Optimization of microwave-assisted extraction of dyes from brown seaweed (*Sargassum duplicatum*) using response surface methodology

Optimization of microwave-assisted extraction of dyes from brown seaweed (*Sargassum duplicatum*) using response surface methodology

P A Handayani, S Pujianti and A H Al'adl

Department of Chemical Engineering, Universitas Negeri Semarang, Semarang, Indonesia

prima@mail.unnes.ac.id

Abstract. Batik is an Indonesian heritage that can use natural or synthetic dyes. Synthetic dyes are quite dangerous for the environment and human health. To reduce the use of synthetic dyes, brown seaweed (*Sargassum duplicatum*) extraction was conducted as a natural coloring. Brown seaweed is a plant that has the potential to an abundant amount of natural coloring in Indonesia. This research aims to obtain optimum conditions in the process of extracting brown seaweed using the Microwave-Assisted Extraction (MAE) method. The material used is brown seaweed obtained from Gunungkidul, Yogyakarta Indonesia, and ethanol as a solvent. The research variables are power 200; 400; 600 watts, 15 extraction time; 30; 45 minutes, and seaweed concentration 0.06; 0.12; 0.18%. To get the optimum conditions in the process of extracting brown seaweed, it uses the Response Surface Methodology (RSM). The results showed that there were interactions between variables that affected the yield. Yield tends to increase with increasing power, time, and concentration of seaweed in the solvent. The optimum conditions of the extraction process in this study were at 400 watts power, 30 minutes, and seaweed concentration 0.12% with a yield of 3.87%..

1. Introduction

Sargassum sp. is a seaweed that belongs to the class of phaeophyceae that grows wild in abundance throughout the world, especially along with the coastal areas in Southeast Asia [1]. In Indonesian waters, there are 15 types of *Sargassum sp* with varying pigment positions, such as *Sargassum duplicatum*. *Sargassum duplicatum* contains photosynthetic pigments, including chlorophyll a, chlorophyll c, carotene, and fucoxanthin [2]. Fucoxanthin pigment gives dark brown to golden brown color [3].

The process of taking color pigments by extraction methods. Conventional extraction methods such as maceration, percolation, reflux, and soxhletation have been replaced by non-conventional methods to increase efficiency [4]. The latest alternative extraction method is Microwave-Assisted Extraction (MAE). This extraction is an extraction that utilizes microwaves in the heating process based on direct collisions with polar or solvent material [5]. The Microwave Assisted Extraction (MAE) method has advantages including the shorter time needed, higher yields, environmentally friendly and less energy consumption and less needed solvents [5][4]. Based on these advantages MAE is a good alternative method for extraction processes such as solid-liquid extraction in plants

The best method for the optimization process is to use Response Surface Methodology (RSM), this process not only determines the optimum conditions but also provides the information needed for the design process [6]. RSM is an efficient method of solving using mathematics and statistics to evaluate interactions and responses between process variables while reducing the number of experiments [7][8].

The purpose of this study was to obtain the optimum operating conditions for extraction of brown seaweed for yield and obtain second order polynomial equations.

2. Material and method

2.1 Material

The main ingredients used in this study are ethanol and brown seaweed obtained from Gunung Kidul, Sleman, Yogyakarta.

2.2 Method

2.2.1 Extraction of dyes by the microwave assisted extraction (MAE) method

Brown seaweed powder with a certain weight is put into the extractor and 250 ml of ethanol. The extractor is put in the microwave and set to a certain power. The extraction process was done for 25-45 minutes. The results of the extraction process obtained two layers, the upper layer in the form of filtrate and the lower layer in the form of residue. Then the filtrate and residue are separated using filter paper. The filtrate is distilled at 80ºC to separate the dye from the solvent. The extract was oven-dried at 80 ºC until its mass was constant. Calculate the yield of the dye by weighing the mass of the extract and weighing the mass of the sample material, the yield calculation uses equation [1].

