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Optimization of Supercritical Co_2 Extraction of Swietenia Mahagoni Seed by Response Surface Methodology

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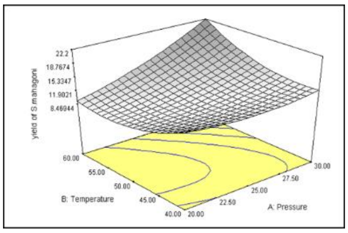
Graphical abstract



Abstract

Three operating parameters were pressure, temperature and particle size of supercritical carbon dioxide extraction of oil from *Swietenia mahagoni* have been optimized by response surface methodology to obtain high yield of oil. Results showed that data were adequately fitted into the second-order polynomial model. The linear and quadratic terms of independent variables of temperature, pressure and particle size have significant effects on the oil yield. Optimum conditions for oil yield within the experimental range of the studied variables were 29.99 MPa, 55.29ºC and 0.75 mm, and the oil yield was predicted to be 20.76%.

Keywords: *Swietenia mahagoni* seed; supercritical CO_2 extraction; response surface methodology

Abstrak

Minyak *Swietenia mahagoni* telah diekstrak menggunakan kaedah superkritikal karbon dioksida di mana tiga parameter iaitu tekanan, suhu dan sais zarah telah dioptimumkan menggunakan teknik gerak balas permukaan untuk mendapat hasil minyak yang tinggi. Keputusan telah diperolehi dengan memasukkan data kedalam model polinomial tertib kedua. Terma linear dan kuadratik menunjukkan bahawa pemboleh ubah tekanan, suhu dan saiz zarah mempunyai peranan yang penting pada penghasilan minyak. Keadaan optimum dalam julat eksperimen ialah 29.99 MPa, 55.29℃, 0.75 mm dan hasil minyak yang diramal ialah 20.76%.

Kata kunci: Benih Swietenia mahagoni; pengeluaran CO_2 superkritikal; kaedah permukaan response

■1.0 INTRODUCTION

Swietenia mahagoni (Linn.) Jacq. (Meliaceae) grows mainly in tropical areas of Asia, such as India, Malaysia, Indonesia and southern mainland China. The seeds have been applied as traditional medicine for treatment of hypertension, diabetes, and malaria, while the decoction of its bark has been used as a febrifuge [1]. The therapeutic effects associated with the seeds are mainly caused by the biologically active ingredients, fatty acids and tetranortriterpenoids [2]. There are reports of *S. mahagoni* seeds having anti-inflammatory, antimutagenicity, and antitumour activities [3]. The plant extracts have been accounted to possess antibacterial and antifungal activities. Limnoid obtained from *S.mahagoni* has antifungal activity and diabetes therapy [4]. The seeds of *S. mahagoni* are good agricultural products and have been found to be potentially rich in fat (64.9%) [5].

Conventional procedures for the extraction of plant materials include hydrodistillation and organic solvent extraction using percolation, maceration or Soxhlet techniques. However, there are drawbacks with these methods such as time and labour consuming operation, and involves large volumes of hazardous solvents. Nevertheless, there is increasing interest in alternative extraction methods that consume smaller quantities of organic solvent due to the rising solvent acquisition and disposal costs,as well as regulatory restrictions [6]. Therefore, it is highly desired to develop alternative extraction techniques with better selectivity and efficiency. Consequently, supercritical fluid extraction (SFE) as an environmentally responsible and efficient extraction technique for solid materials was introduced and extensively studied for separation of active compounds from herbs and other plants [7].

Carbon dioxide (CO_2) is the most common choice of supercritical fluid due to its advantages of being non-toxic, non-flammable, cost effective, and can be easily removed from the extract following decompression [8, 9]. Currently, SFE has become an acceptable extraction technique and being used in many different areas. SFE of active natural products from herbs or more generally, from plant materials has become one of the most important areas of application. Supercritical carbon dioxide (SC-CO_2) was successfully used in the extraction of edible oils from a wide range of seeds, including amaranth [10], hiprose [11], cuphea [12], flax [13], sunflower and rape [14].

