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Extraction Process and Analysis of Components in Essential Oils of *Piper longum* Linn. Harvested in Dak Lak Province, Vietnam

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Nowadays, plants containing biological activities are increasingly interested for their antioxidant and antibacterial properties. In particular, *Pepper longum* Linn. was known as one of the popular types of pepper family in Vietnam with many different uses in food and medicine. In this study, *Pepper longum* fruits were studied for extraction to essential oil by hydrodistillation through two survey methods such as single factor investigation method and optimization by response surface methodology (RSM). The analysis results by design expert software program version 11 shown that at the condition that the ratio of water to raw material 6:1 (mL/g), during 225 min extraction at 130 ºC, the essential oil yield was obtained 0.8%. The model predicts this result for F-value, P-values and Lack of Fit are mathematically significant and values for reliability R^2 = 0.9963, C.V. % =1.99. Essential oil obtained was evaluated by gas chromatography-mass spectrometry (GC-MS) technique, which accounted that β-caryophyllene contains the highest amount among the other 43 components present.

Keywords: *Piper longum* Linn., Essential oil, Gas chromatography, Mass spectrometry, Response surface methodology.

INTRODUCTION

Box and Wilson method [1] based on the suitability of experimental model, developed a response surface methodology so that the experimental data obtained relates to the experimental design of a group of mathematical and statistical techniques. Normally, when optimizing parameters for extraction process, the parameter at a time method is commonly used, however, this method has some limitations. First, because it does not consider the interactive effect between the variables in the research experiments, number of experiments could be large [2]. As such, the time required to carry out investigation and the cost to buy materials will increase proportionally. To overcome these inconvenience, response surface methodology (RSM) has been applied to the optimization of parameters in chemical analysis, which to achieve the highest efficiency [1]. Secondly, the application of RSM in the process can help replace each failed test; while following the traditional method, it is necessary to repeat all the previous experiments [3]. In order to optimize parameters for the process, RSM has been applied in several different areas in extraction of essential oils [4-10], synthetic materials [11-16], cosmetics [17] and food processes [18].

Piper longum Linn. is one of the four most popular and valuable types of economics and medicine in Vietnam. All parts of *Piper longum* (root, stem, fruit and leaf) can extract essential oils, of which the fruit gives the highest essential oil yield. However, previous research showed the yield of *Piper longum* essential oil lower results than the other types [19]. The essential oil in general and the pepper oil in particular have many different uses such as in food, cosmetics, medicines due to its antibacterial, antifungal and antioxidant properties [20-24].

In literature, no research on the response surface methodology and the conditions affecting the extraction process of essential oils from *Piper longum* Linn. grown in Vietnam is reported. Therefore, the purpose of this study is to use RSM by design expert version.11 software to design experiments and optimize the best conditions investigated by single factor investigation method. Finally, the essential oil was analyzed for the active composition by gas chromatography-mass spectrometry (GC-MS) technique.

EXPERIMENTAL

Exraction process: *Piper longum* Linn. (also known as Tieu Lop in Vietnam) was purchased from the local market in Dak Lak Province, Vietnam and carefully stored in air-free bags to avoid spoilage or prevent rapid ripening of *Piper longum*. It was washed several times with water to remove external impurities and then the different sizes was examined to provide high performance on essential oils. *Piper longum* was soaked with 2% NaCl solution for three days in order to break down the cell wall containing essential oils and then subjected to a hydrodistillation process. The composition of the essential oil obtained was evaluated using GC-MS technique.

Single factor investigation method for extraction conditions: In this study, four factors influencing *Piper longum* Linn. fruit essential oil extraction were investigated as the material size (original size: 5-6 cm, then cut into small sizes 3-4 cm, 1-2 cm and < 0.5 cm), the ratio of *Piper longum* Linn. fruit to water (1:2 to 1:6 g/mL), time extraction (30 to 330 min, each 30 min per experiment) and extraction temperature (100 to 140 ºC). All the experiments were repeated three times and reported as average values. The essential oil yield was calculated using eqn. 1:

$$\text{Yield}\left(\frac{\text{mL}}{\text{g}}\right)=\frac{\text{Volume of } P.\,longum \text{ essential oil obtained (mL)}}{\text{Amount of } P.\,longum \text{ originally used (g)}} \quad (1)$$

Optimization of the essential oil extraction: Response surface methodology (RSM) is a method that simultaneously examines three factors (the ratio between water and material, time and temperature extraction). In addition, this method helps to control errors. From the results obtained by the single factor investigation method, the optimization of essential oil extraction was conducted. By using the Design-Expert software with three independent variables: the ratio of water and *Piper longum* (A) 3.3:1-6.7:1 (mL/g), time extraction (B) at 39.5-260.5 min and the microwave power (C) of 113-147 (W) as shown in Table-1.

