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Response surface methodology for optimization studies of hydro-distillation of essential oil from pixie mandarin (*Citrus reticulata* Blanco) peels

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Essential oil extraction technique from mandarin pixie peels by hydro-distillation is optimized by response surface methodology (RSM). Mathematical techniques were used in experimental design to evaluate the impacts of factors that affect the extraction process and improve the yield of the extraction process. A central mixed design based on influencing variables such as water ratio (3–5 mL/g), temperature (110–130 °C) and extraction time (90–150 min) was adopted with essential oil yield as the target function. Correlation analysis of the mathematical regression model showed that the quadratic polynomial model can be used to optimize hydro-distillation of pixie mandarin oil. The results showed that under the optimum extraction conditions, the highest quantity of essential oils was achieved (7.28 mL/100 g materials). In terms of statistical analysis, the significance levels (p-value <0.05) of the model showed that the experimental results had a good impact between factors. The coefficient of determination indicating the match between the experimental value and the predicted value of the model was high (R2>0.9). The chemical composition of the essential oil was analyzed by Gas Chromatography-Mass Spectrometry, revealing the dominance of limonene content (97.667%), which implies that the essential oil of pixie mandarin could be an alternative source of limonene.

Keywords: Hydro-distillation, pixie mandarin oil (*Citrus reticulata* Blanco), RSM, GC-MS.

INTRODUCTION

Essential oils are the aromatherapy source originating from the nature of plants, which are increasingly noticed and favored by people. Each type of essential oil has a specific smell, fragrance and has different effects. For example, peppermint oil has a high mentol content that stimulates nerve endings, causes a cold sensation, and reduces local pain[1], *Plectranthus amboinicus* essential oil, as a popular remedy for cough, contains many important medicinal properties such as antibacterial, antioxidant, anti-inflammatory, larvicidal and analgesic properties[2–3]. The citrus essential oil has been used to stimulate digestion to treat the common cold in traditional medicine and as a common ingredient in industrial fields such as the manufacture of perfumes, cosmetics, and pharmaceuticals[4, 5].

Nowadays, fragrant compounds derived from nature are gaining great attention and becoming a trend, thus research on them is growing continuously[6–9]. Through previous studies and consumption practice, it has been shown that when using products of plant origin brings great effects with less toxicity and side effects. That is the important reason why today natural products are increasingly preferred[10].

Vietnam has a naturally tropical monsoon climate, which is favorable for fruit growth, as citrus fruit accounts for one-fifth of its production, including many plant species that contain valuable essential oils. In particular, the mandarin tree (*Citrus reticulata* Blanco) is a very popular plant, yet its peels have not been exploited and utilized after use. Mandarin pixie oil exhibits many antimicrobial, antioxidant and anti-inflammatory properties[11, 12], mainly due to the presence of limonene, a compound that has been well established for its chemopreventive activity against many types of cancer[13]. Currently, there are many methods of extracting essential oils from plants such as extraction with solvent, steam distillation, microwave extraction, hydro distillation, and cold pressing methods[3, 14, 15]. Each method has different advantages and disadvantages, for example, cold pressing is a good mechanical method to collect Citrus essential oils with a lot of components in essential oils. However, this method produces a low yield and requires fruit colors removal after the pressing process[14]. The solvent extraction method improves the issues related to yield, yet the downside of this method is the need to remove the solvent after the extraction process as the intermediate stage[15]. To improve the problem, hydrodistillation method is considered. Hydrodistillation is a popular method for recovery of essential oils from plant tissues which is the simplest and the most commonly used method to isolate essential oils from aromatic and medicinal plants[16, 17] thanks to its simplicity in installation and implementation while being feasible for industrial scale. In this method, the plant material and the solvent are completely immersed in the flask, boiled and then collect the essential oil after reflux. Essential oils obtained by this method are protected to a certain extent, and the water surrounding the material acts as a barrier to prevent the essential oils from being exposed to high temperatures[18].

Data on the extraction of essential oils from mandarin peels as well as the use of mathematical techniques in process optimization have yet to be found. Therefore, maximization of essential oil yield from mandarin peels is accomplished by response surface methodology (RSM) using a central composite design (CCD) with extraction conditions such as water-to-material ratio, temperature and extraction time. RSM uses mathematical techniques in process design and optimization based on experimental matrices, the results of analysis of variance and the interaction between factors in the process[19–24]. Compared with the previous methods, RSM has shown many benefits in the experimental layout and process analysis, including reducing the number of test runs, time, implementation costs and integrating systematic treatment statistics as well as the interactions of many factors in the model, which are difficult to evaluate by simple experiments. Furthermore, in this study, Gas Chromatography-Mass Spectrometry (GC-MS) analysis was done to evaluate the quality of the extracted essential oils.

