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# Extraction Optimization of Pigeon Pea Seed Protein and Yield Using Response Surface Methodology

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**Abstract**— Pigeon Pea (*Casanus Cajan L.*) is one of the local foods in South Sulawesi. Pigeon pea seeds contain protein that the body can absorb. This study aims to determine the optimum operating conditions for extracting pigeon pea on protein and yield using Ultrasonic-Assisted Extraction (UAE). Response Surface Methodology (RSM) with Box-Behnken Design (BBD) was used by considering three variables, time (30, 40, and 50 min), temperature (40, 50, and 60°C), and methanol concentration (50, 70, and 90%). The experiment results show that yield ranges from 8.85663% to 31.4766% and 46.1538 mg/l to 15584.6 mg/l. Response variables were computed and used to create the contour plot graphs. The optimum operating conditions for sonication extraction were a time of 44.760 minutes, a temperature of 60°C, and a 90% methanol concentration.

**Keywords:** *Pigeon Pea; Ultrasonic-Assisted Extraction; Protein; Yield; Response Surface Methodology; Box-Behnken Design*

## I. Introduction

Pigeon Pea (*Casanus Cajan L.*) is one of the local foods in South Sulawesi. The Bugis-Makassar community knows Pigeon Pea as 'Kence' or 'Bintutoeng'. This plant can withstand heat and dryness, so it is suitable as a green plant in dry areas. Local people usually consume it with pigeon pea skin as a cheap vegetable protein because it can grow on dry soil.

According to Asouzu and Umerah [1], pigeon pea seed has 326.20 kcal, carbohydrates 63.78%, ash 2.27%, fiber 0.45%, fat 1.68%, water content 12.81%, and 19.01% protein. The content of dissolved protein is a consideration for consuming grains because the protein can be absorbed by the body [2]. Amino acids are essential for protein synthesis in muscle growth, while

testosterone, an androgen hormone, plays a role in reproduction by increasing libido and spermatozoa creation [3]. Therefore, it is necessary to use it by extracting it from natural materials easily found in the local community, such as pigeon pea seed.

According to Akintayo et al.[4], 21.5% protein was extracted from pigeon pea seed using alkali NaOH at a pH of 8.5. Mizubuti et al.[5] did the same by varying the effect of NaCl on pigeon pea seeds. However, extraction without adding NaCl yielded a higher protein concentration of 75%. Adenekan et al.[6] employed a different technique involving methanol and acetone as solvents. The higher protein content was 91.83% and 91.73 %. In addition to selecting the solvent, selecting the extraction method is also crucial, as it can affect the protein content of the extract. Ultrasonic-assisted extraction (UAE) is one of the extraction methods.

UAE is an extraction method using ultrasonic waves with a higher frequency of 16-20 kHz. Ultrasonic waves have non-destructive and non-massive properties. Extraction with ultrasonic waves and organic solvents on grains will occur more quickly. This is because ultrasonic waves will break the cell walls of the material so that the content in it can come out quickly [7]. UAE has advantages such as being easy to use and safe because it is at atmospheric pressure at room temperature, and the extraction process is fast [8].

Extraction optimization of yield and protein in pigeon pea seed was carried out using Response Surface

Methodology (RSM). RSM is an effective statistical method to optimize the extraction process. RSM can identify various interaction variables by specifying the applicable mathematical equations. Optimizing RSM does not require much data. Optimal response conditions can be obtained quickly and at minimal costs [9]. The design used in this RSM method is Box-Behnken. After obtaining the surface response function, a combination of the existing factors can be sought to produce the expected response [10]. Therefore, in this study, the optimization of pigeon pea seed extraction was carried out with the help of ultrasonic waves using RSM with the Box-Behnken design on the Design Expert software. This study could predict the effect of parameters from temperature, extraction time, and the ratio of methanol concentration to samples in pigeon pea seed using the RSM method as an optimization technique.

## II. Research Methodology

### 2.1 Materials

Dried Pigeon Pea Seeds (Fig. 1) were purchased from the local market. Seeds were ground using a grinder and stored at room temperature until use. Methanol, distilled water, Folin-Ciocalteu reagent, sodium carbonate ( $\text{Na}_2\text{CO}_3$ ), sodium hydroxide ( $\text{NaOH}$ ), Copper Sulfate ( $\text{CuSO}_4$ ), Bovine Serum Albumin (BSA), and Potassium Sodium Tartrate ( $\text{KNaC}_4\text{H}_4\text{O}_6 \cdot 4\text{H}_2\text{O}$ ). Most of the chemicals are used analytical grade and were used without purification.