TABLE-1
OPTIMAL CONDITIONS FACTORS THAT AFFECT THE EXTRACTION PROCESS

Levels	Independent factors		
	Ratio of water and *Piper longum* (mL/g)	Extraction time (min)	Extraction temperature (°C)
	A	B	C
-α	3.3:1	39.5	113
-1	4:1	159.5	120
0	5:1	210	130
1	6:1	240	140
+ α	6.7:1	260.5	147

GC-MS conditions: After the optimized process, 25 µL of essential oil was mixed in 1.0 mL *n*-hexane and dehydrated with Na_2SO_4 for GC-MS analysis. GC Agilent 6890 N (Agilent Technologies, Santa Clara, USA) was used. The device was coupled with MS 5973 inert, HP5-MS column, head column pressure of 9.3 psi. Following conditions were imposed: carrier gas He; flow rate 1.0 mL/min; split 1:100; injection volume 1.0 µL; injection temperature: 250 ºC. From the initial hold at 50 ºC for 2 min, oven temperature progressed to 80 ºC at 2 ºC/min; from 80 ºC to 150 ºC at 5 ºC/min; from 150 ºC to 200 ºC at 10 ºC/min; from 200 ºC to 300 ºC at 20 ºC/min and was maintained at 300 ºC for 5 min.

RESULTS AND DISCUSSION

Optimization of factors by single factor investigation method: Fig. 1 displayed the essential oil yield of *Piper longum* is affected by factors such as size, water and material ratio, extraction time and extraction temperature. These factors are investigated by the method of single factor. Based on Fig. 1a, the essential oil yield strongly depends on the size to be investigated. As the size decreases, the yield of essential oils increases because when hydrodistillation was carried out with a water solvent, the contact area between the water and the surface of the material increased, thus the more effective extraction process of essential oils achieved with higher size. Similarly, when the extraction time increased, the yield of essential oil increased (Fig. 1b). However, when the optimal time (210 min) was exceeded, no increase in the essential oil yield significantly observed. To save production cost, 210 min value is chosen as the optimal time for the extraction of pepper essential oil. Fig. 1c-d presented the dependence of the essential oil yield on the ratio of material to water and the extraction temperature. Thereby, the yield of the essential oil is affected in an increasing trend as the ratio or temperature increases. Increasing temperature stimulates the heat transfer and mass transfer faster, high water ratio helps break the colloids around the essential oils in plants. However, when increases beyond the optimal limit (1:5 g/mL, 130 ºC), the essential oil yield decreases due to the thermal decomposition of the active compounds in *P. longum* essential oil.

Optimization of factors by response surface methodology (RSM): The optimal conditions were designed by Design expert software version 11.0 with 20 experiments formed from 5 value (±α, 0, ±1). The actual experimental results along with the the predicted results made by DX11 software are presented in Table-2.

The Model F-value of 299.56 implies that the model is significant (Table-3). There is only a 0.01% chance that a large F-value could occur due to noise. The *P*-values were used as a tool to check the significance of each coefficient, which in turn may indicate the pattern of the interactions between the variables. The *P*-values < 0.0500 indicate model terms are significant. In present case, all factors (A, B, C, AB, AC, BC, A^2, B^2, C^2) are significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve the current model. In addtion, the R^2 value of 99.63% showed that ANOVA analysis has a high reliability, at the same time, the Predicted R^2 value of 97.09% is in reasonable agreement with the Adjusted R^2 value of 99.30%; *i.e.* the difference is less than 0.2. Finally, the coefficient of

