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Evaluation and Optimization for Extraction Parameters of Allium sativum Extract using Microwave Hydrodistillation (MHD)

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ABSTRACT

Allium sativum L extraction using microwave hydrodistillation (MHD) was chosen as one of the techniques for becoming a safer and greener technology. The yield of garlic oil obtained by MHD using water as solvent. The optimization was designed by response surface methodology (RSM) to evaluate and analyze the effect parameters of raw material mass, microwave power, and extraction time. The highest yield was found in 100 g, 450 W, and 90 min of 0.1392% based on experimental data. In addition, RSM using the quadratic model predicted that optimal condition at 88.09 g, 474.94 W, and 99.53 min was 0.1430%, respectively. An ANOVA analysis resulted in a p-value of less than 0.05 with a high determination coefficient (R^2 = 0.9971). It indicates that this model gives a significant response and has good accuracy. Furthermore, the error rates between experimental data and the predicted model were less than 5%. The model obtained from optimization is close to the reability runs and could be explained for correlating the yield of garlic oil with parameter conditions using optimization.

1. Introduction

Garlic (*Allium sativum L*) is used as an ingredient purpose and has other health improvements such as medicines, both traditional and modern uses [1]. It contains chemical and biological compounds, mostly thiosulfinates, allicin, and alliin, volatile oil compounds. Allicin is the main oxygenated sulphur compound, as well it is one of the bioactive substances for inhibiting potential tumor disease [2]. It was initially reported that isolated extract could be anti-bacterial agent [3]. In most cases, garlic has other beneficial effects that have been much reported in pharmacological fields. The other compounds have been confirmed for general medical properties, i.e., anticancer, antidiabetic, anti-inflammatory, antimicrobial, and antioxidant [4,5].

Nowadays, previous research has often focused on the extraction yield of isolated extract. Thus few studies were providing and evaluating parameter conditions. Afterward, garlic oil extraction was designed by response surface methodology using some solvents. This research was obtained by solvent extraction (SE) by Li et al. (2010) that has added solvents among pure ethanol, n-pentane, ligarine, and ethyl acetate [6]. Besides, the other techniques have been reported by two methods which are supercritical CO_2 extraction (SFE) and salting-out extraction (SOE) using ethanol-ammonium sulphate as solvents [7, 8]. They have also applied response surface methodology (RSM) for process optimization. The term SFE requires a long extraction time and high pressure so that the process needs high capital costs. The techniques of SOE using organic solvent and inorganic salt can possibly become product contamination, hazardous effect on the human body, and pollution to the environment. Therefore, to our knowledge, the investigation about the influence of parameter conditions using microwave hydrodistillation is poorly analyzed, and then performed design parameters is necessary to evaluate.

Similarly, another objective of this study is to understand the effects of extraction parameters using the polynomial quadratic model. Not only based on the polynomial model, but RSM is also designed to obtain validation and be significant responses expected. RSM design looks for a region with important parameters leading to the best response. Besides, process optimization can assist in determining the correlation of experimental runs for producing a maximum yield region. Turning to microwave hydrodistillation is a green technique for obtaining garlic oil without harmful solvent. In that case, it could be suggested for future applications.

2. Research Methodology

2.1. Materials Chemical

Garlic (*Allium sativum* L.) was purchased from Surabaya. *Allium sativum* L in fresh condition then it was finely chopped into 1 cm. After the extraction process, n-hexane ≥ 99.0% from Merck (Darmstadt, Germany) separated garlic essential oil and water.

2.2. Microwave Hydrodistillation (MHD) for Extracting Garlic Oil

This research aimed to conduct the highest yield so that it was used microwave technology that has usually obtained volatile oils. For the extraction process, a modified microwave laboratory (Electrolux model EMM-2007X) was equipped with the Clevenger-type apparatus as described in previous literature [9]. Microwave tool was designed as Electrolux model EMM-2007X with the following specifications: Maximum Power in 800 W; Voltage on 220 V; Irradiation Power in 1250 W; Magnetron Frequency at 2450 MHz (2.45 GHz). A Clevenger apparatus with a cooling system was set on top and used to obtain the extracted outside. To collect the pure oil, n-hexane was added to the garlic oil. Thus, the solution was evaporated over to remove excess n-hexane become pure oil. It was weighed and kept in a vial at low temperature until used. The extraction operated at atmospheric pressure with added 100 ml water. The result of garlic oil yield was determined by the formula given:

$$Y = \frac{V}{m} \times 100 \quad (1)$$

where V and m are reperesented for garlic oil volume (mL) and weight of fresh *Allium sativum* L (g), respectively.

