JURNAL REKAYASA PROSES
VOLUME 18, NUMBER 2, 2024, 109–117

RESEARCH ARTICLE

Enzymatic saccharification of liquid sugar from cassava peel waste (*Manihot esculenta*): Optimization and characteristics

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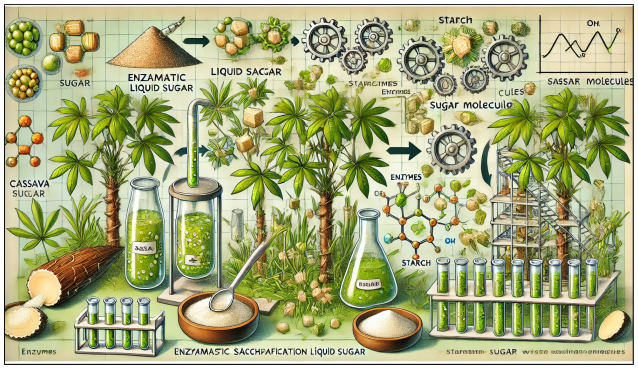
Received 01 June 2024; revised 06 September 2024; accepted 09 September 2024



OBJECTIVES The province of Lampung produced 2.6 million tons of cassava and 0.28 million tons of inner cassava peel waste in 2020, highlighting the direct correlation between production volume and waste generation. The inner cassava peel waste, which contains 44-59% starch, can be utilized as a raw material for producing liquid sugar. METHODS This study employs Response Surface Methodology (RSM) to optimize the saccharification process by varying the duration (2, 4, and 8 hours) and temperature (55, 60, and 65°C). The processes of liquification and saccharification were used to enzymatically convert cassava peel into liquid sugar. RESULTS The study found that the starch yield from cassava peels was 11.54%, with moisture, ash, starch, and crude fiber contents measured of 13.53%, 0.61%, 88.32%, and 1.025%, respectively. The saccharification of cassava peel starch yields 58.36% liquid sugar. The moisture and ash contents were measured of 16.95% and 0.11%, respectively, with the reducing sugar content of 58.07%. This study successfully optimized the saccharification process for liquid sugar production from cassava peel starch at a temperature of 65°C for 6 hours. CONCLUSIONS These optimized conditions resulted in a higher yield of liquid sugar from cassava peel starch, emphasizing the potential of cassava peels as a valuable resources for liquid sugar production.

KEYWORDS α-amylase; cassava peel waste; glucoamylase; hydrolysis by enzymes; liquid sugar

1. INTRODUCTION

Manihot esculenta or cassava peel waste is an indigenous food item rich in carbohydrates. Lampung Province has emerged as one of Indonesia leading cassava-producing regions, contributing an estimated 30% of the nation cassava output. In 2020, Indonesia was ranked among the top five cassava producers globally, with a total production of 18.3 million tons (Teguh et al. 2022). The waste generated from these industries is directly correlated with the high output value, and cassava peel is one of the primary waste materials produced. Based on the research conducted by Syahrir I., M. Syahrir (2017) has reported that the inner peel waste of cassava constitutes approximately 8–15% of the total waste, while the outer peel waste constitutes between 0.5% and 2% of the overall weight of the crop. Consequently, Lampung Province generates approximately 0.28 million metric tons of inner cassava peel waste annually.

The waste generated from the inner layers of cassava peels contains 44-59% carbohydrates, 7.9–10.32% moisture, 1.5–3.7% protein, 0.8–2.1% fat, and 5-27.4% fiber (Syahrir I., M. Syahrir 2017). The high starch content in these peels presents a significant opportunity for their use as a raw material in producing liquid sugar. Additionally, Indonesia is one of the countries that continues to rely heavily on sugar imports, as domestic production has not met the national demand. The country imports approximately 5.6 million metric tons of sugar to satisfy its needs. In 2020, the production of white crystal sugar decreased by 4.52%, further emphasizing the need for alternative sources of sugar. Utilizing inner cassava peels as a raw material for liquid sugar production could help reduce the nation dependence on sugar imports, given the significant starch content in this waste and the pressing need for sugar.

Liquid sugar, widely used as a sweetener in the food and beverage industry, is more soluble compared to sucrose, especially when derived from starch (Godefroidt et al. 2023; Mukarramah et al. 2016). D-glucose, maltose, and polymer molecules of D-glucose are the byproducts of the enzymatic or acid hydrolysis of starch, resulting in the production of liq-

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uid sugar (Jenol et al. 2023; Suripto et al. 2013). The hydrolysis of starch can be achieved using enzymes like glucoamylase and α-amylase. A recent study conducted by Agustina et al. (2024) has reported that the enzyme hydrolysis can produce reducing sugar of 29.668 ± 0.761% in 40% substrate and 60 min liquification and saccharification time. This method yields a higher sugar content compared to acid hydrolysis because it breaks the polymer chain more precisely to create the desired product. Additionally, it produces less ash and fewer by-products, making it safer and more environmentally friendly (Abolore et al. 2024; Gnanasekaran et al. 2023; Martiyana 2022; Sutamihardja et al. 2017). High temperatures are necessary for the gelatinization process, allowing enzymes to effectively hydrolyze amylose and amylopectin (Li et al. 2024; Obadi et al. 2023; Zhong et al. 2022). Enzymes typically function best within a pH range of 5.6 to 6.5 (Dura et al. 2014; Hua and Yang 2015). Furthermore, the α-amylase enzyme, which is utilized to produce dextrin from tapioca, operates within a concentration range of 0.05% to 0.25% and has a hydrolysis duration of 30 to 150 minutes (Handayani et al. 2023). Temperature and reaction duration are two critical variables that significantly influence the efficiency of enzyme activity during starch hydrolysis. Optimal conditions for enzyme function typically include a longer reaction time and a higher temperature. However, enzyme activity declines once it surpasses its optimal level. Therefore, determining the ideal enzymatic reaction duration and temperature is essential, as the results can serve as guidelines for producing liquid sugar (Trithavisup 2020).

