OPTIMIZING THE EXTRACTION OF GINGER ESSENTIAL OIL CULTIVATION IN DAK LAK USING RESPONSE SURFACE METHODOLOGY – FACECENTERED CENTRAL COMPOSITE DESIGN

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TÓM TẮT

TỐI ƯU HÓA CHIẾT XUẤT TINH DẦU GỪNG TRỒNG Ở ĐẮKLẮK BẰNG PHƯƠNG PHÁP ĐÁP ỨNG BỀ MẶT

Mục đích của nghiên cứu này là tối ưu hóa quy trình tách chiết tinh dầu gừng trồng ở tỉnh ĐắkLắk bằng phương pháp chưng cất lôi cuốn hơi nước sử dụng phương pháp CCF - RSM (Response surface methodology - facecentered central composite design or the face-centered cube) với hàm mục tiêu là hiệu suất chưng cất và hai yếu tố ảnh hưởng là thời gian chưng cất (minute) và lượng nước (mL). Kết quả cho thấy mô hình với các yếu tố mã hóa có phương trình dạng $Y = 0.364576 + 0.0528333A + -0.00106667B + -0.01085AB + -0.0201655A^2 + -0.0455655B^2$ *(A thời gian (phút), B lượng nước (mL)) với* R^2 *của mô hình là 0.9982. Hiệu suất tinh dầu tối ưu là 0.39% với thời gian chiết 89 phút, lượng nước là 832 mL (300 ± 0.5 g gừng). Thành phần hóa học tinh dầu được phân tích bằng phổ GC/MS và định danh theo chỉ số lưu RI (Retention Index). Theo kết quả phân tích, có các hợp chất đặc trưng của Zingiber officinale như α-Zingiberene (27.02%), β-Sesquiphellanderene (11.04%), α-Curcumene (8.82%).*

Từ khóa: *Zingiber officinale, chưng cất lôi cuốn hơi nước, RSM, tinh dầu, GC/MS.*

1. INTRODUCTION

The ginger, scientifically named *Zingiber officinale* Roscoe, belongs to the Ginger family (*Zingiberaceae*). The ginger root is the most commonly used part of the plant, utilized as a spice in food, cosmetics, and medicine [1]. It is rich in minerals, vitamins, lipids, proteins, carbohydrates, and essential oils. Ginger is considered a valuable folk remedy due to its antibacterial, anti-inflammatory, and antioxidant properties [2, 3]. Recently, many studies on ginger root and ginger essential oil have been reported [4-7]. Ginger is grown in many regions of Vietnam, but that from Dak Lak is particularly popular for its distinctive spiciness and aroma. However, there have been few studies on the extraction and chemical composition of ginger essential oil from Dak Lak. This article aimed to optimize the extraction process of ginger essential oil from Dak Lak using steam distillation and the CCF-RSM method, considering two influencing factors. The chemical composition of the essential oil was analyzed using GC/MS spectroscopy and identified according to the RI. The steam distillation method for essential oils relies on the absorption, dissolution, diffusion, and steam absorption of organic compounds in essential oils contained within plant tissues when exposed to steam at high temperatures [8, 9] . This method has been employed in recent studies to extract ginger essential oil from various regions, such as Phu Tho [3], Hue [6], Binh Dinh [7], Bac Lieu [8], and

countries like Saudi Arabia and China [9]. However, these studies have not utilized the RSM multivariate optimization method to determine the optimal extraction parameters. Additionally, while ginger essential oil was analyzed using GC/MS spectroscopy in these studies, very few identified the compounds using the RI. Therefore, this study employed the CCF-RSM method, considering two influencing factors, to optimize the essential oil extraction process by steam distillation. The chemical composition of ginger essential oil was analyzed using GC/MS spectroscopy and identified according to the RI proposed.

2. EXPERIMENT

Fresh gingers rhizomes were sourced from Cu Kuin district, Dak Lak province and stored cool refrigerator compartment (10°C) for essential oil extraction.

