic main effect of the operating pressure (x_2^2) was the most significant

Table 4. Regression coefficient of polynomial function of response surfaces of essential oil yields

Model		Unstandardized coefficients		Standardized coefficients		
		B	Std. Error	Beta	t	Sig.
1	(Constant)	13.355	.630		21.208	.000
	X1	−.237	.338	−.143	−.700	.506
	X2	.429	.338	.259	1.269	.245
	X3	.469	.338	.282	1.385	.208
	X1X1	.810	.510	.325	1.587	.156
	X2X2	−1.208	.510	−.485	−2.367	.050
	X3X3	−.192	.510	−.077	−.377	.718
	X1X2	.480	.442	.221	1.086	.314
	X1X3	−.718	.442	−.331	−1.625	.148
	X2X3	.515	.442	.237	1.165	.282

R Squared = .709.

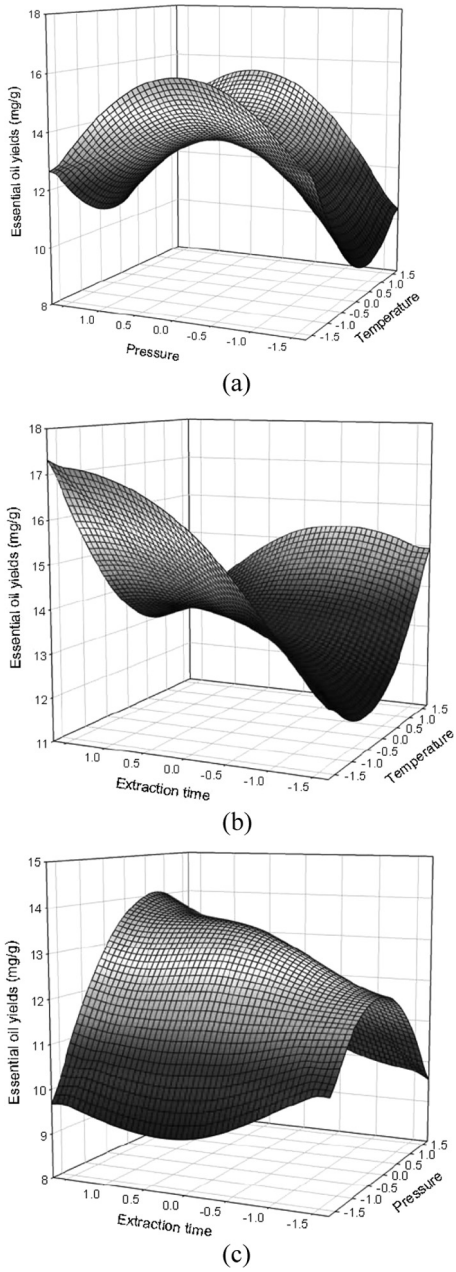

Figure 4. Response surfaces on essential oil yields (for the third coded variable of 0).

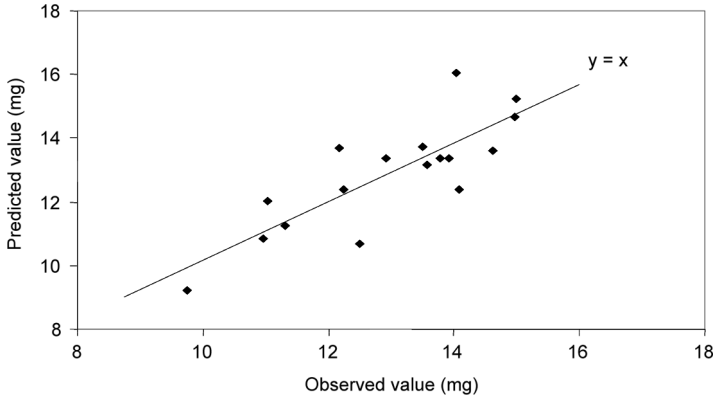

Figure 5. Observed values versus predicted values from response surface model.

(P-value = 0.05). The plot of the observed and the predicted values are shown in Fig. 5.

From the response surface equation for essential oil yields, the optimal condition of this extraction can be determined by taking the partial derivative of the equation with respect to each of the three factors, and set these partial derivatives equal to 0, and solve the equation. The solutions of the resulting equations yield the optimal extraction condition which was 64°C, 277 bar, and 84 min of extraction. The response values for these conditions were 11.90 mg/g dry wt. This condition was the extrapolation from the model which therefore would not be considered the most accurate prediction. Nevertheless, the optimal condition could be obtained by the response surface for the conditions within the experimental range to be at 33°C and 175 bar, and the extraction time was 70 min, which gave the highest amount of essential oil of 17.3 mg/g dry wt.

