Journal of Engineering Science and Technology
Vol. 12, No. 8 (2017) 2106 - 2119

OPTIMIZATION OF PATCHOULI OIL (POGOSTEMON CABLIN, BENTH) WITH STEAM DISTILLATION ASSISTED BY PULSED ELECTRIC FIELD VIA RESPONSE SURFACE METHODOLOGY

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Abstract

The study was aimed to determine the role of pulsed electric field (PEF) treatment before hydro-distillation of the patchouli oil. Response Surface Methodology (RSM) was employed to optimize PEF treatment (voltages, frequencies) and times of distillation of patchouli oil from dried patchouli crops. The experimental design and analysis the result to obtain the optimal processing parameters was a Box-Behnken Design (BBD). Three variables were examined in this study: voltages (1,000-2,000 volt); frequencies (1,000-2,000 Hz); and distillation time (4-8 hours). The results showed that the voltage greatly affects the volume of patchouli oil obtained and optimum condition of PEF was voltages of 2,000 volts, frequencies of 1,874 Hz, and 8 hours distillation. The patchouli oil obtained is 8.037 ml of 300 g of dry material (±2.7%). The verification of the model shows that 96.6% (7.76±0.15 ml) can adequately for reflecting the expected optimization.

Keywords: PEF, Distillation, Patchouli oil, Optimization, Response surface methodology.

1. Introduction

Patchouli (Lamiaceae), is one of the herbal plants and its native tropical and subtropical planted and has been cultivated in Indonesia, Malaysia, China, India, Singapore, Philippines, Brazil for the essential oil production [1-3]. The plant never flowers and the vegetative propagation by stem cutting are slow and sufficient for large scale cultivation, but in the last decades the plant propagation is

Nomenclatures

k	Number of factors were tested
X_i	Code for the treatment of factor *i*
X_j	Code for the treatment of factor *j*
X_1	Actual value of voltage, volt
X_2	Actual value of frequency, Hz
X_3	Actual value of time distillation, hour
Y	Response observations, ml

Greek Symbols

β_0	Intercept
β_i	Coefficient of linear
β_{ii}	Coefficient of quadratic
β_{ij}	Coefficient of the interaction
$\mathcal{E}$	Error

Abbreviations

ANOVA	Analysis of variance
BBD	Box-Behnken design
CCD	Central composite design
E	Electric field strength
GTC	Glandular trichome cell
PEF	Pulsed electric field
RSM	Response surface methodology
SEM	Scanning electron microscope

by tissue culture [4]. Patchouli has a great commercial value and it has been cultivated in various parts of the world because of its economic importance [5]. Patchouli plant (*Pogostemon cablin*, Benth) has great potential to become the most economically important tree crops in the tropics due to its properties which offer patchouli oil found in its glandular trichomes cells [6,7]. Patchouli oil is the result of secondary metabolites and an integral part of the adaptation of plants to environmental disturbances that occur during growth and become an integral ingredient of the product fragrance industry due to its nature as fixative other fragrance material [8]. About 80% of patchouli oil word demand is supplied by Indonesia and applied widely in perfumes, cosmetics, food, beverages, soap and even the last two decades, many medical worlds use as a medicinal ingredient [9]. Patchouli oil obtained by distillation of the dried patchouli crop, using high temperature and pressures to destroy the oil storage cells, and the results are less than the maximum [10]. Hot water extraction is most conventional extraction method for patchouli oil in Asia. It should be noted that traditional hot water extraction is times and solvent consuming and has low efficiency [11].

However, in the Indonesia the lower level of technology of farmer becomes the main hindrance the development of the production of the patchouli plant into plant oil. In Indonesia patchouli plant is processed traditionally to extract the oil. This traditional process, not only time consuming, but also produced a low percentage of oil. Under circumstances to achieve efficient results, it is an at most importance to introduce the use of pulsed electric field (PEF) in order to increase the yield and efficiencies. Recently, major advances in the extraction of bioactive compounds have been achieved with the help of a PEF [12]. It's led to the possibility of increasing yield. In addition, PEF accelerated the kinetic process of the material component due to the discharge of oil barriers have been reduced. However, the application of PEF on patchouli oil before distillation has still minimized information. Several studies have shown that the use of PEF improve the extraction of plant bioactive materials. So that the effect of PEF on extraction of patchouli oil must be investigated with increasing extraction efficiency.

