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Optimization of peppermint (*Mentha piperita L.)* extraction using solvent-free microwave green technology

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ABSTRACT

Peppermint varieties indigenous to Europe and the Middle East are now widely cultivated in various regions, such as Indonesia. Peppermint is the main species of natural raw material in producing peppermint essential oil for its applications in the pharmaceutical, perfumery, and food flavour industries. The main challenge of extracting essential oil lies in optimizing different efficient and sustainable techniques for that proposed conventional extraction, which is usually based on high temperatures for a long time. Alternative green extraction techniques have emerged, such as those based on microwave-assisted extraction. In this study, solvent-free microwave extraction (SFME) has been applied to extract essential oil from *Mentha piperita Linn*, and response surface methodology (RSM) was selected to evaluate extraction conditions. The optimized variable parameter was microwave power (150-450 Watt), feed-to-distiller volume (F/D) ratio (0.08-0.12 g/mL), and particle size (0.5-1.5 cm). The optimum yield obtained was 0.6937% under operational conditions with 450 Watts of microwave power, 0.1 g/mL of F/D ratio, 1cm of particle size, and an extraction time of 60 minutes.

Introduction

Essential oils (EOs) are liquid mixtures of volatile and non-volatile compounds that can be obtained through various extraction techniques and is mainly found in aromatic plants. EOs produced from different parts of plants have a characteristic odour, chemical composition, and physicochemical property that become the main characteristic of each plant's essential oil (Guichard et al., 2016). Odour substances (essential oils) are generally formed in the secretory cytoplasm of particular plant cells located in plant organs (Dhifi et al., 2016). Nowadays, there is increasing attention towards implementing naturally available EOs to be extracted and isolated from aromatic plants using effective and selective methods to increase the quality of the EOs.

Among various EOs, the essential oil derived from peppermint (*Mentha piperita Linn*) is widely known for its use mainly as an additive in the production of pharmaceutical, medicinal, perfume, and food industries (Radwan et al., 2020). Peppermint is a genus of the *Lamiaceae* family, is widely distributed throughout the world's temperate areas, and has been bred into various varieties. In peppermint oil, chemical components can be categorized into several main groups: oxygenated monoterpene, hydrocarbon monoterpene, hydrocarbon sesquiterpene, and oxygenated sesquiterpene (Beigi, Torki-Harchegani and Ghasemi Pirbalouti, 2018). In addition, several biological activities, including antimicrobial (Badea et al., 2019) and antioxidant (Farzaei et al., 2015), are represented in this essential oil or some of its constituents. It is reported to be composed primarily of menthol, menthone, and several minor constituents, including pulegone, furanone, and methyl acetate (Tsai et al., 2013; Beigi, Torki-Harchegani and Ghasemi Pirbalouti, 2018).

The EOs are separated from the aqueous phase by physical technique (Mejri et al., 2018). Essential oil products are obtained from plant materials using several techniques, such as traditional methods. Previous studies regarding the extraction of peppermint leaves *(Mentha piperita Linn)* using steam distillation indicate that this method has several disadvantages, such as consuming much time and energy, high solvents, and low extraction efficiency rates (Liu et al., 2018). In addition, the large volume of solvent and high temperature used

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in conventional extraction methods also may alter the essential oil's physical and chemical quality. Therefore, in the last few years, the research has encouraged more intensification, optimization, and eco-friendly techniques of existing conventional and renewable. Recently several advanced techniques, such as microwave and ohmic heating-assisted extraction (MAE and OHAE, respectively), have been reported as advanced, greener techniques for the extraction of EOs (Gholamipourfard, Salehi, and Banchio, 2021).