$$Yield\ (\%) = \frac{extract\ mass}{sample\ mass} x\ 100\% \quad (1)$$

2.2.2 Optimization Using Response Surface Methodology (RSM)

Optimization using RSM functions was carried out to determine the regression model based on Central Composite Design (CCD) and to determine the effect of the independent variables. The used independent variables is microwave power (200, 400, and 600 watt), extraction time (25, 30, and 45 minutes), and concentration materials with solvents (0.06; 0.12; and 0.18 g / ml) on the dependent variable to produce the yield of dyes [9]. In this study, the independent variables include microwave power (X_1), extraction time (X_2), and concentration of materials with solvents (X_3), as well as the dependent variable was the yield of dyes in which described in the form of a second-order polynomial equation [9] i.e.:

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

Where Y is the predicted response, β_0 constant, β_i linier coefficient, β_{ii} quadaratic coefficient, β_{ij} interaction coefficient, n number of factor studied, $X_i X_j$ variable parameter code, dan ε residue component (error).

2.2.3 Statistical analysis

Data obtained from the extraction of brown seaweed dyes were analyzed using STATISTICA 10 software. This software can display response graphs in 3 dimensions. To find out the independent variable has a significant effect on the dependent variable the degree of significance (confidence level) <0.05 is used. A significant degree <0.05 indicates that this equation model is suitable for experiments.

3. Result and discussion

3.1 Developing model for the brown dye yield

Optimization of the effects of variables on the extraction process follows the RSM method with an experimental design in the form of Central Composite Design (CCD). The design of the experimental optimization using RSM is shown in Table 1. The experimental results are shown in Table 2.

Table 1. Design Optimization Experiments using RSM.

Independent Variable	Level Code				
	-α	-1	0	1	+α
Power (watt)	63,641	200	400	600	736,358
Time (minutes)	4,77	15	30	45	55,22
Concentrations with Solvents (%)	0,019	0,06	0,12	0,18	0,22

Table 2. Design of Experiment Central Composite Design.

Run	X_1	X_2	X_3	
	Power (watt)	Time (minutes)	Concentrations (%)	Yield (%)
1	200	15	0.06	1.40
2	200	15	0.18	1.64
3	200	45	0.06	1.44
4	200	45	0.18	1.90
5	600	15	0.06	2.00
6	600	15	0.18	1.07
7	600	45	0.06	1.53
8	600	45	0.18	3.02
9	63.64	30	0.12	1.70
10	736.36	30	0.12	1.70
11	400	4.77	0.12	1.44
12	400	55.22	0.12	3.87
13	400	30	0.02	1.11
14	400	30	0.22	1.90
15 (C)	400	30	0.12	1.47
16 (C)	400	30	0.12	1.40
17 (C)	400	30	0.12	1.53
18 (C)	400	30	0.12	1.53
19 (C)	400	30	0.12	1.78

RSM analysis results obtained by the regression coefficient values for the polynomial equation are presented in Table 3.

Table 3. Prediction of Regression Coefficients for Multiple Polynomial Order Results.

Factor	Regression Coeff.	Std Err.	t	P
Mean/Interc.	1.5508	0.0640	24.2072	0.00002
X_1	0.1815	0.0776	2.3395	0.07941
X_1^2	0.0143	0.0776	0.1851	0.86215
X_2	0.8591	0.0776	11.0689	0.00037
X_2^2	0.6896	0.0776	8.8829	0.00088
X_3	0.3790	0.0776	4.8839	0.00813
X_3^2	-0.1235	0.0776	-1.5909	0.18684
X_1X_2	0.2950	0.1014	2.9088	0.04372
X_1X_3	-0.0350	0.1014	-0.3451	0.74739
X_2X_3	0.6600	0.1014	6.5079	0.00287
Pure Error				
R^2	0.7705		Adj R^2	0.5411

