Optimization of Ultrasound-Assisted Extraction Parameters from Indigofera Tinctoria L using Response Surface Methodology

By a'yun a'yun

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Abstract
Nowadays, the natural dyes are commonly used in textile industry. One of them is Indigofera tinctoria L leaves extracting natural dyes that produce blue color and have various potential utilization. However, the extraction time required usually takes a long time using conventional extraction. Thus, some researches reported that ultrasound-assisted extraction can accelerate and increase the extraction yield. The mechanism of ultrasonography is able to assist matrix cell rupture through the ultrasound cavitation. The present research aimed to study and to evaluate the Indigofera tinctoria L dye using ultrasound-assisted extraction. Response surface methodology (RSM) in conjunction with box-behnken design (BBD) was applied to optimize and to analyze the extraction conditions. The independent factors were ratio feed to solvent (0.02-0.1 g/ml), ultrasound temperature (50-60°C), and extraction time (50-90 minutes). Further, feed to solvent ratio was the most significant factor of extraction process in comparison with temperature and extraction time. The optimum extraction conditions were 58°C, 0.02 g/ml, and 50 minutes. The predicted model obtained was 6.025% which close to the actual data (5.75%). Moreover, the extract dye compounds were analyzed by FTIR test and the result was 64.07% for presence of Indigo dye. Therefore, the natural dyes can be alternative and sustainable dye as environmentally friendly.

Keywords: *Indigofera tinctoria L., Ultrasound-Assisted Extraction, Yield, RSM, BBD.*

1. Introduction

One of the largest non-oil and gas industries is textile industry. Color design is the most important aspect in textile industry. Many people use clothes with all kinds of colors and patterns. In the process, coloring is a necessary process. The coloring processes commonly used are natural and synthetic dyes. However, the textile industry often interested in chemical and synthetic dyes. The advantages of them are stable, resistance to various environmental conditions and to fading, color strength, and wide color range. On the other hand, synthetic dyes easier to obtain and to practice (Pujilestari, 2017).

In fact, there are also disadvantages caused by synthetic dyes, one of them is impact of dyeing waste on environment (Wahyuningsih et al., 2017). Textile industry waste is classified as liquid waste from the coloring process which is a synthetic chemical compound causing a strong pollutant power and carcinogenic potential contamination. Therefore, as an effort to reduce the coloring waste, alternative synthetic dyes are suggested, namely natural dyes. The natural dyes are extracted from plants, flowers, and leaves (Berradi et al., 2019; Vettumperumal et al., 2018). There are many plants that can be used as natural/synthetic organic dyes, commonly plant materials that have bright colors. One of potential plant is *Indigofera tinctoria* L.

The plant extraction process for coloring is reported by conventional method. This process requires a long extraction time, high cost, and non-optimal extraction yield (Gogus et al, 2005). Therefore, advanced extraction methods are needed as a substitute technique. One method that can be used by ultrasound-assisted extraction (UAE) which is proven to produce three times more extraction compared to conventional methods in extracting natural plants (Rouhani et al., 2009). Ultrasonic-assisted extraction has also confirmed 23 be more efficient in terms of rapidity, cost, and extract quality than the others, such as steam distillation and superheated water extraction (SWE) (Roldán-Gutiérrez, Ruiz-Jiménez, and Luque de Castro, 2008). Besides, the lack of considering the optimal conditions is to obtain high extraction yield. Therefore, the objective studies are to study and to evaluate the extraction conditions of *Indigofera tinctoria* L using ultrasound-assisted extraction. The process factors will be optimized by response surface methodology (RSM). The predicted model from RSM could be assisted to determine the optimum extraction conditions.

2. Methodology

2.1 Materials

The leaves of *Indigofera tinctoria* L were purchased from Surabaya, East Java. The materials were controlled at fresh condition.

2.2 Indigofera tinctoria L Extraction

The ultrasonic mechanism 29 g bath was applied for this extraction process. The tool in KRISBOW model KW1801033, power 100 W, voltage 240 V/50 Hz, frequency 40 kHz, and bath capacity of 2,8 L was performed. Specific amount of *Indigofera tinctoria* leaves dissolved 200mL of aquadest was set in a distiller 500mL, thus placed in ultrasonic bath and system was turned on. At a specific time, the final extraction was resulted an extract and $Ca(OH)_2$ was added for extract. Therefore, it would be consisted of two phases: sediment, and supernatant.

