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Application of Box–Behnken design with Response Surface Methodology for Modeling and Optimizing Microwave-assisted Hydro-distillation of Essential Oil from *Citrus reticulata* Blanco Peel

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Abstract.
In this research, microwave-assisted hydro-distillation and Response Surface Methodology were selected for optimizing factors affecting the yield of essential oil from *Citrus reticulate* Blanco peels. This combined process was successfully modeled and optimized using a Box–Behnken design with response surface methodology (RSM). The effects of the material and water ratios, extraction time and microwave power on the extraction essential oils, efficiency of *Citrus reticulata* Blanco Peel were investigated. The optimal conditions for the *Citrus reticulate* Blanco peels oils yield were found to be a material and water ratios were 3.1:1mL/g, extraction time was 91.24 min, microwave power at 478 (W). Furthermore, analysis of variance indicated that the proposed quadratic model successfully interpreted the experimental data with coefficients of determination of the predicted R^2 of 0.9909 concurred with the adjusted R^2 of 0.9826. A confirmation test of the optimal conditions verified the validity of the model, yielding an essential oil efficiency of 5.5

Keywords: *Citrus reticulate* Blanco peels, Essential Oil, Microwave-assisted hydro-distillation, Response Surface Methodology

1. Introduction

Citrus reticulata Blanco (Rutaceae) is a well-known crop with high economic and culinary value. The fruit is edible and the peel could be extracted for essential oils which play an important role in many industries, from food chemistry to pharmacology and pharmaceutics. Citrus essential oils are composed of different compounds with diverse biological activities including antioxidant, anti-inflammatory, anxiolytic, antimicrobial, and antifungal [1-3]. Such benefits have made *Citrus reticulate* Blanco peels oil increasingly popular and therefore development of new methods for oil extraction, such as supercritical CO_2 and microwave extraction, is necessary to replace traditional methods [4-6].

To obtain high quality compounds, the extraction technique of choice is crucial. The extraction process could be affected by various experimental conditions including temperature, time, and amount of solvent. To investigate the effect of such parameters and optimize the process, response surface methodology (RSM) with Box-Behnken design (BBD) was employed. RSM is a statistical technique that allows for evaluation of the effects of several process variables and their interactions on selected responses [7-14]. This approach is often used to generate second degree polynomial model, which is consequently used in optimizing an experiment process. The method is also advantageous since only a small number of experimental runs is required, minimizing time

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and costs of experiment. Considering the growing interest on the production of these essential oils, the goal of the present study was to extract essential oil compounds from *Citrus reticulate* Blanco peels using the optimized microwave-assisted hydro-distillation (MAHD). In the present study, parameters (material and water ratios, extraction time, microwave power) are optimized for maximum yield of *Citrus reticulate* Blanco peels oils.

2. Materials and Methods

2.1. Material and chemicals

Fresh ripe fruits were purchased from local market in Ben Tre Province, Viet Nam. All samples were collected from the same source and stored in a dry place. The fruits were cleaned, peeled and ground with a home grinder and distilled directly by steam. Anhydrous sodium sulfate (Na_2SO_4) was purchased from Sigma Aldrich (US). Deionized water was used as a solvent to extract *Citrus reticulate Blanco* peels oil by Milli-Q purification system (Millipore, USA).

2.2. Microwave-Assisted Hydro-distillation

A Clevenger type apparatus was connected to a domestic microwave oven MW71E (manufactured by SAMSUNG, Vietnam) for microwave assisted hydro-distillation operation. The power source has the maximum output power of 800W and voltage of 250v-50Hz. In this operation, parameters including materials and water ratio, microwave power and extraction time were determined by Design Expert 11 software.

2.3. Experimental Design

The central composite design was adopted to determine the optimal parameters of MAHD. ANOVA analysis, calculation of coefficients and plotting was carried out with Design Expert 11 software. Predicted yields and actual yields were also compared to evaluate the fitness of the model to the data.

3. Results and Discussion

The experiments were designed according to the design method of complex CCD center. Experimental results (20 experiments) and predictions by Design-Expert 11 are shown in Table 1.

