J Am Oil Chem Soc (2010) 87:1063–1070
DOI 10.1007/s11746-010-1591-0

ORIGINAL PAPER

Optimization of the Extraction of *Alpinia oxyphylla* Essence Oil in Supercritical Carbon Dioxide

Hen-Yi Ju · Kuo-Chuan Huang · Jiann-Hwa Chen · Yung-Chuan Liu · Chieh-Ming J. Chang · Chih-Chen Lee · Cheng Chang · Chwen-Jen Shieh

Received: 28 September 2009 / Revised: 1 April 2010 / Accepted: 14 April 2010 / Published online: 4 May 2010

Abstract The essence oil of the *Alpinia oxyphylla* seed has been used as a vasodilatatory and analgesic agent in pharmacology. The extraction of the essence oil in supercritical carbon dioxide (SC-CO_2) from *Alpinia oxyphylla* seeds was investigated. Small particles were obtained after breaking open, sieving, and drying from the *Alpinia oxyphylla* seeds. The small particles were placed in a 5-L extraction tank in a temperature-controlled system. The CO_2 flow rate of the system was set at 1 L/min in this study. Response surface methodology with a three-factor and three-level Box-Behnken experimental design was used to evaluate the effects of the reaction parameters such as extraction time (1, 2, 3 h), temperature (45, 55, 65 °C), and pressure (20, 30, 40 MPa), on the extraction yield of the essence oil from *Alpinia oxyphylla* seeds. The results indicate that the extraction pressure was the most important parameter affecting the yield of the essence oil. A model for the estimation of the yield was developed. Based on the analysis of ridge max, the optimal extraction conditions were established as an extraction time of 2.8 h, a temperature of 67.5 °C, and a pressure of 28.5 MPa, with an expected yield of 2.78%. Extraction of *Alpinia oxyphylla* essence oil in SC-CO_2 under these optimal conditions was conducted, and a yield of 2.77 ± 0.19% was obtained.

Keywords Essence oil · *Alpinia oxyphylla* · Supercritical carbon dioxide · RSM

H.-Y. Ju · K.-C. Huang
Department of Bioindustry Technology, Dayeh University, 168 University Road, Da-Tsuen, Chang-Hua 51505, Taiwan

J.-H. Chen
Graduate Institute of Molecular Biology, National Chung Hsing University, 250 Kuo-Kuang Road, Taichung 40227, Taiwan

Y.-C. Liu · C.-M. J. Chang · C.-C. Lee
Department of Chemical Engineering, National Chung Hsing University, 250 Kuo-Kuang Road, Taichung 40227, Taiwan

C.-C. Lee
Derlin Biotech Corporation Ltd, 29, Dougong 2nd Road, Douliou city, Yunlin 640, Taiwan

C. Chang · C.-J. Shieh (✉)
Biotechnology Center, National Chung Hsing University, 250 Kuo-Kuang Road, Taichung 40227, Taiwan
e-mail: cjshieh@nchu.edu.tw

Introduction

The *Alpinia oxyphylla* (Sharpleaf Galangal Fruit) seed is an important Chinese medicinal herb. It has been used as a vasodilatatory [1, 2] and an analgesic drug [3], an anticancer agent [4–7], and an anti-hypersensitivity [8], anti-aging, and anti-gastric ulcer [3] agent. Suppression of NO production [9] and prevention of diuresis and dementia [10] have also been documented with the use of this herb. The essence oil of the *Alpinia oxyphylla* seed has been extracted by steam distillation, water distillation, or organic solvent extraction, and the ingredients have been identified. These oils include volatile oils (*ρ*-cymene, valencene, linalool, pinene, etc.) [11, 12], terpenes (nootkato, valencene, nootkatone, oxyphyllol, etc.) [1, 9, 12, 13], flavones (tectochrysin, chrysin, izalpiin, etc.) [9, 14], heptanoids (yakuchinone A, yakuchinone B, neonootkatol), oxyphyllacinol [14], taurine, vitamin B, vitamin C, and microelements (Mn, Zn, K, Na, Ca, Mg, P, Fe, and Cu, etc.) [9]. Traditionally, the essence oil is extracted from various plant materials by steam distillation, water distillation, or organic solvent extraction. While steam distillation and water distillation normally operate at high temperatures, which may lead to a degradation of thermally unsettled compounds and the formation of undesirable compounds [15], organic solvent extraction usually has problems concerning the organic solvent residue left in the essence oil, which may have potentially adverse effects on human health.