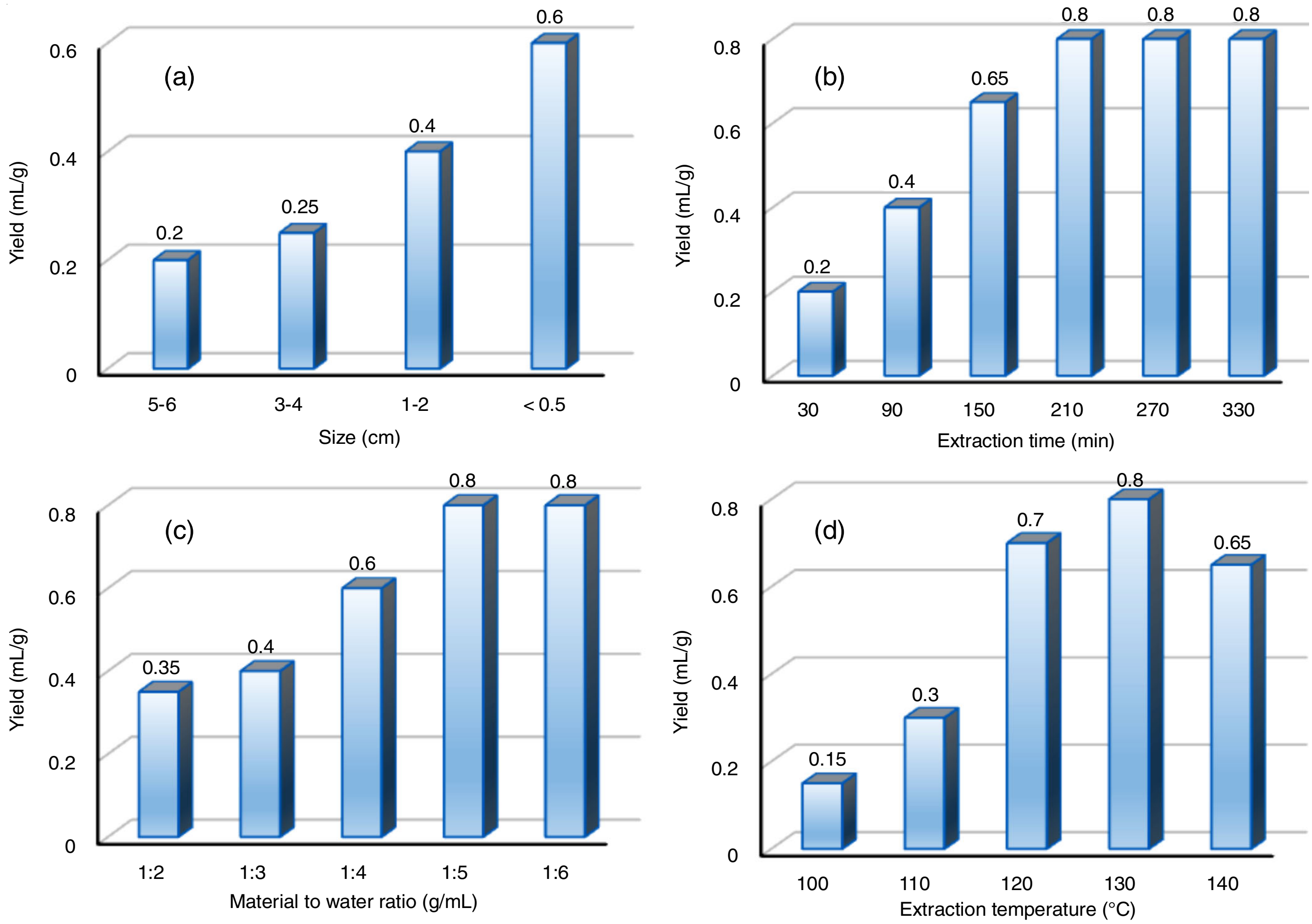


Fig. 1. Factors affecting the yield of extracting essential oils from *P. longum*

TABLE-2
ACTUAL AND PREDICTED RESULTS OF 20 EXPERIMENTS

No.	Independent variables			Y (%)		
	A	B	C	Actual	Predicted	Residual
1	4:1	180	120	0.35	0.36	-0.01
2	6:1	180	120	0.55	0.55	0.00
3	4:1	240	120	0.5	0.51	-0.01
4	6:1	240	120	0.65	0.64	0.01
5	4:1	180	140	0.5	0.52	-0.02
6	6:1	180	140	0.5	0.44	0.06
7	4:1	240	140	0.8	0.80	0.00
8	6:1	240	140	0.7	0.70	0.00
9	3.3:1	210	130	0.65	0.63	0.02
10	6.7:1	210	130	0.7	0.71	-0.01
11	5:1	159.5	130	0.45	0.44	0.01
12	5:1	260.5	130	0.75	0.75	0.00
13	5:1	210	113	0.4	0.39	0.01
14	5:1	210	147	0.6	0.59	0.01
15	5:1	210	130	0.8	0.80	0.00
16	5:1	210	130	0.8	0.80	0.00
17	5:1	210	130	0.8	0.80	0.00
18	5:1	210	130	0.8	0.80	0.00
19	5:1	210	130	0.8	0.80	0.00
20	5:1	210	130	0.8	0.80	0.00

variation (CV = 1.99%) less than 10 indicated that the model was reproducible. Since design expert version 11 software program had presented an equation then one can predicts the yield of extracted essential oil using eqn. 2.

$$Y = 0.8003 + 0.0245A + 0.0919B + 0.0576C - 0.0188AB - 0.0563AC + 0.0312BC - 0.0464A^2 - 0.0729B^2 - 0.1082C^2 \quad (2)$$

Predicted *vs*. Actual plot and Residuals *vs*. Run order plot: The predicted values calculated from eqn. 2 were in good agreement with the experimental values as shown in Fig. 2, which shows the interaction between actual value and prediction around the 45-degree line and thus proves that no major errors occur. Hence, this quadratic model is well suited for this experimental set up. On the other hand, Fig. 3 described the arrangement from DE11 for the experiments randomly, without any arrangement and not followed by any rules. This indicate that the produced model could accurately predict the experimental values.