The microwave-assisted extraction, a novel green by Kaufmann and Christen's (2002) method, is a turning point in the development of microwave extraction. Microwaves generate heat by interacting with polar components in the solvent or matrix within the plant according to the mechanism of ionic conduction and dipole rotation (Zhang, Lin, and Ye, 2018). Therefore, the transfers of heat and mass in the extraction process can be carried out quite quickly, which can be completed in minutes rather than hours like the conventional methods. Advanced microwave-assisted extraction is solvent-free microwave extraction, the method that combines non-solvent distillation and microwave-assisted extraction. Solvent-free microwave extraction (SFME) uses heat from microwave irradiation to break the oil glands in the plant's cell wall (in situ) and vaporize the oil along with moisture content from the plant without any solvent whatsoever (Li et al., 2013). Compared with conventional extraction methods, the SFME affords a simple configuration, short extraction time, no solvent residual and contaminants, and creates an eco-friendly environment (Filly et al., 2014; Chemat et al., 2015; Yingngam, Navabhatra, and Brantner, 2021).

Many experimental design methods have been used in different research fields to optimize process parameters. However, many running extractions are needed to find the optimal variable, so it takes a long time for more data to be obtained. The main focus of experimental design techniques is to investigate and establish the interactions between parameters, which can aid in the optimization of experimental parameters by providing a mathematical model. Some scientific problems in research can be solved by integrating technology and mathematical models. Response Surface Methodology (RSM) is one of the experimental designs which can explain both the mathematical and statistical techniques to design, develop, improve, optimize, and formulate in which several variables have the potential to influence product design characteristics (Riswanto et al., 2019).

Moreover, RSM offers the advantage of reduced runs required to evaluate multiple parameters and their interactions (Shamsuddin et al., 2015). In recent years, several reports have been dedicated to the modelling and optimization of essential oil extraction in mint by different techniques. E.g., optimization of EO from mint leaves (*Mentha spicata*) using supercritical fluid extraction (Shrigod, Swami Hulle, and RV, 2016), optimization of MAE parametersinfluence on peppermint yield and antioxidant activity (Pavlić et al., 2019), and optimization of peppermint oil using conventional steam distillation (Kant and Kumar, 2021). However, to date, there is no report available about the application of RSM to achieve optimization of peppermint EO extraction using solvent-free microwave extraction (SFME). This research aims to perform an experimental design in peppermint oil extraction with SFME based on response surface methodology (RSM) to evaluate the effect parameters and obtain optimum conditions. However, due to the limited significant parameters to describe the solvent-free microwave extraction process, several parameters are the most critical operating range that will effectively and efficiently describe the process. First, parameters (i.e., microwave power, particle size, and feed-to-volume ratio F/D) that affect the yield of peppermint EO (response) are performed by face-centred central composite design (FCCD). Then correlate with the results of the empirical mathematical model and the response variables. Accordingly, the optimal yields of essential oil were predicted and verified experimentally.

Research Methods

Material

Peppermint (*Mentha piperita Linn.*) leaves were collected from the Kediri area, East Java, Indonesia. For the initial treatment of materials, peppermint leaves were cut into small pieces, and variables were collected and determined by the size (0.5, 1, and 1.5 cm with ±0.01cm error). Fresh leaves were packed in a plastic bag and stored in a storage refrigerator until used. Then following the pre-specified plant, peppermint leaves' initial moisture is 77%.

Solvent-free microwave extraction (SFME) apparatus and procedures

SFME was performed in a domestic microwave oven model EMM2308X produced by Electrolux;

irradiation from the microwave acts as the heat source of the extraction process. The size of the oven microwave with an internal cavity was 48.5x37x29.25 cm, modified with a set of Clevenger-type extraction apparatus. The raw materials in a round distillation flask (1000 mL) were placed in a microwave chamber. At the same time, the distillation system circuit was located outside the microwave oven, which aims to condense peppermint oil from the water phase.

Fresh peppermint leaves were weighed (80, 100, and 120 grams) for each extraction and put into a flask distiller without any solvent or water. Extraction was carried out for 60 minutes, with time measured when the first droplets were observed. After the extraction process, the oil phase was collected and decanted using a separatory valve. The essential oil accumulation was weighed and stored in a dry place until subsequent analysis. The percentage yield of essential oil extract was determined by (Chen, Zu, and Yang, 2015) given in Equation 1 as follows:

$$\text{Yield (\%)} = \frac{m_{EO}(g)}{m_i\,(g)\,(1\text{-}x)} \times 100\% \qquad (1)$$

Remarks: Whereas (m_{EO}) is the mass of essential oil extracted to (m_i) the mass of the feed material, and x (%) is the initial moisture content feed of materials.