Comparison of SC-CO_2 Extraction and Solvent Extraction

The yields of oil obtained with with SC-CO_2 at all experimental conditions were between 9.74 and 15 mg/g dry wt., which were generally higher than that obtained by solvent extraction (9.74 mg/g dry wt.). As shown in Fig. 6, the component of the oil obtained by both methods and their composition are similar. Three major compounds: 1,8-cineole, β-pinene, and limonene, were compared. The percent composition of these compounds in the oil extracted with SC-CO_2 were 71.65%, 8.64%, and 4.77%, which were similar to those obtained by solvent extraction which were 70.86%, 7.91%, and 4.30%, respectively.

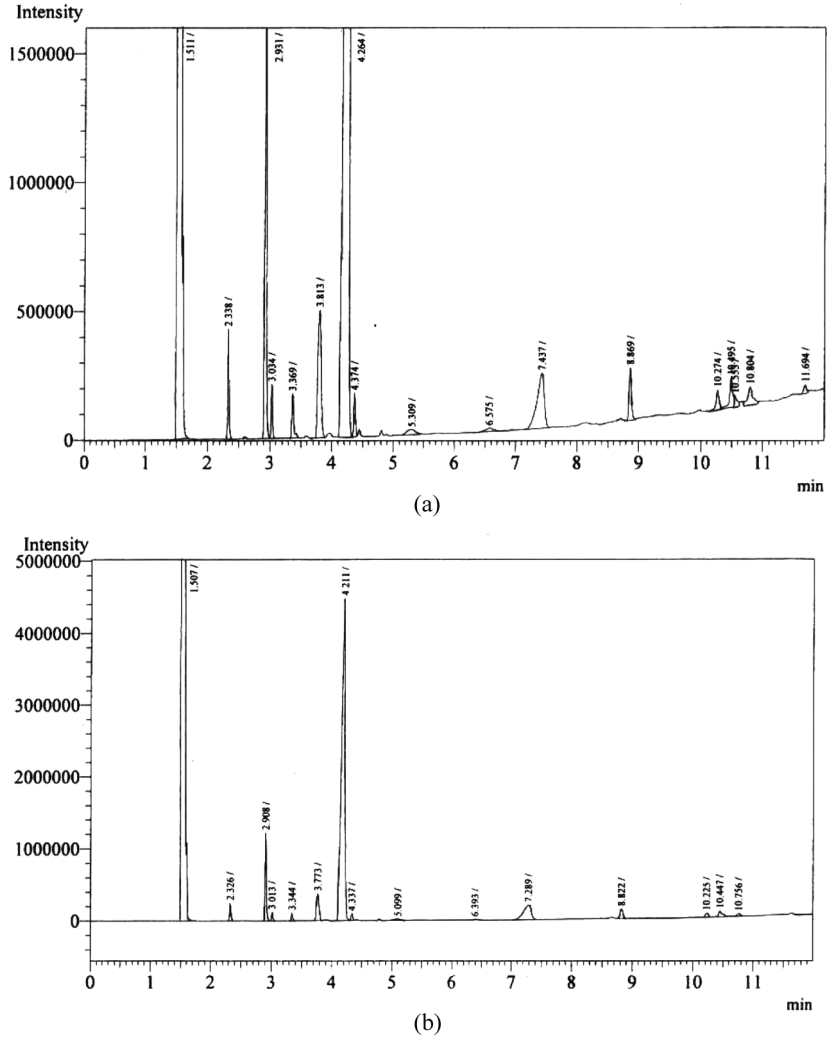

Figure 6. GC analysis of essential oil from *Amomum krevanh Pierre* obtained by (a) SC-CO_2 extraction (at condition: 67°C, 175 bar, and 45 min) and (b) solvent extraction using hexane.

CONCLUSIONS

A the central composite orthogonal rotatable experimental design was employed on extraction of essential oil from *Amomum krevanh Pierre* with SC-CO_2. The analysis of variance (ANOVA) indicated that the main

effect of pressure and the interaction effect between temperature and extraction time significantly influenced the essential oil yields. The optimal extraction condition within the range of experimental study was found based on the polynomial response surface model to be at 33°C, 175 bar, and 70 min extraction time, giving the oil yield of 17.3 mg/g dry wt.

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