MATERIAL AND METHODS

Materials and chemical

The Pixie mandarin fruit used was *Citrus reticulata* Blanco, which was grown in Ben Tre province (latitudes 10º14'54"N and longitudes 106º22'34"E) of Vietnam in March 2020. The peels were carefully removed from fruit flesh and the albedo (about 10–20 g peels in 100 g fresh fruit). The materials were stored cool for later experiments. Before the experiment, fresh peels were ground in a grinder for 1.5 min (Sunhouse SHD 5323, Hanoi, Vietnam).

Anhydrous sodium sulfate (Na_2SO_4) is used for dehydration during essential oil extraction. Experimental chemicals purchased from Sigma Aldrich (US).

Extraction

Hydro-distillation is a method with the same principle as steam distillation used to extract essential oils from plant materials. In this method, the materials are soaked directly with distilled water. This solid-liquid mixture is boiled under atmospheric pressure in a flask. The volatile compounds in the essential oil and water will form an azeotropic mixture, then condense and divide into two layers in Clevenger, where Citrus essential oil with lower density is located in the upper layer). Clevenger device helps to stabilize the ratio of water and materials and quantify essential oils[25]. The flask was immersed in paraffin, which acted as the heat transfer oil, and the oil was heated by using a hotplate stirrer (Daihan MSH-20D). The temperature probe of the stirrer was dipped into the heat transfer oil. This setup is to represent the pilot-scale system which often utilizes heat transfer oil.

A total of 100 g of puree peels samples were added to the Florentine flask with a certain percentage of water. Extraction was carried out until the amount of essential oil in the material reached saturation. Essential oils were dehydrated with anhydrous Na_2SO_4 before GC-MS analysis.

The yield of the process is defined as the amount of essential oil obtained (mL) during the extraction of 100 g of pixie mandarin peel and was calculated using the following equation:

$$\mathrm{Y}\ (\%) = \frac{V}{W} \times 100 \qquad (1)$$

Where Y is the pixie mandarin oil yield (100%, mL/100 g, v/w), V is the amount of essential oil obtained (mL), and W is the weight of materials used (g).

Experimental design with RSM

Response surface methodology (RSM) is known as a statistical method that uses previously designed data to find the optimum process condition. In the work, the optimum conditions of pixie mandarin oil extraction by the hydro-distillation method were determined by RSM method with a matrix of central composite design (CCD). Experimental variables were determined based on preliminary experiments and presented in Table 1.

The range of variables was set based on single-factor experiments. The relationship between response function and three selected variables (A, B, C) were approximated by the following second-order polynomial (Eq. 2) function:

$$\text{Yield (mL/100g)} = \beta_i + \sum_{i=1}^{3} \beta_i X_i^2 + \sum_{i=1}^{3} \beta_{ii} X_i^2 + \\ + \sum_{i<j=2}^{3} \beta_{ij} X_i X_j \qquad (2)$$

Appropriate polynomial equations are presented as 3D surface plots to visualize the relationship between the response function and experimental levels of each factor and to infer optimal conditions. According to the analysis of variance (ANOVA), the coefficients of the model such as the interaction of each factor, linear regression, quadratic regression and the effective coefficient of the process are determined. These regression coefficients are the basis for creating 2D and 3D plots from the regression model. RSM is used for experimental data by Design-Expert version 11 with statistical significance when the p-value is less than 5%.

Optimization procedures were performed to obtain the highest value of the target function (pixie mandin essential oil) at the independent variables (A, B, C) within the model's estimated range. The three-dimensional diagrams (3D) are presented to better visualize the effects of the independent variables on the process properties, and also predict the best values from the target function. The 3D graph is displayed with independent variables changing as a fixed variable at the central range and of two other variables changing in the experimental range.

Chemical composition of the essential oil

A GC-MS is used to analyze the composition of the essential oils of plants. 25 μL sample of essential oil was mixed in 1.0 mL n-hexane. Name of the equipment: GC

Table 1. Independent variables and their levels used in the model design

No.	Name	Code	Levels				
			–α	–1	0	+1	+α
1	Ratio of water and material (mL/g)	A	2.32	3	4	5	5.68
2	Extraction time (min)	B	70	90	120	150	170
3	Temperature (°C)	C	103	110	120	130	137

Agilent 6890 N, MS 5973 inert with HP5-MS column, head column pressure 9.3 psi. GC-MS system was performed hold under the following conditions: carrier gas He; flow rate 1.0 mL/min; split 1:100; injection volume 1.0 μL; injection temperature 250 °C; oven temperature progress included an initial hold at 50 °C for 2 min, and a rise to 80 °C at 2 °C/min, and them to 150 °C at 5 °C/min, continue rising to 200 °C at 10 °C/min and rise to 300 °C at 20 °C/min for 5 min.

