# Optimization of Supercritical $CO_2$ Extraction Process to Improve the Quality of Patchouli Oil by Response Surface Methodology Approach

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## ABSTRACT

*Until now, the patchouli oil is the most substantial commodity export of essential oil for Indonesia. But the price of patchouli oil is often unstable due to the quality of oil which fluctuates depending on the components. To improve the performance and quality of patchouli oil had been carried out the purification process using supercritical $CO_2$ fluid extraction method. Optimization conditions of the extraction process were calculated using the approach of Response Surface Methodology (RSM) including the influence of independent variables such as temperature (35–45 °C), pressure (80–150 atm) and the time of extraction (60–300 min).The optimum condition was attained by using a Box-Behnken Design (BBD). Analysis of the components in the extract was carried out by using GC-MS and GC-FID to detect the changes of compositions of the oil components before and after the extraction process. The optimum condition of supercritical extraction within experimental range of the studied variables was at 38°C, 146.35 atm and 74 min for predicted oil yield of 6.41% and at 41.45 °C, 135.17 atm and 252.62 min and the predicted patchouli alcohol content was 25.34%. The extracted oil is enriched with the main components such as patchouli alcohol compared to the original patchouli oil. The results of RSM evaluation showed consistency between the variables contained in the experiment and the prediction.*

***Keywords***: *supercritical $CO_2$; patchouli oil; Response Surface Methods (RSM)*

## ABSTRAK

*Sampai sekarang minyak nilam adalah komoditas eksport paling besar dari minyak atsiri Indonesia. Tetapi harga pasar minyak nilam ini seringkali tidak stabil dan mengalami fluktuasi yang disebabkan oleh kualitas dan komposisi dari komponen-komponennya. Untuk memperbaiki kualitas minyak nilam telah dilakukan proses esktraksi dengan menggunakan metoda ekstraksi fluida $CO_2$ superkritik. Kondisi optimum proses ekstraksi diperhitungkan melalui pendekatan Metoda Permukaan Respon (MPR) dan pengaruh variable-variabel bebas meliputi temperatur (35–45 °C), tekanan (80–150 atm) dan waktu ekstraksi (60–300 menit), kemudian dievaluasi dengan menggunakan Box-Behnken Design (BBD). Komposisi komponen-komponen dalam esktrak dianalisis menggunakan GC-MS dan GC-FID baik sebelum maupun sesudah proses ekstraksi. Proses ekstraksi $CO_2$ superkritik pada kisaran variabel yang dipelajari menunjukkan bahwa pada 38 °C, 146.35 atm dan 74 menit menghasilkan ekstrak minyak nilam optimum sebesar 6,41% sedangkan pada 41,45 °C, 135,17 atm dan 252,62 menit menghasilkan kandungan patchouli alkohol optimum sebesar 25.34%.*

***Kata Kunci***: *$CO_2$ superkritik; minyak nilam; Metoda Permukaan Respon (MPR)*

## INTRODUCTION

In Indonesia, patchouli oil is one of the most valuable commodities of essential oils exclusively exported to other countries for pharmaceutical and perfumes. According to Indonesian National Standard (SNI), the good quality of patchouli oil must be matched with SNI 06-2385-2006 [1-3] in which the physico-chemicals properties involving two type of color (yellow to reddish brown), density (0.950–0.975 at 25 °C), content of patchouli alcohol (min. 30%), and refractive index ($n_D^{20}$ 1.507–1.515). Especially for patchouli alcohol, this constituent is the most important chemical should be present in enough amounts to meet the

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demand of the global market. Unfortunately, patchouli oil produced by traditional refineries in rural and or modern manufacturing is still lack of quality following the requirements of SNI. Therefore, to improve the quality of patchouli oil that be acceptable in the international market, the oil quality must be enhanced by an appropriate method. Thus, selecting the right method for oil refining is a crucial factor. Refining patchouli oil by using distillation or redistillation method is very risky due to the loss of some important components of the oil. In addition, the liquid-liquid extraction method using conventional solvents such as n-hexane, dichloromethane is incompatible with the needs of the perfume, pharmaceutical and cosmetic industries based on patchouli oil. Beside the solvents left some traces of aroma after separation due to toxic to mammals, its use is insufficient. In the advanced process of eco-green extraction, non-toxic supercritical solvent has been widely applied by many herbal and essential oil industries. The supercritical fluid is an unconventional solvent exhibiting gas-like transport properties, and depending on the pressure of solvent that has properties gas-like to liquid-like [4]. So far, extraction process using non-toxic solvent have been conducted to refine products, such as essential oil [5-10], flavor extraction [11], extraction for flavonoids of *Strobilanthes crispus* [12], herbal antioxidant [13-14] solvent for green synthesis [15], refining on palm oil [16-17]. The supercritical $CO_2$ was also used to separate carotenoid from algae, palm oil and several legume [18-20].

