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Response Surface Methodology-Based Parameter Optimization of Candlenut Seeds (*Aleurites moluccana* Willd) Extraction

Yeni Variyana[a*], Dewi Ermaya[a], Shintawati Shintawati[a], Devy Cendekia[a], Mahfud Mahfud[b]

[a]*Program Studi Teknologi Rekayasa Kimia Industri, Politeknik Negeri Lampung, Bandar Lampung, Indonesia 35144*

[b]*Department of Chemical Engineering, Institut Teknologi Sepuluh Nopember, Surabaya, Indonesia 60111*

[*]Corresponding author: yenivariyana@polinela.ac.id

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ABSTRACT. *Aleurites moluccana* Willd, a known candlenut plant, has the potential to be used for vegetable oil, pharmacological purposes, and biofuel. However, there is a lack of knowledge on the optimal extraction conditions for this extraction. The current study aimed to use response surface methodology (RSM) to optimize the Microwave Hydro-diffusion Gravity (MHG) conditions for extraction yield. A three-factor-three-level Box-Behnken design (BBD) was used to investigate the effects of three independent parameters: material size (A), microwave power (B), and extraction time (C). The experimental data for the candlenut seed extraction were analyzed to obtain quadratic polynomial equations. The effects of various parameters on the yield of extraction yield were then examined and analyzed using plots and contours. The results showing extraction yield significantly influenced all independent parameters were $p < 0.0001$. Further, The study predicted the optimum conditions for extracting candlenut seeds, which included using a material size of 1.378 cm, microwave power of 599.359 W, and extraction time of 66.076 min, resulting in a yield of 5.015%. Based on experimental data conditions, the highest extraction yield was 5.5% of 1 cm, 600 W, and 60 min, respectively, which were in good agreement with the predicted model. The study concluded that the optimization using MHG method could be useful in industrial extraction processes. Further, the statistical methods can optimize the extraction process and reduce the number of experiments required.

1. INTRODUCTION

The candlenut plant (*Aleurites moluccana* Willd) is a plant that belongs to the Euphorbiceae family. Candlenut originally came from Hawaii and then spread to several countries, including Indonesia [1]. In Indonesia, this plant is widely distributed almost throughout the entire region. The growth of candlenut plants in various regions has increased its production from year to year, making candlenuts a leading spice commodity and export product in Indonesia. Generally, candlenuts are exported to Singapore, Hong Kong, the United States, Australia, and Europe. Furthermore, the candlenut seed is the most economically valuable part of the plant, but its use in Indonesia is still limited to seasoning and partially exported in the form of whole or peeled seeds. Its use as a raw material for vegetable oil industries has not developed as much as in Japan, the Philippines, and some other countries.

Candlenut seeds, which contain a high oil content of about 30% to 60%, are composed mainly of this oil [2], [3]. The oil mainly consists of unsaturated fatty acids characterized by a greater proportion of polyunsaturated acids (>89%) and also contains a very small amount of aromatic oils, such as essential oils [4], [5]. This means that candlenut seeds are quite potential as raw material for vegetable oil industries. In addition, the oil contained in candlenut seeds has many benefits, such as being used as a material for making soap, medicines, paints, cosmetics, and biofuels [1], [2]. Nevertheless, the methods that have been experimented with by researchers to extract oil from candlenut seeds are limited to the mechanical press method [6], Soxhlet extraction technology [7], and supercritical carbon dioxide (CO_2) extraction technology [8]. Therefore, the application of technology for extraction using microwaves can be reported based on this research. The advantage of microwave-assisted extraction is the minimization of the use of organic solvents, time efficiency, and environmentally friendly technology [9]–[11]. Additionally, the combination of gravity force and microwave-assisted extraction can increase the yield of candlenut oil extraction as aromatic oil. The Microwave Hydro diffusion and Gravity (MHG) method is a novel extraction method without distillation and evaporation processes, resulting in lower energy consumption [12]–[15]. Furthermore, the extraction method of MHG demonstrated exceptional outcomes across diverse applications that primarily involve extracting active compounds, including antioxidant molecules, essential oils, colorants, pectin, and polyphenols from medicinal and aromatic plants [16]–[18]. However, the process of extracting candlenut oil using microwaves has not been explained in terms of modelling and optimization. The application of the response surface methodology (RSM) is recognized as an effective technique for optimizing processing conditions to obtain desired outcomes [15], [19]–[22]. The process of optimizing accelerated solvent extraction to obtain bioactive compounds from Eucalyptus intertexta employing RSM for assessing the phenolic composition and biological activities to optimal operating conditions [23]. To the best of the authors' knowledge, no studies have applied RSM for optimizing the process of extracting candlenut oil as aromatic oil using MHG.