The aims of this study is to optimize the saccharification process of liquid sugar production from cassava peel starch using Response Surface Methodology (RSM), with a focus on identifying the ideal conditions for enzymatic hydrolysis. Specifically, the research investigates the effects of two critical variables temperature (55, 60, and 65°C) and saccharification duration (2, 4, and 6 hours) on the efficiency of liquid sugar production. By determining the optimal combination of these variables, the study seeks to enhance the yield and quality of liquid sugar, thereby providing a sustainable solution for reducing Indonesia dependence on sugar imports and adding value to cassava peel waste.

2. RESEARCH METHODOLOGY

2.1 Materials

The apparatus used in this study included a 500 mL glass beaker, a 250 mL Erlenmeyer flask, a 100 mL flask, a 25 mL burette, a 100 mL measuring cup, a 10 mL measuring pipette, a thermometer, a centrifuge, an oven, a furnace, a porcelain cup, and an analytical balance. Additionally, the materials utilized in this study comprised distilled moisture, α-amylase, glucoamylase, solution A (i.e. potassium sodium tartarate ($NaKC_4H_4O_6.4H_2O$), sodium phosphate ($Na_3PO_4.12H_2O$), copper (II) sulfate pentahydrate ($CuSO_4.5H_2O$) and potassium iodate (KIO_3)), solution B (i.e. potassium oxalate ($K_2C_2O_4.H_2O$), potassium iodine (KI)), and C (i.e. sulfuric acid (H_2SO_4), as well as starch solutions. The inner cassava peel was sourced from a local home industry in the Natar sub-district, South Lampung Indonesia, which specializes in producing cassava chips.

2.2 Preparing cassava peel for starch production

The clean inner peel of the cassava was ground using a blender (Panasonic, Thailand) to achieve a smooth consistency with a particle size of 80 mesh. The mixture was then squeezed through a filter cloth and allowed to settle, enabling the starch to separate from the moisture above. The separated starch dried in the sun until completely dry. The resulting starch was tested for its moisture content, ash content, starch content, and fiber content.

2.3 Producing liquid sugar by combining saccharification time and temperature

There are two primary steps in the enzymatic process of producing liquid sugar from cassava peel, such as saccharification and liquefaction. Reaction mechanisms of saccharification and liquifications, as presented below:

$$(C_6H_{10}O_5)_n + nH_2O \xrightarrow{\alpha\text{-amylase}} \text{Dextrins + Maltose (Saccharification)}$$

$$\text{Dextrins} + {}_nH_2O \xrightarrow{\text{Glucoamylase}} \text{Glucose (Liquification)}$$

The starch suspension, a heated mixture of starch and moisture, undergoes gelatinization. The mixture is homogenized at 60°C using a water bath with a 3:1 moisture-to-starch ratio (Prio DWB-6H-22L). A quantity of Novozymes α-amylase enzyme (1 mL/kg of starch) is introduced. If the solution appears liquid and clear brown in color after 30 minutes of heating on a hot plate (Thermo), the process can be terminated, and Dextrin is produced because of starch breakdown. After cooling the sample to 50°C, 1 mL/kg of starch of the glucoamylase enzyme (Liquozyme Supra, Denmark) is added. This enzyme further breaks down the chains of dextrin into glucose.

The experimental design of this inquiry utilized Design Expert Version 13 and Response Surface Methodology (RSM), specifically Central Composite Design (CCD). This study identified temperature and time as independent variables associated with the saccharification process. The dependent variable in this study is the reduction of sugar content. The research examines two factors: optimal hydrolysis times (2, 4, and 6 hours) and temperatures (55, 60, and 65°C). The experimental design is presented in Table 1.

To optimize the process, Design Expert software was employed, setting the goals to "in range" for each combination of temperature and time. The output aimed to maximize the reduction of sugar content, with specific goals for each experimental run determined by the input conditions. The use of CCD in RSM allowed for an efficient exploration of the relationship between the variables and the response, ensuring a thorough investigation of the optimal conditions for the saccharification process.

2.4 Examining liquid starch and sugar properties

The tests for moisture, ash, starch, and fiber content comply with the SNI 01-2891-1992 protocol. Starch-based products are assessed for their moisture, ash, starch, and fiber contents, while products containing liquid sugar are evaluated for their moisture, ash, reducing sugar, and organoleptic properties.

2.4.1 Moisture content

Weigh 2 grams of the sample, which was then baked for 3 hours at 105°C in an oven (Kern, Germany) (Agustina et al. 2024; Herlambang et al. 2023). After baking, the sample was allowed to cool for 15 to 30 minutes in a desiccator (Duran, Germany). To calculate the moisture content, refer to Equation 1, where W, W_1, and W_2 represent the mass of the empty cup, the mass of the cup plus sample before baking, and the mass of the cup plus sample after baking, respectively.

TABLE 1. Combination experiment using RSM with various of temperature and time during Saccharification processes.