Table 1. Experimental and predicted extraction efficiencies under different conditions for the RSM model

	Experimental Parameters			Y (%)			Experimental Parameters			Y (%)	
	A	B	C	Actual	Predicted		A	B	C	Actual	Predicted
1	2.0	60	300	2.8	2.89	11	3	39.60	450	2.8	2.87
2	4.0	60	300	4.8	4.63	12	3	140.5	450	3.4	3.27
3	2.0	120	300	4.6	4.52	13	3	90.00	198	4.4	4.41
4	4.0	120	300	2.2	2.37	14	3	90.00	702	4.8	4.73
5	2.0	60	600	2.2	2.07	15	3	90.00	450	5.6	5.54
6	4.0	60	600	4.6	4.72	16	3	90.00	450	5.6	5.54
7	2.0	120	600	4.6	4.81	17	3	90.00	450	5.4	5.54
8	4.0	120	600	3.6	3.56	18	3	90.00	450	5.4	5.54
9	1.3	90	450	3.6	3.56	19	3	90.00	450	5.6	5.54
10	4.7	90	450	4.0	3.97	20	3	90.00	450	5.6	5.54

The results were analyzed using Analysis of Variance (ANOVA). Table 1 presents the experimental parameters produced by CCD design and associated yields corresponding to four maximum points, four minimum points, six axial points, and six center points. RSM software showing no significant difference when comparing actual and predicted values. To determine the factors effects in the model are statistically significant, the p-values less than 5% indicate model terms are significant, whereas the lack of fit (LOF) was not significant at the 5% α-level

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only for the quadratic model described by the following as in equation: Y= 5.54 + 0.1225A + 0.1178B + 0.0932C – 0.9750AB + 0.2250BC + 0.2750 BC – 0.6245 A^2 – 0.8720 B^2 – 0.3416C^2

Table 2. Analysis of variance results for ANOVA model

Source	Sum of Squares	dF	Mean Square	F-value	p-value		Comment
Model	24.92	9	2.77	120.40	< 0.0001	Significant	SD = 0.1517
A	0.2049	1	0.2049	8.91	0.0137		Mean =4.28
B	0.1896	1	0.1896	8.24	0.0166		CV (%) =3.54
C	0.1196	1	0.1186	5.16	0.0465		R^2= 0.9909
AB	0.1186	1	7.61	330.65	<0.0001		AP =32.2665
AC	7.61	1	0.4050	17.51	0.0018		Adj R^2 = 0.9826
BC	0.4050	1	0.6050	26.30	0.0004		Pred R^2= 0.9358
A^2	5.62	1	5.62	244.35	<0.0001		
B^2	10.96	1	10.96	476.40	< 0.0001		
C^2	1.68	1	1.68	73.13	< 0.0001		
Residual	0.2300	10	0.0230				
Lack of Fit	0.1767	5	0.0353	3.31	0.1073	not significant	
Pure Error	0.0533	5	0.0107				
Cor Total	25.15	19					

The results in Table 2 displayed the Analysis of Variance results for the quadratic model of essential oils yield. Three independent factors in the ANOVA table include: water and material ratio (A), extraction time (B), microwave energy level (C), interaction terms (AB, AC, BC) and second-order (A^2, B^2, C^2) terms. The model is significant as demonstrated by significant model F-value of 120.40. There is only a 0.01% chance that an F-value of this large could occur due to noise. Moreover, low p-value of all variables indicated that the response factor A, B, C, AB, AC, BC, A^2, B^2, C^2 are recognized as significant model terms at 95% confidence level. The Lack of Fit F-value of 3.31 implies that the LOF was not significant relative to the pure error. There is 10.73% chance that a LOF this large could occur due to noise. The predicted R^2 of 0.9909 concurred with the adjusted R^2 of 0.9826. A ratio greater than 4 is desirable. Ratio of 32.2665 indicated an adequate signal. Therefore, this model could be used to navigate the design space.

