Emir. J. Food Agric. 2012. 24 (1): 25-30
http://ejfa.info/

NUTRITION AND FOOD SCIENCE

Application of response surface methodology (RSM) in condition optimization for essential oil production from *Citrus latifolia*

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Abstract

This research shows the dependence of distillation time and ratio of water/peel on amount of essential oil after distilling. Researching and optimizing the factors for essential oil production by laboratory – scale distillation and the yield was evaluated using the Response Surface Methodology (RSM) with Central Composite Face-Centered (CCF) model. The regression equation obtained was shown below:

$$y = 0.997 - 0.015x_1 + 0.04x_2 - 0.107x_1^2 - 0.042x_2^2.$$

There is the contrast between distillation time and ratio of water/peel with volume of essential oil received. Optimal result is volume of essential oil was 1 ml (y) with quantity of lemon peel/batch of 50g, ratio of water/peel (x_1) was 6/1, distillation time (x_2) in 20 minutes at temperature at 100°C. The essential oil from the peel of *C. latifolia* was analysed by GC-MS method. A lot of compounds were identified of which limonene, β–pinene and γ–terpinenen were major components. Small amounts of trans-caryophyllene, 4-terpineol, α-terpinenen…were also detected.

Key words: CCF, *C. latifolia*, Essential oil, GC-MS, RSM

Introduction

The advantage of *Citrus latifolia* was high productivity and large size containing a large amount of water (Baomoi, 2008; Wikipedia, 2011). *C. latifolia* was chosen as stable materials in food industry, especially in the field of beverage. However, *C. latifolia* has been known as fruit containing essential oil source from peel which has not been paid attention properly.

Optimization of conditions for processing is one of the most critical stages in the development of an efficient and economic bioprocess. Statistical methodologies involved used mathematical models for designing fermentation processes and analyzing the process results (Bas et al., 2007). RSM is a powerful mathematical model with a collection of statistical techniques where in, interactions between multiple process variables can be identified with fewer experimental trials. It is widely used to examine and optimize the operational variables for experiment designing, model developing and factors and conditions optimization (Cheynier et al., 1983).

Therefore, our group carried out researching subject "Application of response surface methodology (RSM) in condition optimization for essential oil production from *C. latifolia*" to obtain high content of essential oil, contributing to the development of the oil production industry in general and lemon essential oil in particular in Vietnam.

Materials and Methods

Sample collection

C. latifolia: large circular fruit, diameter 4.5 – 5cm, 70–100 gram piece of fruit, ratio of water/peel of 6/1, dark green peel and no insects. *C. latifolia*'s source is from Binh Thanh Hamlet, Binh Phu Commune, Ben Tre Province.

Experimental equipment

The grinding apparatus Philip (Volume of tank: 2000 ml, 500W, 15 000 rounds/minute, China).

The distilling apparatus Clevenger (Volume of boiled container: 500 ml, Germany).

The apparatus Hewlett-Packerd HP 5890 GC/5972 MS (USA) uses for GC-MS method. Samples were subjected to the GC column (Rt×5MS- 29m×250micron, 0.25micro film), at flow rate of 1ml/min at 250°C. Detector temperature was set at 280°C. MS scan mode: full (35-450 amu) Atune. The oven temperature was held at 40°C for 5min, programmed at 3°C/min to

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Received 26 October 2011; Revised 08 December 2011; Accepted 28 December 2011

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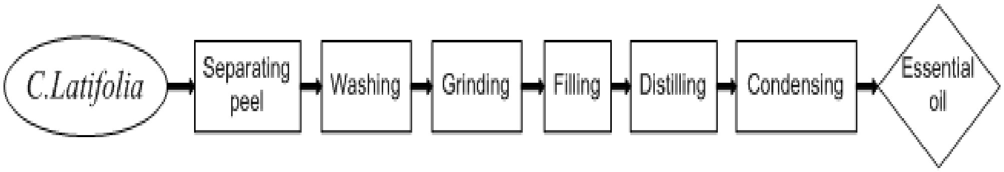
200°C and second programmed at 10°C/min to 300°C then held at this temperature for 5 min.

Methods

Essential oil processing



50g of peel was washed with water and ground in 30 seconds at 28±1°C. The ground peel fill by water and it distilled at 100°C in the apparatus Clevenger to obtain essential oil which was then condensed by cold water and measured in the small cylinder that determines the volume. The chemical composition of the essential oil from *C.Latifolia* peel was analyzed by using GC-MS methodology.