Carbon dioxide is often regarded as a sustainable solvent, because $CO_2$ is not flammable, exhibiting relatively low toxicity and is naturally abundant. However, the use of carbon dioxide in any process improperly can reduce rather than increase overall sustainability. Under the supercritical condition, namely the critical temperature of 31 °C and critical pressure of 74 bar (72.2 atm), molecules of $CO_2$ are acted as an excellent nonpolar species [21-22]. Pure supercritical carbon dioxide is a relatively non-polar solvent that only dissolves many compounds which have suitable polarity. The essential oil contains many compounds with polarity from moderate to non-polar. An important aspect of carrying out extraction using supercritical carbon dioxide as the solvent is solubility [23]. These properties are particularly suitable when the extraction of essential oils is carried out by using supercritical $CO_2$ fluids. Optimization of the extraction cycle requires the maintenance of temperature, pressure and time duration of each operation using response surface methodology approach [8,24].

The research would perform a supercritical $CO_2$ fluid extraction to refine crude patchouli oil in order to increase the quality and enrich the patchouli alcohol content. Response surface methodology with Box-Behnken Design (BBD) would be used in this experiment [25,27].

## EXPERIMENTAL SECTION

### Materials

Commercial high pure $CO_2$, All other chemicals were of analytical reagent grade.

### Instrumentation

The extraction process was carried out at the Institute of Chemical Research Center of the Indonesian Institute of Sciences, using a supercritical fluid extractor model 46-19360 by Newport Scientific, Inc. which is equipped with the $CO_2$ gas cylinder, air compressor, high-pressure stainless steel extractor tube, extracted separator, heater, and chiller.

The composition of patchouli oil was determined by gas chromatography using a GC HP5890), equipped with an HP-5MS capillary column (60 m.0.25 mm i.d.; film thickness 0.25 mm) and FID detector. Each sample (2 µL) was injected into port injector that was set at 250 °C. The programmed temperature was set at 50 °C for 5 min, then increased for 10 °C/min to 300 °C and finally kept constant at that temperature for 5 min. The total of analysis time was 35 min. Nitrogen was used as carrier gas, and dry air and hydrogen gas were used as an oxidizing agent in FID detectors.

The result of GC analysis was confirmed with GC-MS analysis using GC-MS Shimadzu QP-2010S, equipped with an RTx-5MS capillary column (60 m.0.25 mm i.d.; film thickness 0.25 mm) Helium was used as the carrier gas. The mass spectrometry (MS) conditions were as follows: scan 40 to 500 amu, threshold 100, MS temperature 150 °C (quad) and 250 °C (source). The constituents of the extract were determined qualitatively based on the retention time and mass spectra using matching with the Wiley 9 MS libraries.

### Procedure

#### *Patchouli oil preparation*

Patchouli oil was prepared through the process of steam distillation of patchouli leaves that harvested from patchouli plantations in the village of Kesamben, Blitar regency of East Java. The leaves were withered for 2 weeks, and then put into a burlap sack and stored for 5 months. The dried patchouli leaves still contain water around 20±0.1% on average. Distillation of dried patchouli leaves was done using steam distillation at a fixed pressure of 2.5 Bar at 100 °C for 8 h. The physicochemical properties of the patchouli oil were characterized involving specific gravity, color and refraction index.

