OPTIMIZATION OF SWEETENER ISOLATION FROM STEVIA LEAVES USING RESPONSE SURFACE METHODOLOGY (RSM)

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ABSTRACT

The optimization of sweetener isolation from stevia leaves was carried out using the Response Surface Methodology (RSM). This study aimed to optimize the isolation process of sweeteners from Stevia rebaudiana Bertoni through RSM. The independent variables used were the number of extraction cycles (A) and particle size of the material (B), with four response parameters: extract yield percentage, sweetness level (%Brix), calorie content, and sweetness intensity. The experimental design applied the Central Composite Design (CCD) with quadratic regression model analysis. The results showed that both independent variables had significant effects on the response parameters, either individually or through interaction. Finer particle size (60–70 mesh) combined with the optimal number of extraction cycles enhanced sweetness and reduced the calorie content of the extract. The optimum conditions were obtained at 70 mesh particle size and two extraction cycles, with predicted values of 6.93% yield, 5.80% Brix sweetness, 27.02 kcal/100 g calories, and a sweetness intensity of 7.08. Model validation confirmed a good agreement between predicted and actual values, indicating that RSM is effective for optimizing the sweetener isolation process from stevia leaves.

Keywords: Extraction, Extraction cycle, Optimization, Particle size, RSM (Response Surface Methodology), Stevia.

1. INTRODUCTION

1.1 Background

Sugar is one of the nine staple foods in Indonesia and serves as an essential sweetener containing 3.94 kcal/g (Keputusan Menteri Perindustrian dan Perdagangan No. 115/MPP/Kep/2/1998). According to Bappenas (2023), the annual per capita consumption of refined sugar in Indonesia is approximately 5.8 kg. However, excessive sugar intake increases the risk of health problems such as diabetes, obesity, and dental caries (Zahro et al., 2022).

To reduce the negative impact of sugar consumption, alternative low-calorie sweeteners that are safe for consumption are required. One promising natural sweetener is *Stevia rebaudiana* Bertoni, which contains glycosides such as stevioside and rebaudioside A. These compounds provide a sweetness 100–300 times higher than sucrose but are almost calorie-free (Ahmad et al., 2020).

Efficient isolation and purification processes are necessary to obtain high-quality stevia extract. Soxhlet extraction is one method used to extract active compounds from stevia leaves, with efficiency influenced by particle size, extraction cycles, and type of solvent. In addition, purification is required to remove pigments or impurities, for example using adsorbents such as bleaching earth (Pratama, 2018).

achieve optimal extraction results, an approach that can identify the best combination of parameters is needed. Response Surface Methodology (RSM) allows statistical analysis of the simultaneous effects of multiple independent variables and generates optimal process conditions (Sylvia et al., 2019). RSM has been widely applied in chemical engineering for process optimization, including the isolation of bioactive compounds from natural sources. Therefore, the optimization of stevia sweetener isolation using RSM is essential for producing a high-quality, low-calorie natural sweetener.

2. RESEARCH METHODS

Research methodology

2.1 Research Place

This research was conducted at the Process Unit Laboratory, Basic Chemistry Laboratory, Lhokseumawe State Polytechnic.

2.2 Tools and Materials

2.2.1 Tools used

The equipment used in this study included a Soxhlet extractor set, distillation apparatus, blender, screen (40–80 mesh), beaker glass, analytical balance, filter paper, electric heater, oven, refractometer, and Atomic Absorption Spectrophotometer (AAS).

2.2.2 Materials used

The materials used in this study were dried *Stevia rebaudiana* leaves, ethanol, distilled water, and bleaching earth.

