Optimization of operating conditions of Lemon (*Citrus aurantifolia*) essential oil extraction by Hydro-distillation Process using Response Surface Methodology

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Abstract. In this study, extraction of essential oils from the peels of lemon (*Citrus aurantifolia*) was conducted and optimized. Response Surface Methodology (RSM) was adopted to optimize four process parameters including the ratio of water and lemon peel (2:1 to 4:1 mL/g), extraction time (1 to 2 h) and extraction temperature (110 to 130 °C) to achieve maximal oil yield. The results showed a ratio of 3:1 (mL/g), extraction time of 1.6 hours, a temperature of 120 °C produced an oil yield of 2.097%. In addition, GC-MS results revealed a total of 15 identified compounds in lemon essential oil with the major compound being Limonene, accounting for 66% of total content. The study applied surface response methodology to optimize the hydrodistillation conditions for extracting lemon essential oil. Application of this computer software is particularly advantageous in reducing the experiment number while producing reliable results.

1. Introduction

Recent scientific attention in healthcare provision, especially in developing countries, has shifted to utilization of medicinal plants and traditional medicine in disease treatment [1-9]. There have been many studies showing the importance of natural compounds in plants that play an important role in improving human health [10-14]. In addition, these natural extracts are also used as flavors and fragrances in food [15-20]. Therefore, natural compounds are highly important in social life.

*Citrus aurantifolia* is a small citrus fruit of Rutaceae family and has been well-known for important functions across many countries [21-24]. The peel of *Citrus aurantifolia* contains around 4% of essential oil (EOs), contributing to sweet and delicious taste of the fruit and determining its role in manufacture of aromatic agents in cosmetics and food. Medicinal applications of *Citrus aurantifolia* essential oils include treatments for nausea, urinary disorders, digestive and skin problems and obesity. Numerous methods have been developed for extracting EOs from plants, ranging from conventional methods such as mechanical pressing, hydrodistillation, steam distillation and solvent extraction to more novel methods including supercritical fluid extraction and microwave- and ultrasound-assisted extraction [25-28]. The extraction efficiency of essential oil is influenced by several factors, including extraction time, extraction temperature...
and solvent to solid ratio. Optimization of these factors could lead to a more efficient extraction process, thereby conserving manufacturing cost and resulting in essential oils of higher quality. Response surface methodology (RSM) has been a popular routine to quantify and evaluate the effects of experimental conditions on a specified outcome. The technique involves establishing of a function describing interrelations between input variables and an outcome response, which was then used for determining the optimal solution with respect to a target [29-35]. However, there are limited studies on optimization the operating conditions of hydrodistillation extraction of lemon essential oil. Therefore, the present study performed essential oil extraction from Lemon (Citrus aurantifolia) peels with an attempt to optimize hydro-distillation conditions via RSM.

2. Material and method

2.1 Materials and chemicals

Lemon fruits (Citrus aurantifolia) were collected in the province of Tien Giang, Vietnam. Anhydrous sodium sulfate (Na₂SO₄) was obtained from Sigma Aldrich (US). Deionized water provided by the Milli-Q (Millipore) purification method (Massachusetts, USA) was used as a solvent in the extraction phase.

2.2 Method and Process

The lemon after being purchased was treated by washing several times with water. The lemon peels were separated and crushed to increase the yield of the essential oil extraction. 50 g of lemon peels was added to a flask with a volume of 1000 mL and extracted by hydrodistillation method. The hydrodistillation system was heated by Genlab heating device (China, 98-IC model 1000 mL), which was placed under the flask containing the plant materials and solvent. A condenser was connected to the top of the flask to collect the vapor, which was then transferred into a separator to separate essential oil from water. The obtained essential oil is anhydrous with Na₂SO₄ to remove residual water in mixture. The yield of EOs on lemon peels was calculated by the following equation (1)

\[ y = \frac{v}{m} \times 100 \]  

\( y \) is the lemon oil yield (mL/g), \( v \) is the volume of extracted lemon oil (mL) and \( m \) is the weight or mass of lemon peels (g).

2.3 Experimental Design by RSM

To conduct RSM procedure, Design Expert software version 11.0 (DE11, Minneapolis, USA) was used. Three independent variables including material/water ratio, extraction time and extraction temperature were considered in establishing the model. The value ranges of these variables were selected based on previous studies [36-38]. Specific conditions of A, B, C are presented in Table 1, including 2:1 to 4:1 mL/g, 1 to 2 h and 110 to 130 °C, respectively. Each independent factor was varied in 5 levels: minus and plus alpha (axial point), minus and plus 1 (factorial point) and the center point. All experiments were performed in triplicates.

| Independent factors | Levels | Ratio of water and basil leaves A (mL/g) [36] | Time B (hour) [37] | Temperature C (°C) [38] |
|---------------------|--------|-----------------------------------------------|-------------------|------------------------|
| -α                  | 1.32:1 | 0.66                                          | 103                |
| -l                  | 2:1    | 1.0                                           | 110                |
| 0                   | 3:1    | 1.5                                           | 120                |
| 1                   | 4:1    | 2.0                                           | 130                |
| +α                  | 4.68:1 | 2.34                                          | 137                |
2.4. Identify compounds with GC-MS
For the determination of chemical composition of the EOs obtained, GC-MS was applied.

