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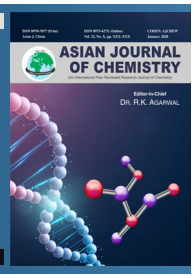

# Optimization of Essential Oil Extraction Process of White Pepper (*Piper nigrum* L.) Harvested in Phu Quoc Island, Kien Giang Province, Vietnam

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Vietnam is a leading producer and exporter of products harvested from plants of the family Piperaceae such as green pepper, black pepper, red pepper and white pepper. In this study, an attempt is made to examine the essential oil extraction process with the material of white pepper grown and harvested in Phu Quoc island, Vietnam. To maximize essential oil production, three factors consisting the ratio of materials and solvents, extraction time and extraction temperature were selected and optimized. The surface response methodology optimization resulted the highest yield of 3.6%, achieved at the ratio of material and water ratio of 1:20 g/g, extraction time of 96 min and at 130 ºC. A high F values, low P values (< 0.0001), the determination coefficient ($R^2$ = 0.9993) and a non-significant lack of fit suggested a strong correlation between actual and predicted values of the responses. The essential oil obtained was determined for chemical composition by gas chromatography-mass spectrometry (GC-MS) method. The GC-MS results showed that major constituents existing in the oil sample were limonene, 3-carene, sabinene, β-pinene, α-pinene and α-phellandrene, accounting for 27.059, 23.345, 17.903, 9.996, 5.167 and 4.588%, respectively.

**Keywords: Optimization, Essential oil, Extraction process, White pepper (*Piper nigrum* L.).**

## INTRODUCTION

Prevention of oxidative degradation is among the most important processes in treatment, preservation and transportation in various industries [1-5]. This urges for development of natural antioxidants derived from plants and aromatic plants [6-10]. Essential oils, one of secondary metabolites obtained from herbal plants or aromatic plants, has been the common ingredient in antioxidative measures due to their diverse biological properties, including positive effects on cardiovascular diseases, analgesic, anti-inflammatory, antibacterial activity and capability of clearing free radicals, inhibiting lipid peroxidation and stimulating activity of antioxidant enzymes [11-15]. *Piper nigrum* L. (black pepper), a popular plant in the family Piperaceae, has been widely cultivated for its fruit for production of pepper spice and seasoning. The peppercorn, in both bare and peeled forms, finds wide application in food, culinary and medicines. In addition, pepper essential oil, abundantly found in its seeds, contains various aromatic compounds with biological activities [16,17]. There are various techniques to obtain pepper essential oil including hydrodistillation with water or organic solvent, microwave or ultrasonic assisted hydrodistillation, or $CO_2$ supercritical method. However, for industrial scale production, the hydrodistillation method offers various advantages including low production cost and operation simplicity.

In order to efficiently employ hydrodistillation for essential oil production, extraction conditions should be optimized. In

this study, extraction of essential oil from Vietnamese white pepper, a common name for peeled black pepper, was attempted. Following that, response surface method (RSM), a common method in optimization of various processes [18,19], was utilized to determine appropriate parameters for maximal essential pepper essential yield. The experiment design consisted of 20 experiments for 3 independent factors and 1 response factor of essential oil yield. Lastly, optimized samples of essential oils were subjected to determination of bioactive components by gas chromatography-mass spectrometry (GC-MS).

## EXPERIMENTAL

The white pepper (*Piper nigrum* L.) seeds used in this study was harvested in Phu Quoc Island, Kien Giang Province, Vietnam (9°53′ to 10°28′N and 103°49′ to 104°05′E) in 2019. Selected pepper seeds was about 3-4 mm in diameter with white milk or gray colours. The pre-treatment process of materials is as follows. Firstly, the white pepper was washed several times with water to dispose of impurities outside the seeds and was soaked in 2% NaCl solution for 3 h. Finally, the solution was removed and the materials was ground by a grinder (Sunhouse SHD5322, 220 W, Vietnam) to produce materials with size of mesh 18. Ground pepper was transferred to zipper bags to prevent sample contact with air and stored at 4 °C by a cooling instrument (Alaska, LC-743H, Vietnam) for hydrodistillation.

