Application of Response Surface Methodology in the Process of extracting essential oil from the Calamondin (Citrus microcarpa) Peels

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Abstract. Calamondin (Citrus microcarpa) essential oil is applied in different fields, including medicine, food, and cosmetic. This study aimed to determine the extraction yield of essential oil from Calamondin peels and optimize the extraction process using Response Surface Methodology (RSM). A three-level three-factors Box Behnken design with three variables including extraction temperature (103-137°C), time (95-145 min), and water to the material ratio (1.32-4.68 mL/g) was adopted. The aforementioned factors are identified to exert significant influence on the essential oil efficiency. Adopting a central composite design, optimal processing conditions were determined. The maximum yield of essential oil was 3.2%, achieved at conditions of the ratio of water and raw materials (3.20:1 mL/g), the temperature of 120.84°C and time of 21.27 min. The results showed good fits with the proposed model for the essential oil extraction (R² = 99.71%).

1. Introduction

Essential oils, or ethereal oils, are liquids that contain essential compounds for maintaining the life of the plant, which are secreted in plant organs and play a key role in resistance of parasites and infections [1–6]. The oil is characteristically hydrophobic (immiscible with water), typically lipophilic (oil or fat soluble) and contains volatile compounds and aromatics that contribute to the scent of the origin plant. Other figured characteristics of many essential oils might include antibacterial, anti-fungal, and anti-parasitic activities. Such features have made plant essential oils common ingredients in production of perfumes, cosmetics, scenting incense, household cleaning and medicinal products. In recent decades, the interest in essential oil has become increasingly stimulated due to the new belief hold by aromatherapy, which emphasizes the importance of essential oil aromas in disease treatment.
Citrofortunella microcarpa (Calamondin), whose fruit was referred to as citrus fruit, is a crop that is widely cultivated in Asian countries, including Vietnam, having enormous commercial value and acting as a chief source of Vitamin C in daily diet [7–10]. The plant is grown principally for its fruits, which was used to produce juice. Since the production of the juice only involves in squeezing the pulp, the peels are considered as by-products.

In recent years, the cosmetic industry has been increasingly developing thanks to the application of natural compounds to the production process, specifically the natural substances in essential oils. Following that development was the introduction of many methods to extract essential oils from different plants. However, the use of computer software tools to optimize extraction processes has not yet been focused. In a hydrodistillation process, experimental parameters that influence the Calamondin (Citrus microcarpa) Peels oil extraction may include water-to-material ratio, temperature and extraction time. To optimize such parameters, dynamics optimization and response surface methodology (RSM) could be employed [11–15]. These methods have been recently used to describe complex processes such as temperature steam, pipe diameter, and steam duty. To assess parameter significance, analysis of variance (ANOVA) is commonly used. The present study aims to optimize to essential oil extraction from Vietnamese Calamondin (Citrus microcarpa) Peels using RSM. Three experimental variables including water-to-material ratio, temperature, and extraction time was considered. The central composite design (CCD) was adopted to develop the optimization. The optimal solution offered by RSM was statistically verified by the coefficient of determination ($R^2$) based on the validation data set.

2. Materials and Method

2.1. Plant materials
The fresh Citrus microcarpa (Calamondin) were gathered during February 2019 from at Ben Tre province (latitudes 10°14’54”N and longitudes 106°22’34”E), Vietnam. Calamondin were peeled to separated the external part, resulting in the peel yield of 15-20% (w/w) relative to the whole fruit. All experimental attempts in this study utilized plant materials in fresh form.

2.2. Extraction procedure
Essential oil was extracted from Calamodin peels using hydro-distillation with a Clevenger apparatus. The extraction was performed with one hundred grams of calamondin peels and 1 L of distilled water for about 120 min starting from the first drop of essential oil to no distillate was obtained. The obtained oil was, dried using anhydrous sodium sulphate and stored in sealed vials at 0°C prior to GC-MS analysis. Percentage yield of calamondin oil in each sample was determined using following formula (1):

$$\text{Yield of essential oil was obtained } (\%) = \frac{\text{Volume of essential oil obtained (ml)}}{\text{amount of raw materials used (g)}} \times 100\%$$