Combinations	Temperature (°C)	Time (hours)
1	65	6
2	55	6
3	65	2
4	55	2
5	60	4
6	60	1.17
7	52.92	4
8	60	4
9	60	4
10	60	6.82
11	67.07	4
12	60	4
13	60	4

$$\text{Moisture content}\ (\%) = \frac{(W_1 - W_2)}{(W_1 - W)} \times 100 \quad (1)$$

2.4.2 Ash content

Weigh 2 grams of the material using a Pyrex porcelain cup with a known mass (Herlambang et al., 2023). The samples were treated with ash for three hours at a maximum temperature of 550°C in a furnace (B-One, China). After cooling the sample for 15 to 30 minutes in a desiccator (Duran, Germany), the sample was weighed until a consistent weight was achieved. The ash content can be calculated using Equation 2, where W, W_1, and W_2 represent the sample mass, the mass of the empty crucible, and the mass of the sample after being in the furnace, respectively.

$$\text{Ash content}\ (\%) = \frac{(W_1 - W_2)}{W} \times 100 \quad (2)$$

2.4.3 Fiber content

Weigh 2 grams of the sample and place it in an Erlenmeyer flask. Add 50 mL of H_2SO_4 (Merck) to the flask and keep it upright while cooling. Next, immerse the flask in boiling moisture for 30 minutes. After removing the flask from the boiling moisture, add 50 mL of NaOH solution (Merck, Indonesia) and boil for an additional 30 minutes. Remove the flask and filter the mixture using filter paper while the sample is still hot. Rinse the precipitate on the filter paper with 50 mL of hot H_2SO_4, 250 mL of hot distilled moisture, and 50 mL of 95% ethanol. Dry the filter paper containing the residue for 3 hours at 105°C. Finally, cool the filter paper in a desiccator for 30 minutes or until it reaches a constant weight. Weigh the filter paper to determine the fixed weight (Agustina et al. 2024). To calculate the crude fiber content, refer to Equation 3, where W, W_1, and W_2 represent the sample mass, the ash mass after combustion, and the mass of the precipitate on the filter paper after drying, respectively.

$$\text{Fibre content}\ (\%) = \frac{W - W_1}{W_2} \times 100 \quad (3)$$

2.4.4 Reducing sugar content and starch content

The modified Somogyi method was employed to determine the starch content. Weigh 1 gram of the sample and place it in an Erlenmeyer flask. Add 50 mL of distilled moisture to the flask and homogenize the mixture. 0.1 mL of α-amylase enzyme (Novozymes) to the mixture and homogenize slowly. Cover the flask with aluminum foil. Incubate the flask in a moisture bath (Prio DWB-6H-22L, China) at 95–100 °C for 30 minutes. After incubation, allow it to cool to 60 °C. Add 0.1 mL of glucoamylase enzyme (Liquozyme Supra, Denmark) to the flask and incubate for 60 minutes at 60 °C in the same moisture bath. Following incubation, add distilled moisture to the flask to achieve a total volume of 100 mL using a 100-mL measuring flask. Filter the mixture using filter paper and collect 10 mL of the filtrate. Pipette 10 mL of the filtrate into a separate container and add 10 mL of distilled moisture. Incorporate 10 mL of Solution A into the container and heat it on a hot plate (Thermo) for 3 minutes. Remove the container from the hot plate and cool it under running moisture. Next, add 10 mL of Solution B and 10 mL of Solution C to both the blank solution and the sample solution. Shake the solutions thoroughly to ensure even mixing. Titrate the solutions with a 0.05 N $Na_2S_2O_3$ solution (Merck, Indonesia) until the color turns a slightly bright green. Add 5 drops of a 1% starch solution indicator and continue the titration with the 0.05 N $Na_2S_2O_3$ solution. Stop the titration when the color changes to a light blue for the first time and record the volume of $Na_2S_2O_3$ solution used (Agustina et al. 2024). Finally, calculate the reducing sugar and starch content using Equations 4, respectively, where B, S, and fp represent the reference solution, sample solution, and dilution factor, respectively.

$$\text{Reducing sugar content}\ (\%) = \frac{(B - S) \times 1.449\ fp}{10000} \times 100 \quad (4)$$

$$\text{Starch content}(\%) = 0.9 \times \text{Reducing sugar content}\ (\%) \quad (5)$$

TABLE 2. Comparison of the quality of cassava peel starch and tapioca flour (SNI 3451-2011).

Parameter	Cassava peel starch (%)	Tapioca flour (%) (SNI 3451-2011)
Yield	11.54	-
Moisture	13.535 ± 0.205	Max.14
Ash	0.610 ± 0.028	Max. 0.5
Starch	88.810 ± 0.693	Min. 75
Fiber	0.515 ± 0.007	Max. 0.4