Response Surface Methodology (RSM) involves optimizing the settings of factorial variables to achieve a desired maximum or minimum response value. Further, ANOVA methods are utilized to model the effects in more detail. Additionally, ANOVA also shows a crucial role in modelling the response [24]. Combining RSM with an experimental design is a strong statistical technique that can help optimize the extraction process and minimize the number of experiments needed. The RSM approach is less time-consuming and less labour-intensive compared to other methods because it requires fewer experimental trials to analyze multiple parameters and their interactions. The most commonly used RSM designs are central composite design (CCD) and Box-Behnken design (BBD). BBD is specifically designed to fit a second-order model, which is the primary focus of most RSM investigations. Moreover, BBD requires only three levels of each factor to fit a second-order regression model, while CCD requires five levels for each factor. Additionally, BBD typically requires fewer experimental runs. As a result, this study aims to utilize RSM to optimize the MHG extraction parameters (material size, microwave power, and extraction time) and determine the optimal conditions for the extraction of candlenut oil using MHG.

2. MATERIALS AND METHODS

2.1 Materials

The main ingredient is used whole round candlenut seeds of the candlenut (*Aleurites moluccana*), which is commonly used as a cooking spice and has had its shell peeled off. Candlenut seeds used for all extraction experiments were bought from a local market in Surabaya, Indonesia. The species obtained from *Aleurites moluccana* (L.) *Willd*. The material treatment for the candlenut seeds was without any pre-treatment.

2.2 Extraction of candlenut oil

This research aims to obtain essential oil products through the extraction process of candlenut seeds using a combination of micro and gravity wave techniques. A total of 100 gr of the sample was extracted without treatment. The extraction method used was microwave hydro-diffusion and gravity (MHG) using an Electrolux microwave model EMM2308X. The operating conditions for this method were microwave power of 300 , 450, and 600 W, and material sizes of 1, 2, and 3 cm with atmospheric pressure (1 atm). The extraction time for the candlenut was 45, 60, and 75 minutes. Solvents were not utilized in the study as the MHG method does not require the use of solvents. The steam produced passed through a condenser and was collected in a separating funnel to separate the oil and water. Subsequently, the oil was stored in a vial bottle at a temperature of 4°C for further analysis. One of the analyses conducted is the calculation of the yield of candlenut oil. The calculation of the extracted oil yield obtained in each experiment was performed using the following formula:

$$yield\ (\%) = (\text{mass of candlenut oil}/\text{mass of candlenut seeds}) \times 100 \quad (1)$$

2.3Response surface methodologyin optimization of candlenut extraction processes

The process of extracting oil from candlenut seeds using the MHG method was modelled and optimized using a BBD approach. This involved using a three-factor-three-level design, resulting in 17 experimental runs with 5 centre point being carried out. The optimization process focused on three independent variables: material size (cm), microwave power (W), and extraction time (min). The chosen ranges for these variables were material size of 1 to 3 cm, microwave power of 300 to 600 W, and extraction times of 45 to 75 min. The factor levels of the independent variables were showed in Table 1. To evaluate the effects of the independent variables on the dependent variable (% oil yield), multiple regression analysis was used. The response surface regression procedure used a second-order polynomial model (as shown in Eq. (2)) to predict the response variable. This helped to analyze the effects of the independent variables on the % oil yield.