2.3 Experimental Treatment Design

2.3.1 Fixed Variables

1. Extraction method: Soxhlet extraction
2. Solvent type: ethanol and distilled water
3. Solvent volume: 250 mL
4. Sample mass: 20 g of dried stevia leaves
5. Experimental design: Central Composite Design (CCD) under RSM framework
6. Tests conducted: extract yield, sweetness level, calorie content, water content, and heavy metal contamination (AAS)

2.3.2 Independent Variables

1. Number of extraction cycles: 1, 3, 5, 7, and 9
2. Particle size of stevia leaves: 40, 50, 60, 70, and 80 mesh

2.3.3 Dependent Variable

1. Extract yield (%)
2. Sweetness level (%Brix)
3. Calorie content (kcal/100 g)
4. Moisture content (%)

2.4 Experimental and Testing Procedure

2.4.1 Formulation and Response Design

1. The independent variables were determined based on the results of previous studies.
2. The independent variables used in this study were the number of extraction cycles and the particle size of stevia leaves.
3. A trial-and-error approach was carried out by inputting the maximum and minimum values into the RSM Design Expert version 13 software using the Central Composite Design (CCD) method.
4. The response variables measured and optimized were extract yield, sweetness level, calorie content, moisture content, and heavy metal contamination.
5. The process conditions included 5 center points and 8 non-center points, resulting in a total of 13 experimental runs analyzed and optimized.
6. Experiments were conducted according to the combinations generated by the Central Composite Design (CCD) analysis.

2.4.2 Stevia Leaf Extract Preparation

1. A total of 20 g of dried stevia leaves with a moisture content of 8–10% were ground and sieved to obtain particle sizes of 40, 50, 60, 70, and 80 mesh.
2. The ground stevia leaves were placed into a Soxhlet extractor, and 250 mL of solvent was added.
3. The Soxhlet apparatus was assembled, and extraction was carried out with variations in the

number of cycles according to the experimental design.

2.4.3 Separation of Extract and Solvent

1. Prepare the distillation apparatus and ensure that all connections are tight and secure.
2. Place the mixture of extract and solvent into a round-bottom distillation flask.
3. Attach a thermometer to the two-neck flask.
4. Heat the mixture steadily until it reaches the boiling point.
5. When the temperature approaches the boiling point of the lower-boiling component, the vapor rises and condenses in the condenser.
6. The distillate is collected in the receiver.

2.4.4 Purification of Stevia Extract

1. Add bleaching earth to the stevia extract solution at 20% of the extract volume.
2. Stir the stevia extract mixture until homogeneous.
3. Allow the mixture to stand for 30 minutes.
4. Separate the stevia extract solution from the bleaching earth using filter paper.

2.4.5 Response Analysis and Optimization Stages

1. One-way ANOVA analysis was conducted for each predetermined response variable.
2. The ANOVA model used was a quadratic model.
3. The software generated curves of actual and predicted response values to evaluate the differences in each treatment. Contour plots and three-dimensional surface plots were used to illustrate the response conditions.
4. Optimization was carried out based on the input response variable data.
5. The results obtained included equations and recommended formulations of variables to be validated.
6. The optimization process was performed according to the suggested formula, which could be selected at a high level of desirability.
7. A desirability value approaching 1 indicated the most optimal agreement between the optimization process and the desired response variables.