The supernatant was evaporated over to make a solid dye extract. After separation, the product was kept in vial. Finally, the extraction yield was calculated from Equation 1.

$$\text{Yield of extract} = \frac{m}{M} x\ 100\% \quad (1)$$

where m and M were weight of natural dye extracts divided by the weight of fresh *Indigofera tinctoria* leaves (g/g). Furthermore, the extract obtained from the extraction was analyzed by Fourier-Transform Infrared Spectroscopy for identifying extract composition in Laboratory Test of Material and Metallurgical Engineering in Surabaya.

Response Surface Methodology

Response Surface Methodology (RSM) is an optimization tool that could assist researchers to analysis and to evaluate the independent and interaction factor effects. This optimization has been useful for reducing the number of running factors in order that research could be relatively effective in time and operating conditions (Bezerra et al., 2008). Box-behnken design using three-level factor responses was performed for three parameters (decision variables) (Cheng et al., 2017; Cui et al., 2018; Variyana, Mahfud, et al., 2019; Variyana and Mahfud, 2019). Table 1 showed the actual, and coded levels, of three factors, were designed to, maximize the yield of extraction. The designs consist of 17 sets of exeperiments and 5 center points using Design Expert (Trial Version 11, Stat-Ease Inc., Minneapolis, MN, USA). The coded and experimental units was displayed in Table 2. On the other hand, a second order model was chosen of regression analysis as followed in Equation 2.

Table 1. Box-behnken Design (BBD) Coded Factors

Coded	Independent Factor		
	A Ultrasound Temperature (°C)	B Feed/solvent Ratio (g/mL)	C Extraction Time (min)
-1	50	0.02	50
0	55	0.06	70
+1	60	1	90

Table 2. Box-behnken Experimental Design in Coded and Actual Units

Temperature (°C)	F/S Ratio (g/ml)	Extraction time (min)	Yield extract (%)		
			Actual	Predicted	Residual
50	0.06	50	2.417	3.053	-0.637
55	0.1	50	1.800	3.127	-1.327
50	0.1	70	1.650	1.565	0.085
55	0.06	70	2.750	2.670	0.080
60	0.06	50	2.583	4.116	-1.532

Temperature (°C)	F/S Ratio (g/ml)	Extraction time (min)	Yield extract (%)		
			Actual	Predicted	Residual
55	0.06	70	2.583	2.670	-0.087
55	0.06	70	2.667	2.670	-0.003
55	0.06	70	2.583	2.670	-0.087
55	0.02	90	5.250	3.931	1.319
50	0.02	90	1.650	2.645	-0.995
55	0.02	50	6.000	6.257	-0.257
60	0.1	70	1.900	0.913	0.987
60	0.02	70	5.500	5.593	-0.093
60	0.06	90	2.750	4.116	-1.366
50	0.06	90	2.417	3.053	-0.637
55	0.06	70	2.750	2.670	0.080
55	0.1	90	1.550	1.301	0.249

$$Y = \beta_0 + \sum_{i=1}^{k} \beta_i x_i + \sum_{i=1}^{k} \beta_{ii} x_i^2 + \sum_{1 \le i \le j}^{k} \beta_{ij} x_i x_j + e \quad (2)$$

The yield of natural dye extract as predicted response was denoted by Y; β_0 is regression coefficients for intercept, β_i is represented for linear regression, β_{ii} and β_{ij} are square and interaction terms; x_i and x_j are coded values the independent factors; k is variable number; and e is the residual between model fitted and experiment. Moreover, the fitted model was employed statistically, using analysis of variance (ANOVA).

The results would be evaluated by p-value for significance, F-testing for knowing error rates, lack of fit for diagnose the model fitted, and coefficient of determination (R^2) for measuring the amount of variation. These factors were designed to obtain the suitability of predicted model. Besides, the mathematical model was confirmed the effects of independent and interaction parameters. The response would be provided by contour and 3-D surface plots. Therefore, predicted model and experimental data were compared to verify the model optimization.