Model adequacy checking: By constructing a normal probability plot of the residuals, a check was conducted for the normality assumption as shown in Fig. 4. The normality assumption was satisfied as the residual plot approximated along a straight line. Fig. 5 presents a plot of residuals *versus* the predicted response. The general impression is that the residuals scatter randomly on the display suggested that the variance of the original observation is constant for all values

TABLE-3
RESULT OF ANOVA FOR THE QUADRATIC MODEL

Source	Sum of squares	Degree of freedom	Mean square	F-value	Prob. > F	Comment
Model	0.4429	9	0.0492	299.56	< 0.0001	Significant
A-A	0.0082	1	0.0082	49.76	< 0.0001	Significant
B-B	0.1152	1	0.1152	701.59	< 0.0001	Significant
C-C	0.0453	1	0.0453	275.65	< 0.0001	Significant
AB	0.0028	1	0.0028	17.12	0.0020	Significant
AC	0.0253	1	0.0253	154.10	< 0.0001	Significant
BC	0.0078	1	0.0078	47.56	< 0.0001	Significant
A^2	0.0310	1	0.0310	188.59	< 0.0001	Significant
B^2	0.0765	1	0.0765	466.00	< 0.0001	Significant
C^2	0.1688	1	0.1688	1027.79	< 0.0001	Significant
Residual	0.0016	10	0.0002	–	–	–
Lack of fit	0.0016	5	0.0003	–	0.2744	Not significant
Pure error	0.0000	5	0.0000	–	–	–
Std. Dev. = 0.0128		Mean = 0.65	C.V. % = 1.99			R^2 = 0.9963
Adjusted R^2 = 0.9930		Predicted R^2 = 0.9709	Adeq precision = 49.5314			