Extraction via supercritical carbon dioxide (SC-CO_2) is a new method for the extraction of essence oil. At or above the critical point of CO_2 (a temperature of 31.3 °C and a pressure of 7.4 MPa), CO_2 changes its physical properties from a gas to a fluid. SC-CO_2 extraction technology utilizes the dissolving power of the CO_2 fluid for extraction. Compared to steam extraction, SC-CO2 extraction operates at relatively low temperatures and could prevent thermal damage of the product. SC-CO_2 extraction is terminated by discharging the pressure of the system, and the liquid CO_2 immediately gasifies and spreads into the atmosphere. Therefore, extraction products do not contain any CO_2 residues. Essence oil extracted by SC-CO_2 technology can be well separated from by-products and hydrolytes and is considered to be of the highest quality [16].

While SC-CO_2 is a hydrophobic solvent, its polarity can be adjusted by including various polar reagents as co-solvents during the extraction. Therefore, hydrophilic compounds such as oxygenated terpenes and sesquiterpenes could be extracted by the SC-CO_2 extraction technology as well [17]. Because of its low toxicity, non-flammability, and environmentally benign character, SC-CO_2 is regarded as a green solvent [18].

There have been several reports investigating the extraction of seed essence oil in SC-CO_2. Grosso et al. [19] reported the extraction of volatile oil from Italian coriander seeds in SC-CO_2. The extraction was conducted at 40 °C and 9 MPa for 30 min with a CO_2 flow rate of 1.1 kg/h. The integrants in the volatile oil were identified as linalool (65–79%), γ-terpinene (4–7%), camphor (3%), geranyl acetate (2–4%), α-pinene (1–3%), geraniol (1–3%), and limonene (1–2%). Gomes et al. found that the best condition for the extraction of rose geranium oil in SC-CO_2 was to perform the extraction at 40 °C and 9–10 MPa for 15–30 min with a CO_2 flow rate of 1.6 kg/h. However, a yield of only 0.22% of rose geranium oil was obtained under these optimal extraction conditions [20]. Shao et al. tested the extraction of wheat germ oil in SC-CO_2 with combinations of different temperatures (40, 50, and 60 °C), pressures (20, 27.5, and 35 MPa), and CO_2 flow rates (15, 20, and 25 L/h). They found that a maximum yield of 10.15% could be obtained by performing the extraction for 1 h at 50 °C, 35 MPa, and a flow rate of 22.5–25 L/h [21]. Thus far, there have been few reports on the extraction of *Alpinia oxyphylla* seed essence oil using SC-CO_2.

Response surface methodology (RSM) is a collection of mathematical and statistical techniques for designing experiments, building models, evaluating the relative significance of several independent variables, and determining the optimum conditions for desirable responses. The Box-Behnken design is considered as an efficient option in response surface methodology and an ideal alternative to central composite designs [22, 23]. The Box-Behnken design has three levels per factor but avoids the corners of the space and fills in the combinations of center and extreme levels. Overall, it combines a fractional factorial with incomplete block designs in such a way that the extreme vertices are avoided and an approximately rotatable design with only three levels per factor is presented [24]. The experimental design is more efficient for simplifying experiments and understanding mutual relationships among experimental parameters.

The present research focused on the reaction (manipulated) parameters that may affect the extraction of essence oil in SC-CO_2 from seeds of *Alpinia oxyphylla*. The aims of this study were to establish the relationships between the manipulated variables (extraction time, temperature, and pressure) and the response (yield of essence oil). The response surface methodology (RSM) using a Box-Behnken experimental design was applied to determine the optimal conditions for the extraction of *Alpinia oxyphylla* essence oil in SC-CO_2.

Materials and Methods

Materials

A supercritical fluid system (2 + 5 L SFE) was purchased from Applied Separation Inc. (Allentown, PA, USA). For all of the extractions performed in this study, the CO_2 flow rate of the system was set at 1 L/min. Carbon dioxide was purchased from the Yi-Zhan Gas Enterprise Co. (Yunlin, Taiwan), and *Alpinia oxyphylla* seeds were purchased from the Tian-Yi Chinese Herb Co. (Yunlin, Taiwan).

Preparation of Small Particles of *Alpinia oxyphylla* Seed for Extraction using SC-CO_2

Alpinia oxyphylla seeds were broken in a crusher (7.5 H.P. with a capacity of 200 kg/h, Thermo, USA) at room temperature for 1 min (3 times), and small particles were sieved out using a 100-mesh sieve and collected. About 700 g of the small particles was weighed (W_1) and dried at 121 °C for 24 h. The dried small particles were weighed a second time (W_2), and the water content of the small particles (A) was determined according to the following Eq. (1).

$$A = (W_1 - W_2) \div W_1 \quad (1)$$

Preliminary Experiment

The dried small particles of *Alpinia oxyphylla* seeds were put into the supercritical fluid system, and extraction was carried out according to the manual supplied with the system. The optimum extraction of the experimental design was performed after the preliminary experiment. In short, the dried small particles were placed in a 5-L extraction tank in a temperature-controlled system. CO_2 gas was fed into the system at a fixed flow rate from a CO_2 cylinder, and the extraction proceeded under stationary pressure in the extraction tank. To terminate the extraction, the CO_2 gas feed into the system was stopped, and the pressure in the tank was manually released. Materials extracted during the process gradually drifted into the collection tank. In this study, the extraction temperature was set at 40, 45, or 50 °C, the extraction pressure was set at 10, 15, 20, 25, 30, 35, or 40 MPa, and the extraction proceeded for 30 or 60 min. In total, 42 extraction experiments were conducted in this study. Each of the 42 experiments was performed two times, and the yields were averaged.