***Supercritical $CO_2$ extraction***

The extraction process was carried out using a supercritical fluid extractor model 46-19360 by Newport Scientific, Inc. which is equipped with the$CO_2$ gas cylinder, air compressor, high-pressure stainless steel extractor tube, extracted separator, heater, and chiller. The $CO_2$ gas was chilled in a chiller (at 5°C) prior to delivering into extractor at 5.5 mL/min. The temperature of extractor was set at 35–45 °C, variously. The pressure of separator was kept at a constant of 500 Psi (34 atm), whiles the pressure of extractor was varied from 80, 115 and 150 atm. At interval time of extraction, the extract was collected and the composition of its component was monitored, especially for patchouli alcohol using Gas Chromatography from HP 5890, and the total ion chromatogram was compromised using Gas Chromatography-Mass Spectrum (GC-MS) from Shimadzu QP-2010S.

***Experimental design of Extraction***

Box–Behnken design (BBD) was applied for determining the optimal condition of temperature, pressure and time for supercritical $CO_2$ extraction of patchouli oil. The temperature ($X_1$), pressure ($X_2$) and extraction time ($X_3$) were independent variables studied to optimize the oil yield (Y) and percentage of patchouli alcohol component. The $CO_2$ mass flow rate value was kept at 5.5 mL/min, the pressure of the extractor was varied at 80, 115 and 150 atm, and the pressure of separator was kept constant at 34 atm. The temperature of extractor was varied at 35, 40 and 45 °C. Then, the extract was collected at 60, 180 and 300 min. Box–Behnken design requires an experiment number (*N*) according to the following eq. 1 [27]:

$$N = 2k(k-1) + C_p \quad (1)$$

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 variables were coded according to eq. 2 [27]:

$$X_i = \left( \frac{Z_i - Z_i^o)}{\Delta Z_i} \right) \beta_d \quad (2)$$

where $Z_i$ is the distance between the real value in the central point and the real value in the superior or inferior level of a variable, $\beta_d$ is the major coded limit value in the matrix for each variable, and $Z°$ is the real value in the central point.

The experimental data were fitted with the second-order response surface model of the following form 3 [24] :

$$Y = \beta_0 + \sum_{j=1}^{k} \beta_j X_j + \sum_{j=1}^{k} \beta_{jj} X_j^2 + \sum\sum_{i<j} \beta_{ij} X_i X_j \quad (3)$$

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

| Independent variable | symbol | level | | |
|---|---|---|---|---|
| | | low (-1) | middle (0) | high (+1) |
| Temperature (°C) | $X_1$ | 35 | 40 | 45 |
| Pressure (atm) | $X_2$ | 80 | 115 | 150 |
| Time (h) | $X_3$ | 60 | 180 | 300 |

**Table 2.** Experimental matrix and values of the observed response of percentage extract yield and patchouli alcohol

| Run | Factor 1 $X_1$ Temperature (°C) | Factor 2 $X_2$ Pressure (atm) | Factor 3 $X_3$ Time (h) | Coded $X_1$ | Coded $X_2$ | Coded $X_3$ | Response 1 Yield (%) | Response 2 Patchouli alcohol (%) |
|---|---|---|---|---|---|---|---|---|
| 1 | 35 | 115 | 300 | -1 | 0 | 1 | 2.19 | 29 |
| 2 | 45 | 150 | 180 | 1 | 1 | 0 | 4.64 | 22.1 |
| 3 | 40 | 80 | 60 | 0 | -1 | -1 | 1.13 | 21.7 |
| 4 | 40 | 150 | 60 | 0 | 1 | -1 | 6.46 | 18.41 |
| 5 | 35 | 80 | 60 | -1 | -1 | -1 | 2.52 | 15.61 |
| 6 | 40 | 150 | 300 | 0 | 1 | 1 | 1.1 | 30.14 |
| 7 | 35 | 115 | 60 | -1 | 0 | -1 | 4.7 | 19.26 |
| 8 | 35 | 80 | 180 | -1 | -1 | 0 | 2.99 | 18.32 |
| 9 | 40 | 115 | 180 | 0 | 0 | 0 | 4.27 | 18.51 |
| 10 | 40 | 80 | 180 | 0 | -1 | 0 | 1.39 | 18.01 |
| 11 | 40 | 150 | 180 | 0 | 1 | 0 | 5.45 | 26.46 |
| 12 | 45 | 115 | 60 | 1 | 0 | -1 | 2.93 | 31.21 |
| 13 | 40 | 80 | 300 | 0 | -1 | 1 | 1.05 | 13.69 |
| 14 | 35 | 150 | 60 | -1 | 1 | -1 | 6.41 | 13.29 |
| 15 | 35 | 150 | 180 | -1 | 1 | 0 | 4.64 | 22.1 |
| 16 | 40 | 115 | 180 | 0 | 0 | 0 | 5.03 | 22.43 |
| 17 | 45 | 150 | 300 | 1 | 1 | 1 | 1.77 | 28.75 |

