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Characteristics of Liquid Sugar from Cassava Flour Using Gelatinization, Liquefaction and Enzymatic Saccharification (*amyloglucosidase and α-amylase*) Processes

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Abstract

Domestic sugar production is still insufficient to satisfy the national demand for sugar, and production costs are expensive. One of the ingredients that can be used to make liquid sugar is starch. This study aims to determine the response of the resulting reduced sugar by optimizing the substrate concentration, liquefaction, and saccharification time. The method used of Box Behnken Design (BBD) with combinations of 20, 30, and 40% substrate concentrations, liquefaction times of 20, 40, and 60 min, and saccharification times of 20, 40, and 60 min. The characteristics of cassava flour, such as moisture (9.208 ± 0.068%), ash (0.987 ± 0.001%), fiber (2.187 ± 0.033%), and starch (79.876 ± 0.000%), were satisfied on SNI 01-2905-1992. Liquid sugar made from cassava flour reduced the sugar content by 28.299 ± 0.101%. The optimal conditions were 40% substrate concentration, 60 min liquefaction time, and 60 min saccharification time. The characteristics of the validation liquid sugar are in accordance with the specifications for the quality requirements of SNI 01-2978-1992 in the form of moisture content, ash content, and reducing sugar of 5.194 ± 0.003%, 0.996 ± 0.000%, and 29.668 ± 0.761%, respectively. These results highlight the effectiveness of optimizing substrate concentration, liquefaction time, and saccharification time in producing high-quality liquid sugar from cassava flour. This optimization addresses the domestic demand for sugar. It provides a cost-effective solution by utilizing cassava flour, thereby supporting the local agricultural economy and potentially reducing reliance on imported sugar.

Keywords *: liquid sugar, cassava flour, liquefaction, saccharification, reducing sugar*

Introduction

Food issues in daily life are highly complicated, and to survive, humans need food. A recent study conducted by (Kusmayadi *et al.*, 2021) has reported that food is the most essential resource for human survival and can be derived from both plant and animal sources. It is anticipated that population growth will continue to accelerate annually. As per the Central Statistics Agency, in 2020, Indonesia will be a populous agricultural nation with around 270 million inhabitants. It is anticipated that increasing land production or developing new food ingredients will be required to meet the world's food needs. According to (Suripto *et al.*, 2013), Sugar imports have increased by 30%. Despite this, Indonesia continues to rely on imports of raw commodities like sugar. Despite high production costs, domestic sugar production cannot meet the country's needs. As of right now, the sugar cane plant is the source of sugar, although there are plenty of other elements that can be substituted for it. One sugar production method developed by the Centre for Agricultural Research and Development uses cassava flour. It is believed that using natural resources entirely derived from non-cane materials will enable us to solve the issue.

Starch is one of the components that can be used to generate liquid sugar. The leading composition cassava of is 60–80% starch, 2–3% protein, and 0.5–1.5% fiber, as well as a range of vitamins and minerals (Egbune *et al.*, 2023; Smole *et al.*, 2019). Indonesia has a plethora of cassava possibilities, including cassava chips, cassava flour, and cassava-based snacks (Abiodun *et al.*, 2023). The versatility of cassava makes it a popular ingredient in Indonesian cuisine and a staple food source for many communities. In addition to its culinary uses, cassava is utilized in various industries, such as paper and textile production. Its high carbohydrate content and resilience to harsh growing conditions make it an essential crop for food security in Indonesia. Cassava contains cyanide acid (HCN), which gives the flesh a dark brown color (Rasulu, 2014). If this pigmentation is left untreated, it will deteriorate and eventually disintegrate. Naturally, this issue poses a serious threat to cassava growers, which is why technology for preservation is required to increase the crop's shelf life. Farmers must think creatively and develop fresh ideas when cassava prices are extremely low to increase the crop's selling price. Cassava production was the first innovative step taken by women farmers in the Bandar Agung Village Group of the Canal Nunyai District of Central Lampung Regency. Dried cassava is a preservation technique that extends the product's shelf life. But cassava is solely used to create tiwul in this village (Howeler, 2009; Suharko & Hudayana, 2020).