RESULTS AND DISCUSSION

Statistical analysis and model fitting

Three parameters such as water-to-material ratio, extraction time, and temperature were selected based on their effects on extraction yield evaluated by CCD. Different ratio water (A: 2.32, 3, 4, 5 and 5.68 mL/g),time (B: 70, 90, 120, 150, and 170 min), as well as various temperature (C: 103, 110, 120, 130, and 137 °C) were selected for code of $-\alpha$, -1, 0, $+1$, $+\alpha$, respectively. After running 20 experiments, the statistical analysis of the studied factors was performed (Table 2). The essential oil extraction yield as the target function was used to determine the parameters of the response surface equation (Equation (2)).

The regression coefficients of the linear, interaction between variables, and quadratic terms of the model which were calculated using the least squares technique and are shown in Table 3. The obtained quadratic polynomial equation (Equation (3)) was found good to represent experimental data ($R^2 = 0.9702$).

Experimental values of the CCD matrix were statistically analyzed and presented in Tables 3 and 4. Experimental R^2 and adj-R^2 values were 0.9702 and 0.9433, respectively. The high correlation coefficient values show a satisfactory fit between the model and the experimental data, 97.02% of the experimental data is compatible with the values obtained from the model. In addition, the coefficient of variation (CV%) from the software is 2.60, which is a statistical measure of the standard deviation as the percentage of the mean. CV% less than 10% indicates that the model is reproducible. The adequate precision value which is used to measure the signal-to-noise ratio was calculated as 19.0802, implying that the range of the predicted values at the design points to the average prediction error is very good. These statistics show the reliability of the model.

The model has an F-value of 36.15 which shows that the RSM design model is statistically significant. In there, p-value = 0.0001, the model shows that there is only a 0.01% chance that F could occur due to noise. To evaluate the significance of each variable as well as their interaction, P-value is used as a tool to perform. According to the model, the P-value <0.05, the corresponding coefficient is statistically significant and p-value> 0.1 reduces the accuracy of the model, so it is necessary to remove these values to improve the model significance. From Table 4 it can be seen that the linear coefficient (B, C), interaction coefficient (AB, BC) and quadratic

Table 2. Matrix of the RSM model and experimental values

Std.	Run	Actual variables			Extraction yield (%)		
		A (mL/g)	B (min)	C (°C)	Actual	Predicted	Residual
1	1	–1	–1	–1	6.20	6.29	–0.0938
2	10	1	–1	–1	5.40	5.59	–0.1892
3	6	–1	1	–1	6.00	6.18	–0.1825
4	19	1	1	–1	6.40	6.58	–0.1778
5	12	–1	–1	1	6.80	6.77	0.0325
6	2	1	–1	1	6.40	6.36	0.0371
7	11	–1	1	1	5.00	4.96	0.0439
8	9	1	1	1	5.60	5.65	–0.0515
9	5	–α	0	0	6.10	6.05	0.0488
10	4	α	0	0	6.20	6.04	0.1567
11	8	0	–α	0	7.00	6.94	0.0568
12	13	0	α	0	6.40	6.25	0.1487
13	14	0	0	–α	6.20	5.89	0.3124
14	16	0	0	α	5.40	5.51	–0.1069
15	20	0	0	0	7.20	7.21	–0.0059
16	18	0	0	0	7.20	7.21	–0.0059
17	7	0	0	0	7.20	7.21	–0.0059
18	3	0	0	0	7.20	7.21	–0.0059
19	15	0	0	0	7.20	7.21	–0.0059
20	17	0	0	0	7.20	7.21	–0.0059

Table 3. ANOVA for the fitted models

Source	DF	Coefficient	Sum of Square	Mean Square	F-value	p-value
Model	9		9.05	0.2513	36.15	<0.0001
Residual	10		0.2781	0.0278		
Lack of fit	5		0.2781	0.0556		
Pure error	5		0.0000	0.0000		
Total	19		2.33			
R^2		0.9702				
Adj-R^2		0.9433				
CV %		2.60				
Predicted R^2		0.7732				
Standard deviation		0.1667				
Adequate precision		19.0802				

coefficient (A^2, B^2, C^2) were significant values ($p < 0.05$). The change in these factors would have a significant effect on the essential oil content obtained.

In addition, several other values are also used to evaluate the model such as model compatibility and experimental data (Fig. 1). As shown in Fig. 1, the predicted values (calculated from Equation 3) and the experimental values are on the same 45° line, showing the suitability of these two values and the suitability of the quadratic model in the experimental set-up.