**Extraction and optimization by response surface methodology (RSM):** In order to obtain white pepper essential oil, the white pepper seeds after pre-treatment, underwent the hydrodistillation procedure at the laboratory scale. In detail, a 1000 mL volumetric flask containing a mixture of the ground white pepper and distilled water in a certain proportion. The flask was connected directly with the condenser and the separator device. The influential factors in the extraction of essential oils derived from white pepper include the ratio of raw materials and water, extraction time and extraction temperature. Optimization of these factors was performed *via* response surface methodology (RSM).

Optimization parameters for maximal essential oil yield included the ratio of water and white pepper seeds (A, g/g), extraction time (B, min) and extraction temperature (C, °C). To generate the experimental matrix design, the central composite design (CCD) approach was employed and the results were displayed in Table-1. The parameter A was allowed to vary from 15:1 to 25:1 g/g. Time range was from 60 to 120 min and extraction temperature was set in the range of 120 to 140 °C. To perform the optimization, Design-Expert® software version 11 was used to generate the experiment design and verify the data. The response factor, yield of white pepper essential oils, was calculated as follows (1):

TABLE-1
LEVELS AND INDEPENDENT FACTORS OF THE LEMON ESSENTIAL OIL EXTRACTION PROCESS

| Levels | Independent factors | | |
|---|---|---|---|
| | Ratio of water and basil leaves A (g/g) | Extraction time B (min) | Extraction temperature C (°C) |
| -α | 11.6:1 | 39.5 | 113 |
| -1 | 15:1 | 60 | 120 |
| 0 | 20:1 | 90 | 130 |
| 1 | 25:1 | 120 | 140 |
| +α | 28.4:1 | 140.5 | 147 |

$$\text{Yield of white pepper oil (mL/g)} = \frac{\text{Volume of essential oil (mL)}}{\text{Amount of seeds used (g)}} \quad (1)$$

**Composition of essential oils by hydrodistillation:** The components presenting in essential oils of white pepper is analyzed by gas chromatography-mass spectrometry (GC-MS). Firstly, 25 µL sample of essential oil was added in 1.0 mL *n*-hexane, followed by dehydration by $Na_2SO_4$. The analysis instrument was GC Agilent 6890 N, MS 5973 inert. HP5-MS column, head column pressure 9.3 psi. Carrier gas in the analysis was He. The flow rate was 1.0 mL/min. the split ratio was 1:100; injection volume 1.0 µL; injection temperature 250 °C; oven temperature progress included an initial hold at 50 °C for 2 min, a rise to 80 °C at 2 °C/min, a rise to 150 °C at 5 °C/min, a rise to 200 °C at 10 °C/min and a rise to 300 °C at 20 °C/min for 5 min.

## RESULTS AND DISCUSSION

**Optimization of factors by response surface methodology:** Table-2 shows the parameter levels for 20 experimental runs generated by CCD and experimental results associated with those conditions. In addition, predicted results calculated from the estimated quadratic model were also presented. It was clear that the oil yield varied drastically with variations of parameters.