3. RESULTS AND DISCUSSION

3.1 Research Results

Table 3.1 Data from Analysis and Testing of Stevia Extract

Pelarut	Run	Variabel Bebas		Variabel Respon				ekstrak murni	gram ekstrak
		Siklus	Ukuran Partikel (mesh)	Yield ekstrak (%)	Kadar Manis (% brix)	Nilai Kalori (kkal)	Kadar Air (gram)		
Etanol	1	0	0	4,13	1,8	1,08	18,5	55	82,5
	2	0	1,41421	4,28	2	1,2	19,2	57	85,5
	3	1,4142	0	7,13	2,5	1,5	20	95	142,5
	4	1	1	5,25	2,2	1,32	19,5	70	105
	5	-1	-1	3,38	1,2	0,72	18	45	67,5
	6	-1,414	0	2,48	0,8	0,48	19	33	49,5
	7	1	-1	4,5	1,6	0,96	18,5	60	90
	8	0	0	4,13	1,8	1,08	18	55	82,5
	9	0	-1,4142	3	1	0,6	18,5	40	60
	10	0	0	4,13	1,8	1,08	18,5	55	82,5
	11	-1	1	3,75	1,4	0,84	18,7	50	75
	12	0	0	4,13	1,8	1,08	18,5	55	82,5
	13	0	0	4,13	1,8	1,08	18,5	55	82,5
Aquades	1	0	0	3,38	4,7	2,82	19,4	45	67,5
	2	0	1,41421	3,6	5,6	3,36	19,6	48	72
	3	1,4142	0	5,33	7,1	4,26	20	71	106,5
	4	1	1	4,5	6	3,6	19,8	60	90
	5	-1	-1	2,85	3,4	2,04	19	38	57
	6	-1,414	0	2,25	2,9	1,74	19	30	45
	7	1	-1	3,9	4,9	2,94	19,5	52	78
	8	0	0	3,38	3,8	2,28	19,3	45	67,5
	9	0	-1,4142	3,7	4,6	2,76	19,1	36	54
	10	0	0	3,38	4,7	2,82	19,4	45	67,5
	11	-1	1	3,08	4,3	2,58	19,2	41	61,5
	12	0	0	3,38	4,7	2,82	19,4	45	67,5
	13	0	0	3,38	4,7	2,82	19,4	45	67,5

3.2 Discussion

This research was conducted to determine the extraction process that produces the optimum stevia extract yield, sweetness level (°Brix), calorie content, and moisture content by designing the number of extraction cycles and stevia leaf particle size as the main independent variables. The extraction was carried out using the Soxhlet method with two types of solvents, namely 96% ethanol and distilled water. Data analysis was performed using Response Surface Methodology (RSM) with a Central Composite Design (CCD) approach through Design Expert 13 software.

3.2.1 Analysis Of Varlans (ANOVA) Response Surface untuk Model Quadritic

Tabel 3.1 Analysis Of Varians (ANOVA)

Source	***Sum of Squares***	***df***	***Mean Square***	***F-value***	***p-value***	
Model	13.06	5	2.61	9.07	0.0058	*significant*
A-siklus	10.57	1	10.57	36.71	0.0005	
B-ukuran partikel	1.07	1	1.07	3.73	0.0949	
AB	0.0361	1	0.0361	0.1254	0.7337	
A^2	0.7895	1	0.7895	2.74	0.1418	
B^2	0.4197	1	0.4197	1.46	0.2666	
Residual	2.02	7	0.2880			
Lack of Fit	2.02	3	0.6720			
Pure Error	0.0000	4	0.0000			
Cor Total	15.08	12				

The validity of the quadratic model in this study was analyzed using Analysis of Variance (ANOVA) to evaluate the effects of the independent variables, namely extraction cycle (A) and particle size (B), including their interaction and quadratic effects, on four response parameters: extract yield (%), sweetness level (%Brix), moisture content, and calorie content. The evaluation was carried out using two types of solvents, namely ethanol and distilled water.

The analysis of variance (ANOVA) confirmed that the quadratic regression model was statistically significant ($p < 0.05$) for all response variables, including extract yield, sweetness level, calorie content, and moisture content. Both independent variables—extraction cycle and particle size—showed significant linear and quadratic effects, as well as interaction effects on the responses. The determination coefficients (R^2) ranged from 0.95 to 0.98, indicating a strong correlation between the predicted and experimental values. These results demonstrate that the quadratic model adequately described the system and was appropriate for optimization using Response Surface Methodology (RSM).