Response surface methodology (RSM), originally described by Box and Wilson [15], is a collection of mathematical and statistical techniques useful for modeling and analysis of problems in which a response of interest is influenced by several variables and the objective is to optimize this response [16]. Recently, RSM has been successfully applied to optimize SC-CO_2extraction of oils from *Salvia mirzayanii*[17], silkworm pupae [18], *Passiflora seed* [19], wheat germ [20], cottonseed [21], *Curcuma longa* [22], rosehip seed [23], *Cyperus rotundus*[24] and amaranth seed [25].

In the present study, SC-CO_2 was used to extract the oil from *S.mahagoni* seed. The aim was to optimize the processing conditions, including pressure, temperature and particle size by applying response surface methodology. The response variables were examined with respect to the yield of oil under different operating conditions.

2.0 MATERIALS AND METHODS

2.1. Plant Material Preparation

S.mahagoni seeds were collected from Indonesia. The seeds were rinsed with tap water to remove any foreign particles and dirt prior to drying. Then, the cleaned seeds were cut into small pieces and dried in an oven at the temperature of 50ºC for one week to remove the moisture. The seeds were then ground by a blender MX-337 (Panasonic Malaysia Sdn Bhd).

2.2 Supercritical CO_2 Extraction

A schematic diagram of SFE apparatus for extraction of *S.mahagoni* seed is illustrated in Figure 1. The ground sample of 5 g was placed in an extractor vessel. The extracts were collected in a glass vial placed in the separator at ambient temperature and pressure. A flow rate of CO_2 was 2 mL/min. The investigated values of pressure, temperature, and particle size were varied from 20 to 30 MPa, 40 to 60ºC, and 0.25 to 0.75 mm, respectively. After each extraction, the obtained extract was placed into glass vials, sealed and stored at 4ºC to prevent any possible degradation.

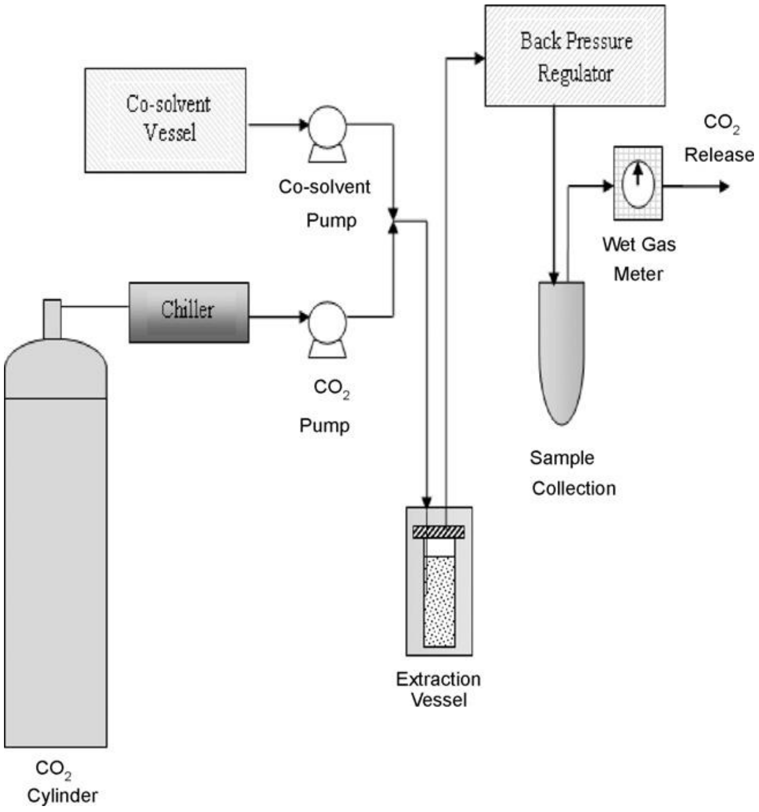


Figure 1 A schematic design of the supercritical fluid extraction (SFE) unit

2.3 Experimental Design

Box-Behnken design (BBD) was applied to determine the optimum extraction pressure, temperature and particle size for supercritical CO_2 extraction of *Swietenia mahagoni* seed. The pressure (A), temperature (B) and particle size (C) were independent variables studied to optimize the oil yield (Y) from *S.mahagoni* seed. The CO_2 flow rate was constant.