2.3. Experimental Design of Garlic Oil Extraction Using Response Surface Methodology

Table 1. Box-Behnken design (BBD) coded parameters

Coded	Independent parameters		
	Mass of raw material (g)	***Microwave power (W)***	***Extraction time (min)***
-1	50	300	60
0	100	450	90
1	150	600	120

Table 1. are presented coded parameters and levels using box-behnken design (BBD), and BBD is usually applied to three independent parameters [10–12]. In these experiments, parameters were set the mass of raw material (g), microwave power (W), and extraction time (min) denoted as A, B, C, respectively. The design consists of 17 experiments that have been shown in Table 2. This design was the same way for extraction field optimization [13]. The experimental data were subjected to regression analysis and the results were fitted into a second-order model. The employed second-order equation is commonly used for optimization studies, as shown in the equation below:

$$Y = \beta_0 + \sum_{i=1}^{3} \beta_i X_i + \sum_{i=1}^{3} \beta_{ii} X_i^2 + \sum_{i=1}^{2} \sum_{j=i+1}^{3} \beta_{ij} X_i X_j \quad (2)$$

Where are regression coefficients in the intercept, regression coefficients in the linear and quadratic, β_{ij} are regression coefficients in the interaction terms; Xi and Xj are the independent parameters, and Y is the predicted model response. The Design Expert 11 software package (trial version; State-Ease Inc., Minneapolis, USA) was designed for extraction.

Table 2. Arrangement and responses from Box-Behnken design

Coded Parameters			Yield (%)	
A (g)	*B (W)*	*C (min)*	*Observed*	*Predicted*
-1	-1	0	0.0622	0.0623
0	0	0	0.1359	0.1356
0	-1	1	0.1121	0.1123
0	1	-1	0.0641	0.0639
-1	1	0	0.0824	0.0810
1	-1	0	0.0696	0.0710
-1	0	-1	0.0551	0.0567
0	0	0	0.1392	0.1356
1	1	0	0.0943	0.0942
0	0	0	0.1371	0.1356
1	0	-1	0.0602	0.0605
0	0	0	0.1354	0.1356
0	-1	-1	0.0521	0.0504
0	1	1	0.1391	0.1408
-1	0	1	0.1192	0.1189
1	0	1	0.1385	0.1369
0	0	0	0.1303	0.1356

3. Results and Discussion

Design-Expert tests are mainly to confirm the significance for each result of p-value, F-test, and lack of fit in accordance with statistical standards. The model summary revealed that the quadratic model was confirmed as statistically significant and polynomial regression. Besides, the lack of fit of the suggested model might be regarded as a reasonable agreement. The evidence indicates that it needs further investigation using the response surface model [14]. The adjusted R^2 and predicted R^2 correlating the accuracy of the polynomial model were determined by them should be one or close to be. The determination and adjusted coefficients, R^2 and Adj-R2, respectively, are also important to evaluate the model adequacy. Moreover, this model is sufficient and acceptable by the model response of $p< 0.05$; lack of fit $p> 0.05$ so that variation of experimental data is around the fitted model well [14, 15]. Therefore, the summary statistics suggest from Table 3 to be chosen from the quadratic model as a polynomial equation.