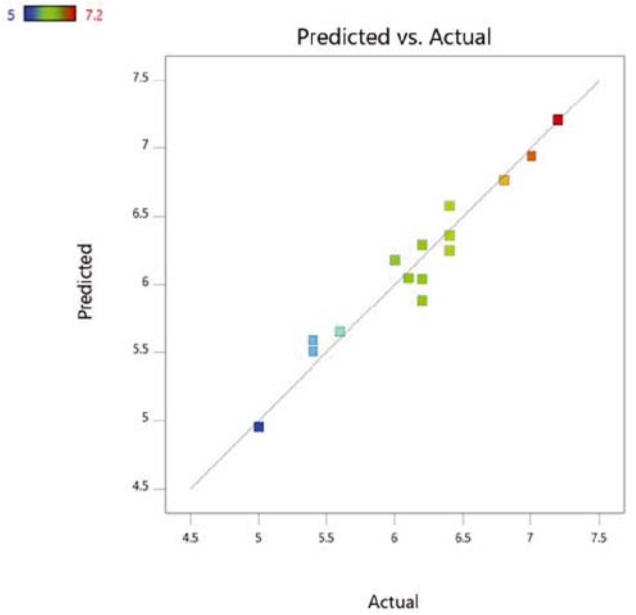

Figure 1. The predicted and experimental extraction yield

Perturbation plot

The perturbation plot of the pixie mandarin oil extraction is shown in Figure 2 with all factors at a selected point are compared in the design space. That is, the graph shows the change of one factor in its range while the other factors are fixed, and the process yield is shown as the objective function to evaluate the influence of the factors in this range. The diagram shows the influence of all factors such as temperature, water-to-materials ratio and extraction time at a central point in the design space. As observed in Figure 2, not all factors showed a positive effect on the yield of pixie mandarin oil. Specifically, the extraction time curve is relatively flat in the range -1 to 0 and tilted to the right when the range across the center point, the yield in this design space is lower. It can be seen from Equation 3 and Figure 2 that the water ratio and temperature strongly influence the curve of the plot. The slope of these factors proves that the change of these factors has accelerated the process. By comparing with the coefficients of Equation 3, the parameters of the process interaction have been determined.

Extraction yield (Y_{MO}) of pixie mandarin oil (mL/g) is determined as follows:

$$Y_{MO} = 7.21 - 0.0023A - 0.2057B - 0.1132C + \\ + 0.2750AB + 0.02750\,BC + 0.0750AC - \\ - 0.4096A^2 - 0.2151B^2 - 0.5334C^2 \quad (3)$$

Where A is a ratio of water and materials (mL/g), B is the extraction time (min), and C is the temperature (°C).

When the effect level has a positive value, the investigating factors and essential oil yield are positively and vice versa correlated. From the above equation, we found the factors that correlate positively with process yield, including time-ratio (AB), ratio-time (AC), and temperature-ratio (BC). Increasing the value of these three pairs of factors increased the amount of pixie mandarin essential oil. At the same time, when the value of the remaining factors increases, the process efficiency tends to decrease. Specifically, the range of influence values of the factors is depicted in Figure 3.

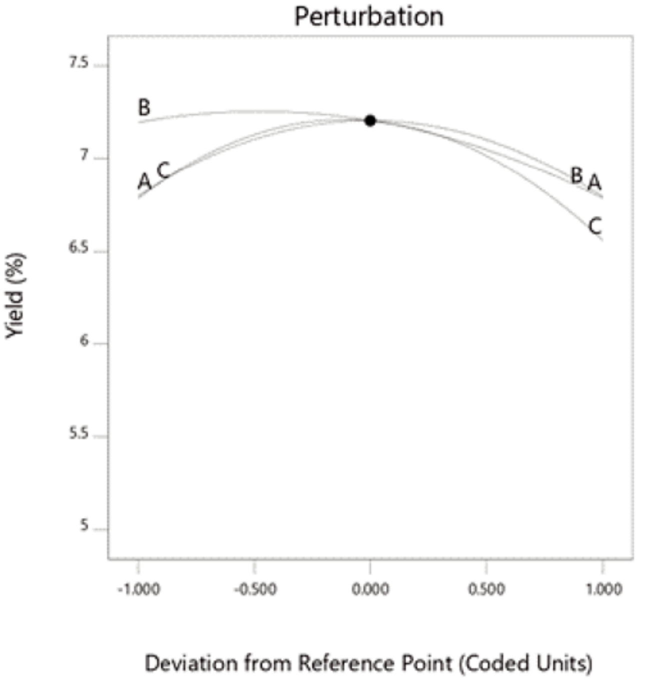

Figure 2. Perturbation plot for rate response (for A: ratio of water and material, B: temperature, and C: extraction time)

Optimization by RSM model

Response surface plots were used to show the effects of factors on the extraction process within the experiment. Charts were exported from Design-Expert software