Experimental design using RSM

In general, the optimization process research begins with determining what things are used as factors that can later be changed based on the object's ability under experimental. The results of the analysis obtained are expected to be used to respond to changing factors as desired. In this study, three factors obtained were tested using a Face-centered Central Composite Design (FCCD) to determine the influence of the parameter factors on the oil yield of extracts from *Mentha piperita Linn* leaves. It was developed using the DX-11 software package (Version 11.1.2.0, Stat-Ease Inc., Minneapolis, USA; trial version), which was used for regression analysis of the data obtained from the experiment. The quadratic response surface model was used to determine the variation of oil yield as a function of the independent variables given in:

$$\text{Y (Response variables)} = \beta_0 + \sum_{j=1}^{k} \beta_j . x_j + \sum_{j=1}^{k} \beta_{jj} . x_j^2 + \sum_{i} \sum_{<j=2}^{k} \beta_{ij} . x_i . x_j + e_i \qquad (2)$$

Remarks: where β_0, β_i, β_{ii}, β_{ij}, x_i, x_j, and e_i mean the regression (intercept value), linear coefficient, quadratic coefficient, interactive coefficient, coded independent parameters, and error, respectively.

In this study, three independent process variables were considered: microwave power (A), feed-to-distiller volume ratio (B), and particle size (C) were investigated using an FCCD matrix. The total number of runs required was $k = 2^n + 2n + k_C$ experiments. Where n, 2^n, 2n, and k_C represent the number of factors, factorial point, axial point, and centre point run, respectively. Suitable values for each variable of extraction factors were selected based on the preliminary study, given in Table 1. This design variable required three levels of each factor. Centre points were the median value between each coded variable's low and high levels, with $\alpha = \pm 1$ being the distance between the axial and centre points based on the face-centred central composite design (FCCD) (Bhattacharya, 2021).

Table 1. Experimental range of independent parameters and levels as per chosen by FCCD

Variable	Coded Variables	Parameter Level		
		-1	**0**	**+1**
Microwave Power (Watt)	A (1)	150	300	450
F/D Ratio (gram/mL)	B	0.5	1	1.5
Particle size (cm)	C	0.08	0.1	0.12

The experimental design for three factors with a total of 20 experimental run results was produced according to the FCCD matrix and the actual response values of the yield obtained from SFME. Thus, a statistical model was established, and a statistical analysis of variance (ANOVA) was performed to evaluate the interaction by analyzing the response surface contour plots. The model fit was determined by evaluating the lack of fit, the determination coefficient (R^2), the adjusted determination coefficient (R^2_{Adj}), and adequate precision. After getting the data verification, then matched again whether the results obtained were still within the range of Confident Interval (CI) or Prediction Interval (PI) was 95%. If verification results were still in the range of CI and PI, then it can be concluded that the model obtained follows what the software shows and can be applied to an actual experiment.

Results and Discussion

Experimental design and fitted model analysis

For peppermint extraction using solvent-free microwave extraction (SFME), the required experimental ranges and responses are given in Table 2.

