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OPTIMIZATION OF ESSENTIAL OIL PRODUCTION FROM *CITRUS MICROCARPA (HASSK.) BUNGE* BY CENTRAL COMPOSITE FACE CENTERED (CCF) MODEL

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ABSTRACT

The aim of this study was the investigation on main factors (the grind time, ratio of water/peel and distillation time) affecting the 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.
There is the interaction between ratio of water/peel, distillation time and grind time with volume of essential oil extracted. Optimal result which is essential oil was 1.83 ml (y) with quantity of kumquat peel/batch of 50 g, grind time (x_1) in 118 seconds, ratio of water/peel (x_2) was 6/1, distillation time (x_3) during 32 minutes at 100 ^{o}C. The essential oil from the peel of *C.microcarpa (Hassk.) Bunge* was analysed by GC-MS method. In which, there are a lot of compounds were useful, it can use widely in the food processing and the cosmetics industry, for instance limonene, α-Pinene, β-Pinene, β-Myrcene, Sabinen.

Keywords: CCF, *C.microcarpa (Hassk.) Bunge*, essential oil, GC-MS, RSM

INTRODUCTION

The essential oil of *C.microcarpa (Hassk.) Bunge* contains a large amount of Limonene which is used wide in food, cosmetics and pharmaceutical industry. *C.microcarpa (Hassk.) Bunge* is harvested year-round, the peel can be in the form of dried, fresh or freeze which is a great advantage in mass production and study of citrus oils. However, *C.microcarpa (Hassk.) Bunge* has been known as fruit containing high essential oil source from peel and leaf but they were not interested in essential oil production **(Hieu *et al.*, 2009)**. In Vietnam, it was used in beverage industry and jam processing. For this reason, essential oil production was potential field in Vietnam.
Optimization of conditions for processing is one of the most critical point in the development of an efficient and economic bioprocess **(Bas *et al.*, 2007)**. Traditional approach for the optimisation of the components production which is time consuming and laborious and often does not yield reliable results because the interactions of the different medium components are neglected. Response surface methodology (RSM) is a very useful tool and a powerful mathematical model for the optimal selection of factors with a collection of statistical techniques, which can provide statistical models aid in understanding the interactions between the process parameters at different levels, and calculating the best level of each factor for the given target. It is widely used in technical process to examine and optimize the operational variables for experiment designing, model developing factor and conditions optimization **(Cheynier *et al.*, 1983; Reddy *et al.*, 2003)**
The objective of this study was therefore to optimise the process parameters in distillation of essential oil. The important parameter that we need to consider during distillation are grind time, ratio of water/peel and distillation time to obtain highest content of essential oil, contributing to the development of the oil production industry in general and citrus essential oil in particular in Vietnam.

MATERIAL AND METHODS

Sample collection

C.microcarpa (Hassk.) Bunge: mass of fruit 101.97 ± 0.21 g, mass of peel 158 g in 1 kg fruits (approximately 15 %, w/w), diameter 2.93 ± 0.06 cm, ratio of water/peel of 6/1, dark green peel and no insects. *C.microcarpa (Hassk.) Bunge*'s source is from Cai Mon, Ben Tre Province, Vietnam.

Experimental equipment

The grind apparatus HR 2068 (Volume of tank: 2000 ml, 500 W, 15 000 rounds/minute - Philips, China).
The distilling apparatus Clevenger (Volume of boiled container: 500 ml - Duran, Germany).
The apparatus HP 5890 GC/5972 MS (Hewlett Packard, USA) uses for GC-MS method. Samples were subjected to the GC column (Rt×5MS- 29 m×250 micron, 0.25 micro film), at flow rate of 1 ml/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 5 min, programmed at 3 °C/min to 200 °C and second programmed at 10 °C/min to 300 °C then held at this temperature for 5 min.

Methods

Peel of *C.Bunge* were separated from fruit, each sample was 50 g of peel which is washed by fresh water and ground at 28±1 ^{o}C. Then the ground peel fills by water and it distilled at 100 °C in the apparatus Clevenger to achieve essential oil which was then condensed by cold water and measured in the small cylinder that determines the volume **(Quoc *et al.*, 2012)**. The chemical composition of the essential oil from *C.microcarpa (Hassk.) Bunge* peel was analyzed by using GC-MS methodology.