Figure 1. The ground peel and the apparatus Clevenger.

Experimental design

Response surface methodology (RSM) was used to determine optimum conditions for distilling essential oil process. Two factors including ratio of water/peel (x_1) and distillation time (x_2) to target distillation time function (obtained volume of essential oil - y) were determined using optimization method (Canh, 2004). Influence of factors to target function was described according to equation below:

$$y = b_o + \sum_{i=1}^{n} b_i x_i + \left(\sum_{i=1}^{n} b_{ii} x_i \right)^2 + \sum_{i<j}^{n} b_{ij} x_i x_j \quad (1)$$

In this study, n-value was 2 so equation (1) can be written:

$$y = b_o + b_1 x_1 + b_2 x_2 + b_{12} x_1 x_2 + b_{11} x_1^2 + b_{22} x_2^2 \quad (2)$$

Table 1. Codes and actual levels of the in dependent variables for design of experiment.

Independent variables	Symbols	Coded levels		
		-1	0	+1
Ratio of water/peel	X_1	5/1	6/1	7/1
Distillation time (minute)	X_2	16	20	24

Using model in this case is Central Composite Face (CCF). The star points are at the center of each face of the factorial space, so α = ±1. This variety requires 3 levels of each factor. CCF designs provide relatively high quality predictions over the entire design space and do not require using points outside the original factor range. Requires 3 levels for each factor (Mary, 2003; Cheynier et al, 1983). A 2^3 three level was used to develop a statistical model for the optimization of process variables such as ratio of water/peel (5/1-7/1, w/w) and distillation time (16-24, minute). The design contains a total of 11 experimental trials with a full factorial design fashion and the replications of the central points.

Statistical analysis

The volume of essential oil was determined by actual response value. The data reported represented its mean. Statistical significance was evaluated using the Analysis of Variance (ANOVA) and $p<0.05$ was considered as significant (Tuan, 2008). Second-order polynomial regressed equations were established on the basis of the experimental data. Optimum parameters were defined by the software Modde version 5.0.

Results and Discussion

Content of essential oil were determined according to Table 2.

Table 2. Three level factorial composite design and experimental responses of dependent variable y (volume of essential oil, ml).

Run No	Coded levels		Real values		Volume of essential oil (ml)	
	x_1	x_2	X_1	X_2	Observed	Predicted(*)
1	-1	-1	5/1	16	0.83	0.8226
2	+1	-1	7/1	16	0.8	0.7926
3	-1	+1	5/1	24	0.9	0.9026
4	+1	+1	7/1	24	0.87	0.8726
5	-1	0	5/1	20	0.9	0.9047
6	+1	0	7/1	20	0.87	0.8473
7	0	-1	6/1	16	0.9	0.9147
8	0	+1	6/1	24	1	0.9947
9	0	0	6/1	20	1	0.9968
10	0	0	6/1	20	1	0.9968
11	0	0	6/1	20	1	0.9968

(*): Running the software Modde version 5.0 to predict obtained model

Based on the RSM method, result of experimental analysis was presented in table 3 and table 4 below.

Table 3. Analysis of variance (ANOVA) for the fitted quadratic polynomial model for essential oil production

y	DF	SS	MS	F	p	SD
Total	11	9.2727	0.842973			
Constant	1	9.21863	9.21863			
Total Corrected	10	0.05407	0.0054073			0.07353
Regression	5	0.05363	0.0107262	121.308	0.000	0.10357
Residual	5	0.00044	8.84E-05			0.0094
Lack of Fit	3	0.00044	0.0001474	--	--	0.01214
Pure Error	2	0	0			--

The p_{value} was used as a tool to check the significance of each of the coefficients, which in turn indicated the pattern of the interactions between the variables. The smaller value of p was more significant to the regression. According to the ANOVA table, the regression model is significant at the considered confidence level (95%) since the regression has $p_{value}<0.05$ and the F value (121.3) were 6 times more than the F listed value (F(4,2)=19.3).