3. Result and Discussion

3.1 The optimization of *Indigofera tinctoria* L Extraction using Box-behnken Design

RSM employing BBD has better prediction accuracy for three levels. Table 2 showed the BBD matrix and the extraction yield for each experimental run. It is applied for optimizing *Indigofera tinctoria* L yield using ultrasound-assisted extraction. The response model can provide coded factor equation to express the extraction yield as follows in Equation 3.

$$Y = 2.66600 + 0.573750\,A - 1.43750\,B - 0.103750\,C - 0.549250\,A^2 + 0.558250\,B^2 + 0.425750\,C^2 - 0.900000\,AB - 0.042500\,AC + 0.125000\,BC \quad (3)$$

Table 3 showed the analysis of variance, the p-value equal to 0.0247 means the overall model of second order equation that is reliable. This result could be used to predict response values on the specific range of factors and F-value equal to 4.84 occurs due to noise. Therefore, a model term is resulted as significant in p-value <0.05. Based on research, the reviewing model terms obtain two significant terms: ratio F/S (B), and quadratic of ratio F/S (B^2). These significant terms were evaluated by the linear and square regressions.

The lack of fit model has an F-value of 189.55 which means the model fits well. Besides, there is 0.01% chance that can be caused by noise. The adequacy for *Indigofera tinctoria* L dye extraction was also evaluated and validated by regression values which close to one in coefficient of determination (R^2) = 0.8616; adjusted coefficient of determination (R^2_{adj}) = 0.6837. The model regressions are sufficient to confirm the adequacy of the model, even though another regression value and predicted coefficient of determination (R^2_{pre}) have a low value of -1.1998. Moreover, adequate precision measures the signal to noise ratio which value is 7.9257. This value is greater than 4 which means desirable model. In addition, this ratio indicates adequate signal so that predicted model can navigate. Figure 1 shows the normal data distribution with following the diagonal line and each point is close to be. Thus, the model from BBD could be represented for *Indigofera tinctoria* L extraction with a fixed model.

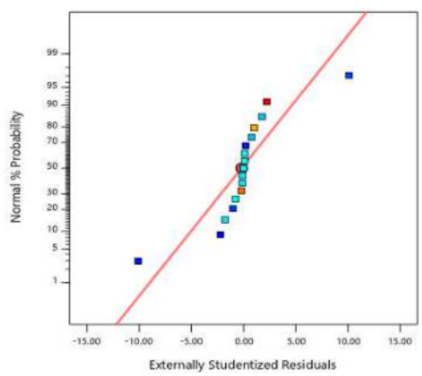
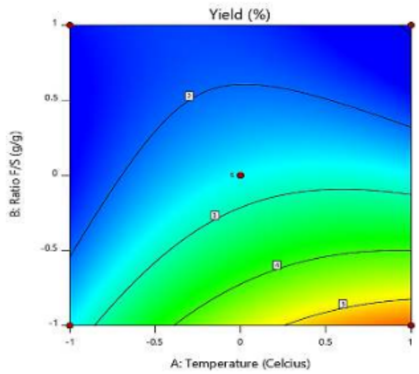


Figure 1. Normal Probability Plot

3.2 Evaluation of Parameter Extraction

Referring to the analysis of variance shows a significant interaction between parameters, the optimization study of *Indigofera tinctoria* L dye extraction would be useful for analysing the influence of the parameters. Besides, RSM provides contour plots that is one of its analytical terms. This plot is represented and given by the model equation, where both contour plots and model equation affect the results on the optimization value of the other constant.



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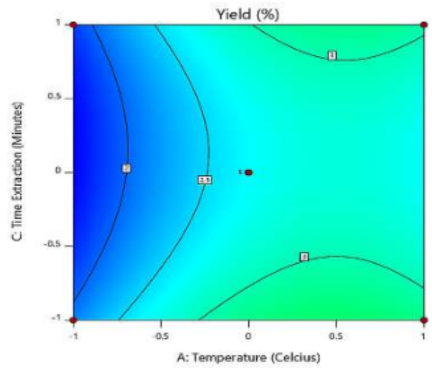

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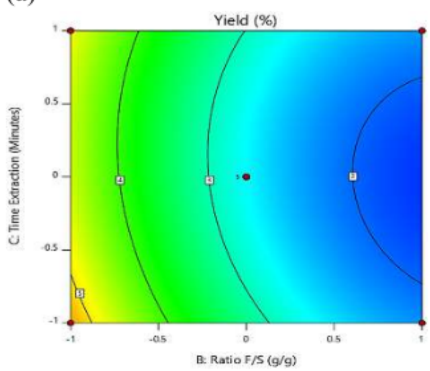