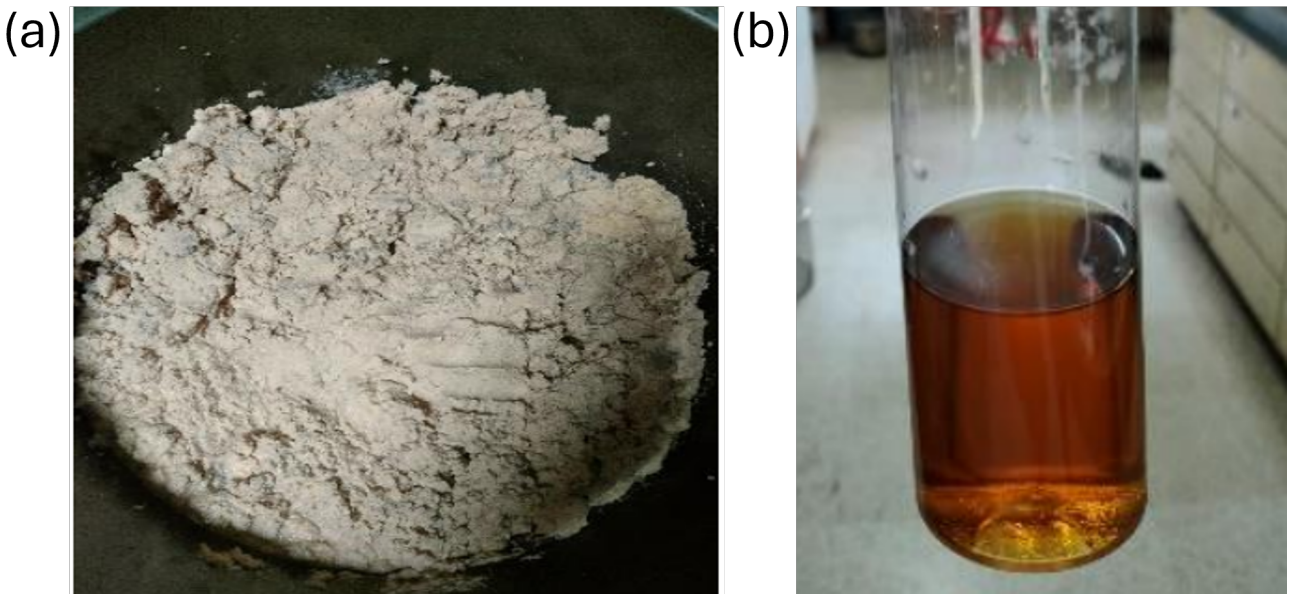

FIGURE 1. Products of (a) cassava pell waste and (b) liquid sugar.

2.5 Statistical analysis

The statistical analysis in this research was carried out using one-way ANOVA with two tailed t-test (with significance p <0.05) using Design Expert version 13. The reducing sugar of the data presented was based on the average value of three replicates.

3. RESULTS AND DISCUSSION

3.1 Cassava peel starch quality

The table 2 compares the properties of cassava peel waste and tapioca flour based on SNI 3451-2011 standards as shown in Table 2. Cassava peel starch has a yield of 11.54%, with a moisture content of 13.535 ± 0.205%, which is slightly higher than the maximum allowable limit of 14% for tapioca flour. The moisture content measured was 13.53%, which is below the standard limit of 14%. A recent study by Alfian et al. (2022) has reported that oven drying reduced the moisture content to 11.13%. The ash content was measured at 0.61%, which is close to the maximum allowable value of 0.5%. The amount of dissolved mineral salts in starch can be inferred from its ash content (Chukwujekwu 2023). The starch content in cassava peel starch is 88.810 ± 0.693%, well above the minimum requirement of 75% for tapioca flour, while its fibre content of 0.515 ± 0.007%, surpassing the maximum allowable fiber of 0.4%. This suggests that cassava peel starch retains more of the fibrous material from the peels, which affects its overall composition. Additionally, cassava peel starch has more fiber and ash, and slightly higher moisture content compared to tapioca flour, but a significantly higher starch content. As compared with other sources (i.e., cassava flour) can generated moisture, ash, fiber, starch content of 9.208%, 0.987%, 2.187%, and 79.876%, respectively (Agustina et al. 2024).

3.2 Reducing the sugar content of liquid sugar by using starch from cassava peels

This study focused on optimizing the temperature and saccharification time to produce liquid sugar from cassava peel starch. Table 3 shows that the highest reducing sugar content of 50.208 ± 0.717% was achieved at a temperature of 65°C for 6 hours. In contrast, the lowest reducing sugar content of 23.836 ± 3.586% was recorded at a temperature of 60°C for 1.17 hours. These findings suggest that reducing sugar levels are significantly influenced by both temperature and saccharification time. Extended reaction times provide enzymes with more opportunity to interact with the substrate, leading to increased starch hydrolysis and enhanced glucose production (Megavitry et al. 2019; Dwi Murtias et al. 2015). On the other hand, raising the hydrolysis temperature results in elevated glucose levels. Higher temperatures facilitate the expansion and breakdown of starch, shortening the glucose chains derived from amylose and amylopectin, and ultimately yielding a greater number of glucose units (Sutanto et al. 2014). However,

TABLE 3. Comparison of the quality of cassava peel starch and tapioca flour (SNI 3451-2011).

Combination RSM	Temperature (°C)	Time (hour)	Reducing sugar (%)
1	65	6	50.208 ± 0.717
2	55	6	30.936 ± 2.152
3	65	2	29.161 ± 1.793
4	55	2	26.372 ± 2.869
5	60	4	30.683 ± 3.227
6	60	1.17	23.836 ± 3.586
7	52.92	4	24.343 ± 0.717
8	60	4	30.175 ± 6.096
9	60	4	29.922 ± 0.717
10	60	6.82	35.501 ± 0.717
11	67.07	4	40.065 ± 0.717
12	60	4	30.936 ± 2.869
13	60	4	30.429 ± 2.152

TABLE 4. Comparison of the quality of cassava peel starch and tapioca flour (SNI 3451-2011).