Table 4. Results of regression analysis of second order polynomial model for optimization of essential oil production.

y	Coeff. SC	Std. Err.	P	Conf. int(±)
Constant	0.996842	0.00482378	5.04E-11	0.0124
x_1	-0.015	0.00383888	0.0113247	0.00986817
x_2	0.04	0.00383888	0.000140312	0.00986817
x_1*x_1	-0.107105	0.00590791	9.38E-06	0.0151868
x_2*x_2	-0.0421053	0.00590791	0.000843875	0.0151868
x_1*x_2	-3.30E-08	0.00470164	1	0.012086
N = 11	Q^2 =	0.923	Cond. no. =	3.0822
DF = 5	R^2 =	0.992	Y-miss =	0
	$R^2_{Adj.}$ =	0.984	RSD =	0.0094
			Conf. lev. =	0.95

The table shows coefficients in the regression equation. Distillation time and ratio of water/peel are absolutely independence and do not interact together ($p_{value}>0.05$) and have strong influence to revoked frequency of essential oil (table 4). Factors have $p_{value}<0.05$, it means these factors effect on the response. Regression equation presents dependence of content of essential oil on two factors quoted above as below:

$$y = 0.997 - 0.015x_1 + 0.04x_2 - 0.107x_1^2 - 0.042x_2^2 \quad (3)$$

Equation (3) showed that the regression coefficients of linear term X_1, X_2 and all quadratic coefficients of X_1 and X_2 were significant at $p_{value}<0.05$ level and none-interactive coefficient of X_1X_2 were significant at $p_{value}>0.05$ level. The variable with the largest effect on volume of essential oil were the linear term X_2 and the quadric term X_1^2.

Table 4 showed that the experimental yields fitted the second order polynomial equation well as indicated by high R^2 (coefficient of determination) value was 0.992. The R^2_{adj} was 0.984 and the Q^2 was 0.923, which indicates that the model is good (for a good statistical model, the R^2 value should be in the range of 0–1.0, and the nearer to 1.0 the value is, the more fit the model is deemed to be; predictive ability of model manifest by Q^2 with fail-safety achieved R^2).

With first-level coefficients: distillation time was covariant and ratio of water/peel was contravariant when comparing its with obtained content of essential oil (influence level of distillation time is stronger than). With second-level coefficients: both distillation time and ratio of water/peel was contravariant when comparing its with obtained content of essential oil (influence level of ratio of water/peel is stronger than).

In Figure 2 and 3, the three-dimensional contour plot and response surface graph are displayed according to Equation (3). The graph determined the contribution of the distillation time and ratio of water/peel on volume of essential oil. The contours around the stationary point were elliptical and became elongated more and more along the distillation time axis, which meant that a small change of the response value would require a small movement along the distillation time axis. Figure 3 indicates that the distillation time and ratio of water/peel exerted a slight effect on volume of essential oil. Distillation time range from 21 to 23 minute and ratio of water/peel range from 5.8 to 6.1 was determined as the optimal condition for achieving the maximal volume of essential oil.

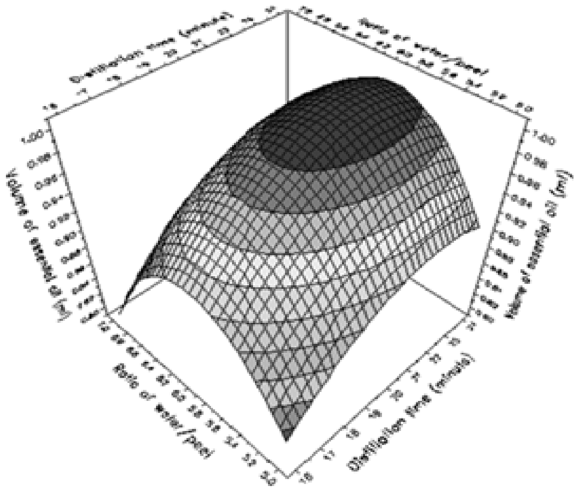


Figure 2. Response surface plot of distillation time versus ratio of water/peel.

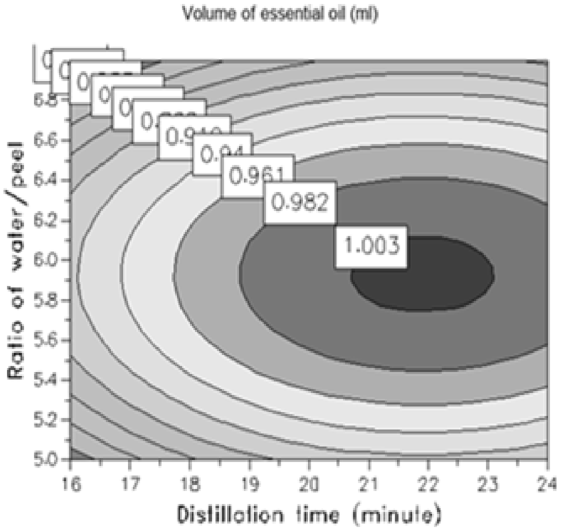


Figure 3. Isoresponse contour plot of distillation time versus ratio of water/peel.

