STEAM DISTILLATION OPTIMIZING FOR LEMONGRASS *(Cymbopogon citratus)* ESSENTIAL OIL EXTRACTION PROCESS

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Abstract: In this study, we optimized conditions of extraction of essential oil from lemongrass *(Cymbopogon citratus)* stalks and leaves, using steam distillation device having an extent of 25 ÷ 30 kilograms of material per batch and approaching industrial scale. Using response surface methodology (RSM) with three factors that affect to the volume of essential oil obtained (mL/kg): material size (mm), water/material ratio (L/kg) and distillation time (minute). In the optimal condition of lemon grass essential oil distillation using steam distillation at 5.66 L water to 1 kg sample, 10.00 mm material thickness size in 180 minutes, the maximum essential oil volume obtained was 2.98±0.02 mL/kg.

Keywords: *Cymbopogon citratus;* steam distillation; RSM; essential oil yield.

1. Introduction

Essential oils are concentrated liquids of complex mixtures of volatile compounds and can be extracted from several plant organs. Essential oils are a good source of several bioactive compounds, which possess antioxidative and antimicrobial properties [1]. Essential oils extracted from a wide variety of plants and herbs have been a source material for the manufacture of foodstuffs, cosmetics, cleaning products, fragrances, herbicides and insecticides.

Lemongrass (*Cymbopogon citratus*), a perennial plant with long and thin leaves, is one of the largely cultivated medicinal plants for its essential oils in parts of tropical and subtropical areas of Asia, Africa and America [3]. The essential oil extracted from lemongrass is often used in pharmaceutical and cosmetic fields [5]. Lemongrass is a tall perennial grass that contains 1 to 2% essential oil on a dry basis [6]. Lemongrass essential oil is characterised by a high content of citral (composed of neral and geranial isomers).

Distillation techniques used to isolate essential oil from medicinal and aromatic plants are classified into three categories on the basis of difference in operation as well as geometric configurations of equipments used. These three distillation techniques are hydro-distillation, water-steam distillation and steam distillation [7]. The aim of the present study was to investigate the applicability of steam distillation technique in isolation of lemongrass (*Cymbopogon citratus*) extracts based on the extraction yield and constituents of oils obtained under optimized condition. The effect of material size such as distillation time and water to raw material ratio were evaluated to identify its optimum condition for distillation and this applicability was appreciated by using the result of subsequent GC/MS analysis.

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Steam distillation is the most widely used method for plant essential oil extraction. Basically, the plant sample is placed in boiling water or heated by steam. The heat applied is the main cause of burst and break down of cell structure of plant material. As a consequence, the aromatic compounds or essential oils from plant material are released. The temperature of heating must be enough to break down the plant material and release aromatic compound or essential oil. A new process design and operation for steam distillation of essential oils to increase oil yield and reduce the loss of polar compounds in wastewater was developed. The system consists of a packed bed of the plant materials, which sits above the steam source. Only steam passes through it and the boiling water is not mixed with plant material. Thus, the process requires the minimum amount of steam in the process and the amount of water in the distillate is reduced. Also, water-soluble compounds are dissolved into the aqueous fraction of the condensate at a lower extent [2], [4].

2. Materials and methods

2.1. Material

The *Cymbopogon citratus* stalks and leaves were collected at Quy Hop District, Nghe An Province and was deposited at the herbarium of School of Chemistry, Biology and Environment, Vinh University.

2.2. Methods

2.2.1. Steam distillation

Steam distillation unit of Chin Ying Fa Mechanical Ind. Co., Ltd., Taiwan, type CYF - R08, volume 180L, was used. Distillation capacity is 25 ÷ 30 kilograms of material per batch.

2.2.2. Experimental design

Response surface methodology was used to determine the optimum levels of material size (mm), water/material ratio (L/kg) and distillation time (min) on the essential oil yield in the *Cymbopogon citratus*. These three factors, namely material size (A), water/material ratio (B) and distillation time (C) were coded into three levels (-1, 0, +1). The coded independent variables used in the RSM design are shown in table 1.