Source	Sum of Squares	df	Mean Square	F-value	p-value
Model	555.12	5	111.02	24.34	0.0003*
A-Temperature	245.26	1	245.26	53.76	0.0002*
B-Time	221.63	1	221.63	48.58	0.0002*
AB	67.92	1	67.92	14.89	0.0062*
A^2	20.00	1	20.00	4.38	0.0745**
B^2	1.27	1	1.27	0.2793	0.6135**
Residual	31.94	7	4.56		
R^2	0.9456		Std. Dev.	2.14	
Adjusted R^2	0.9067		Mean	31.74	
Predicted R^2	0.6192		C.V. %	6.73	
Adeq Precision	16.2863				

Note: (*) p-value< 0.05; (**) p-value>0.01

the mechanistic basis for these observations requires further exploration. The enzymatic reactions involved in saccharification are influenced by temperature and time, with both factors affecting enzyme kinetics and substrate interactions. The rate of enzymatic hydrolysis is generally temperature-dependent, following the Arrhenius equation, which describes how reaction rates increase with temperature up to a certain optimal point. Beyond this point, enzymes may denature or lose activity. In this study, while higher temperatures promote starch breakdown, there is a trade-off as the enzymes' optimal temperature range must be considered. For instance, most α-amylase enzymes, classified as thermolabile (heat-sensitive), have an optimal temperature range of 90 to 105°C (Agustina et al. 2024). In contrast, the glucoamylase enzyme, which belongs to the mesozyme group, has an optimal temperature range of 50 to 65°C. Therefore, achieving high levels of reducing sugars necessitates maintaining the optimal temperature and duration during the enzymatic process.

The analysis of variance (ANOVA) for reducing sugar content based on saccharification temperature and time is presented in Table 4. The p-value indicates the probability that the observed results occurred by chance (Mariana et al. 2018). The model is considered significant, as the p-value is 0.0003, which is less than 0.05 ($p < 0.05$). The R^2 value of 0.9456 and the adjusted R^2 value of 0.9067 indicate a strong model fit (Pawignya et al. 2019), though the predicted R^2 of 0.6192 suggests some discrepancy between predicted and actual values. The Adeq Precision of 16.2863 indicates a good signal-to-noise ratio. Temperature has a significant impact on reducing sugar content, with p-values for the relationship between temperature and saccharification time of 0.0062. The equation for prediction reducing sugars in this research is shown in Equation 6.

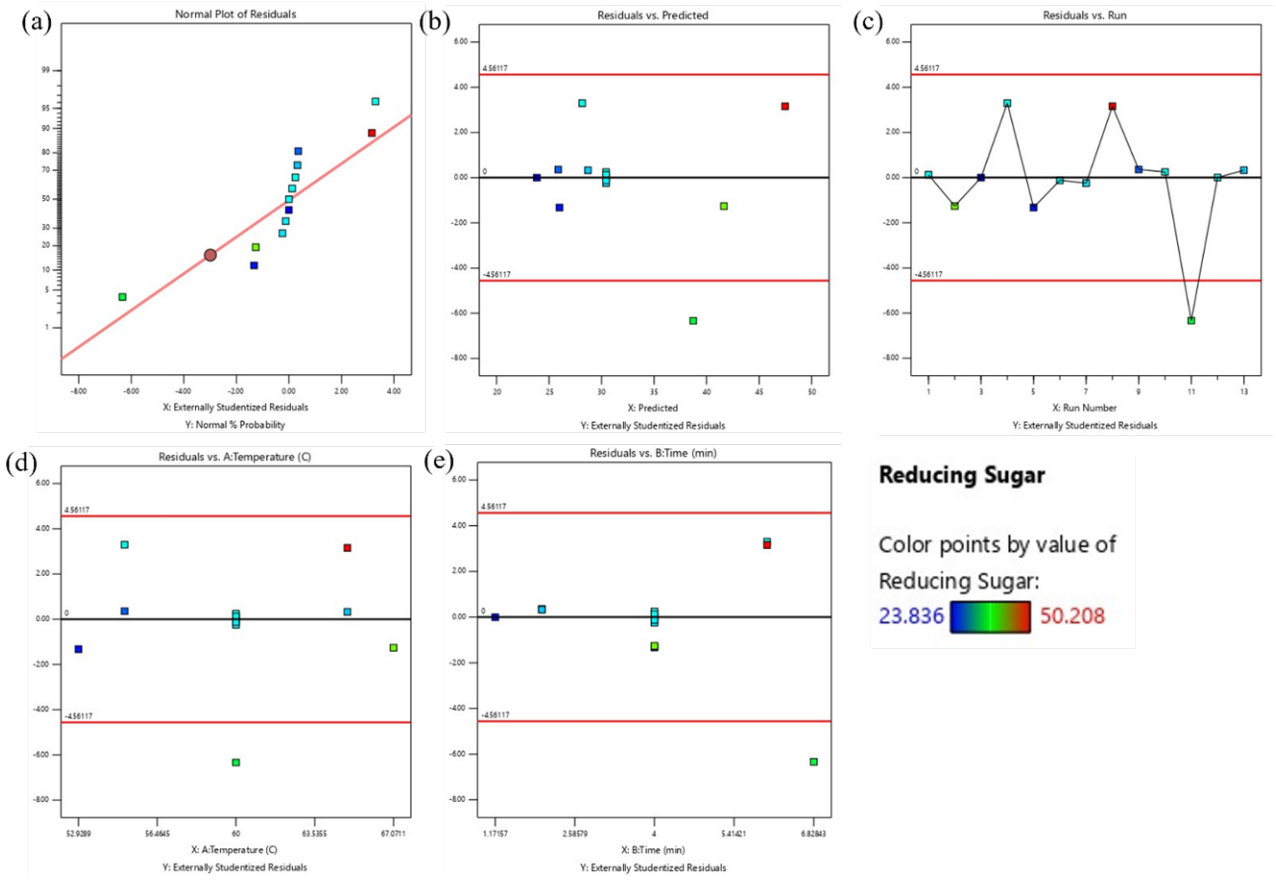

FIGURE 2. Residual diagnostic plots for the regression model predicting reducing sugar, including (a) normal plot of residuals, (b) residuals vs. predicted, (c) residuals vs. run, (d) residuals vs. temperature, and (e) residuals vs. time.