3. Results and Discussion

3.1. Optimization of factors by DE11
Table 2 shows the results of the 20 experiments and the comparison of yields between the actual experimental values and the predicted value made by the software. Only marginal differences (±0.01 to ±0.02) were observed between the two values, which indicates that the experimental results are carried out without errors.

Table 2. Details of experimental optimization attempts used in the RSM.

| No. | Parameters | Yields | | | No. | Parameters | Yields |
|-----|------------|--------|---|---|-----|------------|--------|
|     | (A) (B) (C) | Actual | Predicted | | (A) (B) (C) | Actual | Predicted |
| 1   | 2 1 110    | 1.70   | 1.68      | 11 |  3 0.66 120 | 1.80   | 1.81      |
| 2   | 4 1 110    | 1.80   | 1.81      | 12 |  3 2.34 120 | 1.95   | 1.94      |
| 3   | 2 2 110    | 1.85   | 1.85      | 13 |  3 1.5 103 | 1.80   | 1.81      |
| 4   | 4 2 110    | 1.90   | 1.91      | 14 |  3 1.5 137 | 1.75   | 1.75      |
| 5   | 2 1 130    | 1.80   | 1.79      | 15 |  3 1.5 120 | 2.05   | 2.09      |
| 6   | 4 1 130    | 1.75   | 1.75      | 16 |  3 1.5 120 | 2.10   | 2.09      |
| 7   | 2 2 130    | 1.85   | 1.84      | 17 |  3 1.5 120 | 2.10   | 2.09      |
| 8   | 4 2 130    | 1.70   | 1.72      | 18 |  3 1.5 120 | 2.10   | 2.09      |
| 9   | 1.32 1.5 120 | 1.75   | 1.77      | 19 |  3 1.5 120 | 2.10   | 2.09      |
| 10  | 4.68 1.5 120 | 1.80   | 1.78      | 20 |  3 1.5 120 | 2.10   | 2.09      |

3.2 Statistical analysis
Table 3 illustrates the analysis of variance (ANOVA) for the established model. The F value of the model achieved 102.86, which was highly significant. The lack of fit was 0.4313, which was not significant. This suggests that the model is valid for describing the process and high compatibility of the model with actual experiments. In this study, A2, B2, C2 are significant model terms (P<0.05). The correlation coefficient R2 (R²= 0.9893) was also high, indicating that high fit of the parameters against the response.

Table 3. The result of ANOVA for the quadratic model.

| Source     | Sum of Squares | df | Mean Square | F-value | p-value | R² | Adjusted R² |
|------------|----------------|----|-------------|---------|---------|----|-------------|
| Model      | 0.4198         | 9  | 0.0466      | 102.86  | < 0.0001 | significant | 0.9893 |
| A-A        | 0.0001         | 1  | 0.0001      | 0.1876  | 0.6741  | significant | 0.9797 |
| B-B        | 0.0185         | 1  | 0.0185      | 40.73   | < 0.0001 | significant | 0.9479 |
| C-C        | 0.0040         | 1  | 0.0040      | 8.85    | 0.0139  | significant | Std. Dev. = 0.0213 |
| AB         | 0.0028         | 1  | 0.0028      | 6.20    | 0.0320  | significant | C.V. % = 1.89 |
| AC         | 0.0153         | 1  | 0.0153      | 33.76   | 0.0002  | significant | Mean = 1.13 |
| BC         | 0.0078         | 1  | 0.0078      | 17.23   | 0.0020  | significant | C.V. % = 1.13 |
| A²         | 0.1789         | 1  | 0.1789      | 394.42  | < 0.0001 | significant | 0.9893 |
| B²         | 0.0834         | 1  | 0.0834      | 183.81  | < 0.0001 | significant | 0.9893 |
| C²         | 0.1789         | 1  | 0.1789      | 394.42  | < 0.0001 | significant | 0.9893 |
| Residual   | 0.0045         | 10 | 0.0005      | 1.18    | 0.4313  |             |             |
| Lack of Fit| 0.0025         | 5  | 0.0005      | 1.18    | 0.4313  |             |             |
| Pure Error | 0.0021         | 5  | 0.0004      | 1.18    | 0.4313  |             |             |
| Cor Total  | 0.4244         | 19 |             |        |         |             |             |
3.3 Final Equation in Terms of Coded Factors