Response surface methodology (RSM) was used in this study aimed to optimize the response. The optimization process is often carried out in the industry as an effort to improve the quality of the resulting product. Besides being used in the optimization process of product quality, the method is also used in other fields such as food science, biology, medicine and health [13]. The most popular form of RSM is a central composite design (CCD) and has been used in several studies to optimize the extraction conditions compound [14-17]. The extraction of ginger oil by steam distillation, the optimal values obtained in the comparison of water and powdered ginger for 2,660 ml and 100 g, ginger powder medium size (800 to 2,000 μm) and distillation time of 23.15 hours [18]. Patchouli oil extraction assisted by a PEF, information on the optimal value of the process conditions required is still limited. The purpose of this study was to evaluate the role of a PEF (voltage, frequency) and time of hydro-distillation of the total patchouli oil obtained.

2. Materials and Methods

2.1. Materials

Raw materials used in this study are patchouli plant, harvesting age ±7 months, from the experimental garden in the district of Kesamben, Blitar, East Java-Indonesia. The plant was wind-dried until the moisture content of (20±1) %, and then (10±1) cm long cut (Fig. 1).

Fig. 1. Materials dried patchouli.

2.2. Design of the research

Early research was investigated of the glandular trichomes cell (GTC) damage due to PEF treatment. The data obtained is used as the basis for the PEF treatment before distillation. Optimization results to obtain patchouli oil by response surface methodology (RSM) with a central composite design (CCD). Three variables examined in this study were voltage (1,000-2,000 volt); frequency (1,000-2,000 Hz); and time of hydro-distillation (4-8 hours). Code -1, 0 and 1 is a symbol that shows the value of each variable. A code of -1 indicates the lowest value (minimum), the code of 0 indicates the median (optimum) and code 1 indicates the highest value (maximum). In this experiment, the treatment was presented in Table 1. The entire treatment consists of 20 runs distillation processes, each process condition following the central composite design. Data were analyzed using Design Expert software version 8.0.6. The general model design used to present on Eq. (1):

$$Y = \beta_0 + \sum_{i=1}^{k} \beta_i X_i + \sum_{i=1}^{k} \beta_{ii} X_i^2 + \sum_{i=1}^{k-1} \quad \sum_{j=i+1}^{k} \beta_{ij} X_i X_j + \varepsilon \tag{1}$$

where, Y= response observations, β_0= intercept, β_i= coefficient of linear, β_{ii}= quadratic coefficient, β_{ij}= coefficient of the interaction, X_i= code for the treatment of factor *i*, X_j= code for the treatment of factor *j*, *k* = number of factors were tested.

Table 1. Independent variables and their levels used in the RSM.

Independent variable	Code	Variable level		
		-1	0	+1
Voltages (volt)	X_1	1,000	1,500	2,000
Frequencies (Hz)	X_2	1,000	1,500	2,000
Distillation times (h)	X_3	4	6	8

Box-Behnken design in response surface methodology was used to study the combined effect of three variables: voltage, frequency and time hydro-distillation of patchouli oil. Another parameter that may affect hydro-distillation was ignored from experimental design and focus on the parameters investigated, as done previous researchers [19, 20]. The range level of optimized variables is shown in Table 1. Box-Behnken design suitable for surface exploration quadratic response and produce a second-degree polynomial model, which in turn is used to optimize the process of using a small number of experimental runs. A design developed using Design Expert 8.0.6, resulting in 20 experimental runs as shown in Table 2. A number of 20 running experimental randomized to maximize the effect of the variability described in the responses observed due to outside factors. Level independent variables as shown in Table 1, was chosen based on preliminary experiments, and the GT cell patchouli damage up to 1,000 volts and 1,000 Hz of voltages and frequencies respectively.

2.3. PEF treatment and patchouli oil distillation

Dried patchouli plant weighed 300 g placed in the chamber were treated with PEF 15 seconds, a distance of 20 cm cathode-anode appropriate treatment in Table 1. Further materials incorporated into appliances hydro-distillation using a Clevenger.