Table 2. Experimental result of CCD in coded ranges and responses

Run	Actual Variables			The yield of peppermint oil (% w/w)		
	A (Watt)	B (g/mL)	C (cm)	Experiment	Predicted	Residue
1	300	0.1	1	0.6221	0.6623	-0.0402
2	300	0.12	1	0.5807	0.5713	0.0094
3	150	0.1	1	0.6167	0.5937	0.0230
4	150	0.08	0.5	0.3856	0.3950	-0.0094
5	300	0.1	1	0.6795	0.6623	0.0172
6	150	0.12	0.5	0.5088	0.5122	-0.0034
7	150	0.08	1.5	0.4861	0.4931	-0.0070
8	300	0.1	1.5	0.6896	0.6730	0.0166
9	450	0.08	0.5	0.5535	0.5598	-0.0063
10	300	0.1	1	0.6629	0.6623	0.0006
11	450	0.12	1.5	0.5388	0.5390	-0.0002
12	300	0.1	1	0.6301	0.6623	-0.0322
13	450	0.1	1	0.6937	0.6784	0.0153
14	300	0.1	1	0.6447	0.6623	-0.0176
15	300	0.1	0.5	0.6310	0.6093	0.0217
16	300	0.08	1	0.6047	0.5757	0.0290
17	450	0.08	1.5	0.6588	0.6650	-0.0062
18	300	0.1	1	0.6579	0.6623	-0.0044
19	150	0.12	1.5	0.5311	0.5344	-0.0033
20	450	0.12	0.5	0.5070	0.5096	-0.0026

In the experimental data obtained from solvent-free microwave extraction (SFME), the quadratic model was not aliased or fit into the model using the squares method.

According to data regression analysis, a mathematic model is expressed in a quadratic model is correlated by Eq. (2) in terms of coded factors given by:

$$\text{Yield} = 0.6623 + 0.0424\,A - 0.0022\,B + 0.0319\,C - 0.0418\,AB + 0.0018\,AC - 0.0190\,BC - 0.0263\,A^2 - 0.0888\,B^2 - 0.0212\,C^2 \quad (3)$$

Remarks: A, B, C, and D mean the independent variables following microwave power, feed-to-volume distiller ratio, and particle size, respectively.

The regression model significance test and model coefficients were tested based on lack-of-fit. The primary statistical method for analyzing experimental data and determining the effect of a factor or set of factors on the total variation is based on using a single set ANOVA on the RSM. The ANOVA results for the extraction of peppermint using SFME are summarized in Table 3. Constant terms represent the coefficient parameters of the model: linear coefficients (A, B, and C), interactive coefficients (AB, AC, and BC), and quadratic coefficients (A^2, B^2, and C^2). For each influence coefficient on peppermint oil yield during the extraction process, the fitting model shows that the independent variables A and C, interactive variables AB, and the quadratic variables B^2 have a highly significant effect. The F-value of the model was 22.12, with a low probability indicating that the quadratic model was a statistical significance ($p < 0.0001$) positive effect on the yield obtained. Furthermore, the lack of fit value (LOF) with a probability value was 0.3106 indicate that the LOF is insignificant relative to the pure error.

The fitting of the predicted model was assessed using a coefficient of determination (R^2) and adjusted R^2 (0.9522 and 0.9091, respectively), indicating the second-order polynomial model showed a good fit with the experimental data, and fitted models for yield extract peppermints oil were highly significant with highly satisfactory R^2. Indeed, the predicted-R^2 (0.8448) showed that the model might predict the experimental design. Therefore the adequate precision is 16.4024 (AP > 4), indicating the signal-to-noise ratio and signifies the desirability of the developed model.

Diagnostic plots showed the interaction between independent variables with response and model adequacy, residual normal probability plots (Fig. 1a), and residual versus experimental extraction sequence plots (Fig. 1b).

Table 3. Result from full quadratic models of ANOVA for peppermint extraction using SFME

Source	Sum of Square	df	Mean Square	F-Value	P -Value	
Model	0.1188	9	0.0132	22.12	< 0.0001	R^2 = 0.9522
A	0.0179	1	0.0179	30.05	0.0003	R^2_{adj} = 0.9091
B	0.0000	1	0.0000	0.0833	0.7787	R^2_{pred} = 0.8448
C	0.0101	1	0.0101	17.00	0.0021	Adeq precision = 16.4024
AB	0.0140	1	0.0140	23.46	0.0007	
AC	0.0000	1	0.0000	0.0428	0.8402	
BC	0.0029	1	0.0029	4.82	0.0528	
A^2	0.0019	1	0.0019	3.19	0.1045	
B^2	0.0217	1	0.0217	36.34	0.0001	
C^2	0.0012	1	0.0012	2.07	0.1806	
Residual	0.0060	10	0.0006			
Lack of Fit	0.0037	5	0.0007	1.59	0.3106	
Pure Error	0.0023	5	0.0005			
Cor Total	0.1248	19				