$$y = a_0 + a_1x_1 + a_2 x_2 + a_3 x_3 + a_{11}x_1^2 + a_{22} x_2^2 + a_{33}x_2^2 + a_{12}x_{12} + a_{23} x_{23} + a_{33} x_{33} \quad (2)$$

In this equation, y represents the measured response factors, while x_1 and x_2 correspond to the levels of the independent variables. The constant ao represents the predicted response at the centre, and a1, a2, a11, a22, and a12 represent the linear, quadratic, and two-factor interaction coefficients of the model. The experimental design and analysis of variance (ANOVA) to determine the effects of significant interactions in the model ($p < 0.01$) were performed using the statistical software Design-Expert® (version 22.0.3, Stat-Ease, Inc.).

Firstly, regression terms were evaluated by conducting a fit summary. Only the significant terms were retained and incorporated into the regression model. To determine the significance of the regression models and to test for any lack of fit, analysis of variance (ANOVA) was employed using a significant confidence basis ($p < 0.01$). The coefficient of determination (R^2) was utilized to evaluate the level of appropriateness for each model. To visualize the results from the fitted models in the optimization of extraction conditions for candlenut oil, response surfaces, and contour plots were utilized.

Table 1. Specific factors and corresponding levels utilized in the experimental design

Factors	Units	Levels	
		Low	High
Material size	cm	1	3
Microwave power	W	300	600
Extraction time	min	45	75

3. RESULTS AND DISCUSSION

3.1 Analysis fit summary of model

The complete design comprised of a total of 17 experimental points, which are specified in Table 2. Additionally, three (3) replicates were conducted at the central points of the design to determine the sum of squares for pure error. All the experiments were carried out in triplicate at each design point, following a randomized sequence.

Table 2. The matrix and response for the extraction yield from candlenut seeds using the Box-Behnken Design

	Factor 1	Factor 2	Factor 3	Response 1		
Run	A:Material size	B:Microwave power	C:Extraction time	yield	Predicted Value	Residual
	cm	W	min	%	%	%
1	2	450	60	3.17	3.10	0.0680
2	1	450	45	3.08	3.12	-0.0387
3	1	450	75	3.43	3.44	-0.0137
4	2	450	60	3.02	3.10	-0.0820
5	3	300	60	1.29	1.30	-0.0100
6	3	600	60	3.68	3.72	-0.0425
7	2	600	45	4.68	4.65	0.0288
8	1	600	60	5.5	5.49	0.0100
9	2	450	60	3.22	3.10	0.1180
10	3	450	45	2.35	2.34	0.0138
11	2	300	45	1.29	1.29	-0.0037
12	3	450	75	2.35	2.31	0.0387
13	2	300	75	1.54	1.57	-0.0287
14	2	450	60	3.11	3.10	0.0080
15	2	600	75	4.68	4.68	0.0038
16	2	450	60	2.99	3.10	-0.1120

17	1	300	60	1.49	1.45	0.0425

Based on Design-Expert offers various valuable statistical tables that assist to determine the appropriate model for further examination. The chosen model for candlenut extraction is the final selection made. The quadratic source as suggested the full-order model and satisfied the criteria described in Table 3. This recommended model can be regarded as a reliable starting point for fitting the model.

Table 3. Model summary for model fitting

Source	Sequential p-value	Lack of Fit P-value	Adjusted R²	Predicted R²	
Linear	< 0.0001	0.0297	0.9584	0.9352	
2FI	0.0629	0.0570	0.9731	0.9385	
Quadratic	**0.0207**	**0.2455**	**0.9897**	**0.9533**	**Suggested**
Cubic	0.2455		0.9930		**Aliased**

3.2 Statistical analysis of Box Behnken Design

The current investigation employed RSM with BBD to determine the effects of the MHG method in material (A), microwave power (B), and extraction time (C) influences candlenut oil. Multiple regression analysis of the obtained data resulted in second-order polynomial equations (quadratic model) for the corresponding responses, as presented in Eq. (3).