2.4. Microscopic analyses

Microscopic analyses by Scanning Electron Microscope (SEM) FEI-S25-EDAX Inspect, to investigate the glandular trichomes cells (GTC) of dried patchouli leaf as a place to store essential oil before and after PEF treatment.

3. Results and Discussion

3.1. GT cell changes

Observation of the GTC shape of patchouli leaves was aimed to investigate the effect of PEF on GTC. Figure 2 shows that the changes of the GTC after the PEF treatment by electric field strength (E) and the damage ranging crimped to erupt and patchouli oil is out. The GTC damage to the inside because of patchouli oil as measured turned out to be slightly acidic (pH ±6.3-6.8 or positive pole) so that the movement of the cell wall toward the negative pole of the electrodes.

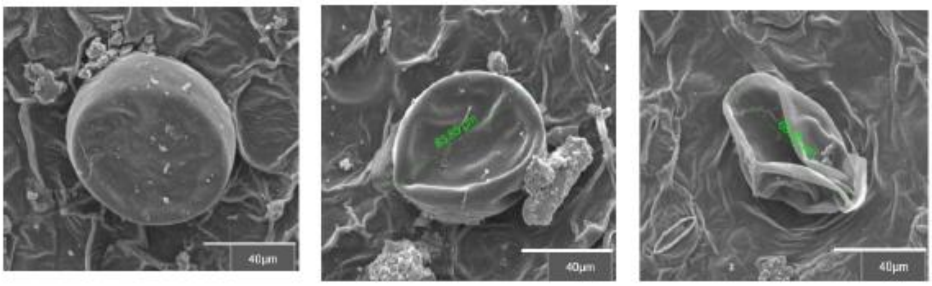

Fig. 2. GT cell before and after PEF 1,000 volt and 2,000 volt (2,500×).

Changes in patchouli GT cell structure are an indication of the successful application of PEF, with the goal of more effective and efficient distillation. Damage to accelerate and simplify the cell wall of water vapor brought patchouli oil out of the material. Rupture of cells is essential for the optimal design process before extraction of the desired compound of plant tissue. A study of Chinese herbs that are treated with the number of pulses, less influence on the results obtained extracts, while the electric field treatment results are almost 3 times higher than without PEF [21]. This is caused by the potential difference between the inside and outside of the cell membrane, so that the cell will undergo electrolysis. The electro-permeability membrane of soybean treated with PEF depending on size and the percentage of porosity increase sharply with increasing electric field strength [22]. The influence of the electric field strength (E) to the outbreak of onion tissue cells, has reported that the number of GT cell rupture was increased. The cells of the onion were broken out after the application of an electric field of 333 V/cm and 100 pulses [23].

3.2. Models fitting

Design optimization using central composite design by the response surface methodology known that patchouli oil obtained was presented in Table 2. The data show that increasing the voltages, frequencies and extraction times, the oil yield also increased. The statistical analysis indicated that the proposed regression model of the patchouli oil volume was adequate, processing to a significant lack of fit and with a satisfactory value of the R^2 for all the response. The R^2 value of patchouli oil yield is 0.98, and model can fit well with the actual data when approaches one. Regression analysis and ANOVA were used for fitting model and for examination the statistical significance of the terms.

3.3. Analysis

The response Y was predicted for the yield of patchouli oil can be expressed by the following second-order polynomial equation in terms of coded values as like as Table 3. Equation. (2) was:

$$Y = -3.45192 + 0.00134981\, X_1 + 0.00433342 X_2 + 1.40131 X_3 + 85 \times 10^{-6} X_1 X_2 + 1.875 \times 10^{-3} X_1 X_3 - 625 \times 10^{-4} X_2 X_3 - 101.995 \times 10^{-5} X_1^2 - 172.672 \times 10^{-5} X_2^2 - 0.090243 X_3^2 \quad (2)$$

The PEF application for electrolysis of biological cells will result in the cell wall membrane and increases the permeability of cell walls and facilitate the release of intracellular compounds [24]. Damage to the cell membrane made of water vapor penetration into the material is shorter and there should be no size reduction process. Damage caused PEF application to directly reach GT storage cells more easily remove the oil so that when the oil distillation. The voltage applied to the PEF will be dealing with the destructive force of the biological cell, but each material has a different resistance so it is necessary that the suitability of the material to be processed and to obtain optimal results [25].