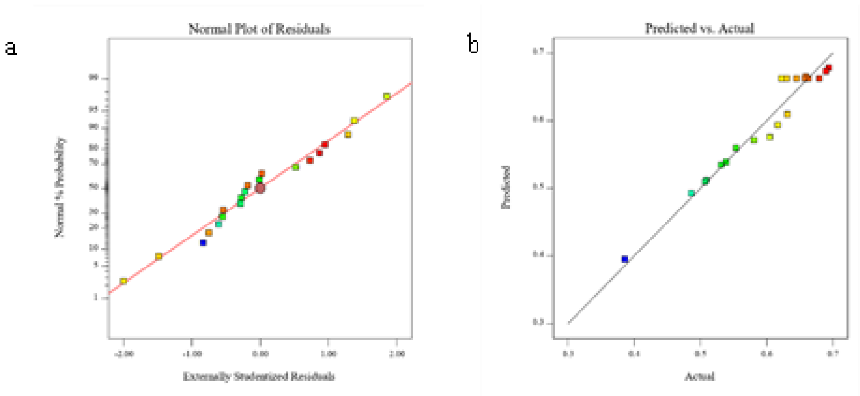

Figure 1. Diagnostic a) Residuals versus normal percentage probability plot and b) Residuals versus experimental plot for extraction of peppermint using SFME

As shown in Figure 1a, it can be observed that the residual for the response of the extracted oil is distributed on both sides. The response transformation can provide a good fit analysis followed by straight lines that reflect the normal distribution. In addition, the plot of residuals versus actual experimental results sequentially in Figure 1b can be examined for variables that might influence the response in the extraction process and assumed to represent a random distribution. The results of this validation indicate that the model obtained is acceptable and can provide an optimal value for the desired response.

3D Response surface analysis

The three-dimensional (3D) response surface plot shown in Figure 2. visualizes the effect of independent variables on yield extract. The entire relationship between the model is represented based on interactive term coefficients (AB, AC, and BC) with the response variable Y. The correlation is assessed through contours and response surface plots generated from the empirical predictive model in Eq. (2). The SFME method for peppermint extraction can also be predicted by this 3D plot using the response as a function of the interaction of the two independent variables while the other variables being maintained constant, are represented by extract yield regarding microwave power, F/D ratio, and particle size.

The results obtained in this study show that microwave power (150 – 450 Watt) has significant positive linear and quadratic effects on the yield of peppermint extract by SFME (Figure 2a). The chosen microwave power intensity limits function from limitations in the microwave oven apparatus. It was seen that the yield of peppermint oil obtained increased with increasing microwave power, which reached a maximum of 300W and then decreased to 450W power. Furthermore, the interaction between microwave power and the particle size ratio shows a significant effect ($P < 0.05$), whereas decreasing the size of the material increased the plant material's surface area, allowing for better *extraction*.

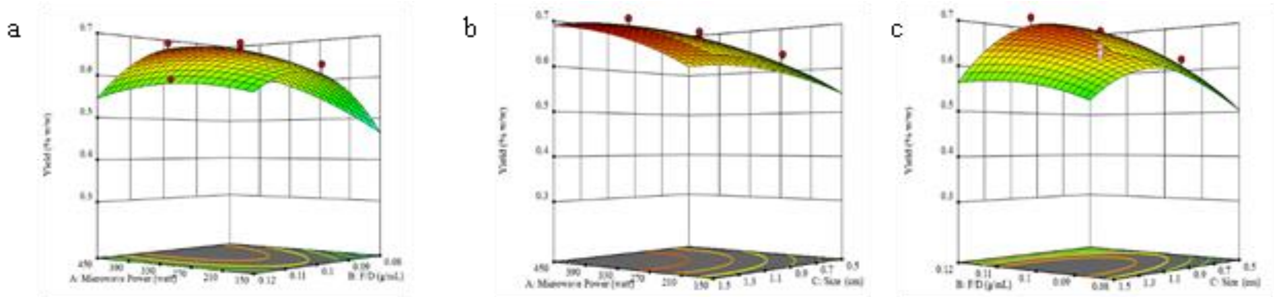

Figure 2. Several response surfaces plots (3D) of the interaction yield with each independent combination factor: a) Microwave power and F/D ratio, b) Microwave power and particle size, and c) F/D ratio and particle size