The effects of the extraction parameters were evaluated using the program Design-Expert®, version 7.0.0. The response variable was fitted be a second-order polynomial model as follows:

$$y = \beta_0 + \sum_{i=1}^{k} \beta_i X_i + \sum_{i=1}^{k} \beta_{ii} X_i^2 + \sum\sum_{i<j} \beta_{ij} X_i X_j$$

where Yi is the predicted response, β_0 is the regression coefficient for main, βi for linear, βii for quadratic and βij for interaction effect of input variables Xi and Xj.

2.2.3. The essential oil yield

The essential oil yield is performed as: Y = v/m (mL/kg); v (mL): volume of *C. citratus* essential oil from distillation; m: mass of raw material.

2.2.4. A GC-MS condition (5973N, Agilent Technologies, Wilmington, DE, UAS) equipped with a mass selective detector operating in the electron impact mode (70eV) was used to study the composition of the essential oil at extracted various group of parameter condition to analyze its quality. The GC part (6890N, Agilent Technologies, Palo Alto, CA, USA) was equipped with an HP-5MS (Agilent BTechnologies) capillary column (30m long, 0.25 mm id and 0.25 mm film thickness). Temperature-programming of the oven included an initial hold at 50 °C for 5 min and a rise to 240 °C at 3 °C/min followed by additional rise to 300 °C at 5 °C/min. A final hold for 3 min was allowed for a complete column clean-up. The injector was set at 280 °C. The samples were diluted with n-hexane (1/10, v/v) and a volume of 1.0 µl was injected to the GC with the injector in the split mode (split ratio: 1/10). Carrier gas, He, was adjusted to a linear velocity of 1 ml/min. The compounds of the extracted essential oils were identified by comparing their mass spectral fragmentation patterns with those of similar compounds from a database (Wiley/NBS library) or with published mass spectra. The components were quantified based on the comparison of compound's retention period, which were similar in both techniques. The normalization method was used; the value of total peak areas is considered 100% and the percentage of each component was calculated using the area of each peak.

3. Results and discussion

3.1. Study on the factors influencing on total essential oil

To determine how material size affects the essential oil yield, we distilled *C. citratus* in different thickness sizes of 10, 15, 20, 25, 30 and 35 mm. The total essential oil amount decreasing when size increased (Fig 1a). Samples were distilled *C. citratus* in different water/material ratio of 3, 4, 5, 6, 7 and 8 liter water for 1 kg material with the material size was 15 mm and the distillation time was 180 minutes. Fig 1b. showed that the total essential oil amount increasing when the water/material ratio increased. To determine the effect of distillation time on the essential oil yield, we distilled *C. citratus* in different times of 60, 90, 120, 150, 180, 210 and 240 minutes with the material size 15 mm and the water/material ratio at 5/1. Fig 1c. showed that the essential oil yield increased significantly to 90 minutes to 180 minutes then increased not significantly.

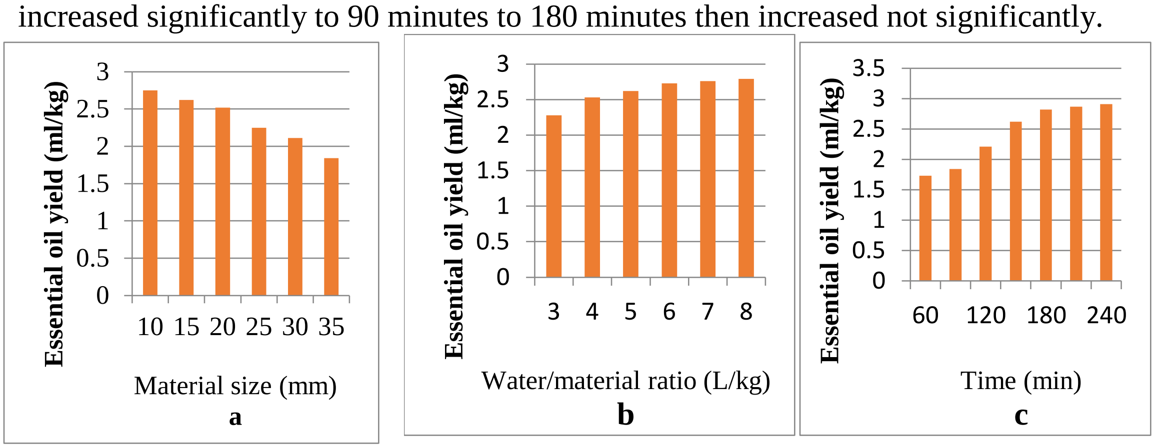


Figure 1: *The effect of material size (a), water/material ratio(b), distillation time (c) on essential oil yield*