In Table 3. the parameter coefficients are used to determine the polynomial equation. It can be seen that there are several variables that significantly influence the response, this is because the p value obtained <0.05 [10]. Based on Table 3 there are two variables that have a significant effect on yield, there is time variable (X_2) and solvent concentration variable (X_3) because the p value <0.05 so that the polynomial equation is presented in the following equation:

$$y = 1,5508 + 0,8591X_2 + 0,3790X_3 + 0,2950X_1X_2 + 0,6600X_2X_3 + 0,6896X_2^2 \quad (3)$$

The R^2 value of this experiment is 0.7705, which means that this experiment has the effect of power, time, and concentration of the material with the solvent on the yield of brown dye by 77.05%. While the rest is influenced by other variables not included in the model or the independent variable. Comparison graphs between research results and predictions are presented in Figure 1.

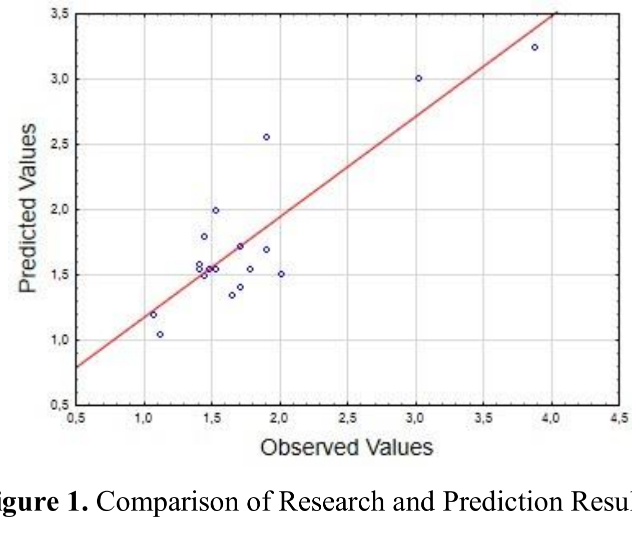

Figure 1. Comparison of Research and Prediction Results.

3.2 Effect of Independent Variables on Yield Results

3.2.1 Effect of power with time

The effect of power and time variables on yield can be seen in Figure 2. that the highest yield is produced at 400 power while the yield yield decreases when the power is increased. It can be concluded that the higher the power does not affect the yield.

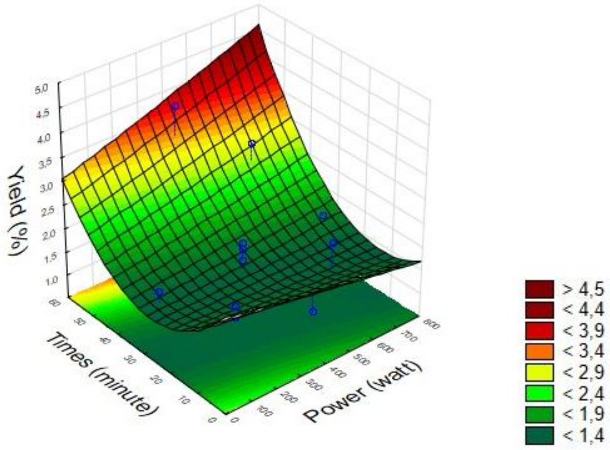

Figure 2. Response Surface Profile of Brown Subtance From Relation Power vs Time Extraction.

3.2.2 Effect of concentration on materials with solvent and power

Figure 3. it can be seen that the yield will increase proportional to the increasing concentration of the material with the solvent. It can be concluded that the concentration of the material with the solvent influences the yield.