$$yield\ (Y) = +3.10 - 0.4175\ A + 1.56\ B + 0.0750\ C - 0.2825\ AB - 0.0875\ AC - 0.0625\ BC - 0.2398\ A^2 + 0.0052\ B^2 - 0.0597\ C^2 \quad (3)$$

In the equation, Y represents the response variable, which is the percentage extraction yield of candlenut oil. A, B, and C are linear terms of factors, while AB, AC, and BC are interaction terms of factors, and A^2, B^2, and C^2 are quadratic terms of factors.

Table 4 indicates the ANOVA results demonstrated significant linear independent effects (A and B), interaction effect (AB), and quadratic effects (A^2) on the extraction yield. The model was significant at $p < 0.0001$, obtaining a high F-value (171.74). Further, As the experiments were conducted with four (4) replicates of centre points, it is necessary to carry out a test to evaluate the significance of the errors resulting from replication in comparison to those caused by pure error. To ensure that the lack of fit test is reliable, RSM suggests having a minimum of three (3) degrees of freedom for lack of fit and four (4) degrees of freedom for pure error. The lack of fit presents the results for both responses, the Prob > F value is greater than (0.05), indicating that there is no lack of fit at this level of significance. Moreover, the coefficient of determination (R^2) and adjusted coefficient of determination (Adj.R^2) were 0.9955 and 0.9897, respectively. The Adj.R^2 was almost equal to R^2, confirming the high significance.

Table 4. The analysis of variance (ANOVA) results for candlenut oil yield

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	21.41	9	2.38	171.74	< 0.0001	significant
A-Material size	1.39	1	1.39	100.65	< 0.0001	
B-Microwave power	19.34	1	19.34	1396.26	< 0.0001	
C-Extraction time	0.0450	1	0.0450	3.25	0.1145	
AB	0.3192	1	0.3192	23.04	0.0020	
AC	0.0306	1	0.0306	2.21	0.1807	
BC	0.0156	1	0.0156	1.13	0.3235	
A²	0.2420	1	0.2420	17.47	0.0041	
B²	0.0001	1	0.0001	0.0084	0.9296	
C²	0.0150	1	0.0150	1.08	0.3322	
Residual	0.0970	7	0.0139			
Lack of Fit	0.0591	3	0.0197	2.08	0.2455	not significant
Pure Error	0.0379	4	0.0095			