Table 4. The significance of each response variable effect

Source	Variables	Df	SS[a]	MS[b]	F-value	p-value	
Linier effects	A	1	0.0001	0.0001	0.0027	0.9598	
	B	1	0.5778	0.5778	20.78	0.0010	Significant
	C	1	0.1749	0.1749	6.29	0.0010	Significant
Quadratic effects	A^2	1	2.42	2.42	86.97	<0.0001	Significant
	B^2	1	0.6673	0.6673	24.00	0.0006	Significant
	C^2	1	4.10	4.10	147.45	<0.0001	Significant
Interaction effects	AB	1	0.6050	0.6050	21.76	0.0009	Significant
	AC	1	0.0450	0.0450	1.62	0.2321	
	BC	1	1.44	1.44	51.97	<0.0001	Significant

[a] Sum of squares
[b] Mean sum of squares

(version 11) to simulate the effect of single factors and their interactions on mandarin essential oil yield. As stated earlier, the process is influenced by three main factors (ratio of materials and water, temperature, and extraction time) and is described more clearly in Figure 3 with the red area denoting the highest recovery efficiency, while the blue area denoting the minimum results.

In Figure 3 (A), the 2-D contour and 3-D response surface plot show the interaction of water-to-materials ratio and temperature on extraction yield. Based on the variation of the color scale in the chart, it can be seen that the change of these two factors also changes the amount of essential oil. First, we consider the effect of temperature on the amount of essential oil obtained. At the 3 mL/g point, when the temperature rises from 110 °C to 130 °C, the yield tends to decrease. At the same ratio, the yield is highest (7%) when the temperature of the process is lower than 115 °C. If the above range is exceeded, the efficiency tends to be the opposite, i.e. the more the temperature increases, the more the process efficiency decreases. Particularly, a ratio of water between 4.75 mL and 5 mL has different interactions with the data presented. As the process temperature increases, the efficiency of the process also increases, however, after 120 °C, the efficiency tends to decrease. The explanation for this might be that the amount of heat used could initially heat the water and not the sample. Next, in terms of the effect of ratio, similar to temperature, the ratio of materials and water inversely interacts with the oil efficiency. As the rate increases, the efficiency of recovered essential oils decreases gradually. Based on the chart (A), the essential oil yield is predicted to reach the highest level when the temperature is between 110–120 °C and the ratio is 3 to 4.5 mL/g.

In Figure 3 (b), 2D and 3D plots show the interaction between extraction temperature and extraction time on extraction yield. The chart also relies on the color scale to evaluate the changes in the factors affecting the extraction process. First, we consider the effect of temperature on essential oil yield. At 90 minutes, when the temperature is increased from 110 to 130 °C, the amount of oil obtained tends to increase. Specifically, using a temperature greater than 115 °C for 90 minutes, the efficiency is greater than 6.5%. However, at this same temperature range, if the time increases by more than 140 minutes, there is the opposite trend: the efficiency of the essential oil decreases as the temperature increases. Next, considering the influence of the extraction time factor, we realize that the interaction time is positively related to yield. As the extraction time is prolonged, a greater amount of the essential oil is yielded, and the optimum duration of the process is close to the center value. Based on the chart (B), the predicted essential oil yield is the highest between 110 and 127.5 °C with extraction time from 110 to 140 minutes.

The plot (C) in Figure 3 shows the interaction between the rate and extraction time at a constant 120 °C on essential oil yield. The variation of the color scale clearly shows the change of two factors to the extraction process. First, we consider the effect of concentration on oil recovery efficiency. At the 90-minute point, when increasing the water-to-material ratio from 3 to 5 mL/g, the oil yield tends to increase. Specifically, the oil yield is high when the temperature is at the central value (4 mL/g). However, if the water ratio increases to 5mL/g, the recovery yield decreases. It is possible that because when the proportion of water becomes too large, more heat is supplied to the water, causing the performance not to be of good value at this range. Next, considering the effect of time, the time has similar interactions with the ratio factor. Yield increases gradually to the center value, then tends to decrease. The explanation for this could be due to heat degradation, the volatile composition of the essential oil is affected by the great heat or prolonged heat exposure. Based on graph 3 (C), the predicted oil yield is the highest when the ratio is between 3.8 and 4.2 mL/g for the time from 112 to 120 minutes.