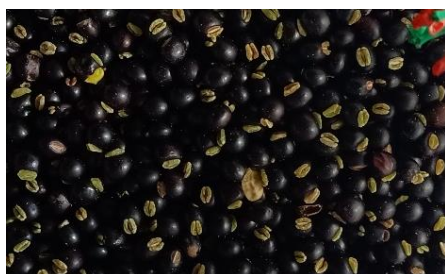


Figure 1. Pigeon Pea Seeds (*Casanus Cajan L.*)

### 2.2 Experimental Design

The effect of four independent variables, X1 (time), X2 (Temperature), and X3 (solvent concentration) at three levels on yield and protein (dependent variable),

were investigated using Box-Behnken Design (BBD) and Response Surface Methodology (RSM). Data were analysed using the software Design Expert 11.

Table 1. Actual and coded levels of the independent variables for BBD

Independent Variable	Code	Treatment Code		
		-1	0	+1
Time (Min)	X <sub>1</sub>	30	40	50
Temperature (°C)	X <sub>2</sub>	40	50	60
MeOH (%)	X <sub>3</sub>	50	70	90

The BBD design assumed that interactions with each other are measured using a second-order polynomial model as below.

$$Y = b_0 + b_1 X_1 + b_2 X_2 + b_3 X_3 + b_{12} X_1 X_2 + b_{13} X_1 X_3 + b_{23} X_2 X_3 + b_{11} X_1^2 + b_{22} X_2^2 + b_{33} X_3^2 + \varepsilon \quad (1)$$

Where Y represents the predicted response value; X<sub>1</sub>, X<sub>2</sub>, and X<sub>3</sub> are the independent variables; b<sub>0</sub> represents the theoretical mean value of the response when all factors are in the level 0; b<sub>1</sub>, b<sub>2</sub>, and b<sub>3</sub> are linear regression coefficients; b<sub>11</sub>, b<sub>22</sub>, b<sub>33</sub> are quadratic regression coefficients; b<sub>12</sub>, b<sub>13</sub>, b<sub>23</sub> are interaction regression coefficients; and ε: is the regression error term.

### 2.3 Extraction of Pigeon Pea Seed and Yield of Extraction

Pigeon Pea Seed (30 g) was extracted for protein with different levels of independent variables (Time 30-50 min, Temperature 40–60 °C, solvent concentration 50-70%) using ultrasonic-assisted extraction methods (Elma Easy Ultrasonic 10 H) with a frequency of 37 kHz. The extracts were concentrated in a rotary evaporator under reduced pressure (Buchi Rotavapor R-100).

The yield (%) of the extraction was evaluated by comparing the weight of pigeon pea seed extract (W<sub>pe</sub>) with the weight of dry pigeon pea seed (W<sub>dp</sub>)

$$\text{Yield (\%)} = (W_{pe}/W_{dp}) \times 100 \quad (2)$$



**2.4 Protein Analysis (Lowry's Method) [11]**

Lowry's reagent A was prepared by mixing Folin Ciocelteau's reagent with distilled water in a ratio of 1:1. Lowry's reagent B was prepared by mixing 50 ml of a solution of 2% Na<sub>2</sub>CO<sub>3</sub> in 0.1 N NaOH with 1 ml of a solution of 1% CuSO<sub>4</sub> and 1% potassium sodium tartrate in distilled water. The protein solution was made using Bovine Serum Albumin (BSA) with concentrations of 30 ppm, 60 ppm, 90 ppm, and 120 ppm.

Place 250 µl of protein solution in a test tube, add 2 ml of Lowry's reagent B, and wait for ten minutes. Then, add 250 µl of Lowry's reagent A, shake, and let stand for twenty minutes. Insert the sample into a spectrophotometer with a 600 nm wavelength and read the sample's absorbance. Absorbance and concentration data are graphed using a linear equation (y= ax+b). The sample undergoes the same process, and the absorbance values are then inserted into the previous equation.

**III. Results and Discussion**

**3.1 Box-Behnken Design Results**

Optimization extraction of yield and protein content from pigeon pea seed was carried out using the Box-Behnken (BBD) design. Table 2 shows the independent variables or factors that influence the extraction in the form of extraction time (minutes), temperature (°C), and solvent concentration (%).