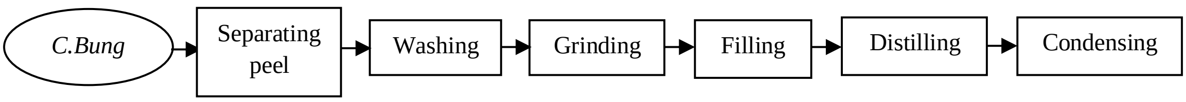

Figure 1 Essential oil processing

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 $\alpha = \pm 1$. 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)**. 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), grind time (90 – 150, second) and distillation time (24-32, minute). The design contains a total of 20 experimental trials with a full factorial design fashion and the replications of the central points.

Experimental design

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

Independent variables	Symbols	Coded level		
		-1	0	1
Grind time (second)	x_1	90	120	150
Ratio of water/peel (w/w)	x_2	5/1	6/1	7/1
Distillation time (minute)	x_3	24	28	32

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 **(Eriksson *et al.*, 2008; 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 Matrix layout experiments and results

Run No	Coded levels			Real values			Volume of essential oil (ml)	
	x_1	x_2	x_3	Grind time	Ratio of water/peel	Distillation time	Observed	Predicted (*)
1	-1	-1	-1	90	5	24	1.4	1.401
2	1	-1	-1	150	5	24	1.37	1.372
3	-1	1	-1	90	7	24	1.47	1.482
4	1	1	-1	150	7	24	1.43	1.433
5	-1	-1	1	90	5	32	1.6	1.591
6	1	-1	1	150	5	32	1.57	1.552
7	-1	1	1	90	7	32	1.63	1.622
8	1	1	1	150	7	32	1.57	1.563
9	-1	0	0	90	6	28	1.63	1.635
10	1	0	0	150	6	28	1.57	1.591
11	0	-1	0	120	5	28	1.63	1.655
12	0	1	0	120	7	28	1.7	1.701
13	0	0	-1	120	6	24	1.73	1.713
14	0	0	1	120	6	32	1.83	1.873
15	0	0	0	120	6	28	1.8	1.791
16	0	0	0	120	6	28	1.8	1.791
17	0	0	0	120	6	28	1.8	1.791
18	0	0	0	120	6	28	1.8	1.791
19	0	0	0	120	6	28	1.8	1.791
20	0	0	0	120	6	28	1.8	1.791

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

Response surface methodology (RSM) was used to determine optimum conditions for distilling essential oil process. The factors including grind time (x_1), ratio of water/peel (x_2) and distillation time (x_3) to target volume of essential oil (y) were determined using optimization method. Influence of factors to target function was described according to equation bellows:

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

(Canh, 2004).

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

y	DF	SS	MS	F	p	SD
Total	20	54.6399	2.732			
Constant	1	54.2192	54.2192			
Total Corrected	19	0.420677	0.022141			0.148798
Regression	9	0.416286	0.046254	105.341	0	0.215068
Residual	10	0.004391	0.000439			0.020955
Lack of Fit	5	0.004391	0.000878	--	--	0.029634
Pure Error	5	2.00E-14	4.00E-15			6.32E-08

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 <0.05. So we can have the regression equation.

Based on the table 4, the grind time, ratio water/peel, distillation time are absolutely independence and do not interact together (p-value > 0.05) and have strong impact to reclaim essential oil. Regression equation presents dependence of content of essential oil above as below:

$y=1.791–0.022x_1+0.023x_2+0.08x_3–0.178x_1^2 – 0.113x_2^2$ (1)