2.3. Experimental Design using RSM
The ultimate objective of RSM is to determine a set of experimental parameters so that the corresponding response is optimized. In this study, a central composite face-centered design involving three different factors: water-to-material ratio, temperature, and extraction time was adopted to determine experimental plan, as shown in table 1. The results were analysed using Analysis of Variance by Design Expert 11 software. Following that, based on the produced quadratic model, three-dimensional plots and their respective contour plots were graphed. From which interaction effects of variables on the response and optimum region could be interpreted. Lastly, the optimized set of parameters was calculated and experimentally validated with five experimental runs. The result was compared with the predicted values to determine the model adequacy.
Table 1. Levels of the variable tested in the central composite designs

| Code | Independent variables | Coded levels |
|------|-----------------------|--------------|
| A    | Water-to-material ratio (mL/g) | -α, 0, +α |
| B    | Temperature (°C) | 103, 110, 130 |
| C    | Extraction time (min) | 95, 105, 120 |

3. Results and Discussion

3.1 Optimization of hydro-distillation parameter by DX11

Table 2 illustrates the design matrix with responses for essential oil yield. The table also summarized predicted and actual yield for each attempt. To be specific, the design consists of 20 experiments of different levels of conditions, which include water-to-material ratio, temperature, and extraction time. The optimum conditions were validated by repeating the extraction at the predicted optimum conditions.

Table 2. Design matrix with responses for essential oil yield

| Std Order | Run Order | Water-to-material ratio (mL/g) | Temperature (°C) | Extraction time (min) | Yield (%) | Actual value | Predicted Value | Residual |
|-----------|-----------|--------------------------------|------------------|-----------------------|-----------|--------------|-----------------|---------|
| 1         | 15        | 2                              | 110              | 105                   | 1.90      | 1.87         | -0.0349         |         |
| 2         | 11        | 4                              | 110              | 105                   | 2.20      | 2.22         | -0.0190         |         |
| 3         | 14        | 2                              | 130              | 105                   | 2.30      | 2.33         | -0.0268         |         |
| 4         | 7         | 4                              | 130              | 105                   | 2.50      | 2.48         | 0.0192          |         |
| 5         | 1         | 2                              | 110              | 135                   | 2.40      | 2.44         | -0.0392         |         |
| 6         | 6         | 4                              | 110              | 135                   | 2.40      | 2.39         | 0.0069          |         |
| 7         | 8         | 1.3                            | 130              | 135                   | 2.50      | 2.50         | -0.0009         |         |
| 8         | 3         | 4.7                            | 130              | 135                   | 2.20      | 2.25         | -0.0548         |         |
| 9         | 10        | 3                              | 120              | 120                   | 2.50      | 2.49         | 0.0094          |         |
| 10        | 17        | 3                              | 120              | 120                   | 2.60      | 2.58         | 0.0187          |         |
| 11        | 5         | 3                              | 103.2            | 120                   | 2.10      | 2.10         | 0.0001          |         |
| 12        | 13        | 3                              | 136.8            | 120                   | 2.40      | 2.37         | 0.0281          |         |
| 13        | 20        | 3                              | 120              | 94.8                  | 2.15      | 2.16         | -0.0145         |         |
| 14        | 2         | 3                              | 120              | 145.2                 | 2.50      | 2.46         | 0.0427          |         |
| 15        | 19        | 3                              | 120              | 120                   | 3.20      | 3.20         | -0.0008         |         |
| 16        | 12        | 3                              | 120              | 120                   | 3.20      | 3.20         | -0.0008         |         |
| 17        | 4         | 3                              | 120              | 120                   | 3.20      | 3.20         | -0.0008         |         |
| 18        | 16        | 3                              | 120              | 120                   | 3.20      | 3.20         | -0.0008         |         |
| 19        | 9         | 3                              | 120              | 120                   | 3.20      | 3.20         | -0.0008         |         |
| 20        | 18        | 3                              | 120              | 120                   | 3.20      | 3.20         | -0.0008         |         |