R^2	0.9955
Adjusted R^2	0.9897

3.3 Effects of extraction conditions

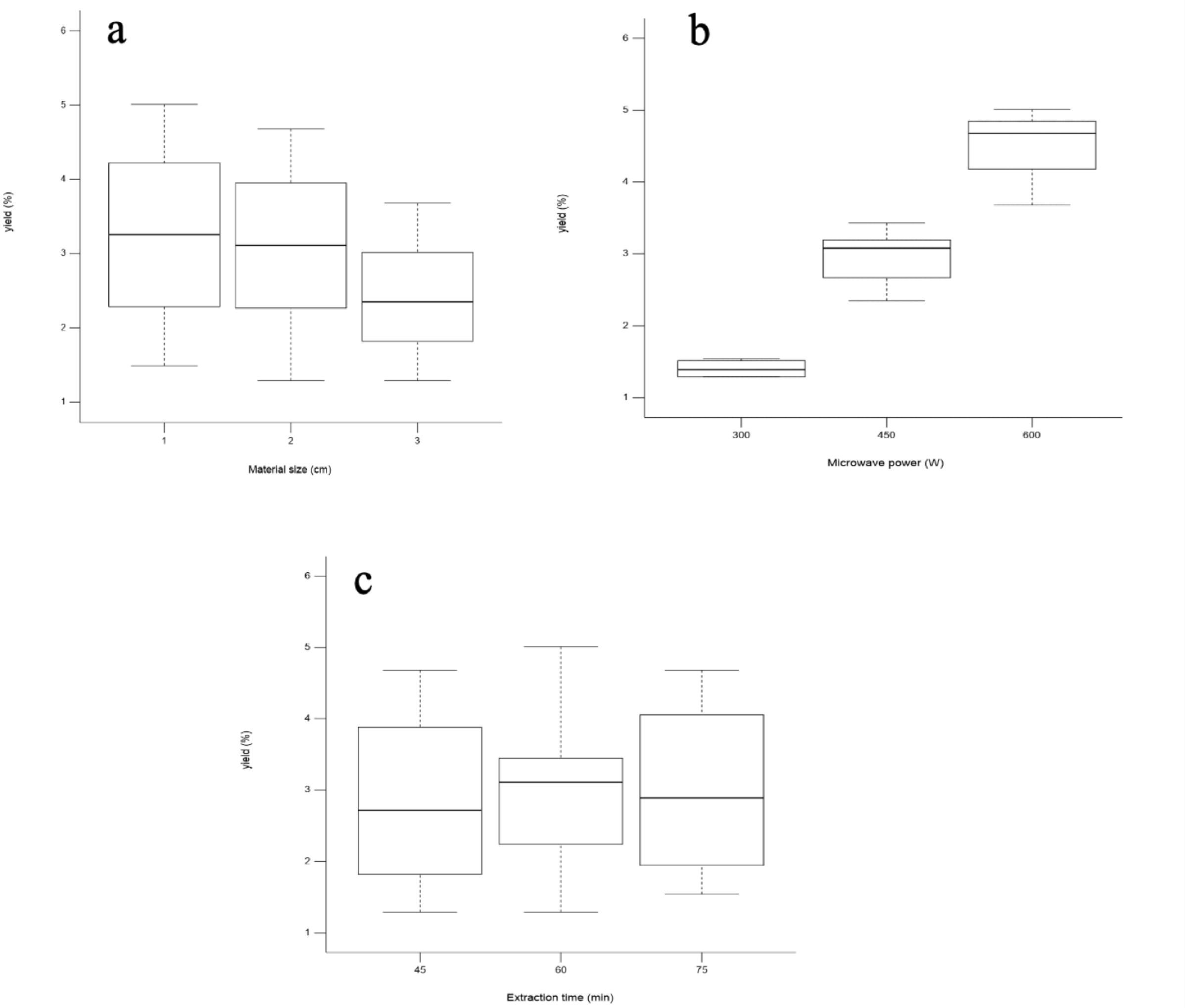

Figure 1. Box plots of experimental data using each factors (a) material size; (b) microwave power; (c) extraction time

The material size of the candlenut seeds needs to be reduced to a small enough size before the extraction process can be carried out. The effect of material size on the extraction yield was investigated of 1, 2, and 3 cm without any pre-treatment, and the results are presented in Fig.1 (a). The results showed that the extraction yield of candlenut oil increased with a decrease in material size. However, no pre-treatment was applied to the seeds before the size reduction. Studies have shown that a smaller particle size can cause severe cell damage and result in a higher ratio of surface area to particles [25]. In the current study, material size of 1 cm was chosen as the optimum independent size because of producing high yield extraction.

The amount of microwave power used in the extraction process is crucial for the efficient extraction of candlenut oil. The appropriate microwave power level can increase the efficiency and shorten the extraction process. Fig. 1 (b) shows the effect of different microwave power levels on extraction. Microwave power between 300 W to 600 W, with a peak at 600 W, resulted the average output the highest yield. The amount of energy provided to the sample is controlled by the intensity of the microwave power, which affects interactions and equilibrium rates and analyte partitioning between sample and solvent. However, increasing the microwave power level beyond a certain point can cause breaking cells, dispersing extracts into the solvent, and alter or degrade the properties of anthocyanin. Therefore, a power level of 600 W was chosen for further research for candlenut seed extraction.

