



Process Optimization by a Response Surface Methodology for Extraction of Essential Oil from Ginger (*Zingiber rubens*) in Vietnam



Le Minh Kha¹, Tran Thi Thanh Tra¹, Nguyen Thi Ngoc Lan¹, Nguyen Bao Viet², Mai Huynh Cang^{1*}, Nguyen Phu Thuong Nhan^{1*}

¹Department of Natural Products, Faculty of Chemical Engineering and Food Technology, Nong Lam University, Ho Chi Minh City, Vietnam.

²Department of Chemical Engineering and Technology, Faculty of Chemical Engineering and Food Technology, Nong Lam University, Ho Chi Minh City, Vietnam.

Abstract

Using response surface methodology (RSM), this study sought to improve the hydrodistillation process for extracting *Zingiber rubens* essential oil. To get the best production of essential oils, tests were carried out utilizing a three-factor central mixture design (CCD) with the following parameters: grinding duration 30 - 50 seconds, material-to-water ratio 1/3 - 1/5 g/ml, and distillation time 240 - 360 minutes. A second-order polynomial regression model, reflecting total extraction efficiency as a function of the key hydro-distillation variables, fit strongly ($p < 0.05$) and had a high coefficient of determination ($R^2 = 0.9628$). The highest extraction efficiency (1.456%) was achieved at 43 seconds, 1/4.3 g/ml, and 316 minutes. The chemical makeup of essential oils was evaluated using gas chromatography-mass spectrometry (GC-MS). The predominant component (concentration $> 5.0\%$) in *Zingiber rubens* essential oil produced by hydro-distillation was discovered to be 7-epi-Sesquithujene (18.71%), which had a high content. The most common compounds were β -Sesquiphellandrene (9.92%), Citral (9.88%), α -Curcumene (9.46%), β -Bisabolene (5.39%), and Camphene (5.08%).

Keyword: Ginger, Essential Oil, Hydrodistillation, GC-MS, *Zingiber rubens*

1. Introduction

Zingiber rubens Roxb, commonly known as red ginger root, is a perennial herbaceous plant of the Zingiberaceae family that grows in moist soil, bright places and under moist cliffs [1]. This plant is grown mainly in Asia [2,3] and the tropics are used as spices and flavoring agents for foods [4]. Its distinctive scent and flavor are mostly derived from essential oils and zingerone [5], shogaols [6] and gingerols constituents [7]. Essential oils are a liquid form of volatile aromatic compounds [8] collected from leaves, stems, flowers, roots, or other parts of plants [9]. Extracting essential oils from the *Zingiber rubens* species to fully exploit the species' essential oil resources in the domains of food, medicines, cosmetics, etc [10-12]. Currently, there are several ways to extract essential oils from various plant sources. They are divided into four categories: hydrodistillation, steam distillation, solvent extraction, and supercritical carbon dioxide. Hydro-distillation produces high-quality essential oils, is simple to use, safe, and ecologically beneficial. The advantage of this process is that the volatile components are condensed into water, and the water vapor substitutes oxygen in the environment, preventing the volatile chemicals from oxidizing [13,14]. Currently, little is known about improving ginger essential oil distillation procedures; research is being undertaken on an individual and uneven basis, and the influence of process factors is minimal. The program has not been assessed yet. Optimizing the distillation process of ginger essential oil allows for improved extraction, management of the distillation process, and cost reduction of raw materials. Regression coefficients and the statistical significance of model variables were ascertained through the use of response surface analysis. For estimating the variance in yield of essential oils derived by hydrodistillation, it offers an empirically significant model ($p < 0.05$). Regression modeling, by fitting the regression model to the experimental data, can yield an overall best design for the response variable under study. The response variable's fluctuation may be explained using the following generic polynomial model:

$$Y_i = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{33} X_3^2 \quad (1)$$