where *Y* is the response of both yield of extract (in %, v/v) and patchouli alcohol (in % relative area of chromatogram)), $\beta_0$, $\beta_i$, $\beta_{ij}$, and $\beta_{ij}$ are constant coefficients of intercept, linear, quadratic, and interaction terms, respectively. $X_i$ and $X_j$ are coded independent variables (temperature, pressure or time). The analysis was performed using software Design-Expert v.6.0.8 portable academically. For evaluating the quality of the fitted model, the analysis of variance (ANOVA) was used and the statistical difference test is based on the total error criteria with a 95% confidence level.

## RESULT AND DISCUSSION

Since various parameters potentially affect the extraction process, the optimization of the experimental conditions represents a critical step in the development of a supercritical fluid extraction method. The experimental design was adopted by 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 atm) and extraction time (in min) with the consideration that these factors are essential factors in the extraction process. The $CO_2$ mass flow rate value was constant (5.5 mL/min).

The effect of linear, quadratic or interaction coefficients on the response was tested for significance by analysis of variance (ANOVA). Regression coefficients of intercept, linear, quadratic, and interaction terms of the model were calculated using the least square method. The degree of significance of each factor is represented in Table 3 by its p-value both for extract yield (Table 3) and patchouli alcohol content in the extract (Table 4). When the p-value of a factor is less than 0.05, the result has a significant influence on the process (for a confidence level of 0.95). It seems for all variable shows a substantial effect on the extract yield and its patchouli alcohol content. But the quadratic term of temperature and also interaction between temperature and time of extraction were not statistically significant against to extract yield. Whiles, there is an only quadratic term of pressure and interaction between the term of extraction time in terms of temperature and pressure significantly effect on the patchouli alcohol content. The second order polynomial model used to

**Table 3.** Regression coefficient of polynomial function of response surface of extracted oil yield and patchouli alcohol content obtained by supercritical $CO_2$ extraction

| Sampling | Factor | Estimate Coefficients | Standard error | F-value | Prob > F |
|---|---|---|---|---|---|
| Yield of Extract | Intercept | 4.44 | 0.29 | 27.60 | 0.0001 |
| | $X_1$ | -0.75 | 0.22 | 11.38 | 0.0119 |
| | $X_2$ | 1.60 | 0.18 | 80.49 | < 0.0001 |
| | $X_3$ | -1.22 | 0.17 | 53.19 | 0.0002 |
| | $X_1^2$ | -0.49 | 0.28 | 3.04 | 0.1246 |
| | $X_2^2$ | -0.89 | 0.28 | 10.27 | 0.0150 |
| | $X_3^2$ | -1.09 | 0.24 | 20.20 | 0.0028 |
| | $X_1X_2$ | 0.85 | 0.29 | 8.61 | 0.0219 |
| | $X_1X_3$ | 0.34 | 0.24 | 1.98 | 0.2022 |
| | $X_2X_3$ | -1.25 | 0.20 | 37.70 | 0.0005 |
| Patchouli alcohol | Intercept | 21.85 | 1.65 | 7.42 | 0.0075 |
| | $X_1$ | 1.05 | 1.24 | 0.71 | 0.4267 |
| | $X_2$ | 3.12 | 1.00 | 9.69 | 0.0170 |
| | $X_3$ | 0.89 | 0.94 | 0.89 | 0.3761 |
| | $X_1^2$ | 1.02 | 1.57 | 0.42 | 0.5358 |
| | $X_2^2$ | -2.01 | 1.56 | 1.67 | 0.2374 |
| | $X_3^2$ | 1.65 | 1.36 | 1.47 | 0.2641 |
| | $X_1X_2$ | -0.10 | 1.63 | 0.00 | 0.9528 |
| | $X_1X_3$ | -5.07 | 1.37 | 13.61 | 0.0078 |
| | $X_2X_3$ | 4.98 | 1.14 | 19.02 | 0.0033 |