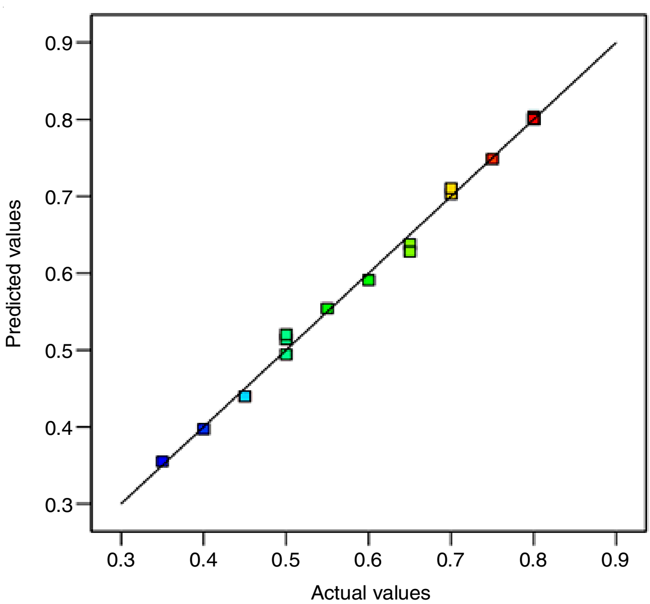


Fig. 2. Predicted *vs.* actual plot

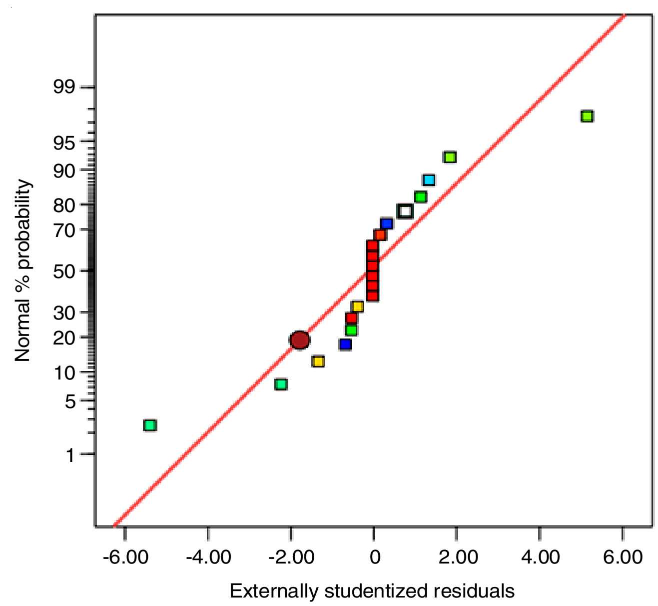


Fig. 4. Normal probability of internally studentized residuals

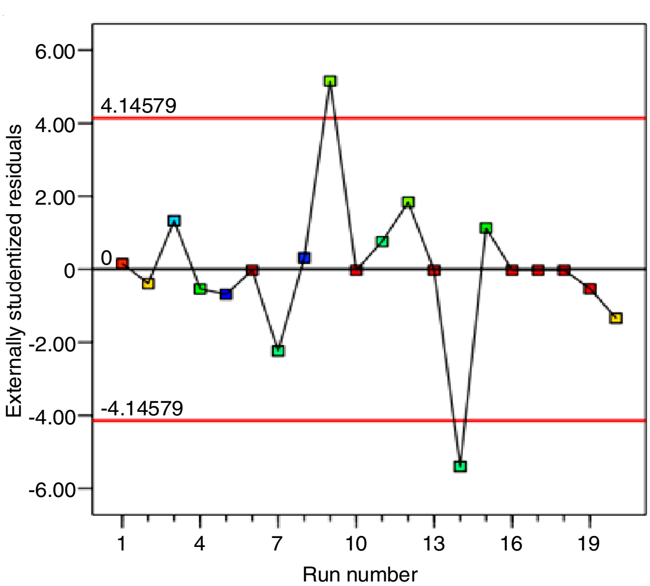


Fig. 3. Residuals *vs.* run order

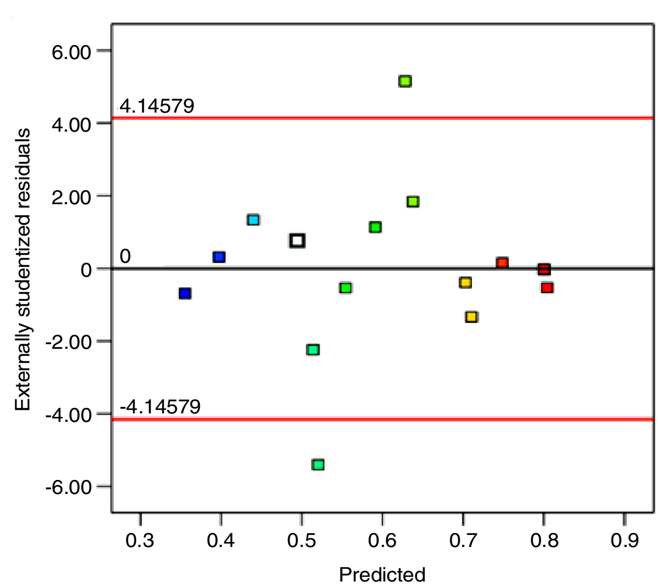
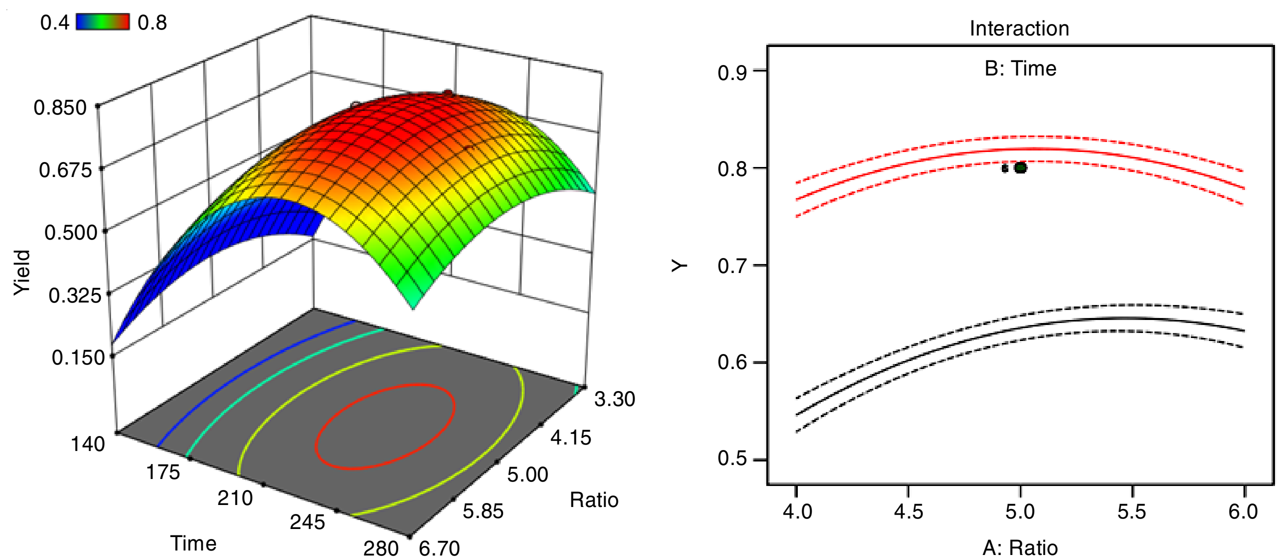


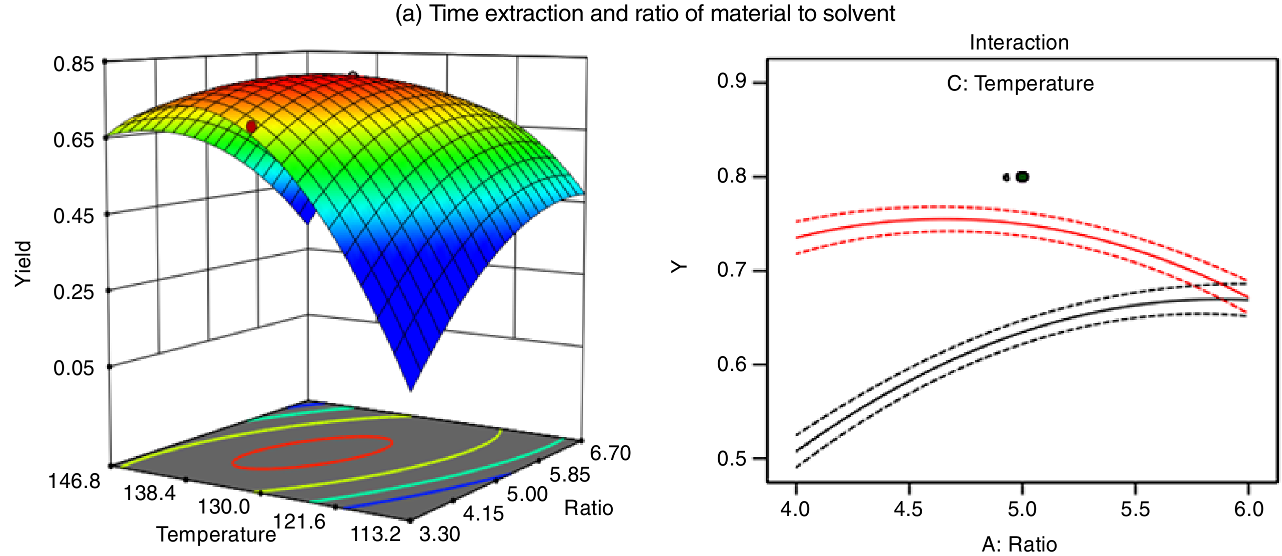
Fig. 5. Plot of externally studentized residuals *vs.* predicted response