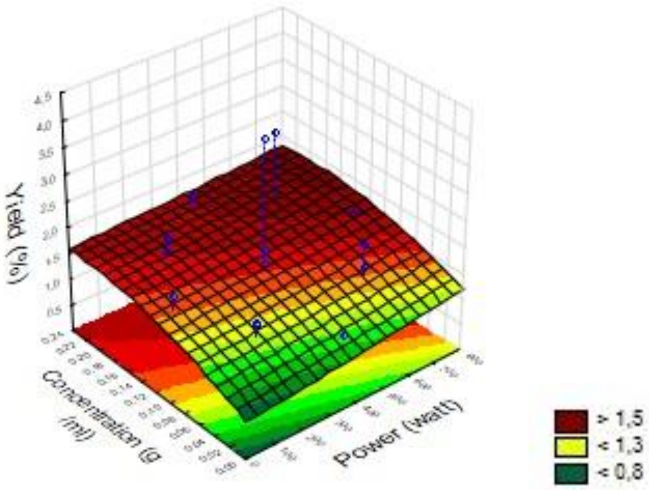

Figure 3. Response Surface Profile of Brown Subtance From Relation Concentration of Material With Solvent vs Power.

3.2.3 Effect of time and concentration on materials with solvents

Figure 4 it can be seen that the yield of brown dyes tends to increase with increasing extraction time, but tends to decrease when the concentration of the material with the solvent decreases. So it can be concluded that the extraction time variable influences the yield of dyes produced.

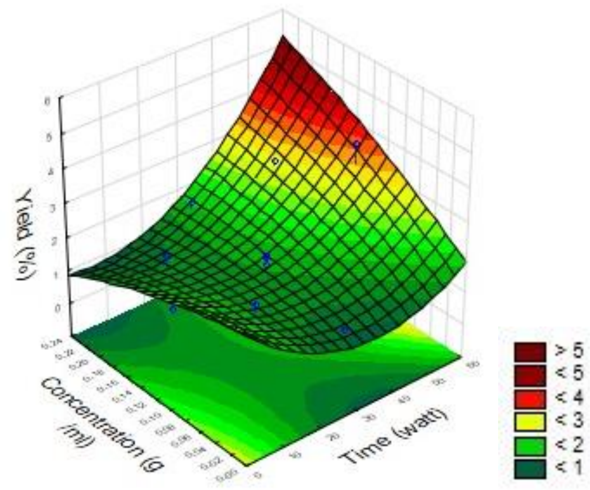

Figure 4. Response Surface Profile of Brown Subtance From Relation Time vs Concentration of Material With Solvent.

3.2.4 Determination of optimum conditions

The optimum conditions can be seen from the straight lines of the three interactions between variables, namely power, time, and concentration of the material with the solvent. Interaction between variables can be seen in Figure 5.