For each extraction experiment, the resulting essence oil collected from the collection tank was weighed. The yield of the essence oil of each extraction experiment was calculated according to Eq. (2).

$$\text{Yield } (Y) = W_3 \div (W_2 \times (1 - A)) \times 100\% \quad (2)$$

where Y is the yield of the essence oil, W_3 is the weight of the resulting essence oil, W_2 is the weight of the dried *Alpinia oxyphylla* seed powder, and A is the water content of the dried *Alpinia oxyphylla* seed powder determined using Eq. (1).

Optimum Extraction Conditions

To determine the optimal extraction conditions, extractions were carried out with combinations of pressures of 20, 30, and 40 MPa, temperatures of 45, 55, and 65 °C, and extraction times of 1, 2, and 3 h. According to the 3-level-3-factor experimental design of Box-Behnken, only 15 extraction experiments (instead of 27 experiments) needed to be conducted. A summary of the pressure, temperature, and extraction times of the 15 experiments is shown in Table 1. The yields of the 15 experiments were calculated according to Eq. (2) and analyzed by the response surface regression (RSREG) to fit the following second-order polynomial Eq. (3) [25]:

Table 1 Three-factor-three-level Box-Behnken experimental design, and the experimental results for the yield of *Alpinia oxyphylla* essence oil in response surface analysis

Treatment no.[a]	Factor			Yield of essence oil Y (%)
	Time (h)	Temperature (°C)	Pressure (MPa)	
1	1 (3)[b]	1 (65)	0 (30)	2.80 ± 0.02[c]
2	1 (3)	−1 (45)	0 (30)	2.05 ± 0.05
3	−1 (1)	1 (65)	0 (30)	2.56 ± 0.06
4	−1 (1)	−1 (45)	0 (30)	1.70 ± 0.09
5	1 (3)	0 (55)	1 (40)	2.40 ± 0.05
6	1 (3)	0 (55)	−1 (20)	2.19 ± 0.02
7	−1 (1)	0 (55)	1 (40)	2.33 ± 0.08
8	−1 (1)	0 (55)	−1 (20)	1.36 ± 0.03
9	0 (2)	1 (65)	1 (40)	2.27 ± 0.06
10	0 (2)	1 (65)	−1 (20)	2.09 ± 0.03
11	0 (2)	−1 (45)	1 (40)	2.42 ± 0.05
12	0 (2)	−1 (45)	−1 (20)	1.53 ± 0.07
13	0 (2)	0 (55)	0 (30)	1.87 ± 0.04
14	0 (2)	0 (55)	0 (30)	1.91 ± 0.05
15	0 (2)	0 (55)	0 (30)	1.93 ± 0.02

Seven hundred grams of dried small particles of *Alpinia oxyphylla* seeds were placed in the 5-L extraction tank in a temperature-controlled system. The CO_2 flow rate of the system was set at 1 L/min

[a] Treatments were run in random order

[b] Numbers in parentheses represent actual experimental values

[c] Each run was performed twice, and the yield shown is the average (±SD) of duplicate experiments

$$Y = \beta_0 + \sum_{i=1}^{3} \beta_i \chi_i + \sum_{i=1}^{3} \beta_{ii} \chi_i^2 + \sum_{i=1}^{2} \sum_{j=i+1}^{3} \beta_{ij} \chi_i \chi_j \quad (3)$$

where Y is the yield of the designated variables, β_0, β_i, β_{ii}, and β_{ij} are constant coefficients, and x is the actual (un-coded) value of the independent variable. The subscripts 1, 2, and 3 denote the reaction time, the extract temperature, and the pressure, respectively. The ridge-max option was employed to compute the estimated ridge of the maximum response for increasing radii from the center of the original design.

Results and Discussion

Preliminary Experiments

Before generating a complete design for the optimization of the extraction of essence oil from *Alpinia oxyphylla* seeds and a model for predicting the extraction yield, a preliminary study was conducted to explore the effects of extraction pressure, temperature, and time on the yield of *Alpinia oxyphylla* seed essence oil.