TABLE-2
RESULTS OF THE ACTUAL AND PREDICTED VALUES FOR THE RSM MODEL

| No. | Independent variables | | | Y (%) | | |
|---|---|---|---|---|---|---|
| | A | B | C | Actual | Predicted | Residual |
| 1 | 15:1 | 60 | 120 | 1.90 | 1.90 | 0.0044 |
| 2 | 25:1 | 60 | 120 | 1.90 | 1.89 | 0.0148 |
| 3 | 15:1 | 120 | 120 | 2.15 | 2.12 | 0.0257 |
| 4 | 25:1 | 120 | 120 | 2.80 | 2.79 | 0.0111 |
| 5 | 15:1 | 60 | 140 | 2.40 | 2.39 | 0.0129 |
| 6 | 25:1 | 60 | 140 | 2.25 | 2.25 | -0.0017 |
| 7 | 15:1 | 120 | 140 | 2.35 | 2.34 | 0.0092 |
| 8 | 25:1 | 120 | 140 | 2.90 | 2.88 | 0.0196 |
| 9 | 11.6:1 | 90 | 130 | 2.55 | 2.57 | -0.0195 |
| 10 | 28.4:1 | 90 | 130 | 3.00 | 3.01 | -0.0145 |
| 11 | 20:1 | 39.5 | 130 | 2.05 | 2.06 | -0.0065 |
| 12 | 20:1 | 140.5 | 130 | 2.75 | 2.78 | -0.0274 |
| 13 | 20:1 | 90 | 113 | 1.70 | 1.72 | -0.0218 |
| 14 | 20:1 | 90 | 147 | 2.20 | 2.21 | -0.0121 |
| 15 | 20:1 | 90 | 130 | 3.55 | 3.59 | -0.0407 |
| 16 | 20:1 | 90 | 130 | 3.60 | 3.59 | 0.0093 |
| 17 | 20:1 | 90 | 130 | 3.60 | 3.59 | 0.0093 |
| 18 | 20:1 | 90 | 130 | 3.60 | 3.59 | 0.0093 |
| 19 | 20:1 | 90 | 130 | 3.60 | 3.59 | 0.0093 |
| 20 | 20:1 | 90 | 130 | 2.40 | 3.59 | 0.0093 |

The quadratic model, after being estimated, was subjected to statistical analysis. Table-3 showed the statistical parameters and ANOVA result of the model. Obtained F-value was 1658.38, suggesting that the produced model was statistically significant and it is unlikely that noise could cause an F-value of this

large. The Lack of Fit F-value of 1.76 suggests the irrelevance of pure error. For individual model terms, none of the included factors exhibited statistical insignificance at 95% confidence. Both predicted ($R^2$) and adjusted coefficient of determination were also in good agreement, suggesting the reasonability of the quadratic model and the unnecessity to re-specify. This was further supported by comparison results in Fig. 1A where predicted and actual yields were approaching each other.

On the other hand, the Fig. 1B showed that the calculated residuals were randomly distributed. The adequacy of model was further investigated as shown in Fig. 2A. It was visually clear that the studentized residuals was positively correlated to probability of being normally distributed. In addition, Fig. 2B also presented no clear relationship between predicted yields and corresponding studentized residuals, indicating that the original observation variance is constant for all values of Y. Therefore, it can be concluded that the statistical model is sufficient to describe the yield of white pepper essential oil.

Further calculations on the quadratic model resulted in the following conditions: A = 20:1 mL/g, B = 96 min and C = 130 °C. This set of optimized conditions correspond to the oil yield of 3.601% with 100% reliability. The following equation 2 describes the relationship between experimental conditions and the oil yield. Response surfaces were illustrated in the Fig. 3.