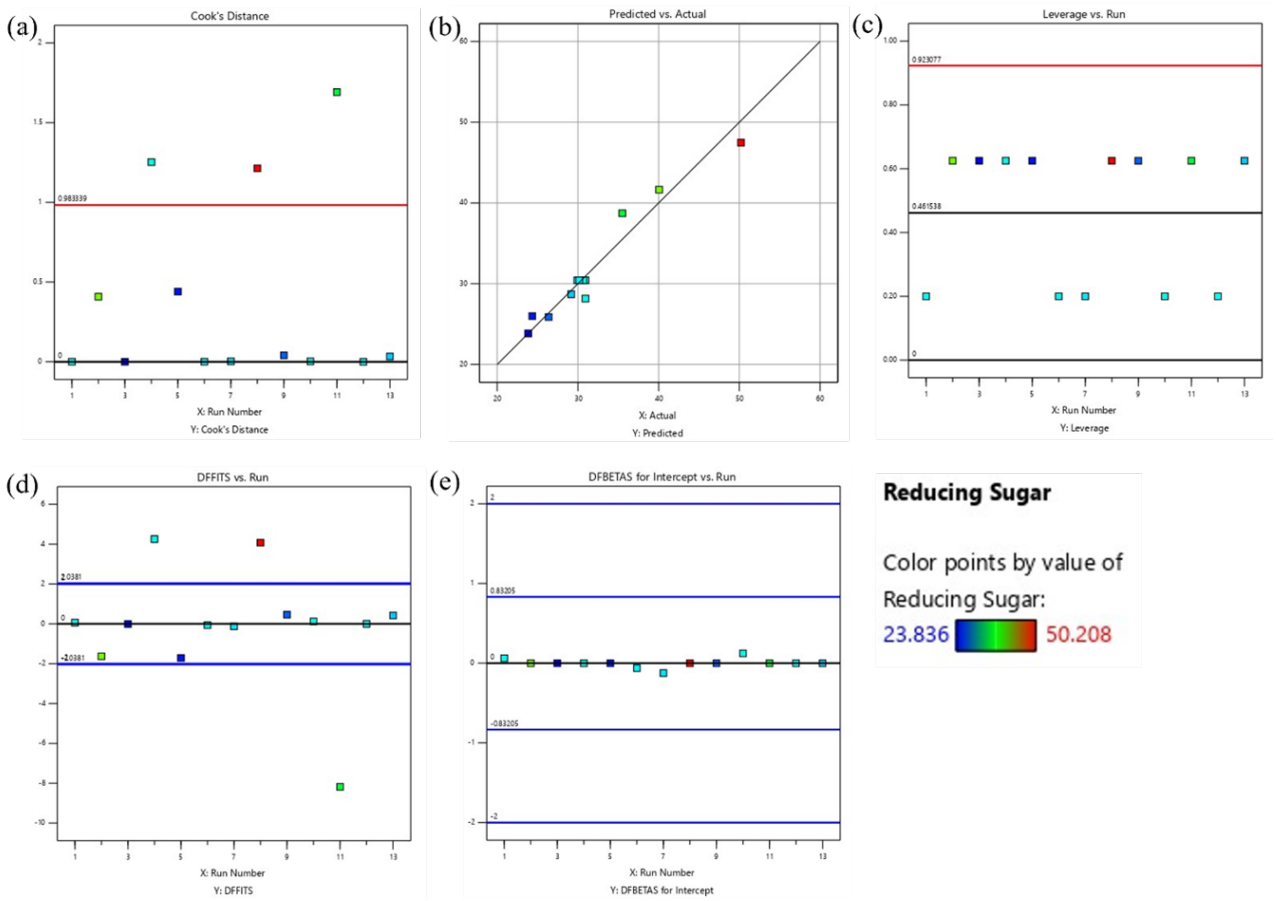

FIGURE 3. Diagnostic plots for regression model predicting reducing sugar, including (a) cook's distance, (b) predicted vs. actual values, (c) leverage (d) DFFITS, and (e) DFBETAs.

$$\text{Reducing sugar} = +30.43 + 5.54A + 5.26B + 4.12AB + 1.70A^2 + 0.4280B^2 \quad (6)$$

Equation 6 in terms of coded factors can be used to make predictions about the response for given levels of each factor. By default, the high levels of the factors are coded as +1 and the low levels are coded as -1. The coded equation is useful for identifying the relative impact of the factors by comparing the factor coefficients.

3.3 Diagnostics and predicted results by RSM

The diagnostic plots provided for the Response Surface Methodology (RSM) analysis indicate a strong model fit, where the assumptions of normality, homoscedasticity, and independence of residuals are largely satisfied as shown in Figure 2 and 3. The residual diagnostic plots for reducing sugar prediction reveal specific areas where the regression model may require refinement. The normal plot of residuals (Figure 2a) shows some deviation from normality, especially for extreme values of reducing sugar, suggesting the model may not fully meet the normality assumption. The residuals vs. predicted (Figure 2b) and vs. run (Figure 2c) plots indicate potential heteroscedasticity and non-random patterns, with larger residuals observed for high reducing sugar values and certain runs. Additionally, the residuals vs. temperature (Figure 2d) and vs. time (Figure 2e) highlight that the model may inadequately account for the effects of these variables, as evidenced by significant residuals at the extremes of temperature and time. These issues suggest that the model may need adjustments, such as transformations or adding interaction terms, to improve its accuracy in predicting reducing sugar levels. When comparing these residual diagnostic findings with other research on predicting reducing sugar, similar challenges are often observed, particularly regarding model assumptions and the need for model refinement. For instance, a study by Bourquard (2018) also identified non-normality and heteroscedasticity in their residual plots when predicting reducing sugars in food processing, indicating that such issues are common in this type of modeling. Their approach involved applying a logarithmic transformation to the response variable, which improved normality and stabilized the variance of residuals. Similarly, van Boekel (2022) found that incorporating interaction terms and polynomial regression improved the model fit, especially for capturing the non-linear effects of processing time and temperature on reducing sugar levels. The current analysis aligns with these findings, suggesting that to achieve more accurate predictions, further refinement of the model is necessary, potentially through transformations or the inclusion of more complex terms to better capture the underlying relationships (Ramandani et al. 2024).