Initially, the dried *Alpinia oxyphylla* seed small particles were extracted in SC-CO_2 at 45 °C for 30 min under seven different pressures (10, 15, 20, 25, 30, 35, and 40 MPa), and the yield for each extraction was determined. As shown in Fig. 1, extraction pressure dramatically affected the yield. With the exception of the extraction at 40 MPa, the extraction yield increased as the pressure increased and reached a maximum at 30 MPa or 35 MPa (1.59%). The yield decreased somewhat at 40 MPa, yet it still was twice of that obtained at 10 MPa (1.4 vs. 0.7%). It appears that high pressure favors the extraction yield. Danh et al. [26] indicated that low pressures resulted in a decrease in the oil yield; however, at high pressures, the oil yield increased with temperature. For example, as pressure increased from 10 to 20 MPa, the oil yield increased from 0.64 to 0.89% at 40 °C and from 0.35 to 1.22% at 50 °C.

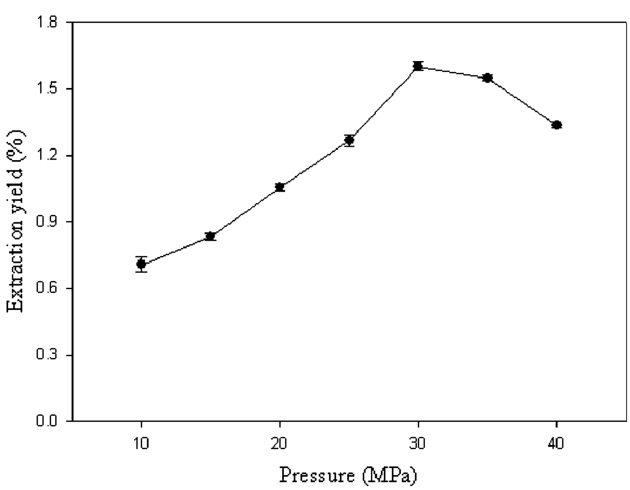

Fig. 1 Effect of SC-CO_2 extraction pressure on the mean (±SD) ($n = 2$) essence oil yield of *Alpinia oxyphylla* seed with SC-CO_2 at 45 °C for 30 min and a CO_2 flow rate at 1 L/min

Next, the effect of temperature on the extraction yield was evaluated. The essence oil of dried small particles of the *Alpinia oxyphylla* seeds was extracted in SC-CO_2 at 35 MPa for 30 min at three different temperatures (45, 50, and 55 °C), and the yield for each extraction was determined. The extraction yields were 1.60 ± 0.01%, 1.75 ± 0.01%, and 1.84 ± 0.01% at temperatures of 45, 55, and 65 °C, respectively. This result indicated that the extraction yield increased only slightly as the extraction temperature increased. In another study, the extraction was conducted at 45 °C and 35 MPa for three different times (30, 60, and 90 min), and the results showed that the extraction yields for the three extraction times did not differ significantly (data not shown).

Optimization of the Extraction Conditions and Model Fitting

The main objective of this study was to develop a statistical model to better understand the relationships between the manipulated variables for the extraction of *Alpinia oxyphylla* essence oil in SC-CO_2 and the response for extraction yield of the essence oil. Compared to the abovementioned preliminary study, which dealt with only the three parameters (time, pressure, and temperature) consecutively, RSM (as employed in this study) dealt with all the experimental treatments simultaneously. RSM was more efficient in reducing the number and time of experiments for optimizing the production of essence oil of the *Alpinia oxyphylla* seed. The conditions and the response values from the experimental Box-Behnken design are listed in Table 1. The manipulated and response (dependent) variables were analyzed to obtain a regression Eq. (3) (model) that could predict the response within the given ranges of the independent variables.

The analysis of variance (ANOVA), as summarized in Table 2, indicated that the second-order polynomial model was highly significant and adequate to represent the actual relationship between the response (yield) and the independent variables, as revealed by a small p value (0.0373) and a high coefficient of determination (R^2=0.9085). From the SAS output of RSREG, the second-order polynomial model, Eq. (4), is given below:

$$\begin{aligned} Y = {} & 1.283125 + 0.174167x_1 - 0.125917x_2 + 0.017375x_3 \\ & + 0.183333x_1^2 - 0.002750x_1x_2 + 0.001908x_2^2 \\ & - 0.0019x_1x_3 - 0.000177x_2x_3 - 0.000001667x_3^2 \quad (4) \end{aligned}$$

Table 2 ANOVA of independent variables performed for the response surface analysis of extraction yield

Source	Degrees of freedom	Sum of squares	Probability > F^a
Linear	3	1.420375	0.0099
Quadratic	3	0.247143	0.2179
Cross-product	3	0.273450	0.1911
Total model	9	1.940968	0.0373
Lack of fit	3	0.193525	0.0143
Pure error	2	0.001867	
Total error	5	0.195392	
R^2	0.9085		

Alpinia oxyphylla essence oil was extracted at a 1 L/min CO_2 flow rate, and the conditions of the 3-factor-3-level Box-Behnken experimental design were time (1, 2, 3 h), temperature (35, 45, 55 °C), and pressure (20, 30, 40 MPa)

[a] Probability > F = level of significance (Probability = 0.05)

where x_1, x_2, and x_3 represent reaction time, extraction temperature, and pressure, respectively.