Table 2. Response central composite patchouli oil volume.

Run	Voltages (volt)	Frequencies (Hz)	Distillation times (h)	Oil yield (ml)
1	1,500	659.104	6	5.8
2	1,000	2,000	8	6.2
3	1,500	1,500	9.36359	7.6
4	1,500	2,340.9	6	5.6
5	1,500	1,500	6	7.4
6	2,000	2,000	8	8.2
7	1,500	1,500	2.63641	4.2
8	1,500	1,500	6	7.2
9	2,000	1,000	4	5.2
10	1,500	1,500	6	7.1
11	1,000	2,000	4	5.2
12	1,500	1,500	6	6.8
13	1,000	1,000	4	4.8
14	1,500	1,500	6	7.2
15	659.104	1,500	6	5.6
16	2,000	2,000	4	5.4
17	1,500	1,500	6	6.9
18	2,340.9	1,500	6	6.8
19	1,000	1,000	8	7.1
20	2,000	1,000	8	7.2

Analyses of variance (Table 3), showed a significant model and lack-of-fit was not significant. In addition, the results of this study also showed that the model was used to adjust the response variable with a significant (p<0.0001) and very adequate to represent the relationship between the response and the independent variables were tested. The analysis model is used to determine the model appropriate in response surface method [26]. Model obtained can be used to predict response patchouli oil for voltages, frequencies and distillation times.

Models are evaluated include linear, interaction, quadratic, or cubic. Process model selection is based on: sequential the model sum of the square, lack of fit test, and model summary statistics. The p-value is a tool to determine the suitability models, the smaller the p-value is, the more significant the model [27]. P-value based on the sequential sum of square models indicate that the model significant and suggested the response was quadratic, because the p-value < 0.0001 (Table 3). This is consistent with the statement if the p-value less than 0.05 indicate the model is a significantly greater effect on response than other models [28].

Table 3. ANOVA for the fitted quadratic polynomial model of extraction of patchouli oil.

Parameters	Sum of Squares	Degrees of freedom	Mean Square	F-value	p-value	
Model	20.95	9	2.33	20.21	< 0.0001	significant
Residual	1.15	10	0.12			
Lack of fit	*0.91*	*5*	*0.18*	*3.80*	*0.0847*	*not significant*
Pure error	*0.24*	*5*	*0.048*			
Cor total	22.10	19				
R^2	0.98					
Adj-R^2	0.96					

Table 4. Regression coefficients estimate and their significance test for the quadratic polynomial model.

Parameters	Sum of squares	Degrees of freedom	Mean Square	*F*-value	*p*-value
X_1	1.63	1	1.63	14.16	0.0037
X_2	0.009.683	1	0.009.683	0.084	0.7777
X_3	13.98	1	13.98	121.43	<0.0001
X_1X_2	0.36	1	0.36	3.14	0.1069
X_1X_3	0.28	1	0.28	2.44	0.1491
X_2X_3	0.031	1	0.031	0.27	0.6137
$X_1^{\wedge 2}$	0.94	1	0.94	8.13	0.0172
$X_2^{\wedge 2}$	2.69	1	2.69	23.32	0.0007
$X_3^{\wedge 2}$	1.88	1	1.88	16.31	0.0024

Lack of fit test is 0.0847 and > 0.05 (Table 3). Based on lack of fit tests, the model suggested in the third the response was quadratic. The model will be considered right a statistically at level certain α when lack of fit test of the model is not significant [29]. In this case α used is 0.05. The process of selecting a model based on a statistical summary of the model indicates that model meet the criteria are quadratic model. Based on the model selection process three models corresponding to an increase of patchouli oil process are quadratic model. The results of ANOVA of response surface quadratic showed a quadratic model has a significant effect on the response.