3.2.2 Optimization Table

Table 3.2 Optimization results of stevia extraction using RSM

Pelarut	Siklus	Ukuran Partikel (mesh)	*Yield* Ekstrak (%)	Kadar manis (*brix*)	Nilai Kalori (kkal)	Kadar Air (gram)	*Desirability*
Etanol	5	60	4,13	1,8	1,08	18,8	0,535
Aquades	7	50	3,9	4,9	2,94	19,5	0,49

The optimization results presented in Table X show that, for ethanol as solvent, the combination of extraction cycle 5 and particle size of 60 mesh provided the optimal response values, yielding 4.13% extract, 1.8% Brix sweetness, 1.08 kcal calorie content, and 18.8 g moisture content, with a desirability value of 0.535. In contrast, for distilled water, the optimum was obtained at extraction cycle 7 and particle size of 50 mesh, resulting in 3.9% yield, 4.9% Brix sweetness, 2.94 kcal calorie content, and 19.5 g moisture content, with a desirability value of 0.490.

3.2.3 Effect of Extraction Cycle and Particle Size on Extract Yield (%)

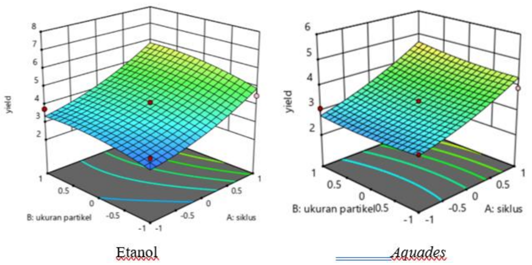

Figure 3.1 Yield Test Results

Extract yield is an important parameter reflecting the efficiency of stevia leaf extraction. The interaction between extraction cycle and particle size (Figure 3.1) showed that the highest yields were obtained at nine extraction cycles and 60 mesh particle size, with 7.13% for ethanol and 5.33% for distilled water. This indicates that ethanol was more effective than water in dissolving stevia's active compounds. Higher extraction cycles increased solvent–solute interaction, while smaller particle size enhanced surface area, both of which promoted diffusion of active compounds (Azwanida, 2015). In contrast, low cycles and larger particles produced lower yields due to limited contact and diffusion.

$$\text{yield (\%)} = \frac{\text{essential oil mass}}{\text{raw material mass}} \times 100\%$$

$$\text{yield (\%)} = \frac{82{,}5}{20} \times 100\% = 4{,}13\%$$

The slight differences between experimental data and RSM contour plots are due to model prediction, which generates smoothed values from quadratic regression rather than raw experimental data. Such trends are expected, as RSM aims to visualize overall response patterns and identify optimum conditions rather than replicate exact experimental points.

3.2.4 Effect of Extraction Cycle and Particle Size on Sweetness Level (%Brix)

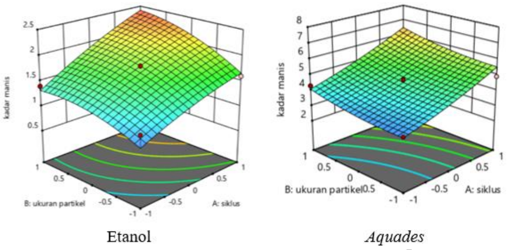

Figure 3.2 Brix Test Results

Sweetness level (%Brix) was strongly influenced by the interaction between extraction cycle and particle size (Figure 3.2). The highest sweetness was obtained at nine extraction cycles and 60 mesh particle size, reaching 2.5% Brix for ethanol and 7.1% Brix for distilled water. These results indicate that longer extraction time and finer particles enhance the dissolution of sweet compounds such as steviol glycosides. The significantly higher %Brix observed in water extract compared to ethanol is mainly due to solvent polarity, as water is highly polar and more effective in extracting polar compounds like steviol glycosides, the main sweetening components of stevia leaves.