2.1. Extraction of essential oil from ginger rhizomes using the steam distillation method

The process of extracting ginger essential oil was performed according to the method of Widayat et al. (2018) [10], with modifications to suit the experimental conditions. Fresh ginger rhizomes were ground using a Philips HR2223 700W blender. Through a preliminary assessment of raw material quantity, water volume, and extraction time was studied. The ground ginger sample, weighing 300 ± 0.5 g, was placed into a 2000 mL round-bottom flask, along with distilled water (700 mL – 1000 mL) and a few boiling stones. The flask was then connected to a steam distillation apparatus and continuously boiled for a duration of (50 minutes – 90 minutes) from the appearance of the first drop of essential oil. The mixture was allowed to stand until it completely separated into two phases. The lower water layer was slowly drained off to remove it, leaving behind the crude essential oil. The crude oil was then collected in a container, anhydrous Na_2SO_4 was added and stirred until Na_2SO_4 crystals began to separate. The upper layer, containing the refined essential oil, was decanted and transferred to a tightly sealed vial for GC/MS analysis.

2.2. Experimental design

The CCF-RSM method with two influencing factors was used to optimize the essential oil extraction process. The objective function is the essential oil yield (Y). The independent factors affecting the objective function (Y) chosen to optimize the conditions for ginger essential oil extraction with the highest yield are: distillation time (A) and water volume (B). The experimental design is arranged around the central point: 70 minutes of distillation time and 850 mL of water. The sequence of experimental runs is determined randomly to eliminate potential errors related to equipment and experimental procedures. The number of experiments were $N=2^k+n_c+2^k$ (N = 13 with k = 2, n_c = 5 (center point experiments)). Here, k represents the number of independent variables, and 2^k denotes the additional experiments at the star points. Therefore, in this study, 13 experiments were performed with 22 factorial design experiments, 5 center point experiments to assess error, and 4 additional experiments at the star points, located at a distance of α=1 from the center experimental point, as detailed in Table 1. The model to predict the value of the objective function (Y) is as follows (1):

$$Y = \beta_0 + \sum_{i=1}^{n} \beta_i X_i + \sum_{i=1}^{n} \beta_{ii} X_i^2 + \sum_{i=1} \sum_{j=i+1} \beta_{ij} X_i X_j + \varepsilon \quad (1)$$

Where Y is the predicted response or dependent variable (predicted efficiency, %), β_0 is constant or model coefficient, βi,

βii, βij are coefficients for the linear, quadratic, and interaction effects, respectively. *Xi, Xj* are the independent variables or factors in coded levels, *n* is the number of factors and *ε* is the model error [11]. The level of values entered in this design can be coded as - 1, 0, and +1 and for the investigated factors each level is assigned a value. The yield of ginger essential oil was calculated using the following equation [12]:

The extraction efficiency = $\frac{m_{essential\ oil}}{m_{ginger\ rhizome}}$ x 100 (%) *(2)*

where

$m_{ginger\ rhizome}$ (g): weight of the ginger rhizome (300 ± 0.5 g)

$m_{essential\ oil}$ (g): weight of essential oil obtained

2.3. GC/MS analysis

Ginger sssential oil samples (25 µL) diluted in 0.5 mL of *n*-hexane were analyzed by GC/MS on an Agilent 6890N GC coupled with an MS 5973, using an HP-5MS capillary column (30.0 m × 0.25 mm × 0.25 µm). The injection volume was 1.0 µL, and helium (1.0 mL/min) served as the carrier gas, operating in constant pressure mode at 9.3 psi. The temperature program started at 50°C, increasing at a rate of 5°C/min to 150°C, then increasing at 3°C/min to 200°C, and finally rising at 10°C/min to 280°C, where it was held for 20 minutes. The RI of the essential oil components was calculated using a standard sample containing a homologous series of *n*-alkanes (C8-C32) purchased from Sigma. The RI was determined using the formula for the Kovats index in temperature gradient mode GC/MS [13]. Essential oil components were identified by comparing their RI values with those in the NIST 3.0.

3. RESULTS AND DISCUSSION

3.1. CCF-RSM (face-centered cube) experimental model using Design Expert

The yield of essential oil

The experimental design was created using the CCF-RSM method in Design Expert 12, with two natural (uncoded) variables: A: time (minutes) with levels at 50 (low, -1), 70 (central, 0), and 90 (high, +1); B: water volume (mL) with levels at 700 (low, -1), 850 (central, 0), and 1000 (high, +1). After conducting the experiments designed randomly using Design Expert 12, the results of the extraction efficiency were presented in Table 1, with the weigh the ginger rhizome was 300 ± 0.5 g.