The values of the yield from the experiments were plotted against those calculated from Eq. (4), and the result is shown in Fig. 2. It indicates that all of the experimental values correspond to the expected values within the 95% confidence interval. The overall effect of the three variables on the yield of essence oil was further analyzed by a joint test using the data from Table 1, and the results are shown in Table 3. The results in Table 3 indicate that the reaction pressure (x_3) was the most important parameter and exerted a statistically significant overall effect ($P < 0.05$) on the extraction yield of essence oil of *Alpinia oxyphylla* seed.

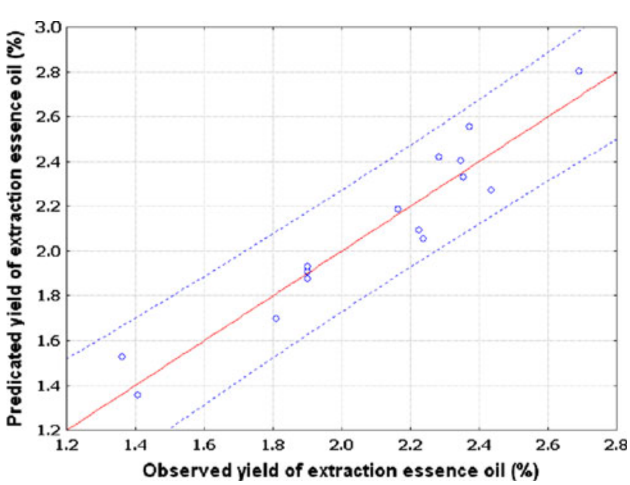

Fig. 2 Correlation between the calculated (predicted) yield (%) and the experimental mean ($n = 2$) yield (%) of SC-CO_2 extraction of the *Alpinia oxyphylla* seed

The relationships between the reaction parameters and the response can be better understood by examining the series of planned contour plots generated from the model (Eq. 4) by holding either the pressure (Fig. 3), the reaction temperature (Fig. 4), or the extraction time constant (Fig. 5). Figure 3 shows the contour plots of yield from the extraction of *Alpinia oxyphylla* essence oil in SC-CO_2 at extraction pressures of either 20, 30, or 40 MPa. The plot clearly indicates that at a fixed temperature to obtain a fixed yield, extraction time could be reduced by increasing extraction pressure. For example, to obtain a yield of 2.1% at 55 °C, extraction time was reduced from 3 to 2 h by increasing the pressure from 20 to 40 MPa. Figure 3 also indicates that at a fixed temperature and extraction time, the yield increased as the pressure increased.

Figure 4 shows the contour plots of yield for the extraction of *Alpinia oxyphylla* essence oil in SC-CO_2 at an extraction temperature of either 45, 55, or 65 °C. The plots show that at 45 °C, the yield increased as the extraction time increased and/or the extraction pressure increased. At 55 and 65 °C, this phenomenon was not significant.

Figure 5 shows the contour plots of yield for the extraction of *Alpinia oxyphylla* essence oil in SC-CO_2 for 1, 2, or 3 h. The yield increased as the extraction temperature and/or the extraction pressure increased for the 1-h extraction. For the 2 and 3 h extraction, this phenomenon was not significant.

Gomes et al. [20] extracted rose geranium oil in SC-CO_2 with an experimental design similar to ours and found that with an extraction temperature of 60 °C and an extraction time of 120 min, the extraction yield increased more than tenfold as the extraction pressure increased from 8 to 16 MPa. Their results also indicated that extraction temperature and reaction time did not significantly affect the yield. It appears that a higher pressure allowed the system to achieve a higher CO_2 density and thus a higher solvent power for extraction.

The optimum point of extraction was determined by ridge max analysis. The method of ridge analysis computes

Table 3 The extraction of *Alpinia oxyphylla* essence oil was estimated in a joint test via response surface analysis of an SAS system

Factor	Degrees of freedom	Sum of squares	Probability > F^a
Time	4	0.549040	0.1004
Temperature	4	0.773564	0.0547
Pressure	4	0.904263	0.0407

Alpinia oxyphylla essence oil was extracted at a 1 L/min CO_2 flow rate, and the conditions of the 3-factor-3-level Box-Behnken experimental design were time (1, 2, 3 h), temperature (35, 45, 55 °C), and pressure (20, 30, 40 MPa)

[a] Probability > F = level of significance (Probability = 0.05)