To carry out optimizing with obtained regression equation and result from the software Modde 5.0 by experimentation.

Table 5. Result of optimum condition.

Result	Distillation time (minute)	Ratio of water/peel	Volume of essential oil
Predictive model	22	6/1	1.0069
Experimentation	20	6/1	1

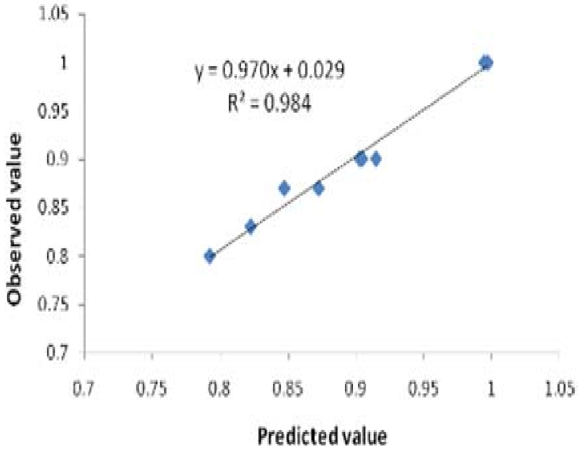


Figure 4. Parity plot showing the distribution of experimental versus predicted values by the mathematical model of the y values.

Table 6. Percentage composition of the essential oil from peel of *C. latifolia.*

No	Compound	Area (%)
1	*Limonene*	56.61
2	*γ–Terpinene*	13.20
3	*β–Pinene*	11.51
4	*E – Citral*	2.09
5	*Alpha – Pinene*	1.83
6	*β-Bisabolene*	1.58
7	*β–Myrcene*	1.57
8	*Neryl acetate*	1.43
9	*Z – Citral*	1.33
10	*Trans α Bergamotene*	1.06
11	*α–Terpineol*	0.96
12	*α–Terpinolene*	0.78
13	*Trans–Caryophyllene*	0.59
14	*4-Terpineol*	0.56
15	*α–Terpinene*	0.42

Response analysis revealed maximum essential oil volume by distilling peel of *C. latifolia* could be achieved at the conditions when distillation time was 20 minutes; ratio of water/peel was 6/1. Under these optimum process conditions a maximum essential oil of 1ml (~2%, w/w) was obtained. The matching quality of the data obtained by the model proposed in Equation (3) was evaluated considering the correlation coefficient, R^2, between the experimental and modeled data. The mathematical adjustment of those values generated a $R^2 = 0.984$, revealing that the model could not explain only 2% of the overall effects, showing that it is a robust statistical model. The parity plot shows a satisfactory correlation between the experimental and predictive values (Figure 4).

GC-MS methodology used for determining the chemical composition and content of the optimization sample. Results showed in the Table 6.

The highest content in the essential oil are limonene, β–pinene and γ–terpinenen. It has characteristic flavour and light yellow. Density of essential oil is lighter than water and it can dissolve absolutely in the alcohol 96^0 (Essential oil/alcohol=1/1, v/v).

Conclusions

From studies quoted above, we come to a conclusion that distillation time and ratio of water/peel have strong influence on revoked frequency of essential oil from *C. latifolia* peel.

Using the optimal method of target function, we exposed regression equation and this equation can be applied on actual model:

$$y = 0.997 - 0.015x_1 + 0.04x_2 - 0.107x_1^2 - 0.042x_2^2.$$

This equation predict optimal result (table 4) with quantity of lemon peel/batch of 50g, however we can accept optimal result according to actual result because between actual result and predictive result have mere error: distillation time of 20 minute, ratio of water/peel of 6/1, optimal content of essential oil 1ml/50g sample with Limonene (56.62%), γ–Terpinene (13.2%), β-Pinene (11.51%). Essential oil after distilling achieves 1ml/50g peel (equivalent 2%, w/w). The productivity is high and we recommended essential oil from *C. latifolia* as a viable possibility in Vietnam.

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