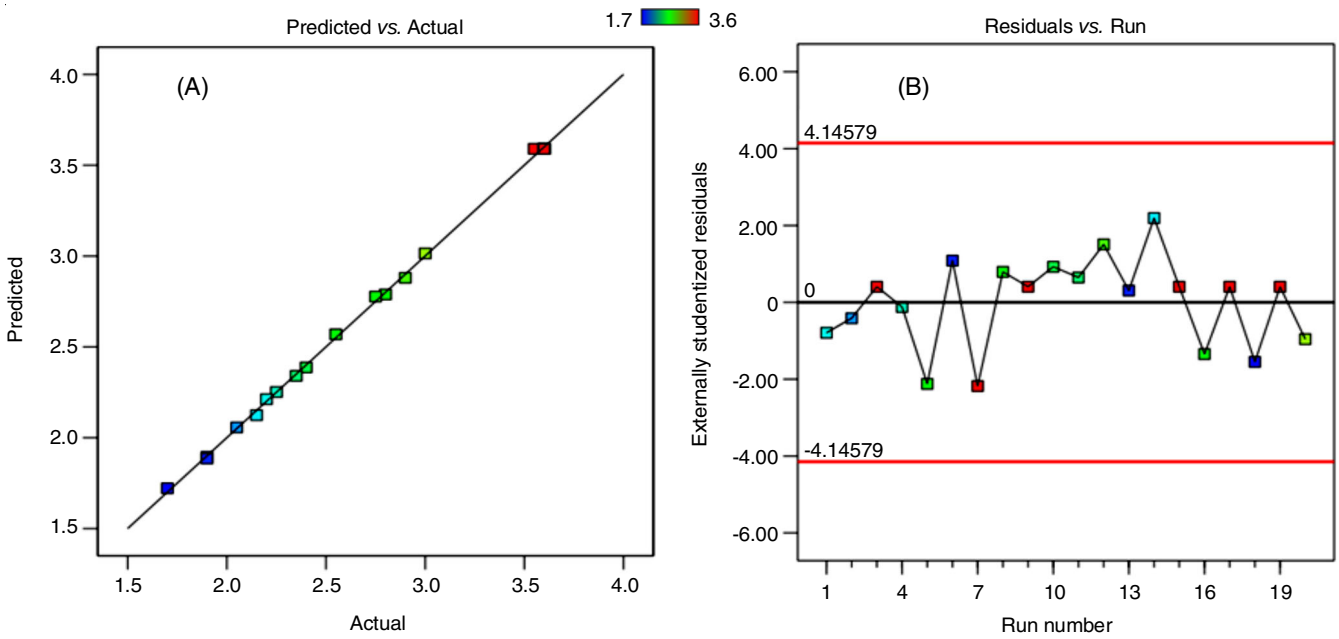


Fig. 1. (A) Comparison between actual values and predicted values, (B) Normal plot of residuals with run number