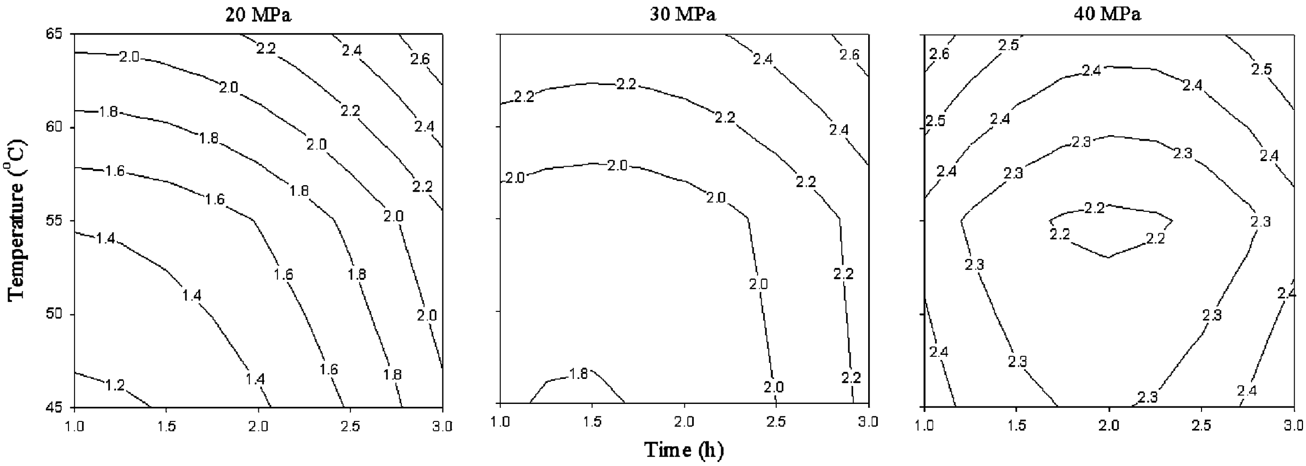

Fig. 3 Contour plots of the mean ($n = 2$) essence oil yields from SC-CO_2 extraction of the *Alpinia oxyphylla* seed at pressures of 20 MPa (**a**), 30 MPa (**b**), and 40 MPa (**c**). *Numbers* inside the contour plots indicate the extraction yield of the essence oil for the indicated reaction conditions

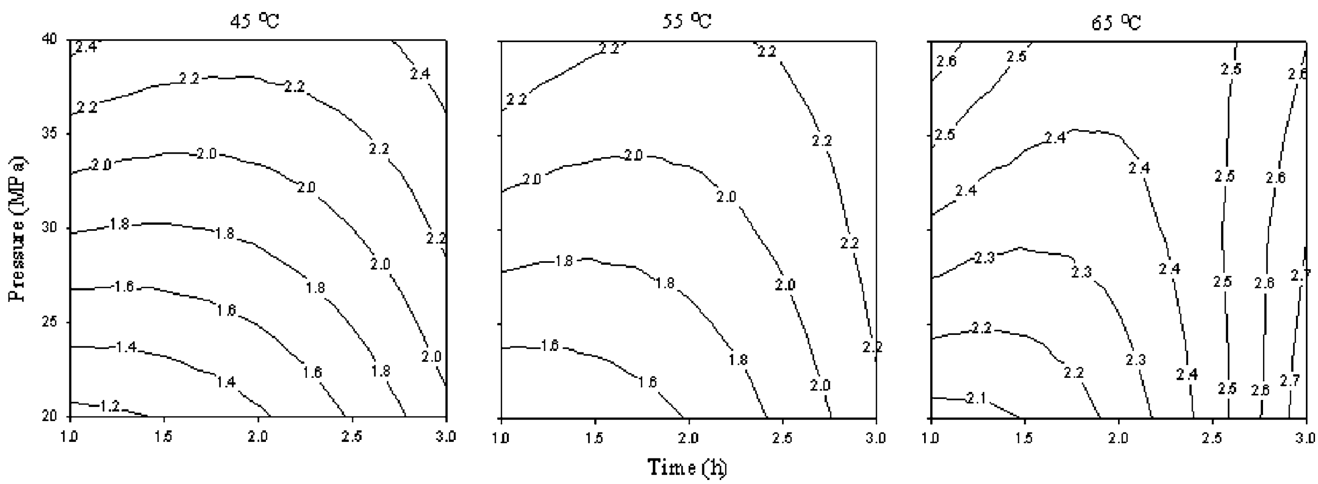

Fig. 4 Contour plots of the mean ($n = 2$) essence oil yields from SC-CO_2 extraction of the *Alpinia oxyphylla* seed at reaction temperatures of 45 °C (**a**), 55 °C(**b**), and 65 °C (**c**). *Numbers* inside the contour plots indicate the extraction yield of the essence oil for the indicated reaction conditions