Table 3. Model summary suggested for garlic extraction yield

Source	Sequential p-value	Lack of fit p-value	Adjusted R^2	Predicted R^2	
Linear	0.0143	0.0003	0.4389	0.3302	
2FI	0.9879	0.0001	0.2796	-0.1302	
Quadratic	**< 0.0001**	**0.7349**	**0.9933**	**0.9848**	**Suggested**
Cubic	07349		0.9912		Aliased

3.1. Model Fitting Parameters

The experimental runs could be analyzed from the fitting parameter to fit the statistical result. Constant terms, linear coefficients for independent parameters, interaction term coefficients, and coefficients of quadratic terms are used to express model coefficients. The ANOVA with the overall model is significant, as shown in Table 4. Independent parameters of A, B, and C have a p-value less than 0.05 ($p< 0.05$). Moreover, the quadratic model also results in each parameter interaction. One of them has an extraction cycle, and microwave power (AB) interactions resulted in significant ($p> 0.05$). It means that the parameters are slightly affected in the extraction process. In contrast, the mass of raw material and extraction time (AC) interactions have a significant response ($p< 0.05$) that both affect the extraction process. Not only optimization for AC, microwave power and mass of raw material (BC) also reported positively influenced on the extraction yield ($p< 0.05$). Therefore, the parameter interaction effects for this study could be accepted to guide the design optimization between experimental data and the predictive model.

Table 4. Analysis of variance (ANOVA) for the quadratic model of garlic oil extraction

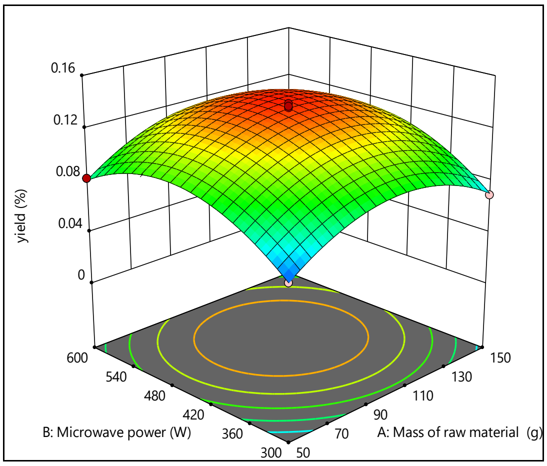
Sources	Sum of Squares	Degree of Freedom	Mean Square	F-value	Prob. > F (p-value)	
Model	0.0197	9	0.0022	264.44	< 0.0001	significant
A-mass of raw material	0.0002	1	0.0002	28.87	0.0010	
B-microwave power	0.0009	1	0.0009	106.42	< 0.0001	
C-extraction time	0.0096	1	0.0096	1163.39	< 0.0001	
AB	5.062E-06	1	5.062E-06	0.6123	0.4596	
AC	0.0001	1	0.0001	6.10	0.0429	
BC	0.0001	1	0.0001	6.80	0.0350	
A^2	0.0034	1	0.0034	414.45	< 0.0001	
B^2	0.0038	1	0.0038	456.12	< 0.0001	
C^2	0.0008	1	0.0008	97.02	< 0.0001	
Residual	0.0001	7	8.268E-06			
Lack of Fit	0.0000	3	4.816E-06	0.4436	0.7349	not significant

R^2 = 0.9971
C. V (%) = 2.83

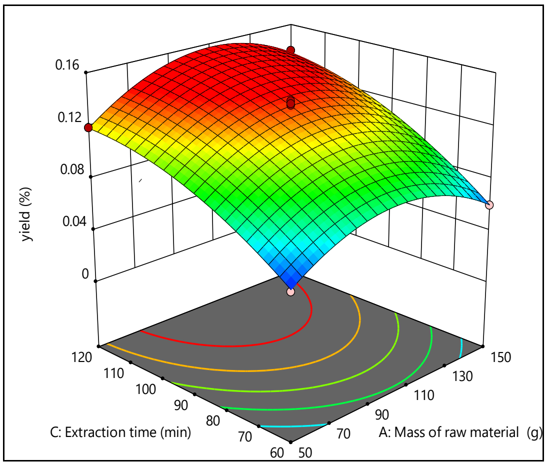
The coefficient of determination (R^2) from ANOVA, as shown in Table 4, is 0.9971. The result is close to 1 in general parameters that are well correlated. F-value of 264.44 implies that the model is giving a significant influence. The coefficient of variation (CV) is less than 5%, indicating the model is an acceptable limit for field experiments [16]. In short, all parameters that RSM completely reported could be adequately represented the feasible relationship among the parameters chosen. Furthermore, the second-order equation for the extraction model is reduced by eliminating non-significant terms as follows:

$$\text{Extraction yield (\%)} = 0.1356 + 0.0055A + 0.0105\,B + 0.0347\,C + 0.0036\,AC + 0.0038\,BC - 0.0286\,A^2 - 0.0299\,B^2 - 0.0138\,C^2 \quad (3)$$