Table 1. Parameters of CCF design

Run No.	Coded variables		Uncoded variables		Y: Yield (%)
	A	B	Time (minute)	Water volume (mL)	
1	0	0	70	850	0.37
2	0	0	70	850	0.37
3	0	0	70	850	0.36
4	+1	+1	90	1000	0.34
5	0	0	70	850	0.37
6	0	+1	70	1000	0.32
7	0	0	70	850	0.36
8	0	-1	70	700	0.32
9	-1	0	50	850	0.29
10	-1	-1	50	700	0.24
11	-1	+1	50	1000	0.26
12	+1	0	90	850	0.39
13	+1	-1	90	700	0.36

The statistical analysis (ANOVA) results shown in Table 2 indicated that the experimental data can be accurately represented by a quadratic model. It was also evident that the factors significantly influence the objective function. The Model F-value of 777.80 indicated that

the model is significant, with only a 0.05% chance that such a large F-value could occur due to random error, necessitating a model change. *P*-value less than 0.05 confirmed the model's significance. Specifically, factors A, AB, A^2, and B^2 significantly affected the model, while factors with *P*-values greater than 0.05, such as B, do not significantly impact the model. The Lack of Fit value, with an F-value of 0.42, indicated that the Lack of Fit is appropriate and did not significantly affect the model compared to pure error, demonstrating the model's statistical accuracy [14]. Table 2 also showed that the experimental data can be well represented by a second-order polynomial model. The optimized parameters of the essential oil extraction process were used to test the model's suitability for predicting performance values. These parameters were validated by performing essential oil extraction under optimized conditions, and the results in Table 3 showed that the experimental values are quite consistent with the predicted values.

Final equation in terms of coded factors:

$$Y = 0.364576 + 0.0528333A + -0.00106667B + -0.01085AB + -0.0201655\ A^2 + -0.0455655B^2 \quad (3)$$

Response surface analysis

The goal of optimizing the essential oil extraction process was to minimize both the extraction time and the amount of water used, in order to achieve the highest yield of essential oil.

Table 2. ANOVA evaluation of factors influencing the objective function

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	0.0275	5	0.0055	777.80	< 0.0001	significant
A-time	0.0167	1	0.0167	2367.70	< 0.0001	
B-water volume	6.827E-06	1	6.827E-06	0.9651	0.3586	
AB	0.0005	1	0.0005	66.57	< 0.0001	
A^2	0.0011	1	0.0011	158.78	< 0.0001	
B^2	0.0057	1	0.0057	810.66	< 0.0001	
Residual	0.0000	7	7.074E-06			
Lack of Fit	0.0000	3	3.954E-06	0.4201	0.7490	not significant
Pure Error	0.0000	4	9.413E-06			
Cor Total	0.0276	12				
R^2	0.9982					

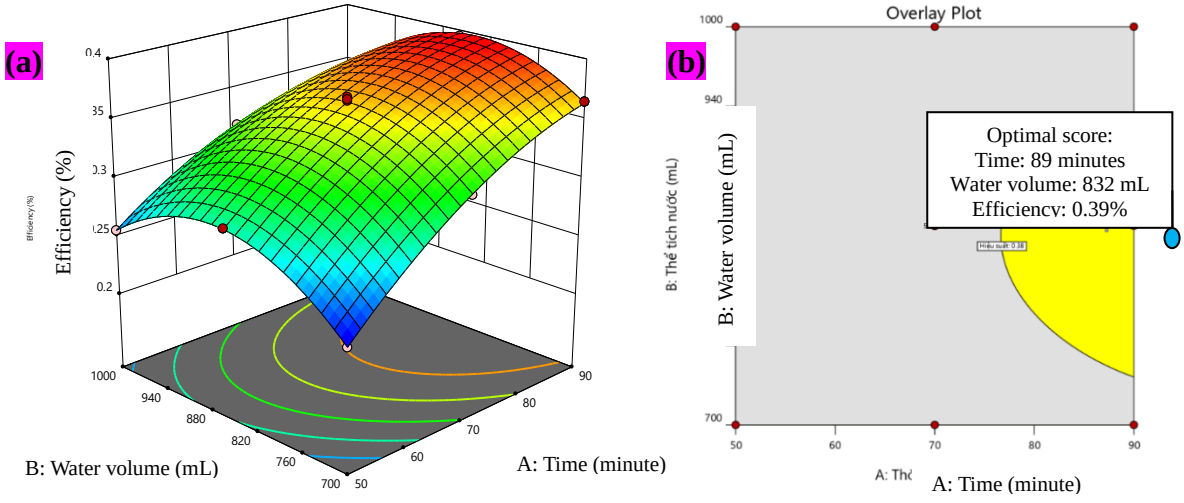

Figure 1. Response surface of essential oil yield (a), optimum parameters for extraction efficiency (b)