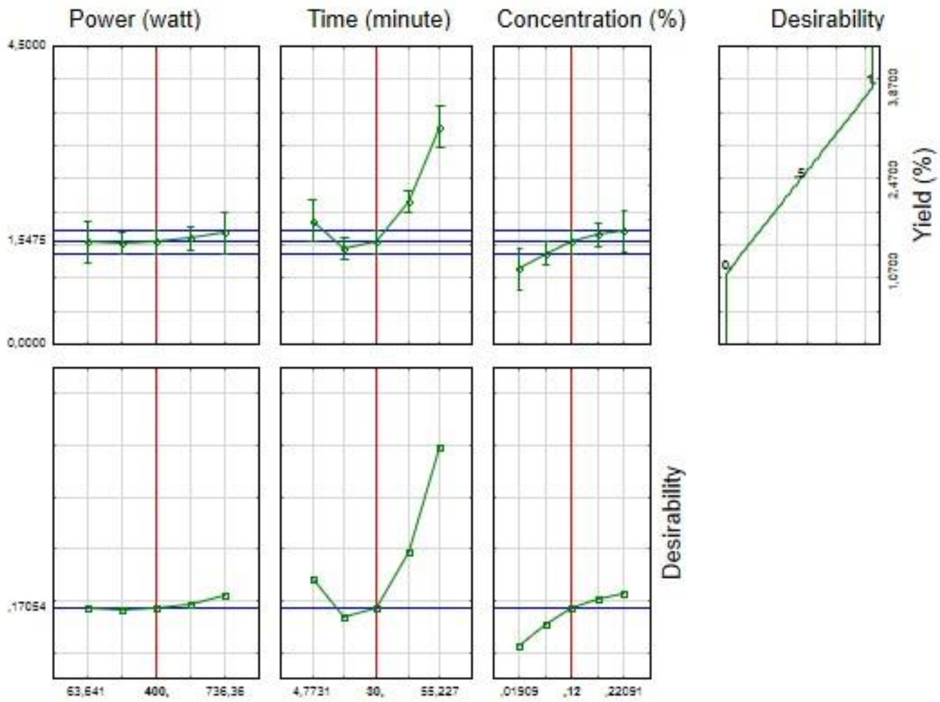

Figure 5. Plot Interaction Between Variables.

Based on Figure 5 it can be seen that the optimum conditions are obtained at 400 watt of power, extraction time of 30 minutes, and the concentration of material with a solvent of 0.12 g / ml with the highest yield of 3.87%.

3.2.5 FTIR test analysis

Functional group analysis is performed to find out the type of functional groups found in fucoxanthin dyes. This analysis was carried out using infrared spectroscopy at wave numbers from 400-4000 cm-1. One of the commonly used spectroscopic methods is the Fourier Transfrom Infrared (FTIR) method which is a modern instrumental method used to detect functional groups of bioactive components based on peak values from the results of the infrared spectrum (Rajeswari and Jeyaprakash, 2019).

To find out the sample has a fucoxanthin content, the results of the FTIR test sample must be compared with the results of the FTIR test of pure fucoxanthin compounds. The absorption area of pure fucoxanthin functional groups can be seen in Table 4. below:

Table 4. Functional Absorption Areas of Pure Fucoxanthin Function

No.	Cluster Uptake Region (cm^{-1})	Functional groups
1	3200-3500	O-H
2	2800-2950	C-H
3	1700-1730	C=O
4	1000-1260	C-O
5	1150-1210	C-C(O)-C
6	~1375	CH_3

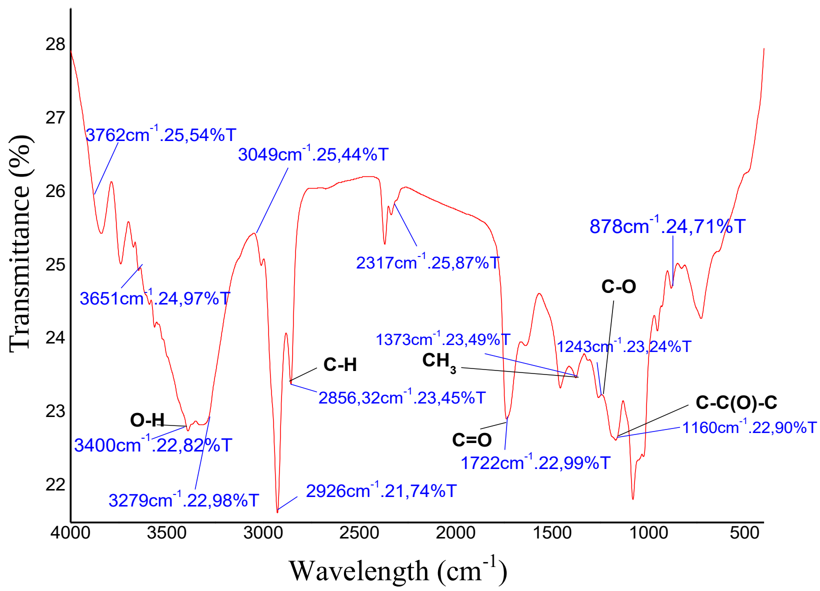

Figure 6. FTIR Test Results for the sample

From Figure 6 we can see the peak absorbance at several wave peaks. The absorbance peak at 3400 indicates the vibration of the O-H bond. Absorbance in 1722 showed the presence of C = O (Carboxylic Acid) bonds. CH stretching alkane bonds occur at wavelength 2856. C-C (O) -C functional group bonds are formed at wavelength 1160 and CH_3 alkene bonds are shown at the peak of the absorbance wave 1373 while at the peak wave 1243 indicates the CO bond. Based on the test results of brown seaweed extract sample results obtained functional group content in accordance with pure fucoxanthin compounds.