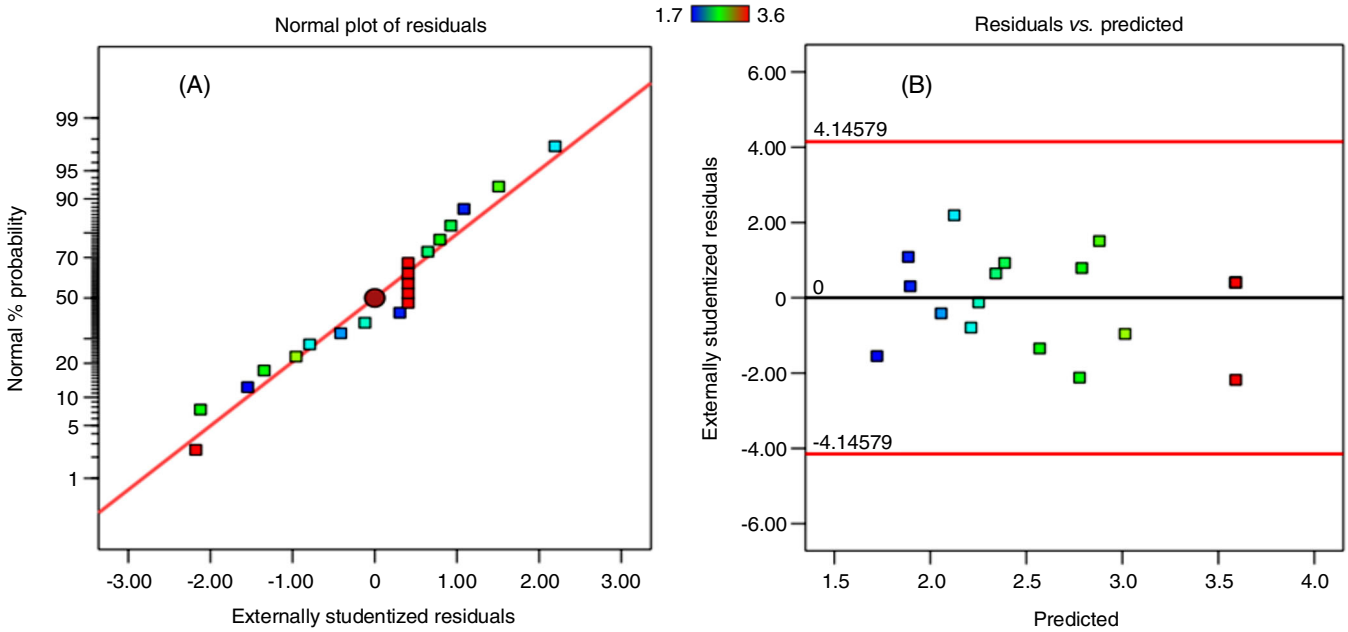


Fig. 2. (A) Normal probability of internally studentized residuals (B) Plot of internally studentized residuals *vs.* predicted response