Based on response surface of essential oil yield in Figure 1.a, it showed that the general parameters of the survey process for factors affecting the experiment, as well as the distribution of experimental points on the surface. The results in Figure 1.b. indicated that the high-efficiency zone is within the time range of 82-90 minutes and a water volume of 767-893 mL (yellow area). According to the default maximize desirability in Design Expert, the optimal value for the essential oil extraction process was predicted to be at an extraction time of 89 minutes and a water volume of 832 mL, with an efficiency of 0.39%.

Model verification

Experiments were conducted under optimal distillation conditions to test the model's suitability in predicting objective function values. The experimental results at these optimal conditions were very similar to the model's predicted values, demonstrating that the model can accurately predict the outcomes of the objective function (Table 3).

Table 3. Optimal conditions, experimental values and predictions of the response at optimal conditions

Optimal conditions	Optimal coded variables	Optimal uncoded variables
Time (minute)	+0.95	89
Water volume (mL)	-0.12	832
Dependent variable (response)	Predicted value	Experimental value
Essential oil yield (%)	0.39	0.40 ± 0.02

3.2. GC/MS analysis

The results of the GC-MS analysis and RI index with comparison of results in the NIST library were shown in Table 4.

Table 4. Chemical components in Dak Lak ginger essential oil

No.	Compound	RI^a	RI^b	Content (%)	Ref.
1	*α*-Zingiberene	1512	1495	27.02	[15]
2	*β*-Sesquiphellandene	1540	1525	11.04	[16]
3	*α*-Curcumene	1499	1486	8.82	[17]
4	*α*-Farnesene	1523	1508	6.57	[15]
5	*β*-Phellandrene	1040	1031	6.23	[18]
6	*α*-Citral	1284	1269	6.19	[19]
7	Camphene	959	951	5.99	[19]
8	*β*-Bisabolene	1525	1517	4.87	[20]
9	*β*-Citral	1254	1237	4.25	[19]
10	*γ*-Cadinene	1517	1513	3.26	[21]
11	Eucalyptol	1042	1031	3.21	[17]
12	*α*-Pinene	944	928	2.14	[22]
13	*endo*-Borneol	1179	1165	1.19	[21]
14	*β*-Myrcene	1002	987	0.96	[20]
15	*γ*-Curcumene	1496	1483	0.94	[23]
16	*α*-Terpineol	1204	1190	0.71	[20]
17	*β*-Elemene	1409	1394	0.61	[17]
18	Linalool	1111	1097	0.55	[20]
19	*α*-Copaene	1393	1376	0.52	[21]
20	(E)-*β*-Famesene	1472	1460	0.51	[18]
21	*2*-Undecanone	1306	1294	0.50	[24]

RI^a: calculated; RI^b: reference.

According to the results in Table 4, the calculated RI^a index and RI^b in the NIST database were very similar. The difference between the RI indices in the experiment and those in the library was no more than 20 units, proving the high reliability of the experimental research results. A typical compound of *Zingiber officinale* is *α*-Zingiberene (27.02%). This result was slightly higher than the previously reported *α*-Zingiberene content of about 24% in ginger grown in Phu Tho [3], or in Saudi Arabia and China [9], this can be related to the raw material source and distillation method.

4. CONCLUSION

Based on the statistical experimental design using RSM, the optimal conditions for steam distillation of *Zingiber officinale* Rose were determined: extraction time of 89 minutes, water volume of 832 mL, and an optimal essential oil yield of 0.39%. Some main chemical components of Dak Lak ginger essential oil were α-Zingiberene (27.02%), β-Sesquiphellandrene (11.04%), and α-Curcumene (8.82%). Because ginger grown in Dak Lak has a higher content of α-Zingiberene in the essential oil compared to ginger grown elsewhere, it exhibits a characteristic spiciness and aroma. This makes it particularly valuable for use in food and cosmetic products.

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