3.2. Model fiting

This paper deals with optimization of yield of *C. citratus* oil in steam distillation using Box-Behnken. The factors considered were mass of material size (mm), water/material ratio (L/kg) and distillation time (min). The input range of the selected variables was determined by the preliminary experiments (Table 1). These experimental values were compared with those of the predicted values to check the validity of the model.

Table 1: *Coded level of independent variables used in the RSM design*

Factors	Symbols	Units	Range and level		
			-1	0	+1
Material size	A	mm	10	15	20
Water/material ratio	B	L/kg	4/1	5/1	6/1
Distillation time	C	min	120	150	180

This design has 17 actual experiments with 3 factors (k = 3), 3 levels with 5 center points to form a central composite design with response: total essential oil (mL/kg)

Table 2: *Experimental design and response values*

RUN	Material size A (mm)	Water/material ratio B (L/kg)	Distillation time C (min)	Essential oil yield Y (mL/kg)
1	+	0	-	2.46
2	0	0	0	2.81
3	0	+	-	2.63
4	-	+	0	2.89
5	0	0	0	2.82
6	-	0	+	3.02
7	0	0	0	2.83
8	0	-	+	2.95
9	-	-	0	2.85
10	+	+	0	2.76
11	0	+	+	2.98
12	+	-	0	2.72
13	-	0	-	2.69
14	0	0	0	2.82
15	0	-	-	2.52
16	0	0	0	2.79
17	+	0	+	2.92

The values of the three evaluation indices for each distilling condition were listed in Table 2. At distilling condition: 10 mm, 5/1 L/kg in 180 mins, the maximal oil yield was 3.02 mL/kg. From the multiple linear regression analysis of the 17 data entries, empirical second-order polynomial models of oil Yield scavenging capacity were derived:

$Y = 2.81 - 0.074A + 0.027B + 0.2C + 0.033AC - 0.02BC - 0.003A^2 + 0.0057B^2 - 0.038C^2$

The R^2 of the model was 0.9946

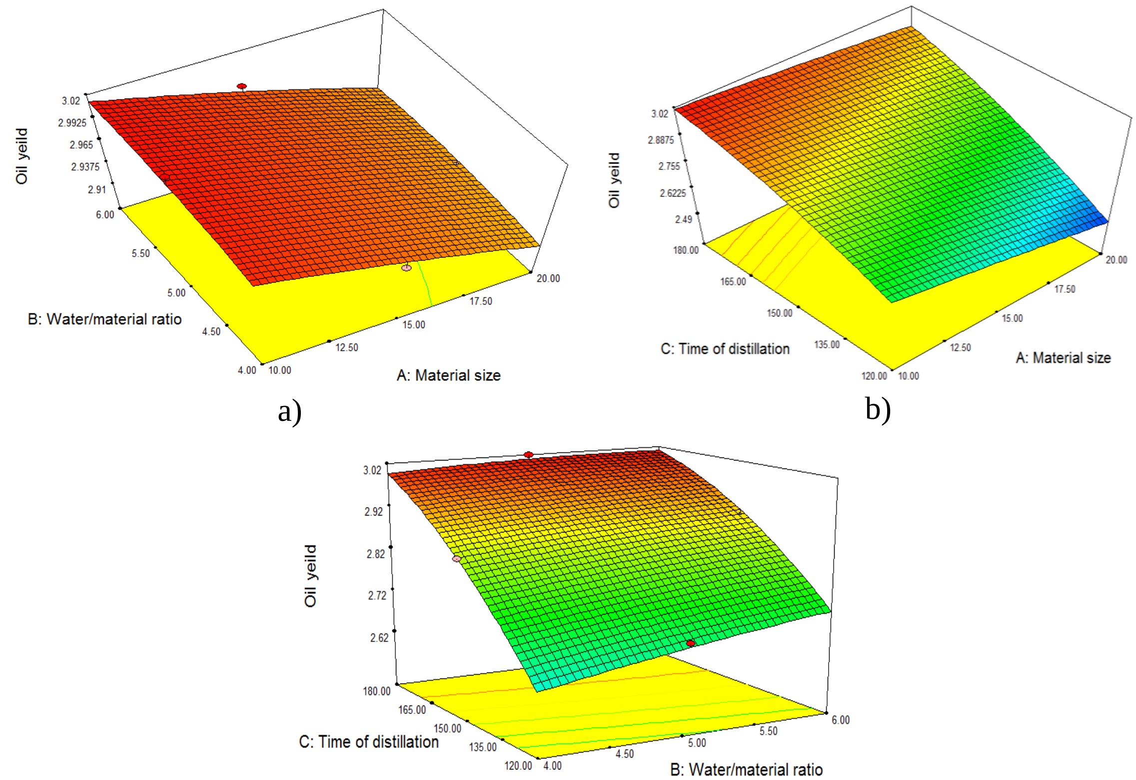
Table 3: *ANOVA for the effect of: material size, water/material ratio and distillation time on total essential oil yield*