Table 2. Matrix of BBD and results for yield and protein

R u n	Independent Variables			Response	
	X <sub>1</sub>	X <sub>2</sub>	X <sub>3</sub>	Yield (%)	Protein (mg/l)
1	-1	-1	0	8,85663	5430,77
2	1	-1	0	12,976	6330,77
3	-1	1	0	22,651	4892,31
4	1	1	0	31,4766	584,615
5	-1	0	-1	17,9126	1407,69
6	1	0	-1	29,8819	507,692
7	-1	0	1	15,5291	5046,15
8	1	0	1	16,4735	9138,46
9	0	-1	-1	17,2321	5969,23
10	0	1	-1	22,9213	9584,62
11	0	-1	1	14,0179	12046,2
12	0	1	1	16,9803	15584,6
13	0	0	0	19,3278	2892,31
14	0	0	0	16,3139	638,462
15	0	0	0	15,8589	46,1538
16	0	0	0	22,7741	3200
17	0	0	0	11,5115	869,231

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To identify the effect of independent variables from the extraction process using the sonication method, an analysis that functions as a response was carried out, such as yield and lowry method protein analysis. The experiment results show that yield ranges from 8.85663% to 31.4766% and 46.1538 mg/l to 15584.6 mg/l. Different responses confirm a relationship between independent variables that can affect the extraction results.

**3.2 Analysis Yield**

Table 3 displays the Analysis of Variance (ANOVA) results for the extract yield response. The p-value is 0.0030, which is below 0.05. Hence the linear model is statistically significant.

Table 3. Statistical Analysis of Variance (ANOVA) yield

Source	Sum of Squares	df	Mean Square	F-value	p-value	
<b>Model</b>	370.96	3	123.65	7.88	0.003	<i>Significant</i>
X <sub>1</sub>	83.58	1	83.58	5.33	0.038	
X <sub>2</sub>	209.58	1	209.58	13.4	0.0029	
X <sub>3</sub>	77.79	1	77.79	4.96	0.0442	
<b>Residual</b>	203.91	13	15.69			
<i>Lack of Fit</i>	133.38	9	14.82	0.84	0.6215	<i>Not Significant</i>
<i>Pure Error</i>	70.53	4	17.63			
<b>Cor Total</b>	574.87	16				

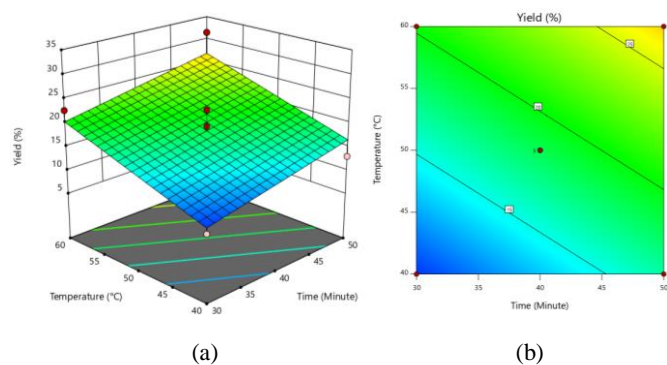


Figure 2. Effect of Time (minutes) and Temperature (°C) at 70% concentration on yield (a) 3D Surface plot, (b) Contour plot

The model selection is based on the adjusted R<sup>2</sup> coefficient, where the linear model's value of 0.5634 is greater than the quadratic model's value of 0.5376. The p-values for the independent variables, such as time,

temperature, and concentration, are 0.038, 0.0029, and 0.0442, respectively, and  $p < 0.05$ . These values indicate that the yield of the extract is affected by time, temperature, and concentration.

The Figure 2 shows a linear surface. High temperatures increase the mass transfer coefficient of a component, hence accelerating the movement of particles toward the solvent [12]. The increase in time and temperature will affect the yield, resulting in a higher yield. It is found at a time of 50 minutes and a temperature of 60 °C with a yield percentage of 31.4766%. At a temperature of 40 °C and a time of 30 minutes, the yield is low, 8.85663%.

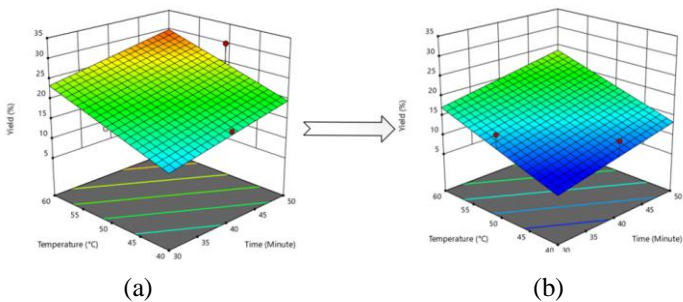


Figure 3. 3D Surface plot Effect of time (minutes) and temperature (°C) on yield (a) at 50% MeOH, (b) at 90% MeOH