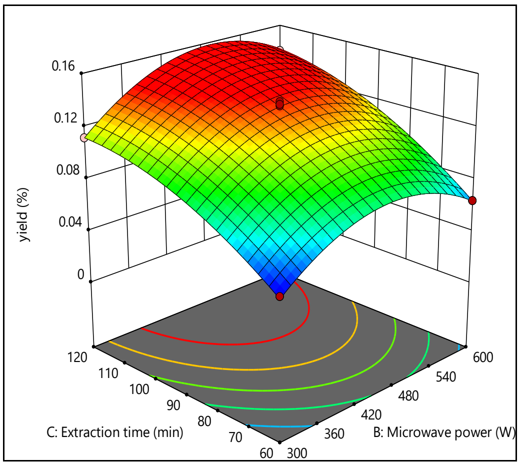
3.2. Evaluation of extraction parameters



(a)



(b)



(c)

Fig. 1.3D response surface of garlic extraction yield (a) AB (b) AC (c) BC

Response surface plotted in Fig. 1(a). Shows insignificant response of raw material mass and microwave power (AB) in contributing to the extraction yield ($p > 0.05$). Nevertheless, the interaction and contour of them could be analyzed to represent the highest yield. According to contour, the highest yield is obtained in the range of parameters at 450 W and 100 g, respectively. In general, the lower levels (300 W and 50 g) would have resulted in a minimum yield. On the other hand, the independent parameter of microwave power showed a greater impact than raw material mass; however, in other cases, at the minimum and maximum levels, oil yield will be decreased. Consequently, it has to be considered to determine a specific parameter to obtain a fitted range [13]. As a constraint of this interaction, increasing microwave power continuously could lead to the degradation of oil compounds [17, 18]. So that to conclude, the effect parameters explain that microwave power is slightly stronger affected than raw material mass.

Similarly, the combined effects of raw material mass and extraction time could respond well in optimal conditions ($p < 0.05$). Fig. 1(b). reported at a constant microwave power of 450 W. As a result, both parameters to produce the highest yield are nearly 100 g and 90 min from optimization design. This response is the maximum point based on the predicted model. According to experimental runs, the best condition in extraction time could be estimated among 90-120 min. The phenomenon usually occurs over 90 min that will accelerate the degradation. For the MHD, the heating transfer will be more stable during process because water is a solvent to resist a significant heat rate. This result was also suggested to be carried out of extraction time at 90 min because plant material is completely immersed in water and boiled perfectly to break down the plant cytoplasm [19]. On the other hand, the contour resulted that extraction time parameter is more sensitive than raw material mass to obtain the extraction yield. Therefore, we suggest focusing on extraction time for further analysis from this contour.

Fig. 1(c). shows the effects between microwave power and extraction time (BC) in garlic oil yield. The response can be concluded that extraction yield was influenced by both parameters. Furthermore, the highest yield based on experimental data is 450 W and 90 min. It confirmed that the response model was also equal to the expected data runs. Based on ANOVA, these interactions were significant responses ($p < 0.05$). Likewise, increasing microwave power was the most effective way of increasing oil yield [18, 20]. Setting microwave power has to be considered because it can affect the quality of the product. In addition, extraction time generally has a positive linear to increase yield. However, the extraction time effect can reduce yield, so it has to concern the definite parameter [13]. Furthermore, the contour showed that parameters of extraction time and microwave power give linear results on the extraction yield as a significant response.