3.2 Analysis of Variance (ANOVA)

The ANOVA approach aims to indicate whether a factor in the model is significant or not by assigning segments of variations observed in experimental data into different determined source of variation.
Table 3. ANOVA table

| Source               | Sum of Squares | dF | Mean Square | F-value | p-value | Significant |
|----------------------|----------------|----|-------------|---------|---------|-------------|
| Model                | 3.66           | 9  | 0.4068      | 386.36  | <0.0001 | significant |
| A-Water ratio        | 0.0099         | 1  | 0.0099      | 9.43    | 0.0018  |             |
| B-Temperature        | 0.0893         | 1  | 0.0893      | 84.85   | <0.0001 |             |
| C-Extraction time    | 0.01035        | 1  | 0.1035      | 98.26   | <0.0001 |             |
| AB                   | 0.0200         | 1  | 0.0200      | 19.00   | 0.0014  |             |
| AC                   | 0.0800         | 1  | 0.0800      | 75.99   | <0.0001 |             |
| BC                   | 0.0800         | 1  | 0.0800      | 75.99   | <0.0001 |             |
| A²                   | 0.7964         | 1  | 0.7964      | 756.44  | <0.0001 |             |
| B²                   | 1.68           | 1  | 1.68        | 1593.05 | <0.0001 |             |
| C²                   | 1.43           | 1  | 1.43        | 1355.03 | <0.0001 |             |
| Residual             | 0.0105         | 10 | 0.0011      |         |         |             |
| Lack of Fit          | 0.0105         | 5  | 0.0021      |         |         |             |
| Pure Error           | 0.0000         | 5  | 0.0000      |         |         |             |
| Cor Total            | 3.67           | 19 |             |         |         |             |

Values obtained from Design Expert 11

Overall, the model is statistically significant, evidenced by the model F-value of 386.36. Further investigations of individual p-value revealed that all nine variables are significant at 5%. In addition, as listed in table 4, both R-squared values achieved the value of larger than 0.9 and the Predicted R-Squared also well agrees with Adjusted R-Squared of 0.9946, indicating that the data could well describe the quadratic model. The adequate precision which measured the signal to noise ratio also reached the value of 58.2, far exceeding the desirable of 4. This indicates that the model is suitable for navigating the design space.

Table 4. ANOVA analysis

|                      |             |
|----------------------|-------------|
| R-Squared             | 0.9971      |
| Adjusted R-Squared    | 0.9946      |
| Predicted R-Squared   | 0.9761      |
| Adeal Precision       | 58.2181     |

3.3 Model Equation

The final equation in terms of coded factors could be described as follows:

\[ Y = 3.20 + 0.0270*A + 0.0809*B + 0.0870*C - 0.0500*AB - 0.1000*AC - 0.1000*BC - 0.2351*A^2 - 0.3411*B^2 - 0.3146*C^2 \]  

Figure 1 displayed the normal plot of residuals, which plots residual values against probability that the residual will follow normal distribution. Overall, the plots show a pattern of an S-shaped curve. This suggests a better model adequacy could be achieved if the response is transformed. In figure 2, residuals were plotted against corresponding predicted yields, which is ascendingly sorted. The plots show the random scattering of data points relative to increasing predicted response, indicating that the variance is constant.
Figure 1. Normal Plot of Residuals

Figure 2. Residuals vs Predicted Plot

Figure 3 shows a plot of the residual versus the experimental run order. The graph indicates that data points are scattered randomly, suggesting the absence of time-related influence. On the other hand, figure 4 plotted predicted values against actual values, showing a close proximity of data point to the 45-degree line. This indicates the produced model could accurately predict the experimental values.

3.4 Model Graphs

Interaction effects of independent variables on essential oil yield were demonstrated through contour plots as in figure 5. Visually, all three examined factors showed clear and profound impacts on the response. In addition, the trend of these influences could be deduced as similar from the plot. In other words, an increase in any of the three variables will induce a rise in essential oil yield. However, after reaching a certain point, this trend starts to reverse, resulting in the inverse relationship between independent variable and the response thereafter. From the quadratic model, the calculation of optimal conditions using DX11 software yielded the conditions of water-to-material ratio of 3.20 mL/g, extraction time of 121.27 min, and temperature of 120.84°C. These conditions correspond with a yield of 3.20% with desirability of approximately 100%.
Figure 5. Model plots of interaction relationship of yield with (A) Water-to-material ratio and Temperature, (B) Water-to-material ratio and Extraction time, (C) Temperature and Extraction time.

4. Conclusions
The research showed the successful application of response surface methodology with the Box-Behnken method to optimize extraction conditions from Citrus microcarpa (calamondin) peels with different variables, including temperature, water-to-material ratio, and time extraction. Maximum obtained essential oil content was 3.2% corresponding to the extraction temperature of 120.84°C,
water to material ratio 3.20:1 mL/g, and time extraction of 21.27 min. Results for essential oil content found in Citrus microcarpa (calamondin) was relatively high with low cost, abundant supplies, the possibility of long-term storage, and simple process saving.

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