The response variable determined by the model is represented by Y_i in the above equation (1); β_0 is a constant; $\beta_1 - \beta_3$ are the regression coefficients for the main variable effects; β_{11} , β_{22} , and β_{33} are the quadratic effects; β_{12} , β_{13} , and β_{23} are the

*Corresponding author e-mail: nhan.maihuynhcang@hcmuaf.edu.vn; (Mai Huynh Cang),
nguyenphuthuong@hcmuaf.edu.vn, (Nguyen Phu Thuong Nhan)

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interaction effects; and $X_1 - X_3$ are the independent variables. Coefficient of determination (R^2) analysis, model analysis, and goodness-of-fit tests were used to assess the response surface model's suitability. The final reduced model was obtained by rescaling the empirical data to only include significant independent variables ($p < 0.05$) and excluding terms that were deemed to be statistically unimportant ($p > 0.05$) from the original model. As such, the simplified model that is ultimately included only contains significant parameters ($p < 0.05$). Vietnam, characterized by its tropical climate, boasts an abundant and diverse array of medicinal plants. Currently, ginger is primarily utilized as a spice in Vietnam, and there has been limited research focused on optimizing the extraction process of ginger essential oil at the laboratory scale. With the help of this study, *Zingiber rubens* essential oil from Binh Phuoc province in Vietnam will be distilled more efficiently. In this work, the optimal parameters for the distillation of essential oil from *Zingiber rubens* were determined by the use of response surface approach [15]. This study aims to establish a foundation for scaling up the extraction of ginger essential oil in Vietnam and to improve its applicability in food and pharmaceutical products.

2. Materials and methods

2.1. Plant Materials

In Vietnam's Binh Phuoc province, ginger root (*Zingiber rubens*) was harvested. The optimal age for harvesting ginger is eight months from the time of planting.

2.2. The extraction of Ginger essential oil using hydro-distillation method

Ginger root (*Zingiber rubens*) is washed to remove dirt and damaged or crushed ginger roots. After that, the ginger root will be dried at room temperature and 100 grams of ginger root will be weighed to puree and then hydrodistilled. Ginger essential oil (*Zingiber rubens*) is extracted by hydrodistillation according to AOAC (*Official Methods of Analysis of AOAC International*). For hydrodistillation, weigh around 100 g of ground material using a basic Clevenger-style setup that includes a 1000 ml round-bottom flask, condenser, and measuring tube with a stopcock. Until no more essential oil can be extracted, the extraction process lasts for almost five hours. Following its collection in the trap and cooling to 30 °C by leaving it to remain at room temperature, the resultant volatile oil is measured to the closest 0.01 milliliter.

2.3. Yield calculation

The essential oil yield was calculated using the following equation to compare the experiment's efficiency after the essential oil was acquired after dehydration with sodium sulphate [16].

$$\text{Yield (\%)} = \frac{\text{Volume of ginger essential oil obtained (mL)}}{\text{Amount of ginger originally used (g)}}$$

2.4. Experimental design

One of the key aspects of the hydraulic distillation process that must be examined is optimizing the process to produce essential oils. It is possible to greatly increase the extraction efficiency recovery of the goal function by choosing values that are consistent with the several independent factors that will influence the hydrodistillation process. To assess the impact of variables in the distillation process, a three-factor central mixture design with $8(2^3)$ factorial points, six star points, six central points, and $\alpha = \pm 1.682$ was employed in this work. hydraulics to the extraction efficiency of *Zingiber rubens* oil, including grinding duration (30–50 seconds), material to water ratio (1/4–1/5 g/ml), and distillation time (240–360 minutes). Table 1: Based on CCD, independent variable levels are established. The method's repeatability was determined by repeating the center point six times [17–19].

Table 1: The levels of independent variables established according to the central composite design (CCD).