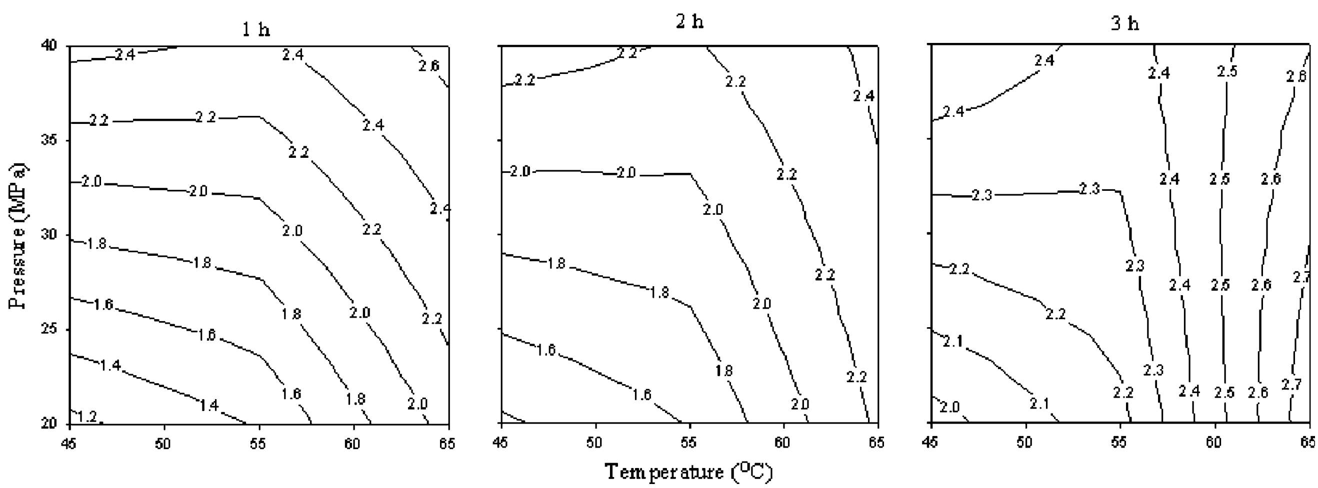

Fig. 5 Contour plots of the mean ($n = 2$) essence oil yields from SC-CO_2 extraction of the *Alpinia oxyphylla* seed at extraction times of 1 h (**a**), 2 h (**b**), and 3 h (**c**). *Numbers* inside the contour plots indicate the extraction yield of the essence for the indicated reaction conditions

Table 4 The estimated ridge of maximum response for the yield of *Alpinia oxyphylla* seed essence oil was extracted at certain conditions in SC-CO_2, and the data analysis was performed with an SAS system

Coded radius	Uncoded factor values			Estimated response[a]
	Time (h)	Temperature (°C)	Pressure (MPa)	
0.0	2.0	55.0	30.0	1.90
0.5	2.3	58.9	31.7	2.12
1.0	2.5	63.4	30.4	2.39
1.5	2.8	67.5	28.5	2.78

[a] Estimated response was the calculated (predicted) yield for the indicated conditions

the estimated ridge of the maximum response for increasing radii from the center of the original design. The ridge max analysis (Table 4) indicates an optimal yield of 2.78% with a reaction temperature of 67.5 °C, an extraction pressure of 28.5 MPa, and an extraction time of 2.8 h. By conducting the extraction under these optimal conditions, a yield of 2.77 ± 0.19% was obtained.

Conclusions

A model for predicting the yield of essence oil from the extraction of *Alpinia oxyphylla* seeds in SC-CO_2 was successfully developed by RSM. Based on the model, an optimal extraction condition (including extraction temperature) of 67.5 °C, extraction pressure of 28.5 MPa, and extraction time of 2.8 h was obtained. Under these optimal conditions, a yield of 2.78% can be expected. Extraction of *Alpinia oxyphylla* essence oil in SC-CO_2 under these optimal conditions was conducted, and an average yield of 2.77 ± 0.19% was obtained. By ANOVA analysis, we found that the extraction pressure was the most important parameter affecting the extraction yield. The model developed and the optimal conditions obtained in this study can be applied to the large-scale production of essence oil from *Alpinia oxyphylla* seeds using SC-CO_2.