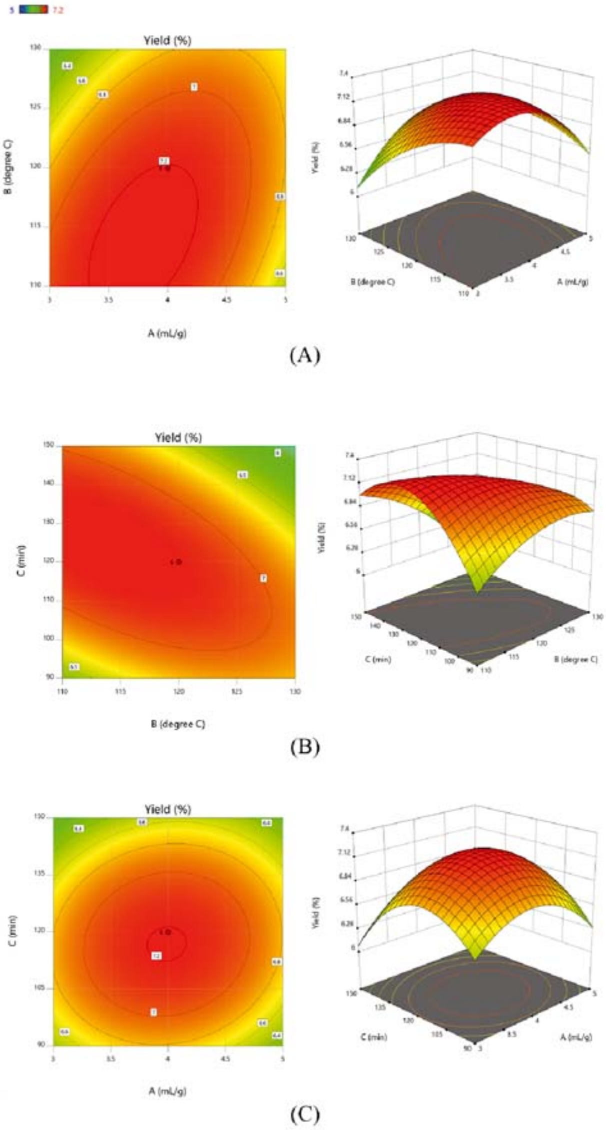

Figure 3. Contour plots (2D) and response surface (3D) show the effect of the temperature and water-to-material ratio (A), temperature and extraction time (B), ratio and extraction time (C) on the pixie mandarin essential oil yield

As observed from Figure 3, it can be seen that the trends of temperature, the ratio of water and materials, and extraction time are similar. Any increase in any factor causes the recovery efficiency to increase until the amount of essential oil in the material reaches saturation, and begins to decline due to the opposite effect of the factors. A possible explanation for this is that during the extraction process, the mixture of water and the material is heated to create steam which seeps through the epidermis (where the essential oil is stored), breaking and attracting the essential oil by steam. There are two issues to consider such as the proportion of water and the extraction temperature. Firstly, considering the ratio of material and water: when the amount of water is not enough or excessive, it will adversely affect the extraction process. An insufficient content of water is unable to dissolve the glue in the essential oil-containing enclosures, which will cause the device to burn or cause wasting heat to the water instead of the samples during the heating process. For this reason, a suitable amount of water is essential in the experimental design as well as the process experiment to avoid the above problem. Secondly, the factors of extraction temperature and time which represent the amount of heat impacting the tank containing the materials and water mixture. These two factors help essential oils escape more, improving the process efficiency if suitable parameters are found. The biggest impact on the process is that an excessive heat supply and prolonged heat exposure would cause the volatile components in the essential oils to denature[26]. Under certain conditions, the essential oil exits the storage bag to the saturation stage, so it is necessary to set up the right process parameters to avoid adverse effects of the factors as well as the efficiency of the extraction process.

From the consistency of the data above, optimization was carried out based on the surface response graph. The optimum extraction conditions of pixie mandarin oil are the temperature 113.12 °C, the ratio of water and material 3.73 mL/g and the extraction time of 126.65 minutes (Cube chart in CCD matrix, Fig. 4). Under these optimal conditions, the predicted oil yield was 7.28% with the desirability of 1.00.

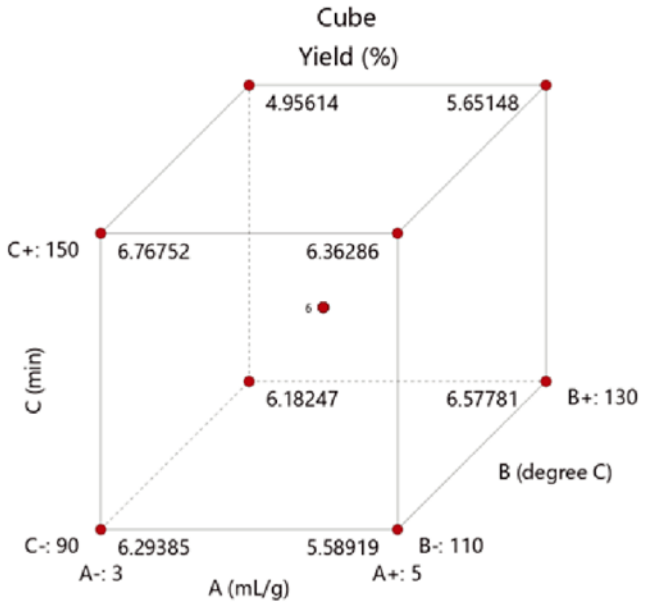

Figure 4. Graphical optimization of extraction yield of pixie mandarin oil (mL/100 g)

Model adequacy checking

Usually, evaluation of the model adequacy is necessary as it ensures that the provided values of the model are close to the actual value. This helps to minimize bias when optimizing the surface. By graphing the normal probabilities of residues, checks are performed, as shown in Fig. 5. The residues which are represented by multicolored squares are used for evaluation of completeness of the model, showing approximate values along a line. It is also likely to say that this is the fulfillment of these normal probability values. For further tests for authenticity, a plot of residuals versus predicted values is given (Fig. 6). From the graph, it can be seen that the residues are randomly distributed within the limit, showing that the variance of the initial observation is constant for all values of Y. Both graphs (Fig. 5 and 6) are both satisfactory, so we conclude that the empirical model is sufficient to describe the yield of extraction of the pixie according to the reaction surface.