4. Conclusions

Optimization in the process of extracting brown seaweed dyes with the help of Microwave Assisted Extraction using Response Surface Methodology (RSM) is based on Central Composite Design (CCD). The results showed that the extraction time variable and the concentration of the material with the solvent had a significant effect on the yield of the dye, while the microwave power variable had no significant effect on the yield of the dye. Optimum operating conditions were obtained at 400 watt microwave power, extraction time of 30 minutes, and the concentration of material with a solvent of 0.12 g / ml obtained a yield of 3.87% and obtained a systematic model as follows: $y = 1{,}5508 + 0{,}8591X_2 + 0{,}3790X_3 + 0{,}2950X_1X_2 + 0{,}6600X_2X_3 + 0{,}6896X_2^2$. Second order polynomial equations were obtained to predict the accuracy of the model of the study.

References

[1] Ale MT, Mikkelsen JD, and Meyer AS 2012 Designed optimization of a single-step extraction of fucose-containing sulfated polysaccharides from Sargassum sp. *J. Appl. Phycol.* **24** 715–723

[2] Resita D, Merdekawati W, Susanto A, and Limantara L 2010 The Content and Pigment Composition of Sargassum Sp. in Awur Bay Territorial Waters, Jepara by Fresh and Dry Treatment *J. Perikan. (J. Fish. Sci.)* **12** 11–19

[3] Ruslan and Wiraningtyas A 2018 Extraction of Pigment Color from Sargassum sp. seaweeds *J. Redoks (J. Pendidik. Kim. Dan Ilmu Kim.)* **2** 1–10

[4] Belwal T, Ezzat SM, Rastrelli L *et al.* 2018 A critical analysis of extraction techniques used for botanicals: Trends, priorities, industrial uses and optimization strategies *TrAC - Trends Anal. Chem.***100** 82–102

[5] Ingrath W, Nugroho WA, and Yulianingsih R 2015 Ekstraksi Pigmen Antosianin dari Kulit Buah Naga Merah (*Hylocereus Costaricensis*) Sebagai Pewarna Alami Makanan dengan Menggunakan Microwave (Kajian Waktu Pemanasan dengan Micrawave dan Penambahan Rasio Pelarut Aquades dan Asam Sitrat) *J. Bioproses Komod. Trop.* **3** 1–8

[6] Geiger EO 2014 *Statistical Methods for Fermentation Optimization* Third Edition. Elsevier Inc.

[7] Gao W, Wang Y, Basavanagoud B, and Jamil MK 2017 Characteristics studies of molecular structures in drugs *Saudi Pharm. J.* **25** 580–586

[8] Huerta-Ochoa S, Castillo-Araiza CO, Guerrero AR, and Prado-Barragán A 2019 Whole-Cell Bioconversion of Citrus Flavonoids to Enhance Their Biological Properties *Studies in Natural Products Chemistry* **61** 335-367

[9] Behera SK, Meena H, Chakraborty S, and Meikap BC 2018 Application of response surface methodology (RSM) for optimization of leaching parameters for ash reduction from low-grade coal *Int. J. Min. Sci. Technol.* **28** 621–629

[10] Simic VM, Rajkovic KM, Stojicevic SS *et al.* 2016 Optimization of microwave-assisted extraction of total polyphenolic compounds from chokeberries by response surface methodology and artificial neural network *Separation and Purification Technology* **160** 89–97