Specifically, the cook's distance (Figure 3a) and leverage (Figure 3c) highlight that certain data points exert a disproportionate influence on the model's predictions. For example, if a particular sample has a high leverage value and a significant cook's distance, it suggests that this sample has an unusual combination of features that strongly affect the model's output. In real-world predictions, such influential points could lead to inaccurate estimates of reducing sugar if not properly addressed. These findings are consistent with the research by Vasconcelos et al. (2020), who also observed that high leverage points and elevated cook's distances in their regression models significantly skewed the prediction of reducing sugars in agricultural products. In their study, they mitigated this by applying robust regression techniques, which down-weight the influence of these outlier points, leading to more reliable predic-

TABLE 5. Characteristics of liquid sugar by validation processes.

Parameter	Results	SNI 01-2978-1992
Smell	Odorless	Odorless
Taste	Sweet	Sweet
Color	Colored	Colorless
Moisture (%)	16.954 ± 0.206	Max. 20
Ash (%)	0.115 ± 0.020	Max. 1
Reducing sugar (%)	58.069 ± 0.414	Min. 30

tions. The predicted vs residual plot (Figure 3b) assesses the homoscedasticity assumption, where the spread of residuals should be constant across all levels of predicted values. The scattered points around the zero line should be random with no clear pattern. Here, we observe that some points, particularly those representing high reducing sugar values (colored in red), show larger deviations, suggesting potential issues with the model's fit at these levels. The DFFITS and DFBETAs plots (Figure 3d) and 3e further emphasize this by showing that some samples significantly alter the predicted value of reducing sugar or the regression coefficients. This indicates that the model may be sensitive to specific data points, potentially compromising its generalizability. Similarly, the DFFITS and DFBETAs plots from the current analysis show that certain samples substantially alter the predicted values and regression coefficients, a phenomenon also reported by Ramandani et al. (2024) in their work on waste problem and removing influential points and re-estimating the model, which enhanced its generalizability and accuracy across different data sets. These comparisons underscore a common theme in predictive modeling of reducing sugars. The identification and adjustment for influential data points are crucial steps in refining the model. Robust methods or careful examination of influential observations, as demonstrated in both the current analysis and the cited studies, are necessary to ensure that the model remains accurate and generalizable across various conditions.

3.4 Optimization, validation, and characteristic of Liquid Sugar Saccharification Process

Response Surface Methodology (RSM) was utilized to optimize the data on reducing sugar levels, with the goal of identifying the optimal temperature and time parameters for saccharification. Figure 4 illustrates the results of the optimization process for liquid sugar saccharification. The contour colors in the graphic represent the relationship between saccharification temperature and duration in relation to the reduction in sugar concentration. The dark green area signifies the highest reducing sugar content, exceeding 55%. In contrast, the dark blue area indicates a reducing sugar content of less than 25%.

The contour plot illustrates that reducing sugar levels increase with higher temperatures and longer saccharification times, reaching values exceeding 55%. According to the optimization graph, the optimal temperature and duration for saccharification are 65°C and 6 hours, respectively, with an anticipated reducing sugar concentration of 47.47%. To validate these optimal conditions, data validation was conducted to ensure that the achieved reducing sugar content aligned with the predicted value from the Response Surface Methodology (RSM) optimization process. The validated sugar content was measured of 58.07%, while the predicted value of 47.47%. The validation results from the saccharification process optimization were utilized to derive the characteristics of the liquid sugar, which were subsequently compared to the SNI 01-2978-1992 standard. Table 5 displays the quality of liquid sugar from the validation process in relation to the SNI 01-2978-1992 standard.

Table 5 indicates that the liquid sugar attained a moisture content of 16.95%, which complies with the quality criterion of a maximum of 20%. Moisture content is a critical factor in determining the quality of liquid sugar (Sutamihardja et al. 2019). As compared with other study by Agustina et al. (2024) has reported that the liquid sugar from cassava flour can achieved moisture content of 5.194%. Lower moisture content is preferred, as it reduces the risk of microbial growth and enhances product durability during storage. Increased viscosity, associated with reduced moisture content, ultimately results in higher-quality sugar. This lower moisture content also extends the shelf life of the sugar, making it less susceptible to spoilage or rancidity.

In terms of other characteristics, the ash content of 0.11% met the liquid sugar quality standard, which permits a maximum of 1%. Additionally, the reducing sugar content was satisfactory, measuring 58.07% against a minimum requirement of 30%. The physical characteristics of aroma and taste also conformed to the standards, as the liquid sugar was odorless and sweet. However, the color