of Y. Both plots (Figs. 4 and 5) are satisfactory, so it is concluded that the empirical model is adequate to describe the lemon oil extraction yield by response surface.

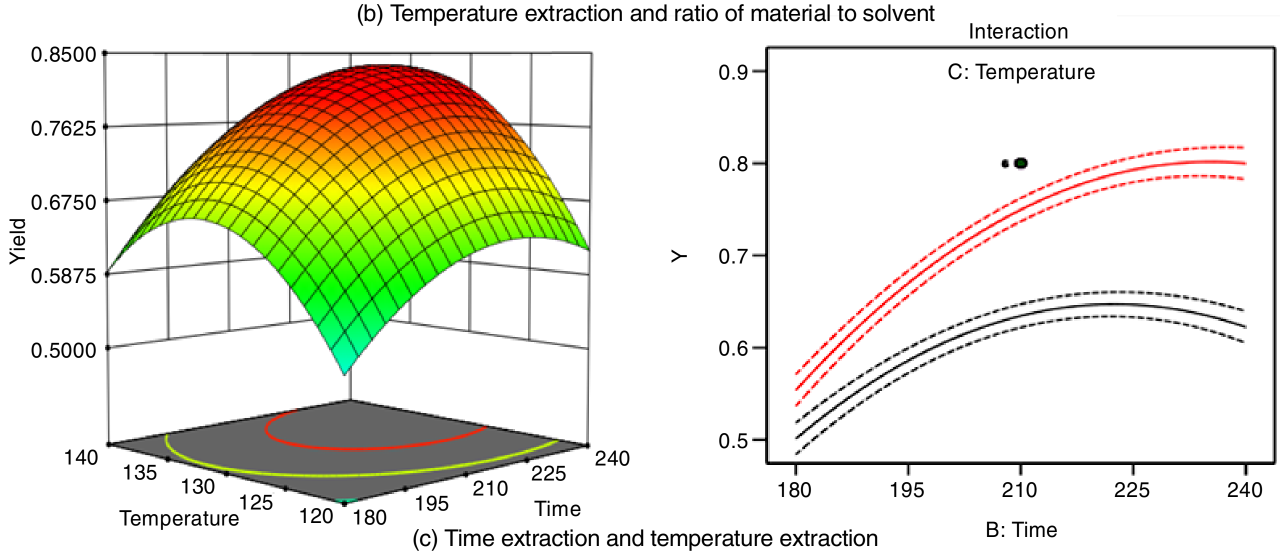
3-D response surface plot: After integrating the data, Fig. 6 shows the 3D models of the effect of factors on the yield of essential oils obtained from the extraction process by hydro-



(a) Time extraction and ratio of material to solvent



(b) Temperature extraction and ratio of material to solvent



(c) Time extraction and temperature extraction

Fig. 6. 3D models and curves represent the simultaneous interaction of two factors on yield Y

distillation. It can be observed that, on the one hand, the yield of the essential oil is directly proportional to the influencing factors including the ratio of material to water, time and temperature of extraction. On the other hand, when these factors exceed the optimum point, the amount of essential yield oil may decrease. The results obtained after optimization were water and raw material ratio of 6:1 (mL/g), 225 min time and extraction temperature of 130 ºC with expected yield of 0.8% (desirability 99.7%). With this result, it is proposed that the experimental values were accurately predicted by the quadratic model.

Influence a factor on yield and perturbation and cube plot: In general, yield of *Piper longum* essential oil has been altered and tends to be extreme when all three factors *viz*. material and solvent ratio, extraction time, temperature of extraction change in the survey area as shown in Fig. 7. This change is quite evident in the two factors of material ratio and solvent and extraction temperature, however, for the extraction time, the change of the target function is not clear.

The perturbation plot shown the comparison between all factors at a selected point in the design space considered. The disturbance diagram for the extraction yield of *Piper longum* essential oil is shown in Fig. 8. Satisfactorily extraction yield is derived by changing only one element in its range while other factors were kept and unchanged. The diagram shows the effect of all the factor at a central point in the design space. The high curvature in all three plant-to-solvent ratio, time and temperature of extraction showed that the reaction of the extract of lemon essential oil changed very quickly because of the effect this element.