Independent variables	Coded variable level				
	$-\alpha$	-1	0	+1	$+\alpha$
X_1 : Grinding time(seconds)	23.1821	30	40	50	56.8179
X_2 : Ratio (g/ml)	1/2.3182	1/3	1/4	1/5	1/5.6818
X_3 : Distillation time(minutes)	199.092	240	300	360	400.908

Along with the quadratic impact and the three most crucial elements, this study will also take into account the interaction between the variables. Consequently, CCD provided twenty tests with three independent variables at five levels of each variable. Table 2 presents the CCD matrix together with the experimentally acquired extraction efficiency data.

Table 2: The matrix of central composite design (CCD) and experimental data obtained for response variable.

No.	Independent variables			Response
	X ₁	X ₂	X ₃	R ₁
	(seconds)	(g/ml)	(minutes)	(%)
1	-1	-1	-1	1.07
2	+1	-1	-1	1.13
3	-1	+1	-1	1.17
4	+1	+1	-1	1.16
5	-1	-1	+1	0.98
6	+1	-1	+1	1.08
7	-1	+1	+1	1.26
8	+1	+1	+1	1.27
9	- α	0	0	1.18
10	+ α	0	0	1.39
11	0	- α	0	0.85
12	0	+ α	0	1.17
13	0	0	- α	1.12
14	0	0	+ α	1.32
15	0	0	0	1.46
16	0	0	0	1.45
17	0	0	0	1.41
18	0	0	0	1.39
19	0	0	0	1.42
20	0	0	0	1.46

2.5. Optimization and validation procedures

The goal of the optimization process was to produce the most essential oil from *Zingiber rubens*. The three-dimensional (3D) surface plot was chosen as the ideal graphic representation for the shortened response model in order to effectively illustrate the distillation's substantial ($p < 0.05$) interaction effects. The study involves hydraulics and response factors. To find the ideal operational parameters (grinding time, material-to-water ratio, and distillation time) for distillation, numerical optimization was also carried out using the Design Expert 11 optimizer. The intended response variable is obtained using hydraulic

distillation. The experimental data are compared with the projected values derived from equation (1) in order to assess the suitability of the regression equation.

2.6. Determination of chemical composition

Gas chromatography-mass spectrometry (GC-MS), using an Agilent Technologies (USA) GC-MS 6890-5873N Mass Selective Detector, was used to investigate the composition and quantity of gooseberries in ginger essential oil. One milliliter of *Zingiber rubens* essential oil was transferred to the Institute of Chemical Technology at the Vietnam Academy of Science and Technology for GC-MS analysis of its constituents. GC Aligent 6890N in combination with inert HP5 – MS and MS 5973 columns is the basic idea. There is 9.3 psi in the head column. After adding 1 ml of n-hexane and using Na₂SO₄ to dehydrate it, the essential oil was added. At 1 ml/min, the flow rate was steady. The nozzle has a separation rate of 30 and a temperature of 250 °C. The sample's thermal processing method: After two minutes at 50 °C, raise the temperature by 2 °C/min to 80 °C, then increase by 5 °C/min to 150 °C. Keep raising the temperature by 10 °C/min to 200 °C, then raise it by 20 °C/min to 300 °C while holding for five minutes.

2.7. Statistical and data analysis

Data were processed and graphed in Microsoft Excel. Data are presented as mean \pm SD. ANOVA and LSD analyses were conducted to compare differences between treatments using Statgraphics Centurion XV software (Statgraphics Technologies, Inc, USA). Design Expert 11 was used to optimize the experimental process and draw correlation charts for the effects of factors.

3. Results and discussion

a. Fitting the response surface models to significant independent variables

Establishment of quadratic regression equation: Grinding time, material-to-water ratio, and distillation time were the three independent variables that were used in the development of the experimental attempts using Central Composite Design (CCD). For the remaining studies, 20 RSM attempts with two replicates were conducted and the results were compiled in Table 2.

Table 3: ANOVA for response surface quadratic model.