**Table 4.** Analysis of variance (ANOVA) for the response surface quadratic model for the oil yield from patchouli obtained by supercritical $CO_2$ fluid extraction

| Source | Sum of squares | Degree of freedom | Mean Square | F-value | Prob > F | |
|---|---|---|---|---|---|---|
| Model | 54.51 | 9 | 6.06 | 27.60 | 0.0001 | significant |
| Residual | 1.54 | 7 | 0.22 | | | |
| Lack of Fit | 1.25 | 6 | 0.21 | 0.72 | 0.7169 | not significant |
| Pure Error | 0.29 | 1 | 0.29 | | | |
| Total | 56.05 | 16 | | | | |

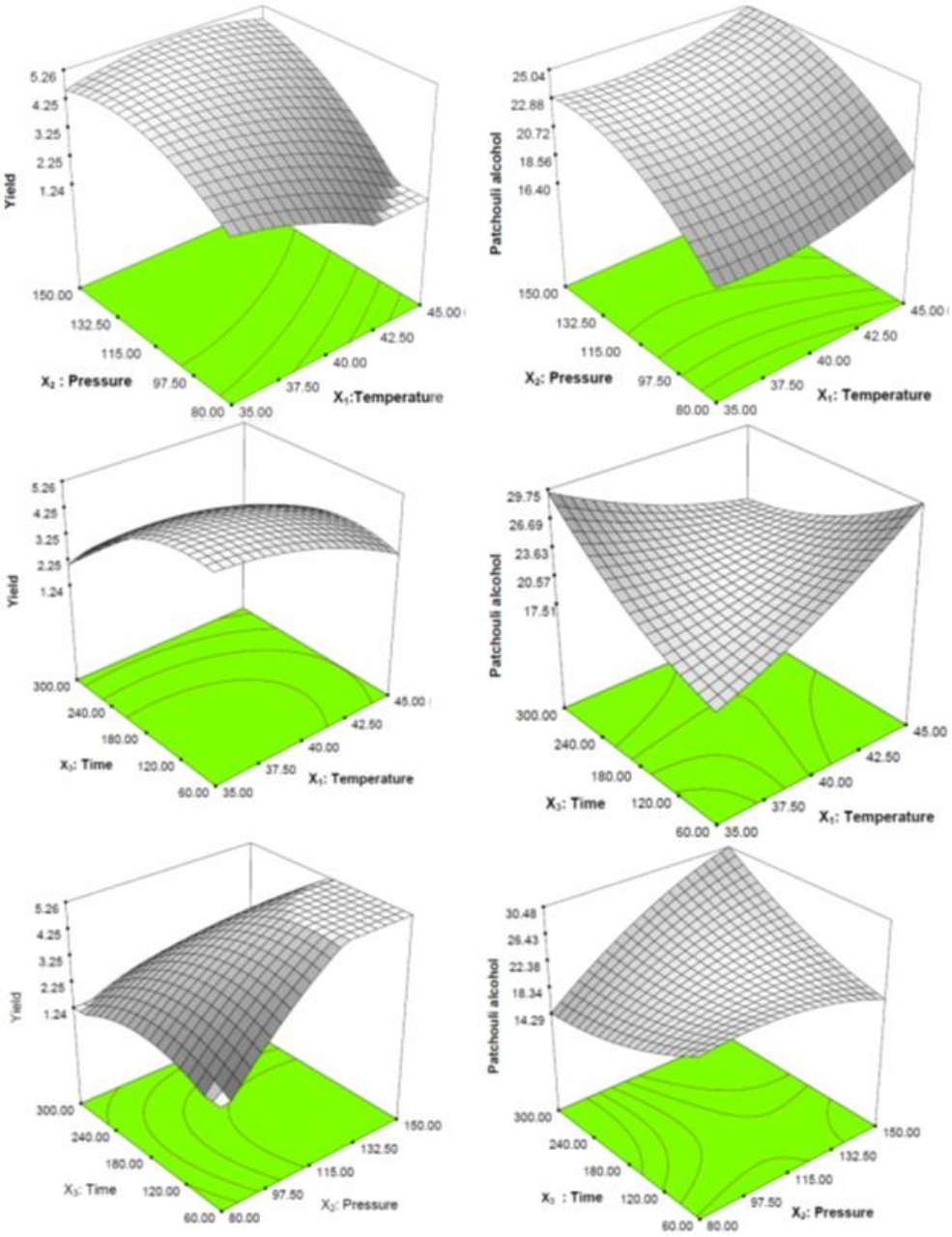


**Fig 1.** Response Surface plot of extracted oil yield (left) and its patchouli alcohol content (right) at a constant of temperature (40 °C), pressure (115 atm) and time (180 min)