The rise in concentration, however, was inversely proportionate to the amount of extract produced. In Figure 3, the surface of the graph will decrease in direct proportion to the concentration rise. At 30 minutes and a temperature of 40°C, the 50% concentration produced more extract than the 90% concentration, 13.22%, and 7.08%, respectively. The type of substance extracted is dependent on the methanol solvent's concentration. In addition, an increase in the water content in the solvent will cause the sample to swell to increase the contact between the sample matrix and the solvent, which contributes to an increase in sample yield [13].

$$\text{Yield (\%)} = 18,3938 + 3,23234X_1 + 5,11831X_2 - 3,11839X_3 \quad (3)$$

Equation 1 shows the linear equation of the extract yield. This equation can predict the conditions of the extraction process to produce the optimum yield of pigeon pea seed extract. The coefficients on time,

temperature, and concentration, respectively, are 3.23234, 5.11831, and -3.11839. The coefficient value for the temperature and time parameters is positive, indicating that an increase in temperature and time can result in an increased extract yield. On the other hand, a negative concentration coefficient gives opposite results.

### 3.3 Analysis of Protein

Based on table 3, the quadratic model is a significant model with a p-value of 0.0126 for the analysis of protein content. The model selection is based on the  $R^2$  coefficient, which has a value of 0.8884 compared to other models, such as the linear model with an  $R^2$  value of 0.2293. The time, temperature and concentration factors have p-values respectively 0.9742; 0.8962; and 0.0068.

Table 4. Statistical Analysis of Variance (ANOVA) protein

Source	Sum of Squares	df	Mean Square	F-value	P-value	Info
<b>Model</b>	2,875E+08	9	3,194E+07	6,19	0,0126	<i>Significant</i>
$X_1$	5798,73	1	5798,73	0,0011	0,9742	
$X_2$	94435,74	1	94435,74	0,0183	0,8962	
$X_3$	7,409E+07	1	7,409E+07	14,37	0,0068	
$X_1X_2$	6,780E+06	1	6,780E+06	1,31	0,2892	
$X_1X_3$	6,231E+06	1	6,231E+06	1,21	0,3081	
$X_2X_3$	1481,07	1	1481,07	0,0003	0,9870	
$X^2_1$	1,676E+07	1	1,676E+07	3,25	0,1144	
$X^2_2$	9,603E+07	1	9,603E+07	18,62	0,0035	
$X^2_3$	8,493E+07	1	8,493E+07	16,47	0,0048	
<b>Residual</b>	3,610E+07	7	5,157E+06			
<i>Lack of Fit</i>	2,802E+07	3	9,341E+06	4,63	0,0866	<i>Not Significant</i>
<i>Pure Error</i>	8,078E+06	4	2,020E+06			
<b>Cor Total</b>	3,236E+08	16				

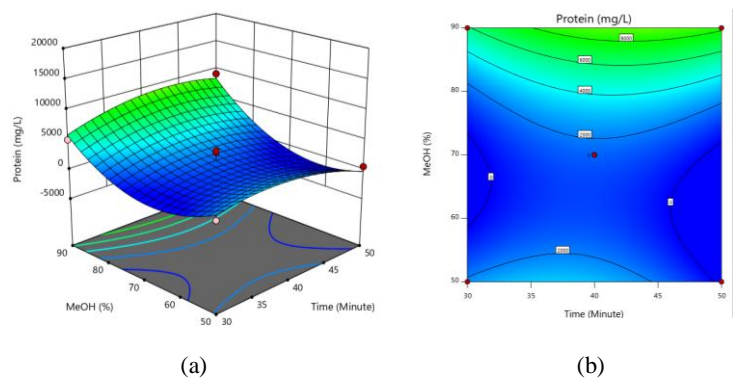


Figure 4. Effect of MeOH (%) and time (minutes) at 50°C on protein (a) 3D Surface plot, (b) Contour plot



The lack of fit is a test value that checks the linear relationship between the independent and dependent variables. The p-value on the lack of fit above 0.05, which is 0.0866, indicates that it is not significant, therefore the model used is appropriate and has a linearity relationship.

Figure 4 depicts the impact of time and temperature on the effect of dissolved protein in a sample. The amount of protein in the sample is affected by the rise in solvent concentration. The sample contains a protein extract between 6000 and 8000 mg/l with a methanol concentration of 90%. At 50 minutes and a concentration of MeOH 90%, the protein content in the sample was 9138.46 mg/l, while at the time of 30 minutes with the same concentration, the dissolved protein was 5046.15 mg/l.