References

1. Shoji N, Umeyama A, Takemoto T, Ohizumi Y (1984) Isolation of a cardiotonic principle from *Alpinia oxyphylla*. Planta Med 50:186–187
2. Shoji N, Umeyama A, Asakawa Y, Takemoto T, Nomoto K, Ohizumi Y (1984) Structural determination of nootkatol, a new sesquiterpene isolated from *Alpinia oxyphylla* Miquel possessing calcium-antagonistic activity. J Pharm Sci 73:843–844
3. Sakai K, Oshima N, Kutsuna T, Miyazaki Y, Nakajima H, Muraoka T, Okuma K, Nishino T (1986) Pharmaceutical studies on crude drugs. I. Effect of the Zingiberaceae crude drug extracts on sulfaguanidine absorption from rat small intestine. Yakugaku Zasshi J Pharm Soc Jpn 106:947–950
4. Itokawa H, Watanabe K, Mihashi S (1979) Screening test for antitumor activity of crude drugs I. Shoyakugaku Zasshi 33:95–102
5. Lee EY, Park KK, Lee JM, Chun KS, Kang JY, Lee SS, Surh YJ (1998) Suppression of mouse skin tumor promotion and induction of apoptosis in HL-60 cells by *Alpinia oxyphylla* Miquel (Zingiberaceae). Carcinogenesis 19:1377–1381
6. Chun KS, Park KK, Lee J, Kang M, Surh YJ (2002) Inhibition of mouse skin tumor promotion by anti-inflammatory diarylheptanoids derived from *Alpinia oxyphylla* Miquel (Zingiberaceae). Onco Res 13:37–45
7. Chun KS, Kang JK, Kim OH, Kang H, Surh YJ (2002) Effects of yakuchinone A and yakuchinone B on the phorbol ester-induced expression of COX-2 and iNOS and activation of NF-kappa B in mouse skin. J Environ Pathol Toxicol Oncol 21:131–139
8. Shin TY, Won JH, Kin HM, Kim SH (2001) Effect of *Alpinia oxyphylla* fruit extract on compound 48/80-induced anaphylactic reactions. Am J Chin Med 29:293–298
9. Morikawa T, Matsuda H, Toguchida I, Ueda K, Yoshkawa M (2002) Absolute stereo structures of three new sesquiterpenes from the fruit of *Alpinia oxyphylla* with inhibitory effects on nitric oxide production and degranulation in RBL-2H3 cells. J Nat Prod 65:1468–1474
10. Kubo M, Matsuda H, Suo T, Yamanaka J, Sakanaka M, Yoshimura M (1995) Study on Alpiniae fructus. I. Pharmacological evidence of efficacy of Alpiniae fructus on ancient herbal literature. Yakugaku Zasshi J Pharm Soc Jpn 115:852–862
11. Lin JM, He W, Wu Y (2000) GC-MS analysis of essential oil components of *Alpinia oxyphylla*. J Chin Med Mater 23:448–453
12. Luo XZ, Yu JG, Xu LZ, Li KM, Tan P, Feng JD, Yang SL, Feng JD, Ou SL (2001) Chemical constituents in volatile oil from fruits of *Alpinia oxyphylla* Miquel. J Chin Mater Media 26:262–264
13. Yamahara J, Li YH, Tamai Y (1990) Anti-ulcer effect in rats of bitter cardamon constituents. Chem Pharm Bull 38:3053–3054
14. Luo XZ, Yu JG, Xu LZ, Li KM, Tan P, Feng JD (2000) Studies on the chemical constituents of the fruits from *Alpinia oxyphylla*. Acta Pharmacol Sin 35:204–207
15. Roy BC, Goto M, Kodama A, Hirose T (1996) Supercritical CO_2 extraction of essential oils and cuticular waxes from peppermint leaves. J Chem Technol Biotechnol 67:21–26
16. Gaspar F, Santos S, King MB (2000) Extraction of essential oils and cuticular waxes with compressed CO_2: effect of matrix pretreatment. Ind Eng Chem Res 39:4603–4608
17. Talansier E, Braga MEM, Rosa PTV, Paolucci-Jeanjean D, Meireles M, Angela A (2008) Supercritical fluid extraction of vetiver roots: A study of SFE kinetics. J Supercrit Fluids 47:200–208
18. Leitner W (2002) Supercritical carbon dioxide as a green reaction medium for catalysis. Acc Chem Res 35:746–756
19. Grosso C, Ferraro V, Figueiredo AC, Barroso JG, Coelho JA, Palavra AM (2008) Supercritical carbon dioxide extraction of volatile oil from Italian coriander seeds. Food Chem 111:197–203
20. Gomes PB, Mata VG, Rodrigues AE (2007) Production of rose geranium oil using supercritical fluid extraction. J Supercrit Fluids 41:50–60

21. Shao P, Sun P, Ying Y (2008) Response surface optimization of wheat germ oil yield by supercritical carbon dioxide extraction. Food Bioprod Process 86:227–231
22. Otto M (1999) Chemometrics: statistics and computer applications in analytical chemistry. Wiley, Chichester
23. Hanrahan G, Zhu J, Gibani S, Patil DG (2005) Chemometrics: experimental design. In: Worsfold PJ, Townshend A, Poole CF (eds) Encyclopedia of analytical science. Oxford 2:8–13
24. Hanrahan G, Lu K (2006) Application of factorial and response surface methodology in modern experimental design and optimization. Crit Rev Anal Chem 36:141–151
25. SAS (1990) SAS user guide. SAS Institute Inc., Cary
26. Danh LT, Mammucari R, Truong P, Foster N (2009) Response surface method applied to supercritical carbon dioxide extraction of *Vetiveria zizanioides* essential oil. Chem Eng J 155:617–626