The coefficient of determination (R^2) obtained was 0.98 indicating that this value is better able to explain the results obtained. Because it has a coefficient of determination 95%, meaning that the model is able to explain the results of 95%, and the rest is influenced by other factors. In addition, the solution obtained optimization is the process of extraction is done by using a voltage of 1,999.997 volts (±2,000 volts), the frequency of 1,874.215 Hz (±1,874 Hz) with an extraction time of 8 hours, and 8.037 ml volumes of oil obtained from plants dried patchouli 300 g. Based on the above data, it can be obtained yield of 2.697% (± 2.7%) and it is higher than the distillation without PEF treatment (±1.8%). Voltage value of 1,999.997 volts also shows that the value of the electric field strength (E) as previously predicted is ±100 V/cm, closer to the truth. It is evident that the cathode-anode distance tested is 20 cm and the solution of 1,999.997 volts indicates the optimal voltage, the electric field strong prediction is correct (Figs. 3-5). Changes in oil yield obtained from the time of distillation as shown in Figs. 6-8.

Figure 5 shows the effect of voltage and frequency against distilled patchouli oil. It is known that the voltage and frequency have a positive influence on patchouli oil produced. Patchouli oil obtained increases with an increase in voltage and frequency. When linked with the distillation time, the longer it will also increase the yield of distilled patchouli oil (Figs. 6-8). Distillation time of 8 hours is the optimum time with the highest yield (8.037 ml) and the results obtained oil increased compared to the untreated PEF. If time distillation continued until 10 hours no change of oil results obtained.

In an effort to optimize variables that affect hydro-distillation patchouli oil, response surface plot generated from the regression model. Three-dimensional (3D) plot generated by maintaining a constant variable at a central point and varying the other within the experimental range. The resulting response surface shows the effect of voltage, frequency, and time distillation in patchouli oil is obtained. Figure 5 shows the response surface and contour plots corresponding to the amount of patchouli oil obtained as a function of the voltage and frequency. An increase in voltage, frequency and time distillation resulting in an increase of patchouli oil to the optimum value of about 8.037 ml/300 g, the voltages were 1999.997 volt, frequencies were 1,874.215 Hz and time distillation were 8 hours. Any further increases in voltage, frequency and time distillation found to be advantageous for the production of patchouli oil as described by the downward trend observed. Extraction results greatly influenced the magnitude of the electrical voltage and the contact time of the material. Electrolysis can cause differences in the stability of the ions in the compound that will result in improved conductivity and tissue disintegration [31].

The important diagnostic tool that is used as described in the normal probability plot of the residuals shown in Fig. 3. The data points of the plot are approximately linear, which indicates that the quadratic model developed is a good representation of the process. Figure 4 shows a plot of the predictions against experimental values actually indicate that actual values are distributed relatively close to a straight line. This suggests that an adequate model to predict the efficiency in the range of variables studied [30]. Modeling factors and responses made by the response surface methodology to predict the likelihood of the highest-level results hydro-distillation of patchouli oil.

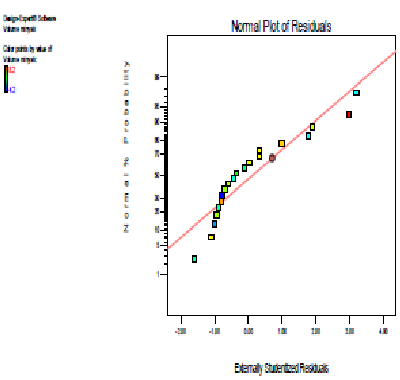

Fig. 3. Normal plot residual.

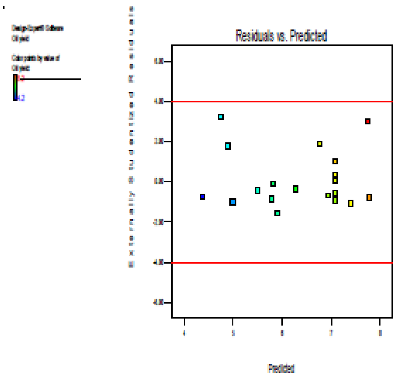

Fig. 4. Residual versus predicted plot.