The proposed function describes the influence of process parameters and their mutual interactions on the attained oil yield. In other words, the response is influenced by both the first and second order of variables including the ratio of materials and solvent (A), extraction time (B) and extraction temperature (C) and their interaction terms. The estimated function shows that, among the three considered conditions, only extraction temperature (C) negatively affects the yield, evidenced by a negative coefficient (-0.0171) while both factor A and factor B exert strong influence on the objective function with positive coefficients of 0.0025 and 0.0368, respectively. All quadratic terms of A, B and C show negative coefficients, which is indicative of the presence of impact thresholds of variables on the response. The final equation is established as follows.

\[
\text{Yield} = 2.09 + 0.0025A + 0.0368B - 0.0171C - 0.0188AB - 0.0438AC - 0.0313BC - 0.1114A^2 - 0.0761B^2 - 0.1114C^2 \quad (2)
\]

The predicted values calculated from Eq. (2) were in line with the experimental values, as shown in Figure 1. It was observed that data points intersected by actual and predicted value were scattered around the 45-degree line, which proves that no major errors occur. Moreover, Figure 2 described that the residuals from experiment attempts were randomly distributed, suggesting the potential of the established model to accurately predict the yield.

3.4 Model adequacy checking

The normal probability plot of residuals is shown in Figure 3. The normality assumption is in line with the residual plot, which shows along a straight line. Figure 4 displays the predicted response versus a plot of residuals. The residuals were found to be scattered randomly within confidence range, implying that the variance of the original investigation is constant for all values of Y (Figure 4). Therefore, we assume that the empirical model is sufficient to describe the lemon oil extraction yield by RSM.
3.5. The 3-D response surface plot

Figure 5 presents 3D representations of the influence of the factors on the yield of lemon EOs obtained. Visually, the yield of EOs increases proportionately to the parameters condition. Nevertheless, rising these conditions exceeding the optimal point, the obtained amount of lemon essential oil stopped increasing, and eventually begin to decrease.

![Figure 3. Normal probability of externally studentized residuals](image1)

![Figure 4. Plot of externally studentized residuals vs. predicted response](image2)

**Figure 4.** Plot of externally studentized residuals vs. predicted response

**Figure 3.** Normal probability of externally studentized residuals

![Figure 5. 3D models describing influencing factors on yield Y](image3)

**Figure 5.** 3D models describing influencing factors on yield Y: a) Time extraction and ratio of material to solvent, b) Time and temperature extraction, c) Temperature extraction and ratio of material to solvent.
The optimal parameters obtained after optimization consisted of the ratio of water and raw materials of 3:1 (mL/g), extraction time of 1.6 hours and extraction temperature of 120 °C. These software-predicted parameters were then used for testing to execute real experiment tests. After a series of three experiments, the mean yield was determined at 2.1%, which approximates the expected yield from the model (2.097%). With this result the experimental values are proposed to be in line with the quadratic model's expected.

3.6. Influence a factor on yield

Variations of essential oil yield with respect to individual changes in experimental factors are illustrated in Figure 6. In general, all three factors seemed to capable of altering the yield when being individually varied. This change is more pronounced in the factor of material/solvent ratio and extraction temperature. However, with varying extraction time, the change of the target function is not clear.

3.7. Perturbation and cube plot

The perturbation plot describes the individual influence of factor variations on the response. The reference point was set at the optimal set of conditions and the impact of their deviations was shown in Figure 7. Clearly, the highest yield was maintained when individually varying one factor while other factors are kept and unchanged. The high curvature in all three curves that represent the three parameters suggests that the oil yield is highly sensitive to the change of any of the elements.

3.8. Chemical analysis of essential oil constituents

Lemon essential oil, after being extracted by hydrodistillation, was anhydrous and tested for analyzed for volatile composition by GC-MS. The results are presented in the chromatography diagram in Figure 8. There are 15 identified components in the oil sample. Of Which the component with the highest content is
Limonene (approximately 66%), followed by α-Terpinen at 13%. Other major observable components were α-Pinene, Sabinen, β-Pinene, β-Cymene and α-Bergamotene (with content ranging from 1.1 to 1.8%). Other minor compounds are determined to be lower than 1.0%.

Figure 8. The chromatography of lemon essential oil.

4. Conclusions
RSM based on Central Composite Design (CCD) has been used to determine the optimum conditions for the hydro-distillation process of Lemon (Citrus aurantifolia) EOs with a reduced number of experiments while producing reliable results. The optimum conditions included the ratio of water and lemon peels of 3:1 (mL/g), extraction time of 1.6 hours and extraction temperature of 120 °C. These parameters gave the maximal essential oil yield of 2.097%. In the obtained lemon essential oil, a total of 15 volatile constituents were identified, with the major components being Limonene at 66%, followed by γ-Terpinen at 13%.

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