Source	Sum of square	DF	F-value	P-value
Model	0.37	9	144.27	<0.0001 significant
A	0.044	1	152.68	<0.0001 significant
B	6.05E-003	1	21.23	0.0025 significant
C	0.31	1	1080.1	<0.0001 significant
AB	0.000	1	0.000	1.0000
AC	4.225E-003	1	14.82	0.0063 significant
BC	1.600E-003	1	5.61	0.0497
A^2	4.447E-005	1	0.16	0.7046
B^2	1.392E-004	1	0.49	0.5072
C^2	6.160E-003	1	21.61	0.0023 significant
Residual	1.995E-003	7		
Lack of Fit	1.075E-003	3	1.56	0.3308 not significant
R^2	0.9946			

ANOVA analysis of the quadratic regression model for total essential oil yield demonstrated the model to be significant ($p<0.05$) with an F-value of 144.27. There is only a 0.01% chance that a “Model F-Value” this large could occur due to noise. Value of “Prob > F” less than 0.0500 indicate model terms are significant. In this case A, B, C, AC, BC, C^2 are significant model terms. Values greater than 0.1000 indicate the model term are not significant. If there are many insignificant model term (not counting those required to support hierarchy), model reduction may improve your model. The “Lack of Fit F-value” of 1.56 implies the Lack of Fit not significant relative to the pure error. There is a 33.08% chance that a “Lack of Fit F-value” this large could occur due to noise. Non-significant lack of fit is good.

Response surface analysis

The X- and Y- axes of the three- dimensional response surfaces represented two factors, for material size and water/material ratio (distillation time 150 min), material size and distillation time (water/material ratio 5/1 L/kg), water/material ratio and distillation time (material size 15 mm). The Z-axes represented one of evaluation indices oil yield. Three dimensional response surfaces were constructed as depicted in Fig 2.



c)

Figure 2: *Response surface of oil yield*

Fig. 2 showed that the slope of the first response surface followed an upward trend with two factors having a significant impact on the oil yield. We speculated that with increasing distillation time, water/material ratio then yield increased rapidly. But material size decreasing then oil yield increased rapidly.

Optimization and model verification

The optimal values of the independent variables were obtained by solving second - order regression equations using a numerical optimization method. Experimental data suggested the existence of optimization of oil yield (2.98±0.02 mL/kg) occurred with 10.00 mm, 5.66 L/kg at 180 minutes.

Table 4: *Optimum conditions, predicted and experimental values of responses of C. citratus crude extraction*

Independent variables			**Dependent variables (Response)**	**Optimum value**	
X_1	X_2	X_3		Experimental$^{(*)}$	Predicted
10	5.66	180	Y	2.98±0.02	3.01244

X_1: Material size (mm); X_2: Water/material ratio (L/kg); X_3: Distillation time (min); Y: oil yield (%).