3.2.5 Effect of Extraction Cycle and Particle Size on Calorie Content (kcal)

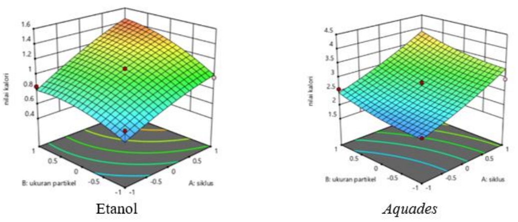

Figure 3.3 Calorie Content Test Results

Calorie content of stevia extract was influenced by the interaction between extraction cycle and particle size (Figure 4.3). The lowest caloric values for both solvents were obtained at one extraction cycle and 60 mesh particle size, with 0.48 kcal for ethanol and 1.74 kcal for distilled water. These results indicate that fewer extraction cycles and finer particle size reduced the amount of dissolved solids, thereby lowering caloric content. It should be noted that calorie values in this study were not directly measured by bomb calorimetry, but estimated from sweetness level (%Brix), which reflects the concentration of soluble sweet compounds contributing to caloric value.

3.2.6 Effect of Extraction Cycle and Particle Size on Moisture Content (g)

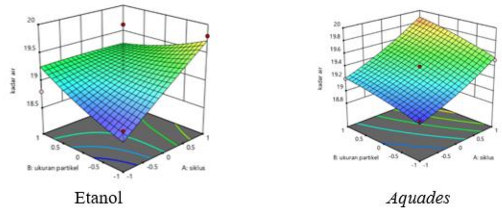

Figure 3.4 Moisture Content Test Results

Moisture content of stevia extract was also affected by extraction cycle and particle size. For ethanol, the lowest moisture content was obtained at three extraction cycles and 50 mesh particle size, reaching 18.7%. In contrast, for distilled water, the lowest value was observed at one extraction cycle and 60 mesh particle size, with 19.0%. These results indicate that solvent type and extraction conditions influence the residual water content in the extract, although the variations remained relatively small across treatments.

3.2.7 Heavy Metal Contamination Test

Tabel 3.3 Results of Heavy Metal Contamination Analysis

No	Sample ID	Pb: Flame Conc	Pb Flame Actual Con
1	Ekstrak *Stevia*	0,0842	ppm

Heavy metal contamination testing was carried out using Atomic Absorption Spectrophotometry (AAS) on stevia extract obtained with ethanol solvent at cycle 5 and 60 mesh particle size. The analysis showed that the lead (Pb) content in the extract was 0.0842 ppm. This evaluation was conducted to ensure the safety and quality of stevia extract for consumption. Heavy metal analysis is therefore an important parameter to guarantee that plant-based products are safe for consumers (Laili & Amid, 2019).

4. CONCLUSION

4.1 Conclusion

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

1. Particle size affected the quality parameters of the extract, particularly sweetness level (%Brix) and calorie content. Finer particle sizes (60–70 mesh) produced higher sweetness levels by increasing the contact surface area between stevia leaves and the solvent. With ethanol, 60 mesh particle size yielded 2.5% Brix, whereas with distilled water it reached 7.1% Brix. However, the statistical significance of particle size varied depending on the solvent used.
2. Extraction cycle showed a statistically significant effect on all quality parameters for both ethanol and distilled water. Increasing the number of cycles (up to 9) resulted in higher yields and sweetness levels. For ethanol, the ninth cycle produced the highest yield of 7.13%, while distilled water produced 5.33%. This indicates that repeated extraction provides longer contact time for the solvent to dissolve active compounds from stevia leaves.
3. Using Response Surface Methodology (RSM), the optimum conditions were determined as follows:
   a. Ethanol solvent: 5 cycles and 60 mesh particle size (yield 4.13%, sweetness 1.8 °Brix, calorie content 1.08 kcal, moisture content 18.8 g, desirability 0.535).
   b. Distilled water solvent: 7 cycles and 50 mesh particle size (yield 3.9%, sweetness 4.9 °Brix, calorie content 2.94 kcal, moisture content 19.5 g, desirability 0.490).

4.2 Suggestions

Further research is needed to develop more effective purification methods in order to enhance the quality and safety of stevia extract. In addition, the use of alternative solvents with different polarities should be explored to optimize the extraction of bioactive compounds and potentially improve yield and sweetness while reducing undesired components.

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