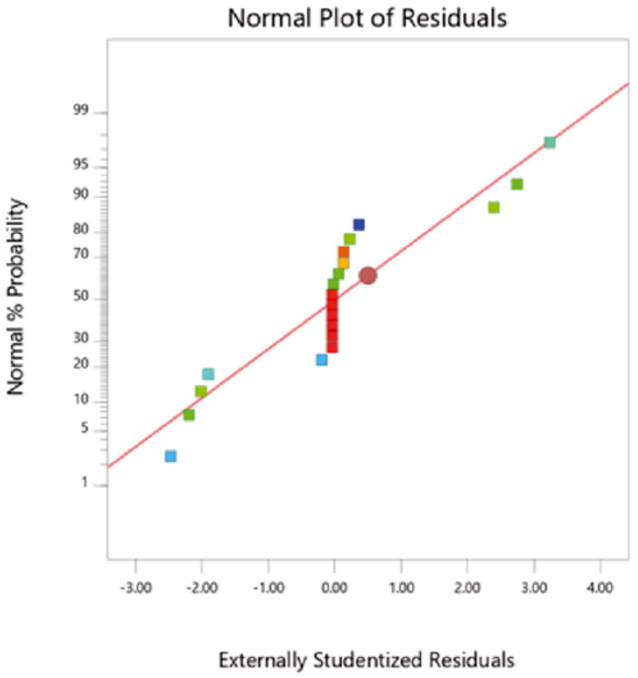

Figure 5. Normal probability externally student residuals

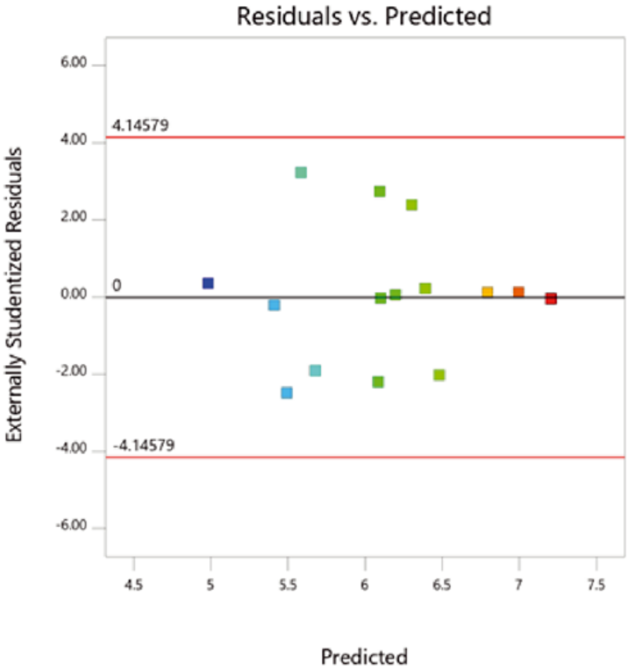

Figure 6. Plot externally studentized residuals vs. predicted response

Validation of the model

The probability of achieving the expected results from the recommended optimal point is 100% or "desirability" = 1.00. From the data shown in Figures 3 and 4 and applying the results of Design expert 11 software, the optimum point including temperature, the ratio of material and water, and extraction time are listed in Table 5. According to the predicted results of the model, under optimal conditions, the yield of the process is 7.28%. However, to match the experimental conditions, these results were experimented to verify the model with the corrected values such as 3.73 mL/g ratio, 110 °C temperature and extracted in 127 minutes. Compared with the model results, the actual yield is 7.15%. Yield deviation is calculated as the percentage difference between the predicted and actual yield. With an error of less than 5% and desirability 1.00, they show that the model's condition is consistent with the experimental values.

Applying the RSM surface response method, ANOVA analysis method, and interactions through 2D & 3D chart of Design expert 11 software, the optimal parameters of the pixie mandarin essential oil extraction by method hydrodistillation is the ratio of 3.73 mL/g, the temperature of 110 °C, and extraction time of 127 min with a process efficiency of 7.15% on 100 g of mandarin peels.