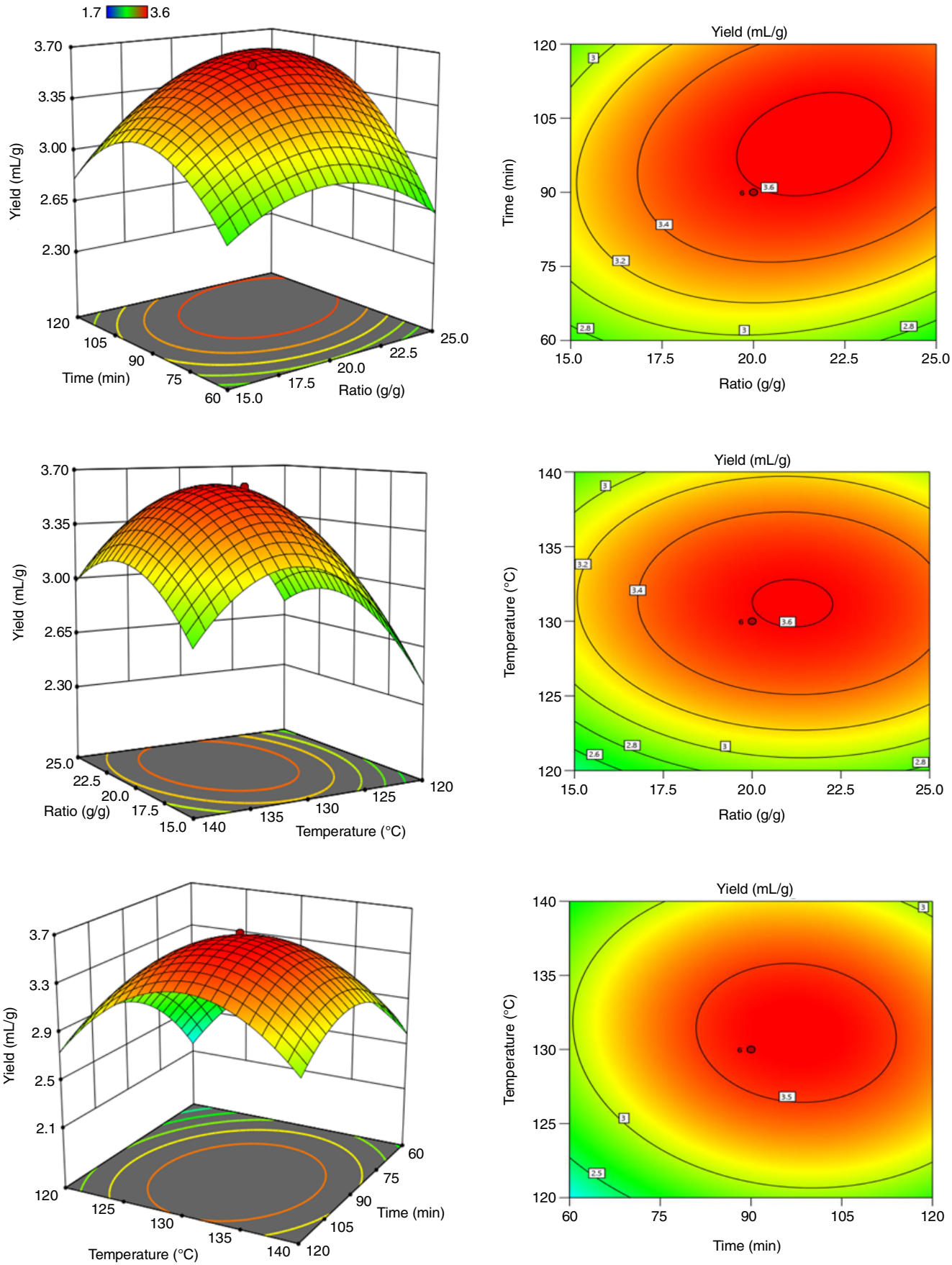


Fig. 3. 3D models and contour of the interaction relationship between yield and factor A (water and seeds ratio), B (extraction time), C (extraction temperature)

TABLE-3
ANOVA FOR QUADRATIC MODEL

| Source | Sum of squares | Degree of freedom | Mean square | F-value | Prob. > F | Comment |
|---|---|---|---|---|---|---|
| Model | 8.5900 | 9 | 0.9546 | 1658.38 | < 0.0001 | Significant |
| A-A | 0.2390 | 1 | 0.2390 | 415.27 | < 0.0001 | Significant |
| B-B | 0.6274 | 1 | 0.6274 | 1089.99 | < 0.0001 | Significant |
| C-C | 0.2902 | 1 | 0.2902 | 504.20 | < 0.0001 | Significant |
| AB | 0.2278 | 1 | 0.2278 | 395.76 | < 0.0001 | Significant |
| AC | 0.0078 | 1 | 0.0078 | 13.57 | 0.0042 | Significant |
| BC | 0.0378 | 1 | 0.0378 | 65.69 | < 0.0001 | Significant |
| $A^2$ | 1.1500 | 1 | 1.1500 | 1996.48 | < 0.0001 | Significant |
| $B^2$ | 2.4800 | 1 | 2.4800 | 4311.23 | < 0.0001 | Significant |
| $C^2$ | 4.7500 | 1 | 4.7500 | 8250.73 | < 0.0001 | Significant |
| Residual | 0.0058 | 10 | 0.0006 | – | – | – |
| Lack of Fit | 0.0037 | 5 | 0.0007 | 1.76 | 0.2744 | Not significant |
| Pure Error | 0.0021 | 5 | 0.0004 | – | – | – |
| Std. Dev. = 0.0240 | | Mean = 2.72 | | C.V.% = 0.8813 | | $R^2$ = 0.9993 |
| Adjusted $R^2$ = 0.9987 | | Predicted $R^2$ = 0.9964 | | Adeq Precision = 110.1609 | | |

$$Yield\ (Y) = 3.59 + 0.1323A + 0.2143B + 0.1458C + 0.1688AB - 0.0312AC - 0.0688BC - 0.2824A^2 - 0.4150B^2 - 0.5741C^2 \quad (2)$$