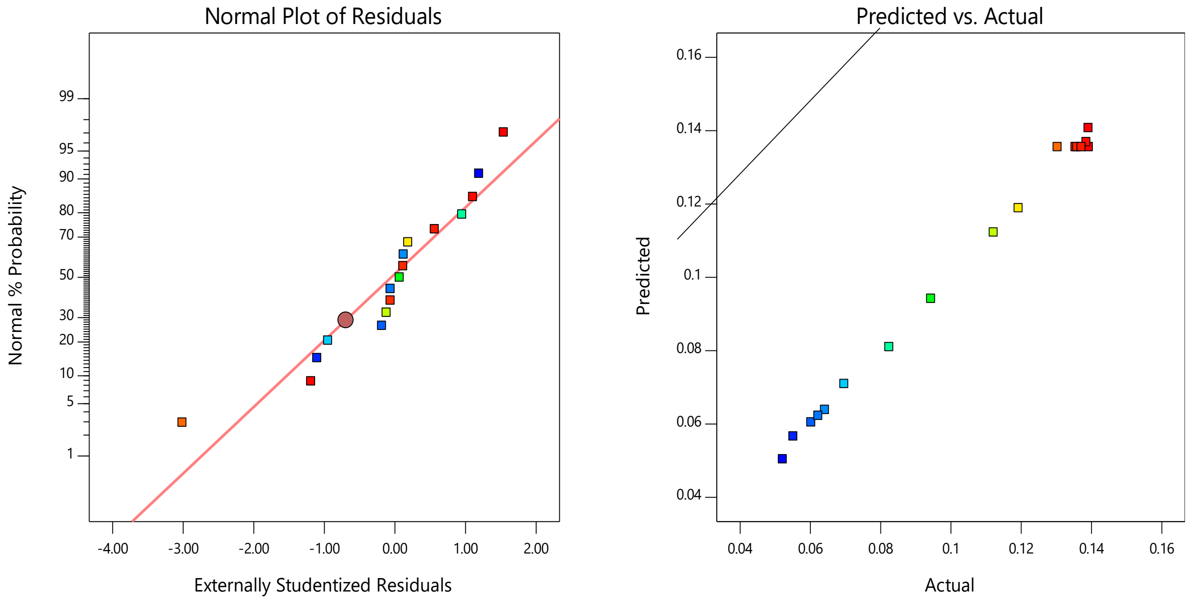


Fig. 2.(a) Normal percentage probability and studentized residuals and plot for extraction yield of garlic oil (The points denoted as square correspond to actual data) (b) Comparison between experimental data and predicted model.

The studentized residual plot's equal variance and normality assumptions are shown in Fig. 2 (a). The normal probability has a function to check the standard deviation of the extraction process that plotted the points are close to the straight line, and then the graph shows the same regressions. It means that equal variances from residuals are reasonable and sufficient evidence. Afterward, the experimental data and the predicted model from Fig. 2 (b) have a well-fitted result. A strengthened quadratic model was suggested for optimizing *Allium sativum* L oil yield. Besides, the graph results clearly show the points between experimental data and the predicted model, which are almost precise.

3.3. Verification and validation for optimization of the garlic extraction yield

RSM design establishes estimated interaction parameter effects to obtain the best extraction yield. According to Raissi and Farsani (2009), the desirability result is one as desired target (D= 1), indicating response in the ideal interval as an ideal approximation [21]. The optimal conditions for garlic oil based on the quadratic model are mass of raw material (88.09 g); microwave power (474.94 W); extraction time (99.53 min), selected by minimizing the std error of yield. At the same time, the highest yield of the experiment in 100 g, 450 W, and 90 min is 0.1392%, which is quite near to the predicted optimum model of 0.1430%. As a result, the error rates between both data, which are less than 5% (desirability= 1), indicating parameters of raw material mass, microwave power, and extraction time, could be accurately predicted optimum conditions using response surface methodology. Similarly, the latest research has been carried out using solvent-free microwave gravity extraction to extract Allium sativum, which reveals that their parameters will affect the extraction yield [22].

4. Conclusion

Another technique can be applied for extracting garlic oil using microwave hydrodistillation (MHD)**.** Besides, the optimization was studied by parameter conditions affecting the yield of garlic more clearly. The quadratic model (second-order) was evaluated to obtain the best fit and find a maximum response region. Based on the experiment, the highest extraction yield is 100 g, 450 W, and 90 min, which is close to the predicted model. The predicted optimal conditions in 88.09 g, 474.94 W, and 99.53 min resulted from response surface methodology. Furthermore, these studies could be expected an improved green technique and be maximized yield of extraction. They would be useful for the potential application involved in garlic oil extraction, which has a better process for an industrial scale.

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