Box-Behnken design requires an experiment number (N) according to the following equation:

$$N = 2k(k-1)+c_p \qquad (1)$$

Where k is the factor number and c_p is the replicate number of the central point. There are three levels of design (-1, 0, +1) with equally spaced intervals between these levels. The investigated factors and tested levels are reported in Table 1.

Table 1 The coded and uncoded levels of independent variables used in RSM design

Independent variable	Symbol	Level		
		Low (-1)	Middle (0)	High (+1)
Pressure (MPa)	A	20	25	30
Temperature (°C)	B	40	50	60
Particle size (mm)	C	0.25	0.50	0.75

The experimental data were fitted with the second order response surface model of the following form:

$$y = \beta_o + \sum_{i=1}^{k} \beta_i X_i + \sum_{i \leq i}^{k} \beta_{ij} X_i X_j \qquad (2)$$

Where y is the response (extraction yield in %); β_0, β_j, β_{jj}, β_{ij} are constant coefficients of intercept, linear, quadratic, and interaction terms, respectively;and X_i and X_j are coded independent variables (pressure, temperature or particle size). Analysis was performed using commercial software Design-Expert® v.6.0.4

The analysis of variance (ANOVA) was also used to evaluate the quality of the fitted model. The test of statistical difference was based on the total error criteria with a confidence level of 95%.

■3.0 RESULTS AND DISCUSSION

3.1 Response surface analysis

Since various parameters potentially affect the extraction process, the optimization of the experimental conditions represents a critical step in the application of SFE method. The experimental design was adopted on the basis of coded level from three variables (Table 1), resulting in seventeen simplified experimental sets (Table 2) with five replicates for the central point. The selected factors were extraction temperature (in °C), pressure (in MPa) and particle size (in mm) with consideration that these factors are important in the extraction process.

The effect of linear, quadratic or interaction coefficients on the response was tested for significance by analysis of variance (ANOVA). The degree of significance of each factor are represented in Table 3 by its probability (Prob) > F. When the values of "Prob>F" was less than 0.05, the factor has a significant influence on the process (for a confidence level of 95%) [23].

Table 3 shows that the linear term of pressure and particle size had significant effect on the extraction yield, followed by the quadratic term of temperature and interaction of pressure and temperature. Meanwhile, the interaction between pressure and particle size, and temperature and particle size on the yield of *S. mahagoni* seed extraction were not statistically significant ("Prob>F" more than 0.05).

Table 2 Experimental matrix and values of the observed response

Run	Pressure (MPa)	Temperature (°C)	Particle size (mm)	Coded pressure variable	Coded temperature variable	Coded particle size	Observed extraction yield (%)
1	20	40	0.50	-1	-1	0	15.52
2	30	40	0.50	+1	-1	0	15.47
3	20	60	0.50	-1	+1	0	7.76
4	30	60	0.50	+1	+1	0	20.68
5	20	50	0.25	-1	0	-1	6.19
6	30	50	0.25	+1	0	-1	11.64
7	20	50	0.75	-1	0	+1	10.97
8	30	50	0.75	+1	0	+1	18.26
9	25	40	0.25	0	-1	-1	5.07
10	25	60	0.25	0	+1	-1	13.42
11	25	40	0.75	0	-1	+1	16.35
12	25	60	0.75	0	+1	+1	17.74
13	25	50	0.50	0	0	0	11.12
14	25	50	0.50	0	0	0	10.91
15	25	50	0.50	0	0	0	10.52
16	25	50	0.50	0	0	0	11.04
17	25	50	0.50	0	0	0	10.82

The second order polynomial model used to express the total extraction yield as a function of independent variables (in terms of coded values) is shown below:

$$Y=149.623-5.381A-3.861B+45.736C+0.051A^2+0.026B^2-6.636C^2+0.064AB+0.368AC-0.696BC \qquad (3)$$