GC-MS analysis: *Piper longum* Linn. essential oil, after extracted by hydrodistillation was analyzed for active compounds by GC-MS technique. In *Piper longum* Linn. essential oil, 43 compounds were found, of which 35 components have identified name (97.66%) and 8 components with unknown names (2.34%) (Fig. 9). β-Caryophyllene, which is the main compound in *Piper longum* essential oil, accounts for 15.248% (Table-4), this result is similar to Zaveri *et al*. [19] that *Piper longum* contains less essential oil when compared to its relatives (about 1%), including β-caryophyllene accounting for 10 to 20% [19], in addition, Kadota *et al*. [31] showed the content

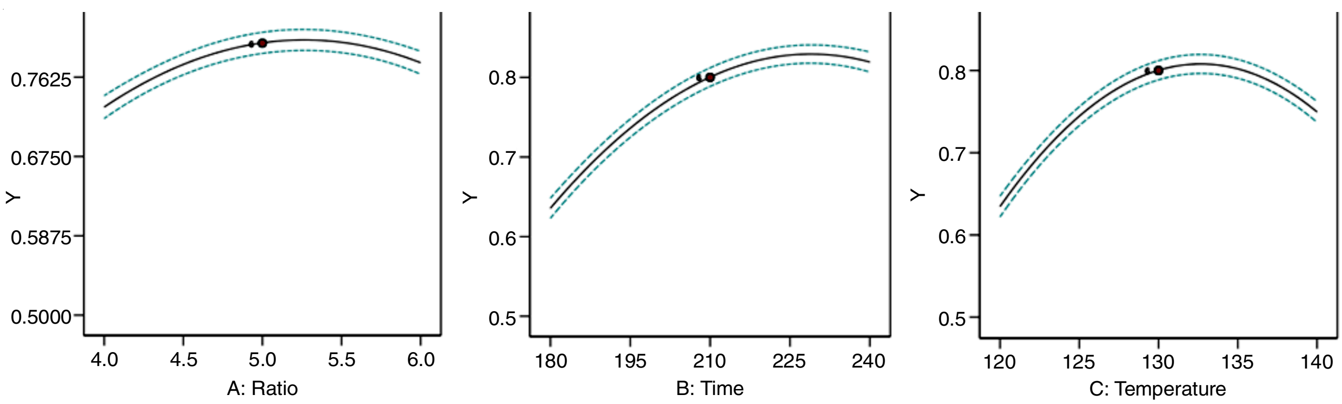


Fig. 7. Influence a factor on yield: Ratio of material to solvent, time extraction, temperature extraction