Result analysis of GC-MS

In this study, from 100 g of fruit rind around 7.15 mL (7.15%, v/w) of essential oil has been obtained by hydrodistillation connected to Clevenger's device at optimal conditions. The chemical composition of the essential oil was analyzed by GC-MS (Table 6 and Figure 7). Analysis results show that there are 5 chemical components in pixie mandarin oil, with the most abundant being limonene (97.667%) followed by β-myrcene (1.411%), α-pinene (0.563%), β-pinene (0.239%), and saninene (0.12%).

Since the peak at 11.967 min has the greatest intensity, it is suggested that Limonene is the most important component in pixie mandarin oil which is reported to be the main component in commercial essential oils. Studies related to mandarin peels oil[27–29] also mentioned that limonene accounts for a relatively high proportion (about 80.3–96.2%). The composition and content of essential oils depend on the species, extraction method, and soil of each region. For example, Marie-Laure Lota et al.[9] The content of essential oils from varieties grown in France with higher levels of limonene, α-Pinene, Myrcene, and Sabinene than before (52.2–96.2%, 0.1–2, 1%, 1.3–1.8%, 0.1–1.3%, respectively). Gulay Kirbaslar et al.[30] announced the composition of tangerine peel oil grown in Turkey obtained 45 compounds, of which, limonene (90.7%), followed by myrcene (2.1%), and α- pinene (0.5%).

As mentioned earlier, the extraction method affects the composition and content of essential oils. Simon Muholo Njoroge et al.[31] identified 43 components in tangerine peel oil extracted by cold-pressing. The main components are Limonene (85.9%), γ-terpinene (6%), myrcene (2.2%), α-pinene (1.1%), linalool (1.0%), ß-pinene (0.3%), terpinolene (0.3%), sabinene (0.4%) and octanal (0.2%). Ibtehal K. Shakir and Sarah J. Salih[32] reported that by analyzing the essential oils extraction by two procedures (i.e. microwave-assisted steam distillation and steam distillation), limonene was found the most abundant in all the samples.

Table 6. Chemical constituents of pixie mandarin oil

Peak	Retention time (min)	Compounds	Percent
1	7.272	1R-α-Pinene	0.563
2	9.008	Sabinene	0.12
3	9.091	L-β-pinene	0.239
4	9.949	β-Myrcene	1.411
5	11.967	Limonene	97.667

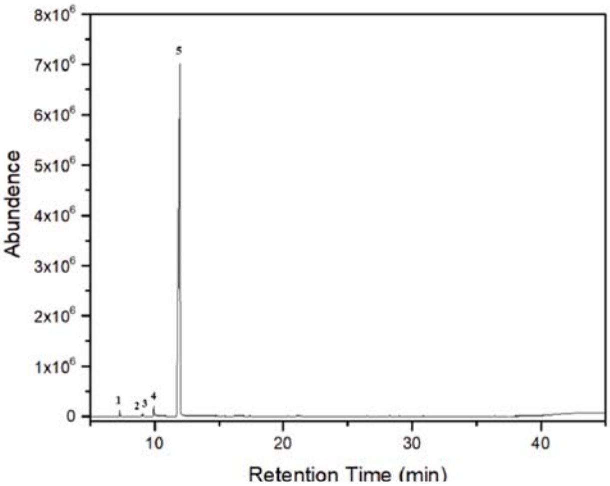

Figure 7. The GC-MS chromatogram of pixie mandarin oil

CONCLUSIONS

This study aimed to investigate and optimize the parameters that affect the extraction of pixie mandarin essential oil. RSM is a mathematical technique that can effectively estimate the effect of the parameters of the extraction process. The experimental results show that the pairs of interaction factors such as temperature-ratio, temperature-time, and ratio-time have statistically significant and positive interactions with process performance. Based on the results of ANOVA and the agreement between experimental and predicted results, it can be concluded that the generated model is suitable for simulating hydro-distillation of peels of pixie mandarin. The optimum conditions are as follows: temperature of 113.12 °C, water and material ratio of 3.73 mL/g, and extraction time of 126.65 min. Under optimal extraction conditions, the essential oil yield is 7.28 mL/100 g of material. In addition, the quality of mandarin peel oil was also assessed through GC-MS results. The results

Table 5. Experimental and predicted value of the responses using optimum condition

Factors/Code	A (Ratio, mL/g)	B (Time, Min)	C (Heat, °C)	Y (Yield, %)	Error (%)
RSM	3.73	126.65	113.12	7.28	
Experiment 1	3.73	127	110	7.10	–2.47
Experiment 2	3.73	127	110	7.20	1.10
Experiment 3	3.73	127	110	7.15	1.79
Average Exp	3.73	127	110	7.15	1.79

showed five main components which are Limonene (97.667%), followed by β-Myrcene, 1R-α-Pinene, L-β-pinene and 1R-α-Pinene. These chemical components are possibly correlated with the extraction method as well as the soil of the plant.

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