Table 3 Response surface of yield obtained by SC-CO_2

Variable	Coefficients	Standard error	F-value	Prob>F
Intercept	10.88	0.78		
A	3.20	0.62	26.94	0.0013
B	0.90	0.62	2.12	0.1884
C	3.38	0.62	29.94	0.0009
A^2	1.30	0.85	2.33	0.1707
B^2	2.68	0.85	9.92	0.0162
C^2	-0.41	0.85	0.24	0.6406
AB	3.24	0.87	13.82	0.0075
AC	0.46	0.87	0.28	0.6142
BC	-1.74	0.87	3.98	0.0863

Table 4 Analysis of variance (ANOVA) for the response surface quadratic model for the yield of *S.mahagony* seed obtained by SC-CO_2 extraction

Source	Sum of squares	Degree of freedom	Mean square	F-value	Prob>F
Model	273.67	9	30.41	9.99	0.0031
Residual	21.30	7	3.04		
Lack of fit	21.09	3	7.03	129.40	0.0002
Pure error	0.22	4	0.054		
Total	294.98	16			

Analysis of variance (ANOVA) results of the model are shown in Table 4. The regression model for the oil yield was significant with “prob>F” of less than 0.05 and satisfactory coefficient of determination (R^2) of 0.9278. Three dimensional (3D) response surface plots as a function of two factors while keeping other factors at fixed levels are helpful in understanding the effect on the response and the interaction effects of these two factors [27]. Thus, in order to gain a better understanding of the influence of independent variables and their interactions on the dependent variable, 3D response surface plots for the measured responses were produced based on the model equation (3) in this study. Figure 2-4 show the 3D response surface plots as the functions of two variables with another variable is kept constant at the center level.

Figure 2 illustrates the response surface and contour plot for the influence of pressure and temperature on the yield of oil for a fixed 0.50 mm particle size. The yield increased at lower pressure and temperature. Lower pressure had a positive linear effect on the oil yield. As the pressure was raised, the density of SC-CO_2 would increase, resulting in enhanced solubility of SC-CO_2. However, there was a negative quadratic effect at high pressure (Table 3). A possible reason was that highly compressed CO_2 facilitates solute-solvent repulsion [18]. Thus, high pressure is not always recommended, as it can potentially induce complex extraction and complicate the analysis [27]. The effect of particle size was not important on the total extract yield for particle size of 0.75 mm; however the oil yield increased by increasing particle size (Figure 3). Particle size needs to be evaluated case by case based on the type of material to be processed. In the case of spice and seeds processing, particle size is generally between 30 and 60 Mesh [28]. According to previous investigations [29, 30], we came to a conclusion that particle size has no influence on the extraction rate in the two outermost cases: fine milled material and coarsely ground plant material. Effects of temperature and particle size on the oil yield at a fixed pressure of 25 MPa are shown in Figure 4. Particle size had positive linear effects on the yield, while the interaction between particle size and temperature was negatively correlated with yield.

It was more difficult to predict the influence of temperature on SC-CO_2 extraction than for pressure because temperature has two opposing effects on the yield of oil. As the temperature increases, the density of CO_2 decreases, which leads to a reduced solvent power. Conversely, higher temperature increases the solute vapour pressure, resulting in enhanced SC-CO_2 solubility. Hence, the solubility of SC-CO_2 is inclined to increase, remain constant, or decrease with increasing temperature at constant pressure, depending whether solute vapour pressure or solvent density dominates [17, 31].

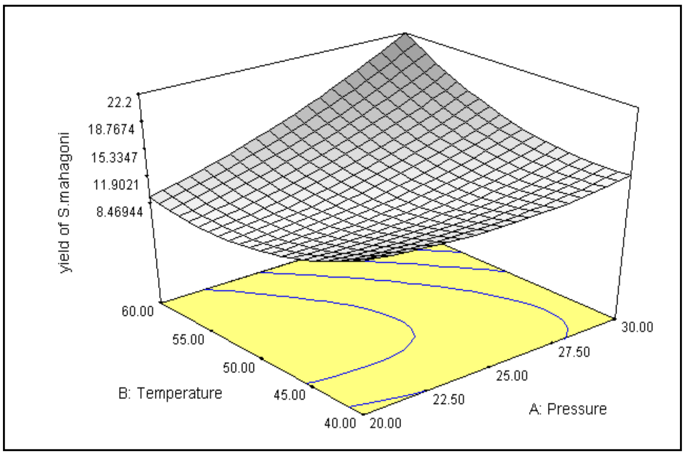


Figure 2 Surface plot of oil yield from *S.mahagoni* as a function of pressure and temperature at constant particle size of 0.50 mm