The regression equation depends on x_1, x_2, x_3; in other words, it depends on the grind time, ratio of water/peel, distillation time do not interact together. Equation showed that the regression coefficients of linear term x_1, x_2 and x_3 and quadratic coefficients of x_1, x_2. The variable with the largest effect on volume of essential oil were the linear term x_3 and the quadratic term x_1. R^2 varies between 0 and 1, where 1 indicates a perfect model and 0 no model at all. Regression equation reflects the accuracy of the empirical model, and this is confirmed by R^2 (the goodness of fit), an accuracy level of 0.989, which means that the equation can explain 98.9 % of the actual data. The main disadvantage of R^2 is that it can be made arbitrarily close to 1. Hence, R^2 alone is not a sufficient indicator for probing the validity of a model. A much better indication of the usefulness of a regression model is given by the Q^2 parameter (the goodness of prediction) and estimates the predictive power of the model. Predictability of this model represented by compatibility Q^2 model with 0.931 reliability. In other ways, according to **Chu (2002) and Eriksson *et al.* (2008)**, both R^2 and Q^2 should be high preferably not separated by more than 0.2-0.3 ($R^2–Q^2 \leq$ 0.2-0.3). A substantially larger difference constitutes a warning of an inappropriate model, $Q^2 > 0.5$ should be regarded as good and $Q^2>0.9$ as excellent. Correspondingly, this model is quite accurate and appropriate.

Based on the results, we used software Modde 5.0 to optimizing of parameters in experiment, the volume of essential oil from the experimentation was lower than the volume of essential oil from the predictive model approximately 2.18 % in the same parameters, the deviation was not significant and it could be accepted in model. In other word, there was not the different results between predictive model and experimentation.

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	1.79127	0.0072	2.72E-20	0.0160506
x_1	-0.022	0.00663	0.007746	0.0147644
x_2	0.023	0.00663	0.006011	0.0147644
x_3	0.08	0.00663	2.76E-07	0.0147644
x_1*x_1	-0.17818	0.01264	6.32E-08	0.0281545
x_2*x_2	-0.11318	0.01264	4.32E-06	0.0281545
x_3*x_3	0.00182	0.01264	0.888459	0.0281545
x_1*x_2	-0.005	0.00741	0.515037	0.0165071
x_1*x_3	-0.0025	0.00741	0.742752	0.0165071
x_2*x_3	-0.0125	0.00741	0.122447	0.0165071
N = 20	Q^2 =	0.931	Cond. no. =	4.5044
DF = 10	R^2 =	0.989	Y-miss =	0
	R^2 Adj. =	0.98	RSD =	0.021
			Conf. lev. =	0.95

x_3*x_3, x_1*x_2, x_1*x_3, x_2*x_3 have p-value > 0.05 so they should be excluded from the equation.

Table 5 Results of optimal conditions

Result	Grind time x_{1opt}	Water/ peel x_{2opt}	Distillation time x_{3opt}	Volume of essential oil y_{max}
Predictive model	117.881	6.0629	31.9999	1.8742
Experimentation	117.881	6.0629	31.9999	1.83