Source	Sum of Squares	Df	Mean Square	F-value	p-value	
Model	0.5538	9	0.0615	28.76	< 0.0001	Significant
A - X ₁	0.0193	1	0.0193	9.01	0.0133	
B - X ₂	0.0949	1	0.0949	44.33	< 0.0001	
C - X ₃	0.0115	1	0.0115	5.38	0.0429	
AB	0.0032	1	0.0032	1.50	0.2494	
AC	0.0005	1	0.0005	0.2103	0.6563	
BC	0.0144	1	0.0144	6.75	0.0266	
A ²	0.0439	1	0.0439	20.53	0.0011	
B ²	0.3349	1	0.3349	156.50	< 0,0001	
C ²	0.0881	1	0.0881	41.18	<0,0001	
Residual	0.0214	10	0.0021			
Lack of Fit	0.0171	5	0.0034	4.00	0.0773	not significant
Pure Error	0.0043	5	0.0009			
Cor Total	0.5752	19				

The analysis of variance (ANOVA) for the extraction of essential oils was displayed in Table 3. Both the regression model $P < 0.0001$ and the model $F = 28.76$ are statistically significant. Since the 0.0773 lack of fit was not statistically significant, the model could be used to extract *Zingiber rubens* essential oil. A², B², and C² were significant model terms in this investigation (<0.05). The regression coefficient (R^2) of 0.9628 indicated that 96.28% of the experimental data matches the data predicted by the model.

Fisher's F-test and P-value were used to assess the importance of factors; larger F values and smaller P values indicated greater accuracy in the coefficients. Table 2 data indicated that all linear components of the steam distillation method's essential oil extraction process, including A, B, C, BC, A², B², and C², were statistically significant ($p < 0.05$). The most

significant factor affecting the efficiency of ginger essential oil extraction was the distillation time, followed by the material-to-water ratio and grinding time.

3.2 The yield of extract

In this study, we aim to develop a predictive equation that incorporates all parameters in the proposed ANOVA table, without excluding factors that may have negligible influence. Although the results may exhibit minor variations, we believe that the findings remain valid to a reasonable extent. The relationship between essential oil content and independent variables A, B, and C from Design - Expert 11 software was shown in the actual equation (Final Equation in Terms of Actual Factors) as follows:

$$R_1 (\%) = -30.74053 + 0.072175A + 42.19046B + 0.003099C - 0.02AB + 0.000012AC + 0.007083BC - 0.000552A^2 - 15.24359B^2 - 0.000022C^2 \quad (2)$$

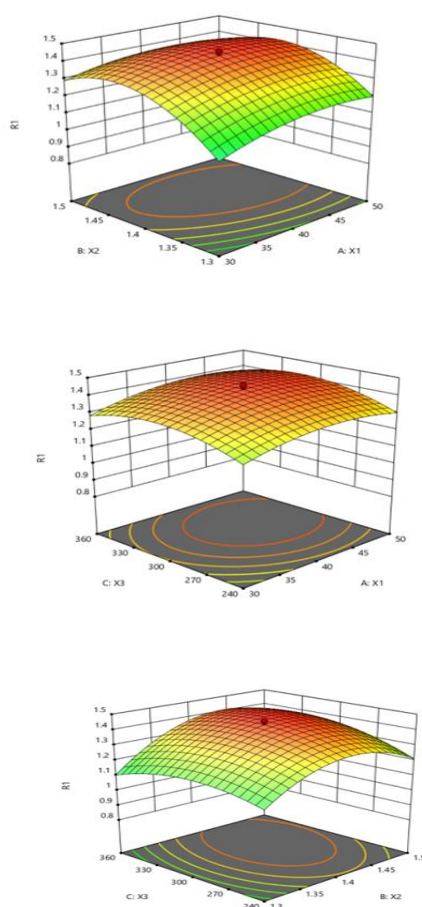


Figure 1: Response surface plots of the essential oil yield (R_1) of the *Zingiber rubens* Where A was the grinding time (s), B was the raw material to water ratio (g/ml) and C was the distillation time (minutes).