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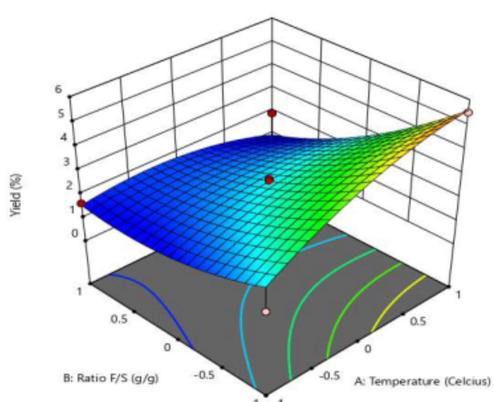
Figure 2. Contour Plots



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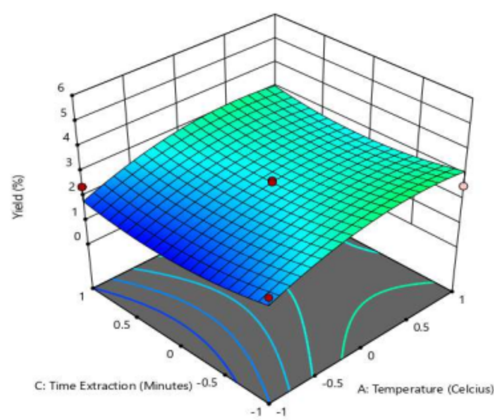

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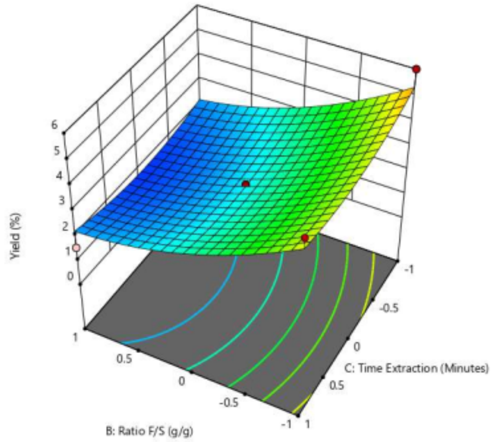

(c)

Figure 3. 3D Surface Plots

Table 3. Analysis of Variance (ANOVA) of Second-Order Model

Source	DF	Adj SS	Adj MS	F-Value	p-Value
Model	9	25.78	2.86	4.84	0.0247
Linear	3	19.25	6.42	7.82	0.0031
A	1	2.63	2.63	4.45	0.0728
B	1	16.53	16.53	27.95	0.0011
C	1	0.0861	0.0861	0.1456	0.7141
Square	3	3.22	1.07	1.82	0.2320
A^2	1	1.27	1.27	2.15	0.1862
B^2	1	1.31	1.31	2.22	0.1800
C^2	1	0.7632	0.7632	1.29	0.2934
2-Way Interaction	3	3.31	1.10	1.5	0.2742
AB	1	3.24	3.24	5.48	0.0518
AC	1	0.0072	0.0072	0.0122	0.9151
BC	1	0.0625	0.0625	0.1057	0.7546
Error	7	4.14	0.5915		
Lack-of-Fit	3	4.11	1.37	189.55	<0.0001
Pure Error	4	0.0289	0.0072		
Total	16	29.92			

$R^2 = 0.8616$; $R^2_{adj} = 0.6837$; $R^2_{pre} = -1.1998$

In Figure 3, there is code which value in range -1 to 1. Value of -1 and 1 mean the lowest and the highest parameter, respectively. Figure 2a and 3a show the contour and 3D plots in range of temperature extraction and feed to solvent with extraction time at 70 min. The higher feed/solvent ratio and temperature, the higher extraction yield obtained from experiments. However, the yield was decreased at temperature of 60°C. Therefore, it suggests to operate process at higher temperature. Further, the effect of increasing feed/solvent ratio would be reduce the *Indigofera tinctoria* L extract. Temperature is one of the factors that influence extraction efficiency and selectivity. Besides, the temperature can also affect the physicochemical properties of water at high temperatures, reducing water polarity and thereby increasing the solubility of less polar compositions in water. This reason explains that water can extract more quantities of the mixture at higher temperatures (Rahman, Tajuddin, and Tumin 2013). In addition, the extraction temperature has a significant influence on the ultrasound-assisted extraction process. Increasing temperature affects in a decreasing of both viscosity and surface tension of the solvent for reducing the cavitation threshold. The lower cavitation threshold makes cavitation bubbles easier to form. Breaking cavitation bubbles can cause the cell wall to be damaged. This phenomenon makes it easier for the solvent to diffuse into the material matrix and to extract the dye so that the extraction yield increases (Mason and Lorimer, 2004). Another study also stated that higher temperature in extraction conditions results in more extracted dyes, however it can affect the extracted substance quality and convert the structures of its composition (Rouatbi et al., 2007).