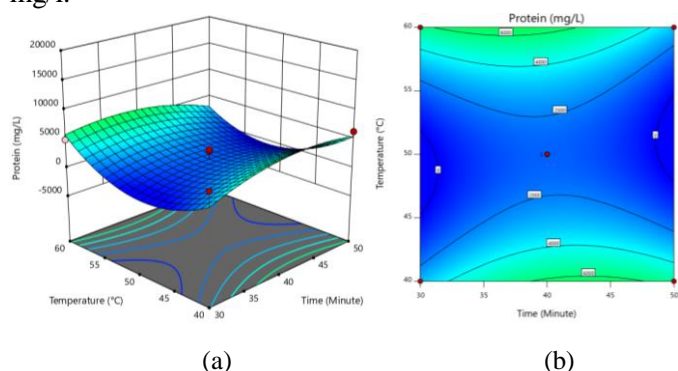


Figure 5. Effect of Temperature (°C) and time (minutes) at concentration MeOH 70% on protein (a) 3D Surface plot, (b) Contour plot

In addition to time and concentration, the temperature can also affect the amount of protein in the sample. Long-term exposure to high temperatures will denature the protein, reducing the sample's protein concentration [14]. In Figure 5, there was a decrease of 2657.69 mg/l protein content at a temperature of 60 °C and a time of 50 minutes. On the other hand, at 40 °C and 50 minutes, there was an increase of 2552 mg/l.

$$\text{Protein (mg/l)} = 1529,23 - 26,9229X_1 + 108,648X_2 + 3043,27X_3 - 1301,92X_1X_2 + 1248,08X_1X_3 - 19,2423X_2X_3 - 1995,39X_1^2 + 4775,78X_2^2 + 4491,16X_3^2 \quad (4)$$

Equation 2 shows the quadratic equation of the protein. This equation can predict the conditions of the extraction process to produce the optimum protein extract of pigeon pea seed. The time, temperature, and concentration coefficients are -26,9229, 108,648, and 3043.27, respectively. The coefficient values for the temperature and concentration components have positive values, indicating that an increase in temperature and concentration can result in a large protein concentration. On the other hand, a negative time coefficient value suggests that a more extended period will result in a lower protein concentration.

### 3.4 Optimization of Extraction Conditions

Time, temperature, and concentration are optimized in 30-50 minutes, 40-60°C and 50-90%. Protein and yield are optimized responses with maximum targets but have different levels of importance. The protein response has a high importance level, 5 (+++++), while the yield has a 3 (+++) importance level. This is because the protein content in the extract is a crucial consideration compared to yield.

Table 4. Optimized Response Components, Goal, Limits, and Importance at the Formula Optimization stage

Name	Goal	Lower Limit	Upper Limit	Importance
A: Time	is in range	30	50	+++
B: Temperature	is in range	40	60	+++
C:MeOH (%)	is in range	50	90	+++
Yield	maximize	8,85663	31,4766	+
Protein	maximize	46,1538	15584,6	+++++

Table 5. Formulas result in the optimization stage

No	Time (Min)	Suhu (°C)	MeOH (%)	Yield	Protein	Desirability
1	44.760	60.000	90.000	21.932	13438.3	0.742
2	46.160	60.000	90.000	22.385	13121.8	0.740
3	44.469	60.000	89.767	21.875	13348.5	0.738

Based on the optimization process, there are three optimum operating conditions. Process conditions with a time of 44,760 minutes, a temperature of 60 °C, and a

concentration of 90% methanol are recommended as the optimal formula solution because this process condition has a high desirability value of 0.742. The desirability value is a value to determine the degree of accuracy of the optimal solution [15]. The desirability value is closer to one, the higher the accuracy value. The operating conditions in formula number 1 have yield and protein response values, 13438.3 mg/l and 21.932%. Formula 1 has a higher value in protein, so the optimal operating conditions for extraction of pigeon pea seed are at 44,760 minutes, a temperature of 60 °C, and a methanol concentration of 90%.

#### IV. Conclusion

Based on the results obtained in this study, the following conclusions were obtained:

1. The experiment results showed that the yield ranged from 8.85663-31.4766%, and protein had a range of 46.1538-15584.6 mg/l. Different responses confirm a relationship between independent variables that can affect the extraction results.
2. Optimal operating conditions for extraction based on yield and protein response in pigeon pea seed extract were at a time of 44,760 minutes, a temperature of 60 °C, and a MeOH concentration of 90% methanol with a desirability of 0.742.

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