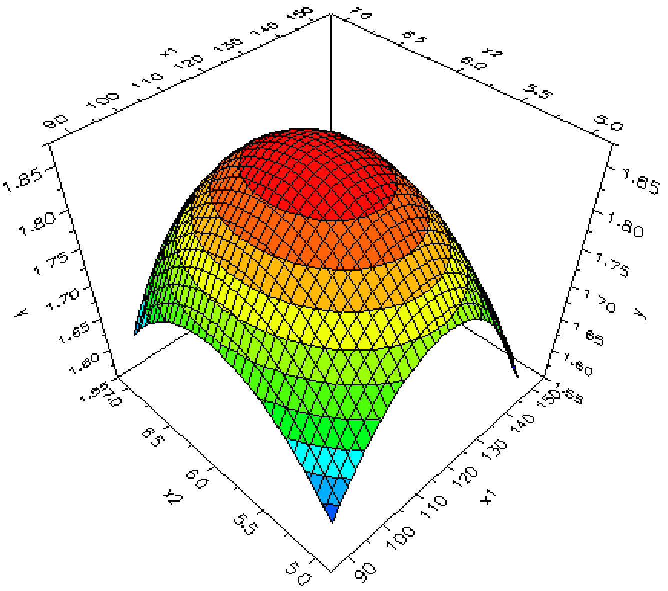

Figure 2 Response surface plot x_3=32 minutes

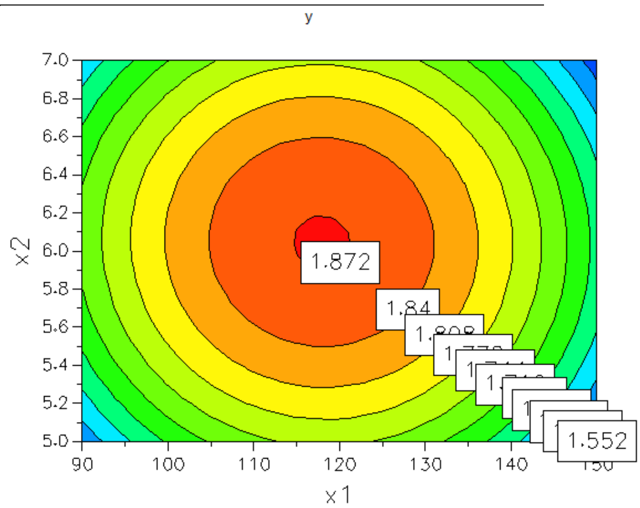

Figure 3 Prediction plot with x_3=32 minutes

The figure 2 and figure 3 show the volume of essential oil increases when the ratio of water/peel and grind time increasing. And predicted y_{max}=1.87, with x_1=(115–122), x_2=(5.9–6.5). Based on the data received from table 2, we get the graph shows the correlation between the amount of essential oil obtained from the experimental and model predicted.

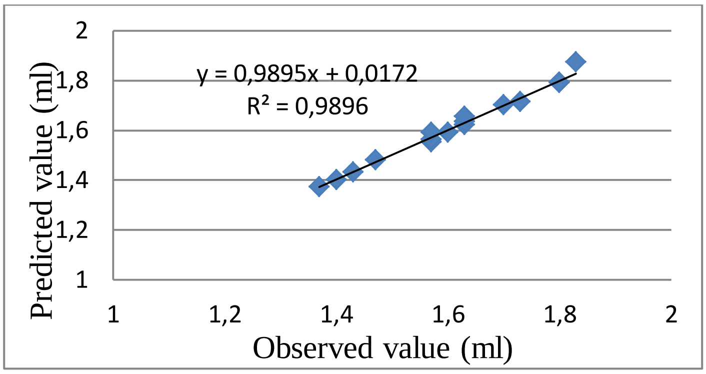

Figure 4 Parity plot showing the distribution of experimental versus predicted values by the mathematical model of the y values.

The matching quality of the data achieved by the model proposed in equation (1) was evaluated considering the correlation coefficient (R^2), between the experimental and modeled data. With a correlation coefficient of R^2=0.989 (Figure 4), it means that the equation can explain 98.9 % of the actual data and this model could not explain only 1.1 % of the overall effects. According to Dung (2010), 0.9<|R|<1, the results between predicted value and observed value had closely linear correlation. Therefor, it is a strong statistical model.

Table 6 Composition of the essential oil from peel of *C.microcarpa (Hassk.) Bunge*

No	Compound	Area %
1	α-Pinene	1.12
2	Sabinene	0.34
3	β-Pinene	1.24
4	β-Myrcene	4.41
5	Limonene	90.87
6	Linalool	0.9
7	Germacrene D	1.12

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 (90.87%). The rate is quite high compared to normal levels of limonene in the essential oil obtained in other documents, for examples limonene of kumquat peel was 14 % **(Yulliasri *et al.*, 2000)** and 88 % **(Thach, 2003)**. This demonstrates that the essential oil distillation process achieve high performance and pure oil.

CONCLUSION

Response surface methodology was a powerful tool for optimisation of parameters. From studies quoted above, the optimum parameters in essential oil production were the grind time, ratio of water/peel and distillation time which had strong influence on the volume of essential oil from *C.microcarpa (Hassk.) Bunge* peel. Using the optimal method of target function, we had the regression equation that can be applied on actual model:

$$y= 1.791 – 0.022x_1 + 0.023x_2 + 0.08x_3 – 0.178x_1^2 – 0.113x_2^2$$

With quantity of kumquat peel/batch of 50 g, grind time (118 seconds), ratio of water/peel (6/1) and distillation time (32 minutes), optimal content of essential oil was 1.8 ml/50 g sample (equivalent 3.6 %, v/w). Compositions of essential oil consisted of limonene (90.87 %), β-myrcene (4.41 %) and small amount of α-Pinene, β-Pinene, Sabinene.

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