According to equation (2), a quadratic mathematical model based on coded variables of essential oil content demonstrates that when the impact level was positive, the survey factor and the obtained essential oil content were the same. Positive, and vice versa. In equation (2), the following components were positively connected with essential oil content: grinding time (A), raw material to water ratio (B), distillation time (C), size factor - distillation time (AC), and scale factor pair - distillation time (BC). The amount of essential oil acquired grew as the value of the five survey parameters listed above rose. Furthermore, when the value of parameters such as grinding time-ratio (AB), grinding time squared (A^2), raw material to water ratio

squared (B^2), and average distillation time. Due to a negative correlation, as the technique (C^2) grows, the resultant essential oil content decreases.

Figure 1 depicts three three-dimensional response plots that indicate how influences impacted essential oil content. The chart's red region indicates the highest essential oil content, while the blue area indicates the lowest essential oil concentration. Essential oil concentration readings range from 0.85% to 1.46%, shown by blue to red hues.

Figure 1A depicts the influence of grinding time and material-to-water ratio on essential oil concentration. The color scale chart shows that altering the values of these two elements affected the essential oil content. First, we look at the effect of grinding time on essential oil content. At a material-to-water ratio of 1/4 (g/ml), when the grinding duration rose, lowering the size of the material, the resultant essential oil concentration remained nearly constant from 35 to 50 seconds. According to equation (2), grinding time was a factor that corresponds favorably with essential oil concentration. Figure 1A shows the effect of the ratio on the amount of essential oil obtained. The highest essential oil content (1.46%) was obtained in the material-to-water ratio range of 1/3.7 to 1/4.7, because the higher the material-to-water ratio, the greater the contact between water and ingredients, from which the water was boiled to saturation temperature, causing the essential oil cells to swell and burst, and the essential oil was diffused out of the ingredients by the water and stored. Steam attracted and repelled. Furthermore, when the raw material to water ratio grew, the amount of essential oil tended to drop significantly owing to the boiling phenomena that caused the loss [17,18]. Furthermore, when there was a lot of water, highly polar cell components dissolved more easily, resulting in less essential oil content. Figure 1A shows that the essential oil concentration was maximum when distilled under circumstances ranging from 35 to 50 seconds of grinding time and a raw material-to-water ratio (g/ml) of 1/3.5 to 1/4.

Figure 1B depicts how pureeing time and distillation time affected essential oil content. The color scale in the illustration indicates that altering the values of these two elements affected the essential oil content. First, we look at the effect of grinding time. At a distillation duration of 300 (minutes), the essential oil content rose minimally or not at all when the grinding time increased from 35 seconds to 50 seconds since the components had reached the lowest size the blender could ground. Take the pureeing time to 40 seconds, and if you continue to raise the distillation duration, the essential oil content will grow until it reaches its greatest value at around 300 minutes when it reaches almost 1.46% and can go past the border. The best distillation time for the procedure was close to the center value. The figure shows that the greatest essential oil concentration was 1.46% when distilling at a grinding time of 35 to 50 seconds and a distillation period of 270 to 350 minutes.

Figure 1C depicts the interplay between the material-to-water ratio and distillation duration. The ideal water ratio for the procedure was 1/4 to 1/5, with a distillation period of 300 to 330 minutes. Figure 1C demonstrates that raising the raw material to water ratio, as mentioned in the survey of each variable, causes the essential oil content to increase somewhat, if not at all. When the distillation period reached 360 minutes, the essential oil content was maximum and remained constant. However, looking at the color scale and curvature of the 3D surface on the response surface graph, it is clear that as the distillation duration increases, the components' boiling points rise, and the essential oil dissolves into the condensate and appears. Oxidation lowers the raw material's essential oil content [19]. Experiments with components in the middle provided the maximum essential oil concentration. The greatest essential oil concentration was about 1.46%.