express the total extraction yield and patchouli alcohol content as a function of independent variables (in terms of coded values) is shown in eq. 4 and 5:

$$Y_{\text{extract yield}} = 4.45 - 0.75X_1 + 1.6X_2 - 1.22X_3 - 0.49X_1^2 - 0.89X_2^2 - 1.09X_3^2 + 0.85X_1X_2 + 0.34X_1X_3 - 1.25X_2X_3 \quad (4)$$

$$Y_{\text{patchouli alcohol}} = 21.85 + 1.05X_1 + 3.12X_2 + 0.89X_3 + 1.02X_1^2 - 2.01X_2^2 - 1.65X_3^3 - 0.10X_1X_2 - 5.07X_1X_3 + 4.98X_2X_3 \quad (5)$$

The optimal conditions to obtain the highest extraction yield of patchouli oil were determined at 38 °C, 146.35 atm, and 74 min, and the predicted extraction oil yield was 6.41%. Whiles, the patchouli alcohol content was at 41.45 °C, 135.17 atm and 252.62 min and the expected patchouli alcohol content was 25.34%. Analysis of variance (ANOVA) results of the model is shown in Table 4. The regression model for the extracted oil yield was highly significant ($p < 0.01$) with a satisfactory coefficient of determination ($R^2$) of 0.9276 for all variables. However, only variable of pressure affected to the patchouli alcohol content in the extracted oil. Nevertheless, the result was supported by the

interaction between variables of time, temperature, and pressure, respectively. These results show that the model predicted for the extracted oil yield and the patchouli alcohol content was adequate, as indicated by error analysis that showed non-significant lack-of-fit (Table 4). Low residual values indicate good agreement of the experimental data with the mathematical model. The best way to determine the effect of the independent variables on the dependent ones is to draw surface response plots of the model which is represented graphically on the three-dimensional surface of the extraction process of patchouli oil obtained by supercritical $CO_2$ extraction as shown in Fig. 1. Response surface of extracted oil yield and its patchouli alcohol content were plotted at a constant of temperature (40 °C), pressure (115 atm) and time (180 min). These curves of the plot indicated that extracted oil yield increased with the increased extraction pressure and with more prolonged extraction time. This phenomenon also occurred in rising of patchouli alcohol content. The obtained oil yield increased with increasing on extraction temperature up to about 38 °C, while a further increase of temperature led to the decrease in the extracted oil yield.

As shown in Fig. 1, the extracted oil yield is greatly influenced by the three independent variables. The significance value is < 0.05 (confidence level of 95%) for all variables as well as the interaction between these variables except the quadratic temperature is not ambiguous. In other words, a quadratic temperature increase does not provide a significant effect on extracted oil yields. However, for changes in patchouli alcohol content, it is seen that only various extractor pressure has a significant impact, while the extraction time variable affected if it is varied with both temperature and pressure.

## CONCLUSION

Supercritical $CO_2$ extraction was proven to be a valuable alternative technology to refining essential oil as techniques for oil processing. In terms of extraction time, supercritical $CO_2$ extraction was the fastest process compared to Soxhlet extraction. The current results show that the second-order polynomial model was sufficient to describe and predict the response variable of extracted oil yield and also its patchouli alcohol content. According to the mathematical (Eq. 4 and 5) the linear and quadratic terms of temperature, pressure, and extraction time highly significantly affected both extracted oil yield and its patchouli alcohol content. This process runs relatively quickly compared to conventional methods such as Soxhlet extraction. The quality of extraction results to be better, the main component content becomes increasing and very profitable because $CO_2$ gas is relatively cheap and not toxic.

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