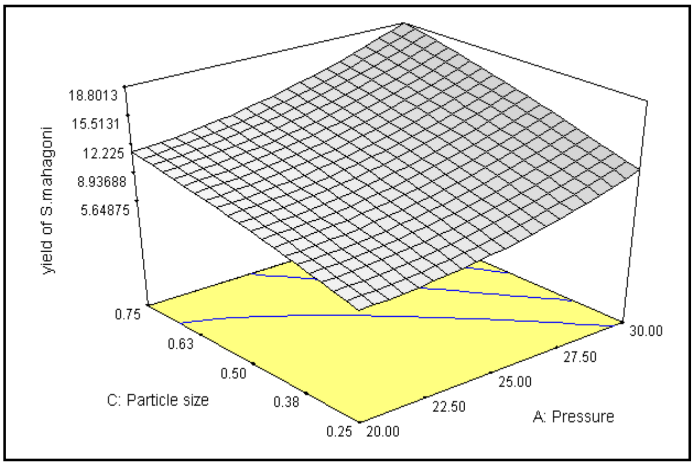


Figure 3 Surface plot of oil yield from *S.mahagoni* as a function of pressure and particle size at constant temperature of 50ºC

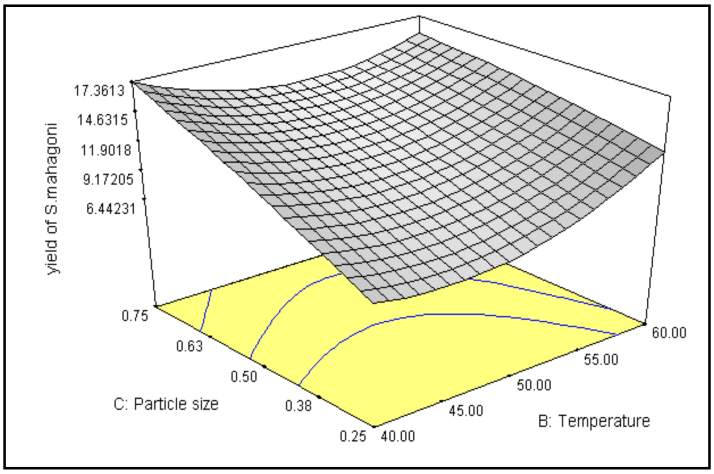


Figure 4 Surface plot of oil yield from *S.mahagoni* as a function of temperature and particle size at constant pressure of 25 MPa

3.2 Optimization of Extraction Parameters

In general, reducing the independent variable will optimize the ratio of oil yield to production cost. The independent variable was further minimized for optimum oil yield using the numerical optimization function of the Design Expert programme. The numerical range specified was set to minimize temperature between 40 and 60^{o}C, pressure between 20 and 30 MPa, and particle size between 0.25 and 0.75 mm, while maximizing oil yield to 5.07-20.76%. Design Expert programme indicated that for an oil yield of 20.76%, the optimum values for each independent variable were temperature of 55.29^{o}C, pressure of 29.99 MPa, and particle size of 0.75 mm. Under these optimum conditions, the experimental value was 20.07%, which is in agreement with those predicted by Design Expert programme.

■4.0 CONCLUSION

Current results showed that second-order polynomial model was sufficient to describe and predict the response variable of the *Swietenia mahagoni* seed yield obtained by SC-CO_2 extraction within the experimental ranges. The linear and quadratic terms of pressure, temperature and particle size significantly affected the yield. Based on the proposed model, the optimum conditions for *Swietenia mahagoni* seed yield within the experimental range were found to be 29.99 MPa, 55.29 ^{o}C and 0.75 mm, and the predicted yield was found to be 20.76 %. Under these optimum conditions, the experimental values were in agreement with the predicted values.

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