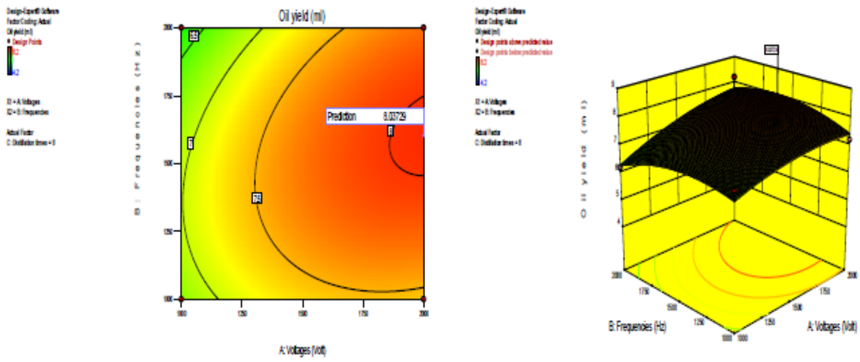

Fig. 5. Plot contour and response surface method 3D solution model.

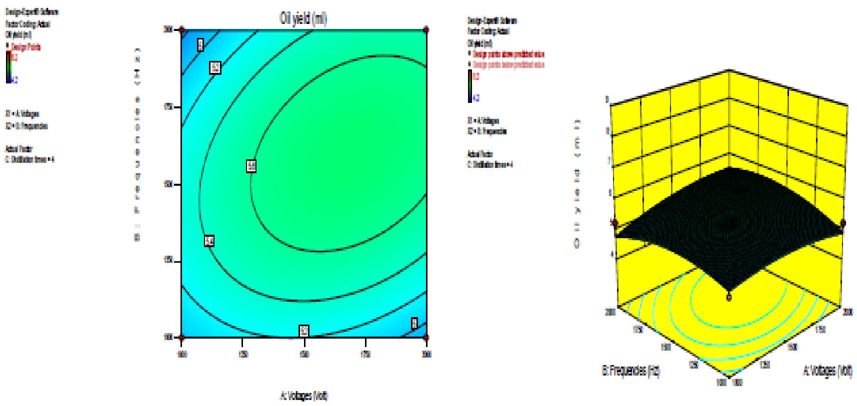

Fig. 6. Plot contour and 3D distillation 4 hours.

The PEF (voltage and frequency) influence and 4 hours distillation on the amount of patchouli oil are presented in Fig.6. The distillation 4 hours still not up to the amount of patchouli oil obtained mean ±5.15 ml/300 g of material (Table 2). It is characterized by blue-green contour plot. In general, changes in the contour plot RSM start from blue to green, yellow and red peak is as previous studies [32-34].

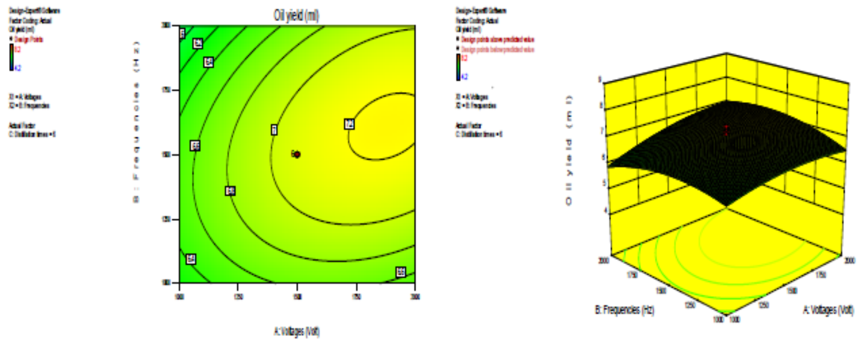

Fig. 7. Plot contour and 3D distillation 6 hours.

Figure 7 shows the effect of PEF and distillation time of 6 hours and a mean of patchouli oil obtained was 6.68 ml/300 g of material (Table 2). An increase in voltage, frequency and distillation times was followed by an increase of the patchouli oil. The central point of optimal values of distillation time and PEF was to get the highest amount of oil. Any further increases in all three-treatment voltage, frequency and distillation times causes a high effect on patchouli oil produced. Observed a higher rate of distillation at the voltage and frequency at a higher level than at lower voltage and frequency. However, the distillation 6 hours also still shows the contour plot of yellowish green color, it indicates that the distillation 6 hours has not yet reached its maximum. Distillation time is very influential on the results of the extraction yields [15]. A notable increase in the extraction yields was observed with an extension of extraction time from 4 to 8 hours. At longer times, the yield kept a steady level. The yields were lower when the extraction time was too long, and the target product ware not stable [15].