Response optimization was used to determine the ideal amount of independent variables that will result in the desired response target. Numerical and graphical optimization techniques were utilized to determine the best treatment strategy. For graphics optimization, a 3D response surface and overlay technique was highly suggested [20]. As a result, the best condition was determined by stacking all of the cells. To determine the precise ideal points of the independent variables that led to optimal circumstances, numerical optimization was also performed. Based on response surface plots and the response optimizer, the overall optimal area was predicted to be obtained by hydraulic distillation at a combination of grinding time of 43 seconds, material-to-water ratio of 1/4.3, and distillation time of 316 minutes, with a desirability of 0.990. The resulting simplified model predicted a yield response value of 1.454%.

3.3 Verification of the final reduced model

Verification of Optimized Condition and Predictive Model: The optimal levels of the parameters that were investigated and determined from the established model are displayed in Table 4. To verify these results, the experiment was run three times using the ideal settings. Assumed to be in good agreement with the anticipated essential oil recovery efficiency in Table 1 were the experimental data. With a triplicate's average performance of 1.456%, the relative error between the experimental and projected values was 0.58%. The verified value further demonstrated that the findings of the *Zingiber rubens* essential oil obtained were within 99% of the expected value, suggesting that the established model was optimized and could accurately predict the verification tests.

Table 4: Influence of optimal extraction conditions on the essential oil extraction

Table 4. Influence of optimal extraction conditions on the essential oil extraction						
Optimal point	R ₁ predicted	Total essential oil yield (%)			Mean**	Relative error (%)
		Experiment value				
		1	2	3		
Essential oil yield						
X ₁ = 43 (s)	1.454	1.45	1.46	1.46	1.456	0.58
X ₂ = 1/4.3 (g/ml)						
X ₃ = 316 (min)						

3.4 Chemical composition of essential oils

The GC-MS study of *Zingiber rubens* is presented in Table 5, a plant species grown in Binh Phuoc province, yielded 78 compounds, or 99.97% of the total compounds. The remaining compounds were unidentifiable. They consist of six primary compounds: 7-epi-Sesquithujene (18.71%), β - Sesquiphellandrene (9.92%), Citral (9.88%), α - Curcumene (9.46%), β - Bisabolene (5.39%) and Camphene (5.08%). The most prevalent constituents of *Zingiber rubens*, in contrast to the findings of Isiaka et al., were (Z) -Citral (30.1%), Camphene (9.7%), β - Phellandrene (7.5%), 1,8-careole (7.0%), and Zingiberene (5.3%). Z-Citral (26.1%), Camphene (16.3%), Sabinene (14.6%), Zingiberene (7.2%), and Lavanduglyl Acetate (6.7%) make up the majority of *Zingiber Zerumbet*'s oil content. The zerumbone content of this species was modest (1.2%) according to the research conducted by Dai et al. (2013) [1].

Table 5: Chemical components (% of total peak area) of *Zingiber rubens* essential oil obtained by hydrodistillation method.

No.	RT	Component	Area (%)
1	6.302	Camphene	5.08
2	9.714	Citral	9.88
3	13.530	α -Curcumene	9.46
4	13.825	7-epi-Sesquithujene	18.71
5	14.115	β -Bisabolene	5.39
6	14.481	β -Sesquiphellandrene	9.92
7		Other compounds	41.56

4. Conclusion

Response surface analysis revealed significant regression equations for the analyzed response variables, with R^2 values greater than 0.9. This shows that the response surface models fit the experimental data adequately. In general, CCD was regarded as a viable experimental design for determining the effect of hydrodistillation conditions on the efficacy of *Zingiber rubens* essential oil. Blending time, material-to-water ratio, and distillation duration all had a substantial impact on essential oil performance. This study also showed that adjusting the hydraulic distillation parameters of grinding time, material-to-water ratio, and period of distillation may improve the efficiency of essential oil extraction. Understanding the connections between essential oil extraction factors can therefore aid in the effective hydrodistillation of *Zingiber rubens* essential oil.

5. Conflict of interest

Authors declare there is no conflict of interest.

6. Acknowledgment

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