One of the most significant influences on extraction yield of candlenut oil is microwave extraction time showed Fig.1 (c). The extraction time has an important impact on the contact between solvents and solids. The findings from RSM indicate that the amount of extract obtained from candlenut seeds using MHG was affected by extraction times between 45-75 minutes, which was a significant effect. This demonstrates one of the primary benefits of microwave energy, which is the ability to produce a substantial quantity of extract within a short time. Moreover, Numerous studies have indicated that an increase in extraction time can lead to a higher extraction yield [26], [27]. Since extraction is a process of mass transfer, longer irradiation times are expected to increase both the extraction yield and the amount of oil extracted up to the equilibrium point [28]. As for the quantity of the extract, extraction time had an important impact on the yield extraction, resulting extraction time of 75 min to produce the highest yield.

Further, based on Fig. 2 presented a diagnostic plot comparing the actual and predicted responses of the experimental runs. The plot indicates that the actual responses were closely clustered around the trend line representing the predicted responses. These experimental runs were conducted with precision. Furthermore, the optimization responding surface quadratic model was the most suitable model for fitting the data of this research.

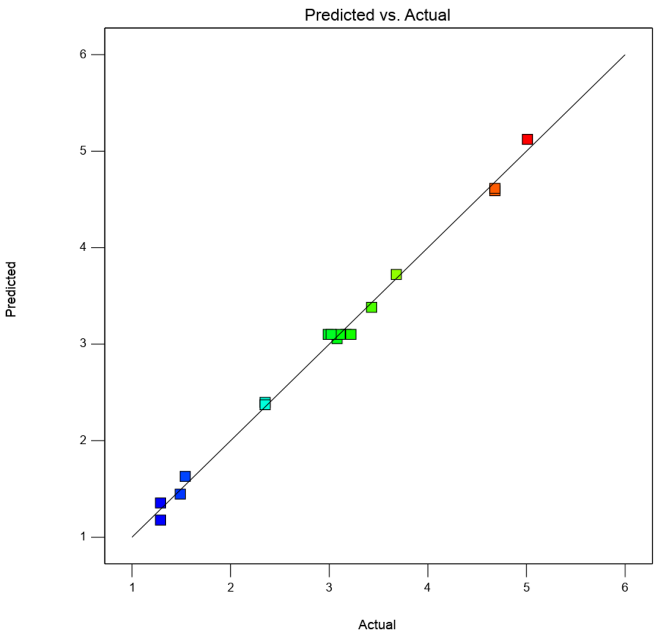

Figure 2. The comparison between the actual and predicted extraction yield (%) for the experimental runs

The results revealed that the extraction yield ranged from 1.29% to 5.5%. Based on Fig. 3 demonstrated the interaction effect between factors, where Fig. 1 (a) showed that the interaction effect between material size (A) and microwave power (B) on the extraction yield was significant at $p < 0.0001$. The highest percentage extraction yield (5.01%) was optimized by the material size of 1 cm, microwave power in 600 W, ad extraction time at 75 min. Besides, the most significant independent parameters affecting the extraction yield were material size (A) and microwave power (B). This finding describes in Fig. 3 contours resulting in interaction between material size and microwave power as a significant model. The response indicates that increasing the microwave power and reducing the particle size of candlenut seeds can improve the extraction yield. Additionally, the results shown in the image regarding the interaction between material size and extraction time (AC) do not demonstrate a significant effect, the same is shown for the interaction between microwave power and extraction time (BC) resulting insignificant responses.