As reported, the power of microwaves plays an essential role as a driving force in breaking the structure of the oil gland cells in plants so that the oil can diffuse and evaporate. Thus, higher microwave power generally increases the yield and shortens the extraction time (Racoti et al., 2017). In addition, our observation of a correlation between microwave power and peppermint extraction yield indicates that high power and long extraction times will cause exposure to microwaves to be absorbed in solvents and materials. Previous studies on *Eucalyptus robusta* (Bhuyan et al., 2015) show that using very high microwave power causes damage to the cell structure of plants, leading to a decrease in the yield of the essential oil obtained.

The effect of the feed-to-distiller volume ratio on oil extraction is shown in Figure 2b, and it was seen that the peppermint essential oil yield increased as the F/D ratio changed from 0.08 - 0.1 g/mL, but the yield obtained decreased by 0.5070% at an F/D ratio of 0.12 g/mL. The interaction between microwave power and feed-to-distiller volume ratio did not show a significant effect ($P > 0.05$) on the yield of peppermint extract. It can occur because the increase in the mass of the material used causes the density of the material to be higher, which affects the rate of evaporation of the essential oil to be slow due to the inhibition of the movement of steam to evaporate into the condenser (Kusuma et al., 2019).

The effect of particle size of the extracted peppermint leaves (Figure 2c) on the yield obtained was significant, and the yield would increase with the change in size from 1.5 – 0.5 cm. However, the yield obtained decreased at 0.5 cm leaf size with a yield of 0.3856%. Reducing particle size can increase surface contact, which benefits the extraction process (Stéphane et al., 2021). However, the size of the material that is too small can reduce the yield of essential oils due to the complex mechanism parameters process (Cui et al., 2018). This explanation supports our studies of the negative correlation between particle size and mass transfer mechanism. Where indeed, the particle size dramatically influences the duration of the process if the mass transfer resistance can be reduced, and thus the extraction yield was obtained.

Specifically, an increase in yield due to one of three factors (i.e., microwave power, feed-to-distiller volume ratio, and particle size) causes the oil yield to increase until it reaches a certain optimum point and eventually decreases the variable factor, giving an insignificant effect.

Optimization and confirmation of peppermint extraction

Thus, the simultaneous effects of independent variable parameters and their interaction on the extraction of peppermint using SFME can be evaluated. The process has to maximize the peppermint yield obtained using SFME under the range parameter condition chosen. To further test the experimental models, extraction experiments were carried out by adjusting the operating conditions with 60 minutes extraction process at 450 Watt, 1cm, and 0.1 g/mL, which were microwave temperature, particle size, and F/D ratio, respectively. With these optimized conditions, the predicted response for peppermint essential oil yield was 0.6120 - 0.7448%, and the experimental yield confirmed was 0.6937%. Thus, the study's results indicate that the experimental results obtained are close to the model's predicted value with PI (95%), and it can be concluded that the selection of extraction techniques is adequate.

Conclusion

In this study, an experimental design using RSM was successfully implemented to optimize the extraction of the SFME method from the yield of essential oil derived from peppermint. A total of

20 trials running following the FCCD design were generated and attempted to generate data for the experimental design procedures. It was also found that the parameters of the operating condition, especially microwave irradiation power, had the most significant impact on the extraction yield. This research serves as a precursor to industrial-scale production by finding the optimal conditions for peppermint extraction.

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Declarations

Conflict of interests The authors declare no competing interests.

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