**Composition of white pepper essential oils:** Fig. 4 and Table-4 showed the GC-MS chromatograms and chemical composition of the obtained essential oil, respectively. Determination of compounds and their content in white pepper essential oil showed that 22 compounds were present in the essential oil composition. Out of which, 13 compounds, accounting for approximately 94% of total content, was identified and nine remaining compounds remained unidentified. The component with highest content was limonene (27.059%), followed by 3-carene (23.345%), sabinene (17.903%), α-pinene (5.167%), β-pinene (9.996%) and α-phellandrene (4,588%). For comparison, contents of most of the major constituents, including limonene, sabinene, 3-carene, α-pinene and α-phellandrene, were higher than those reported by Singh *et al*. [20]. In present work, limonene content, a main compound in pepper essential oil, also exceeds that of essential oils derived from different types of pepper harvested from Sri Lanka (10.2-19.7%). However, in comparison with Indian white pepper oil, current result falls short of caryophyllene, which accounted for 16.0% of

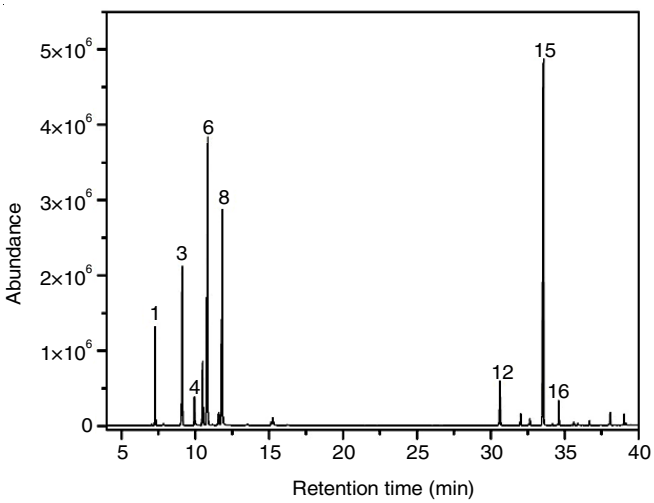


Fig. 4. GC-MS spectrum of white pepper essential oil

TABLE-4
RESULTS OF THE COMPOUNDS DETERMINATION IN WHITE PEPPER OIL

| Peak | R.T. | Name | Content (%) |
|---|---|---|---|
| 1 | 7.282 | α-Pinene | 5.167 |
| 2 | 7.857 | Unknown compound | 0.137 |
| 3 | 9.112 | β-Pinene | 9.996 |
| 4 | 9.949 | β-Myrcene | 2.001 |
| 5 | 10.503 | α-Phellandrene | 4.588 |
| 6 | 10.838 | 3-Carene | 23.354 |
| 7 | 11.591 | Benzene | 1.066 |
| 8 | 11.831 | Sabinene | 17.903 |
| 9 | 15.135 | Unknown compound | 0.266 |
| 10 | 15.250 | Unknown compound | 0.797 |
| 11 | 30.538 | Unknown compound | 0.13 |
| 12 | 30.633 | Unknown compound | 2.767 |
| 13 | 32.023 | Unknown compound | 0.716 |
| 14 | 32.651 | Unknown compound | 0.508 |
| 15 | 33.571 | Limonene | 27.059 |
| 16 | 34.617 | α-Caryophyllene | 1.413 |
| 17 | 35.631 | β-Selinene | 0.21 |
| 18 | 35.903 | α-Selinene | 0.2 |
| 19 | 36.698 | Naphthalene | 0.294 |
| 20 | 38.088 | Caryophyllene oxide | 0.749 |
| 21 | 39.009 | Unknown compound | 0.573 |
| 22 | 39.155 | Unknown compound | 0.107 |

content in a previous study [21]. These results suggests that Vietnamese white pepper essential oil is rich in limonene and suitable for limonene-intensive applications in food or pharmaceutical industries.

## Conclusion

This study has successfully extracted the essential oil from white pepper materials collected from Vietnam. Response surface methodology-optimization of the extraction process resulted in the optimal yield of 3.6% and optimal conditions consisting of ratio of water and materials of 1:20 g/g, extraction time of 96 min and temperature of 130 °C. GS-MS determination of the obtained oil sample revealed a high content of limonene.

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## CONFLICT OF INTEREST

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

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