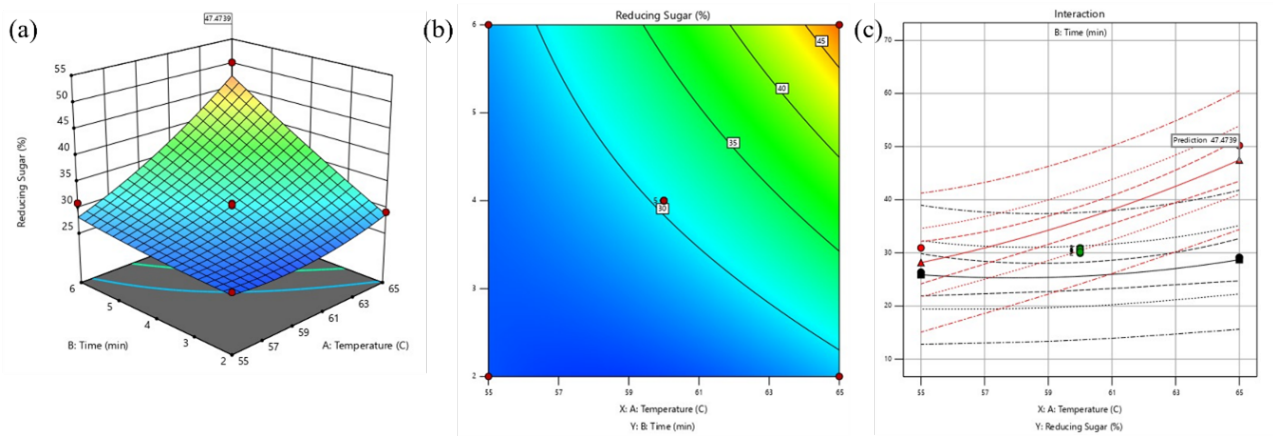

FIGURE 4. Diagnostic plots for regression model predicting reducing sugar, including (a) cook's distance, (b) predicted vs. actual values, (c) leverage (d) DFFITS, and (e) DFBETAs.

TABLE 6. Characteristics of liquid sugar by validation processes.

Raw materials	Methods	Results	Optimizing and validation	Limitation	References
Cassava flour	Three methods, such as gelatinization, liquification, and enzymatic saccharification	Reducing sugar between 18.004 ± 0.254% and 28.299 ± 0.101%	Substrate 40%, liquification 60 min, saccharification 60 min can produce reducing sugar of 29.6735%	Focused only on liquid sugar yield	(Agustina et al., 2024)
Cassava peel	0.05 M sodium hydroxide at 121°C for 15 min	Glucose yield of 4.53±1.20 mg/ml	-	Optimizing proses to enhance glucose yield	(Zulkifli & Karim, 2023)
Cassava starch waste	Solution plasma process for 300 min in 0.08 H2SO4	Glucose production of 47.9%	-	High cost; requires economic analysis	(Prasertsung et al., 2019)
Cassava and sweet potato roots	Enzymatic hydrolysis with pH 5.4 and Spezyme® XTRA (0.025% v/w for cassava and 0.02% v/w for sweet potato)	Reducing sugar of 97.23 ± 0.81 and glucose yield of 90.41 ± 0.40	-	High cost; complex process	(Johnson et al., 2009)
		Fructose syrup of 9.67 ± 0.05 g/100g cassava and 5.96 ± 0.05 g/100 g sweet potato			
Cassava peel waste	Isomerization process (i.e., hydrolysis and saccharification) in 450 mg glucose for 72 hours	Yield fructose of 26.05 %	Isomerization time 66.5051 hour and 450 g glucose can produce fructose yield of 26.8060%	Limited analysis of reducing sugar and glucose yield	(Debora et al., 2024)
Wheat Straw	Hydrolysis using cellulase with pretreatment using NaOH	Reducing sugar	Optimum conditions were achieved at 8% solid content b/v and a hydrolysis time of 96 hours	Long hydrolysis time, product produced is reducing sugar with physical properties that have not been clearly described	(Wang et al., 2021)
Cassava Peel	Hydrolysis using -amylase and glucoamylase followed by isomerization using glucose isomerase	Fructose	Highest fructose content was 26.8% with an isomerization time of 66.5 hours and isomerase enzyme weight of 450 mg	The glucose yield from the hydrolysis process in the first stage is not explained	(Debora et al., 2024)
Cassava Starch	Hydrolysis using pectinase, α-amylase, and amyloglucosidase	Fermentable sugar	Reaction time of 7 hours; solid/liquid ratio of 1:1	Reducing sugar concentration of 16%, slightly lower than this study	(Collares et al., 2012)
Cassava peel waste	Liquification and saccharification at temperature 65°C for 6 hours	Reducing sugar of 50.208 ± 0.717 %	Temperature of 65°C for 6 hours, with reducing sugar of 47.47%	Requires economic analysis	This study

characteristic did not meet quality standards, as the product exhibited a brownish hue due to inadequate purification. Therefore, further purification is necessary to comply with quality standards, particularly to eliminate the brownish color. Table 6 presents a comparative analysis of methods for sugar production from various cassava-based raw materials. The comparison highlights different approaches and their respective outcomes, optimization strategies, limitations, and references.

4. CONCLUSIONS

The study identifies that at temperature of 65°C for 6 hours are optimal for saccharification, yielding a liquid sugar content of 47.47%, but the optimizing proses can produce liquid sugar content of 55%. To further enhance the efficiency and quality of the saccharification process, future research should focus on optimizing additional variables such as enzyme concentration, pH, and substrate concentration. Detailed analysis of quality attributes including moisture, ash, and reducing sugar content should be conducted to ensure compliance with SNI 01-2891-1992 standards. Moreover, it is essential to investigate the stability and shelf life of the liquid sugar, evaluate the feasibility of scaling up the process, and examine any by-products or side effects. These targeted studies will provide a comprehensive understanding needed for industrial application and process improvement.

5. ACKNOWLEDGEMENTS

In this section the authors can dedicate the article to a scientist of outstanding merit or acknowledge financial support, technical assistance and other contributions or advice from persons who are not coauthors. The grant number of financial support must be provided.

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