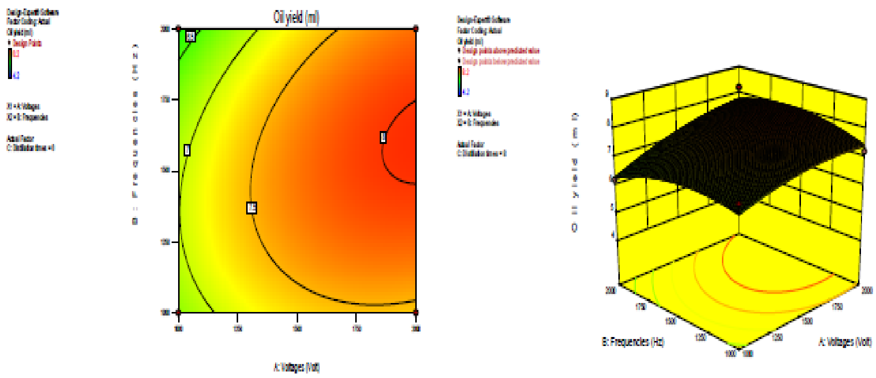

Fig. 8. Plot contour and 3D distillation 8 hours.

In order to select the optimum conditions and the level of each model are analyzed. The maximum response of the model is the amount of patchouli oil obtained from 300 g of patchouli. Contour plots already red and an indication of optimization has been achieved (Fig. 8). To determine the optimal level of the test variable for the patchouli oil yields, the 3D response surface described by the regression models is presented in Figs. 6-8. By solving the inverse matrix, the optimum value of the variable affecting the yield of patchouli oil given by the software where the amount of voltage 2,000 volt, the frequencies 1,874 Hz and 8 hours of distillation time. Under this optimal condition, the model gave the maximum predicted value of patchouli oil (8.037 ml). Extraction times influence of the response value and this study could be useful for the development of industrial extraction processes [17].

3.4. Validation of the models

In order to validate the adequacy of the model equation (Eq. 2), a verification experiment was carried out using the recommended optimum conditions. The maximum predicted yield and experimental yield of patchouli oil were shown that under the conditions, the experimental yield of patchouli oil was 7.76±0.15 ml (96.6 %) which was close to the predicted value (Table 5). The good correlation between these results confirmed that the model was adequate for reflecting the expected optimization.

Table 5. Predicted and experimental value of the responses at optimum modified conditions.

Conditions	Voltages (volt)	Frequencies (Hz)	Distillation times (h)	Yield (ml)
Optimum	2,000	1,874	8	8.037
Experimental	2,000	1,870	8	7.76

In order to select the optimum conditions and the level of each of the parameters studied, performed analyzed models. The estimated maximum response of the model is the amount of patchouli oil produced 8.037 ml. Response surface method optimization results need to be validated in order to test the accuracy of prediction models produced. As did some previous researchers, deviation validation results showed less than 5% produced an excellent model [34-36]. The validity of the results predicted by the regression model, confirmed by repeated experiments in hydro-distillation optimal conditions (i.e., voltages 2,000 volts, frequencies of 1,870 Hz and hydro-distillation of 8 hours). The results obtained from the three replications showed that the average result of 7.76 ml (96.6%) of patchouli oil obtained is close to the predicted value (8.037 ml). The very good correlation between predicted and measured values of these experiments have confirmed the validity of the model the appropriate response.

4. Conclusion

In this work, the PEF treatment before hydro-distillation of patchouli oil was studied quantitatively with three variables using a Box-Behnken design for response surface methodology. The following conclusions can be drawn from the study.

- The use of response surface methodology to determine the optimal conditions of patchouli oil distillation have been demonstrated.
- The early study concluded that voltages 1,000 volts, and the frequencies 1,000 Hz of PEF treatment were damaging the GT cell wall.

- RSM optimization solution with PEF by 2,000 volts, the frequency of 1,874 Hz with 8 hours of distillation obtained oil 8.037 ml of dried patchouli 300 g (±2.7%) and it is higher than the distillation without PEF (±1.8%).
- The verification of the model shows that 96.6% can adequately for reflecting the expected optimization.

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