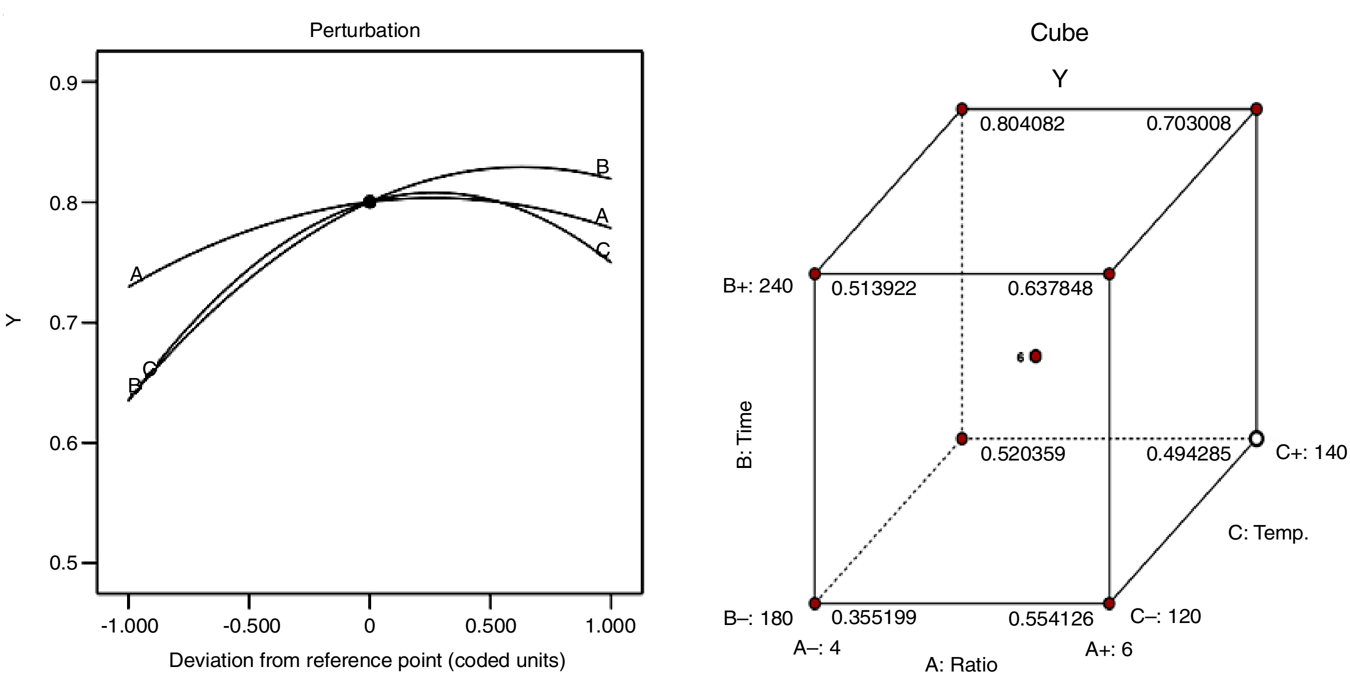


Fig. 8. Perturbation and cube plot

TABLE-4
COMPOUNDS IN *Piper longum* Linn ESSENTIAL OIL

Peak	RT	Compounds name	Content (%)	Peak	RT	Compounds name	Content (%)
1	7.261	α-Pinene	0.123	23	32.588	Unknown name	0.511
2	25.289	Tridecane	0.742	24	33.121	β-Spathulenol	0.388
3	26.481	*d*-Elemene	0.365	25	33.173	Unknown name	0.246
4	27.694	Copaene	0.584	26	33.226	Sesquisabinene hydrate	1.951
5	28.008	Unknown name	0.133	27	33.341	Unknown name	0.656
6	28.175	β-Cubebene	0.367	28	33.738	Cyclodecene	0.721
7	28.227	β-Elemene	2.964	29	33.811	Neointermedeol	0.218
8	28.928	α-Bergamotene	0.389	30	33.916	Selin-6-en-4α-ol	0.283
9	29.064	β-Caryophyllene	15.248	31	34.094	Isospathulenol	0.338
10	29.524	α-Farnesen	0.725	32	34.292	Unknown name	0.479
11	30.047	α-Caryophyllene	9.576	33	34.533	α-Cadinol	0.173
12	30.151	β-Farnesene	1.333	34	34.627	Naphthalenol	0.221
13	30.841	Hexadecen-1-ol	13.752	35	34.836	Isoheptadecanol	4.344
14	30.967	β-Eudesmene	5.973	36	34.972	Tridecane	5.650
15	31.207	α-Selinene	3.079	37	35.160	Heptadecane	6.271
16	31.302	Pentadecane	6.475	38	36.990	Unknown name	0.137
17	31.396	*g*-Elemene	1.624	39	37.147	Unknown name	0.102
18	31.563	β-Humulene	6.167	40	37.377	Unknown name	0.076
19	31.772	α-Selinene	3.108	41	37.419	Eicosene	0.453
20	31.919	β-Sesquiphellandren	0.696	42	37.513	Tetradecene	0.863
21	32.107	γ-Bisabolene	0.419	43	37.618	Pentadecane	0.570
22	32.337	α-Bisabolene	1.507				

of β-caryophyllene in essential oil for 10.2%. Similarly, Liu *et al*. [32] reported that β-caryophyllene accounted for 33.44%, while Varughese *et al*. [33] reported 5.7 ± 0.2%. *Piper longum* essential oil has been researched and published to show that it has insecticidal and acaricidal activity, bioavailability enhancers, antifungal activity, antiamoebic activity, adulticidal activity thanks to the bioactive compounds was displayed in Table-4 [20-22,34].

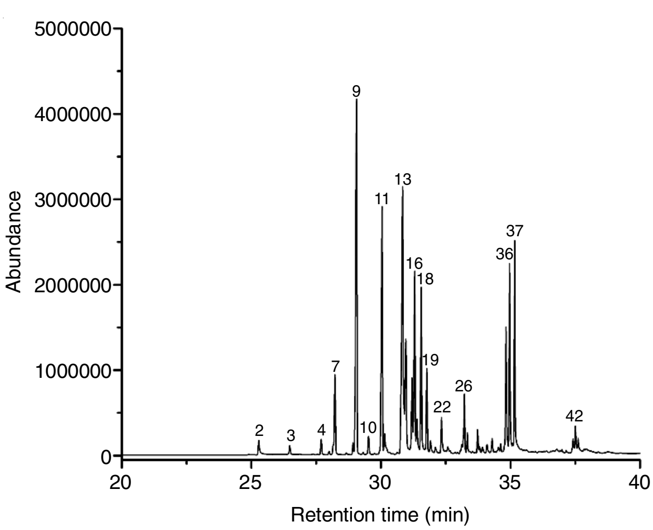


Fig. 9. Chromatography of *Piper longum* Linn essential oil

Conclusion

In this study, the essential oils extracted from the fruits of *Piper longum* Linn. were obtained at 0.8% under optimized conditions having R^2 = 99.63% and reliability of 99.7%. Compared to essential oils extracted from different types of pepper, *Piper longum* Linn. have lower essential oil yield. In addition, GC-MS results show that β-caryophyllene was the main active compouds in *Pepper longum* Linn. essential oil, which accounts for 15.248%.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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