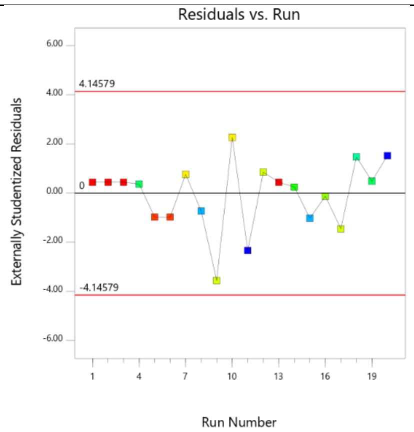


Figure 1. Normal plot of Residual vs Predicted

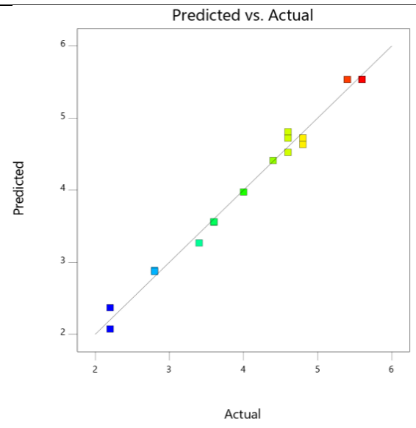
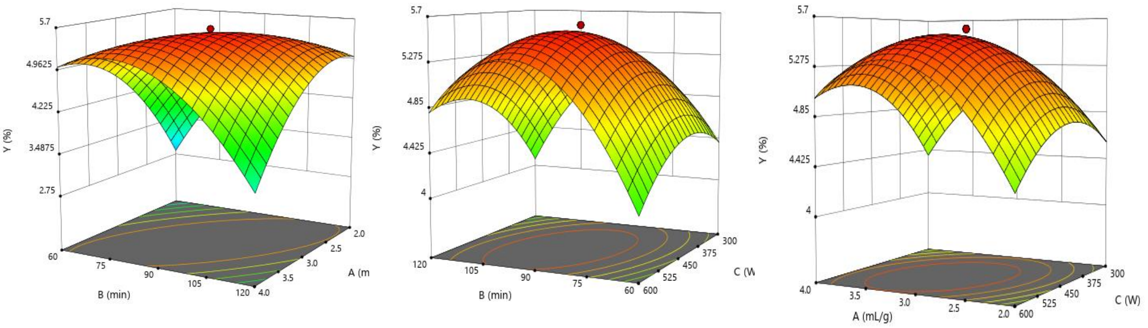


Figure 2. Comparison of predicted and experimental

reponse for yield of essential oil	extraction efficiencies

To validate the resulting model, residuals of 20 runs and yields of oil were plotted in the figure 1(a) and 1(b), respectively. Figure 1 indicated that residuals of experimental yields clearly follow a random pattern. This suggested that there was no violation of assumptions regarding independence of variables and constant variance. Figure 2, which plotted predicted versus against actual values, also indicated a close proximity of scattered data points to the 45 degree line suggesting the reasonable predicting accuracy of the model. The interaction between the essential oil yield and three process factors are shown in Figure 3. Based on the graphs, it could be observed that general trends of the three factors are similar, when the quantities of microwave power, extraction time, material and water ratio gradually increased, the yield of the *Citrus reticulate Blanco* peels oil obtained is also increased. However, this only holds to a certain point where the yield stops rising and eventually, starts diminishing. Optimization of the estimated statistical model yielded following optimal conditions: material and water ratios 3.1:1, extraction time 91.24 min, microwave power 478W.
The data from Table 3 shows the optimum conditions resulted from optimization. Accordingly, material and water ratios of 3.1, the time of 91.24 minutes and 482.06 W operating power yielded highest efficiency of 5.55%. This number approximates to the actual yield, conucted with almost identical conditions, of 5.5%



(a) (b) (c)

Figure 3. 3D response surface plots of interaction of Y with **(a)** B-extraction time and A-material and water ratio, **(b)** B-extraction time and C-microwave power, **(c)** A-material and water ratio and C-microwave power

Table 3. The experimental results using optimum condition comparison with predicted results

	Material and water Ratios (g/mL)	Extraction time (min)	Microwave power (W)	Yield of essential oil (%)
Predicted	3.1:1	91.2365	478.01	5.55228
Actual	3.1:1	91.24	478	5.5%

4. Conclusions

In this study, A Box–Behnken design with Response Surface Methodology was successfully applied to the extraction essential oil from *Citrus reticulata* Blanco Peel using the microwave-assisted hydro-distillation technology. Under the optimization performed by RSM, we found that the optimal level of three influencing conditions for extraction of the *Citrus reticulate* Blanco peels oil using microwave oven heat are as follows: the ratio of water to raw materials of 3.1:1 mL/g, the extraction time at 91.24 min, and microwave power of 478W with yield of 5.5523 %. The model fitted the experimental data well, with a coefficient of determination, R^2, of

0.9909 and an Adj-R^2 of 0.9826. In addition, the p-value of this model was less than 0.0001, which indicates that the model is very significant.

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