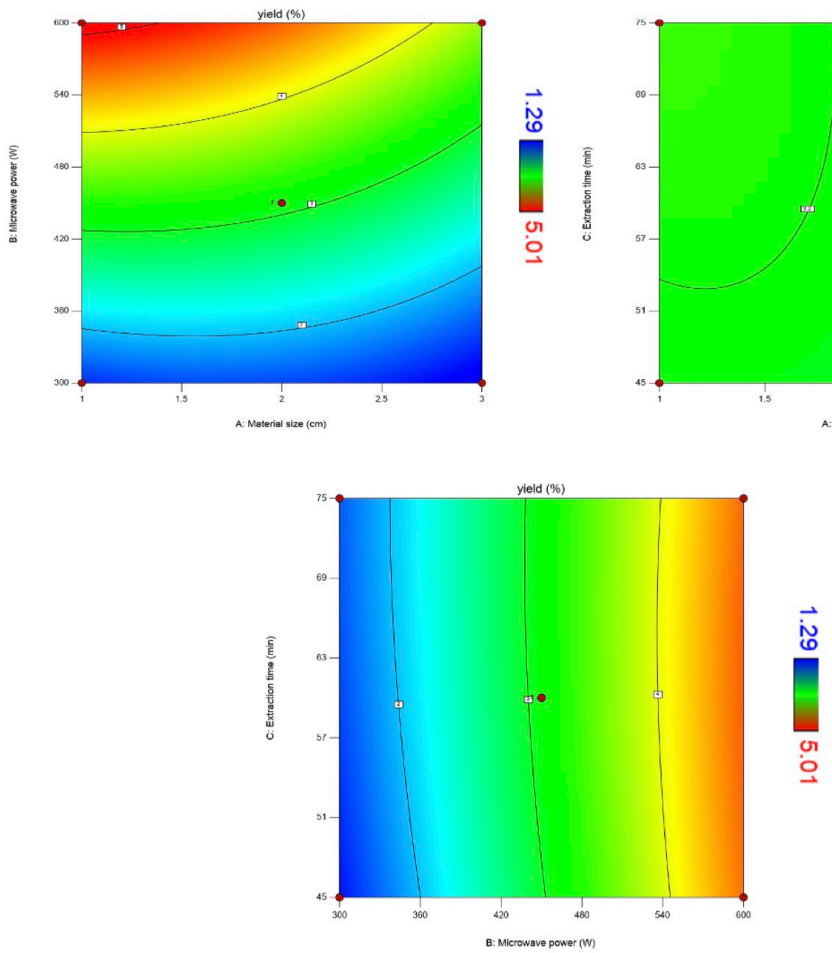

Figure 3. Interaction factors of (a) material size and microwave power (AB); (b) material size and extraction time (AC); (c) microwave power and extraction time (BC) based on contour responses

3.4 Optimum condition and verification

The initial approach involves manually response contour plots to obtain the desired value. According to Granato et al. [29], the graphical method is inefficient and cannot be automated. Thus, an alternate approach was employed to obtain the desirability function and Design Expert Software version 22.0.3 was used to compromise between responses. The optimization process assigned desirability values ranging from 0 to 1 to each minimum and maximum responses, resulting in an overall desirability of 1. The optimum conditions were determined by maximizing the desirability of the responses, and these conditions were used for the extraction process. The results showed that under the optimal conditions of a material size of 1.378 cm, microwave power of 599.359 W, and extraction time of 66.076 min of 5.015%, the experimental values were in agreement with the predicted values for 1 cm, 600 W, and 60 min of 5.5%. The residual standard error (RSE) percentages were used to compare the experimental results with the predicted values, and the obtained RSE values for the extracts of candlenut oil demonstrated no significant result between the experimental and predicted values. These results suggest that the model obtained by BBD can accurately predict the optimal conditions of material size, microwave power, and extraction time in the candlenut oil from candlenut seeds.

4. CONCLUSION

The authors conducted microwave hydro-diffusion gravity (MHG) of candlenut oil from candlenut seeds and carried out a prospective study to optimize the extraction conditions of extraction time. The method aimed to find the optimal extraction conditions for the extraction process and successfully applied RSM to analyze the effects of material size, microwave power, and extraction time. The optimized condition was determined using RSM obtaining 1.378 cm, 599.359 W, and 66.076 min of 5.49%, which was in close agreement with the experimental validation yield of 5.5%. Besides, The polynomial model resulted in the best fit, with an R^2 value of 0.9955. Thus, it is recommended to conduct additional research to fully comprehend the behaviour of the candlenut seed extraction from parameters and enhance the efficiency of extraction for industrial applications.

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