$^{(*)}$Mean ± standard deviation (SD) of three determinations (n= 3) from three distillations.

3.3. *Composition of essential oil from C. citratus*

Table 5: *Composition of essential oil from Cymbopogon citratus*

No	Compound	RI[a]	%[b]	No	Compound	RI[a]	%[b]
1	Tricyclene	926	0.09	22	β-Caryophyllene	1419	0.88
2	α-Pinene	930	0.21	23	longifolene	1402	3.84
3	Camphene	953	0.37	24	α-Cadinol	1654	0.87
4	β-Myrcene	990	6.98	25	γ-selinene	1484	0.51
5	Trans-fanesol	1741	0.09	26	α-eudesmol	1652	0.22
6	Limonene	1032	0.53	27	Tau-Muurolol	1646	0.58
7	(Z)-β-ocimene	1043	2.08	28	β-selinene	1486	0.24
8	(E)-β-ocimene	1052	1.75	29	epi-bicyclosesquiphellandrene	1489	0.03
9	γ-Terpinene	1061	0.05	30	ledene	1492	0.04
10	Linalool	1100	1.27	31	δ-selinene	1493	0.23
11	Alloocimene	1120	2.02	32	α-muurolene	1500	0.15
12	Cis-carveol	1142	0.31	33	β-elemene	1391	0.21
13	α-Terpineol	1189	0.24	34	β-elemene	1391	0.21
14	β-maaliene	1732	0.74	35	δ-Cadinene	1525	0.46
15	Citronellal	1223	0.53	36	β-elemene	1391	0.21
16	Juniper camphor	1691	0.47	37	β-elemene	1391	0.21
17	α-humulene	1454	0.39	38	γ -Cadinene	1541	0.21
18	Neral (Z-citral)	1318	61.62	39	elemol	1550	0.07
19	α-beganotene	1435	0.34	40	Germacrene-D-4-ol	1574	0.13
20	Geranic acid	1355	0.25	41	Caryophyllene oxide	1583	0.22
21	Geranyl acetate	1363	0.76	42	β-elemene	1391	0.21

[a] Retention indices on HP-5MS capillary column

[b] Percentage content of total essential oil

According to the result, there are two typical compounds of lemon grass (*C. citratus*) such as β-Myrcene and Z-citral, especially Z-citral takes 61.62%.

4. Conclusion

Based on the statistical experimental design using response surface and desirability methodology, the optimal conditions for steam distillation of *C. citratus* were determined: material size was of 10.00 mm, water/material ratio was 5.66 L/kg, distillation time was of 180 minutes and the maximum *C. citratus* essential oil was predicted to be 2.98±0.02 mL/kg.

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TÓM TẮT

TỐI ƯU HÓA QUÁ TRÌNH CHƯNG CẤT TINH DẦU TỪ CÂY SẢ (*Cymbopogon citratus*)

Trong nghiên cứu này, chúng tôi đã tối ưu hóa điều kiện chưng cất tinh dầu từ thân và lá sả (*Cymbopogon citratus*) bằng phương pháp chưng cất lôi cuốn hơi nước, mỗi mẻ 25-30 kg nguyên liệu. Dùng phương pháp bề mặt đáp ứng (SRM) với ba yếu tố ảnh hưởng đến thể tích tinh dầu thu được (mL/kg): kích thước vật liệu (mm), tỷ lệ nước/vật liệu (L/kg) và thời gian chưng cất (phút). Kết quả tối ưu hóa quá trình chưng cất tinh dầu sả bằng phương pháp chưng cất lôi cuốn hơi nước ở điều kiện 5,66 L nước, 1 kg mẫu, kích thước độ dày vật liệu 10,00 mm trong 180 phút, thể tích tinh dầu thu được lớn nhất là 2,98 ±0,02 mL/kg.

Từ khóa: *Cymbopogon citratus;* chưng cất hơi nước; RSM; tinh dầu.