In Figure 2b and 3b show the contour plot at 0.06 g/mL of feed/solvent ratio. It means that the increasing of ultrasound temperature and time extraction will cause a significant decrease in extraction yield. While increasing the extraction time at low temperature levels will increase the yield. In general, the experiments that have been carried out have increased yield with increasing extraction time. Increasing extraction yield was due to the initial duration of exposure to ultrasonic waves which increased swelling and hydration of the material due to the cavitation effect. Cavitation induces the formation of microjets on the material surface and it can damage the material with causing the solvent to easily diffuse into the material matrix. In these conditions the ability of the solvent to dye extract increases so that the extraction yield obtained is greater. Furthermore, after 70 minutes there might be reported by continuous ultrasonic waves through the solvent that will disrupt the structure of the dye (Rahman et al. 2013; Tayade and Adivarekar 2014).

On the other hand, in Figure 2c and 3c represent the constant variable of 60°C. Extraction yield will change according to extraction time and temperature levels. The greater the ratio of raw materials to solvents, the smaller the extraction yield obtained as shown Figure 3c. The decrease in extract yield at a high feed to solvent ratio was due to the large number of leaves in the distiller during *Indigofera tinctoria* L dye extraction. The large mass of raw material with a solvent volume of 200 ml causes a low extract yield. The solvent has the maximum capacity to extract the leaf as raw material.

3.3 Verification and Validation for Response Surface Optimization

The extract with high yield in parameter range from contour plots can be optimized by determing the critical point of the model equation where the model differential is equal to zero (Khajeh and Ghanbari, 2011; Syafaatullah and Mahfud 2021). The optimization conditions were obtained by RSM with the response as a maximum condition region of 50°C-60°C for ultrasound temperature, 0.02g/mL-0.1g/mL for feed to solvent ratio, and 50 min-90 min for extraction time. The optimum condition predicts in 58°C for temperature, 0.02g/mL for feed to solvent ratio, and 50 min for extraction time. The predicted model resulted the optimum extraction yield is 6.025%. Besides, the actual experiment in the best extraction yield is 5.75%. This actual yield has the residual value of 0.275 which means close to the predicted extraction yield. Therefore, the process optimization could optimize the extraction yield of *Indigofera tinctoria* L using ultrasound-assisted extraction.

3.4 Analysis compounds of Indigofera tinctoria L Dye

To obtain the quality of *Indigofera tinctoria* extract, fourier-transform infrared spectroscopy is required to establish the characteristics of the functional groups and chemical bonds contained of *Indigofera tinctoria* L extract. Table 4 shows the spesific compounds from *Indigofera tinctoria* extract, whereas the presence of *indigo* dye is 64.07%. This study summarizes that ultrasound assisted extraction could be applied for extracting a dyestuff.

Table 4. FT-IR Spectra for *Indigofera tinctoria* L Extract

Similarity	Name of compound
64.07	Indigo, Synthetic
39.72	Penicilin G Potassium in Kbr
35.29	Sucrose in Kbr
34.28	Dexbrompheniramine Maleate in Kbr
31.00	Opium Powder in Kbr
29.60	1,2-Cyclohexanedione Dioxime, 97%
29.45	Diphenylglyoxime, 97%
28.73	2,2,6,6-Tetramethyl-3,5- Heptanedione,98+%
28.62	Methyl Alcohol, 99,9%
28.57	Cellophane

4. Conclusion

Ultrasound-assisted extraction was employed to extract *Indigofera tinctoria* L dye. The extraction yield was maximized at optimized extraction conditions to obtain high extract dye by temperature, F/S ratio, and extraction time. To maximize the extraction yield, BBD was applied for determining the optimum extraction regions. The interaction between factors on the responses can evaluate the parameter effect, however F/S ratio has the most significant effect. Optimal conditions of process extraction were 58°C, 0.02 g/mL, and 50 min. These results have been validated from the actual data yield (5.75%) approached the predicted yield (6.025%) with an acceptable error rate of 0.275. Besides, FT-IR analysis showed the presence of *Indigofera tinctoria* L extract was Indigo dye (64.07%), which is the most percentage from the compounent. Therefore, natural dyes from *Indigofera tinctoria* L could be alternative and sustainable substitute dye for textile industry.

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