Cassava can substitute raw material for liquid sugar if further processed. The enzymes α-amylase and glucoamylase can hydrolyze starch to produce liquid sugar, often known as glucose syrup (Balakrishnan *et al.*, 2019; Hua & Yang, 2016). The goal of hydrolysis is to use the α-amylase enzyme to turn starch into dextrin (liquefaction), which is subsequently turned into simple sugars like glucose by the glucoamylase enzyme (saccharification) (Cereda, 2024; Hua & Yang, 2016). This process is commonly used in various food and beverage industries. The resultant sugar compound optimization can be affected by several parameters, including temperature, stirring factor, enzyme solution pH, substrate concentration, enzyme activity, and purity (Wagner *et al.*, 2015; Wang *et al.*, 2020; Zhang *et al.*, 2021). Our project aimed to produce liquid sugar from cassava flour in collaboration with the Women's Farmers Group in Bandar Agung Village. This may be the most recent study because few have addressed the production of liquid sugar from cassava flour. This research aims to determine how responsive the reducing sugar produced by optimizing the hydrolysis duration and substrate concentration is. Next, a test to see if the product satisfies the quality standards for glucose syrup will be conducted to determine which one is the best.

The duration also has an impact on how well the enzyme functions. Since the outcomes might be used as parameters for producing liquid sugar, it is crucial to ascertain the ideal enzymatic reaction time. The α-amylase enzyme facilitates the conversion of starch into dextrin, a process known as liquefaction that takes between thirty and fifty min. Next, the glucoamylase enzyme is used to continue the saccharification stage, which should take 30 to 50 min (Ayu Ratna P & Yulistiani, 2015). The most ideal substrate, according to Ayu Ratna P and Yulistiani (2015), is 33.3% in 150 mL when 0.3 mL of a 1:1 enzyme is added.

Methodology

This research was carried out from February to July 2022 at the Anak Tuha National Research and Innovation Agency (BRIN) Laboratory and the Industrial Chemical Engineering Technology Analysis Laboratory. The apparatus utilized included Erlenmeyer flasks (Pyrex, USA), an analytical balance (Sartorius, Germany), an upright cooler (Thermo Fisher, USA), an oven (Memmert, Germany), a hot plate (Corning, USA), a pH meter (Hanna Instruments, USA), measuring flasks (Pyrex, USA), filter paper (Whatman, UK), aluminum foil (Reynolds, USA), a glass funnel (Kimble, USA), a thermometer (Fisher Scientific, USA), a furnace (Carbolite, UK), a porcelain cup (CoorsTek, USA), a spatula (Fisherbrand, USA), a desiccator (Bel-Art, USA), a centrifuge (Eppendorf, Germany), and a refractometer (Atago, Japan).

Cassava flour from the Women's Farmers Group of Bandar Agung Village, potassium nitrate trihydrate ($NaKC_4H_4O_6.4H_2O$), tri-basic sodium phosphate ($Na_3PO_4.12H_2O$), copper (II) sulphate pentahydrate ($CuSO_4.5H_2O$), potassium iodate (KIO_3), aquades, concentrated sulfuric acid (H_2SO_4), sodium thiosulphate ($Na_2S_2O_3.5H_2O$), sodium carbonate (Na_2CO_3), 1% starch, activated charcoal, and enzymes amyl glucosidase and α-amylase (Liquozyme®). All the chemicals used were of analytical grade and purchased from Sigma Aldrich (USA).

Experimental procedures

The Response Surface Methodology (RSM) approach from Minitab software version 18 has been utilized in this research design. With the support of this software, statistical techniques that will be applied to ascertain the best value formulation can be found. liquefaction and saccharification procedures by the optimization stage of substrate concentration with time. Response Surface Methodology (RSM) was the technique employed, specifically Box Behnken Design (BBD) with three factors, such as 20 min, 40 min, and 60 min for saccharification, 20%, 30%, and 40% for substrate concentration, and 20 min, 40 min, and 60 min for liquefaction. **Table 1** shows the range of values for the independent variable in this experiment.

Table 1. Experimental Desain using RSM to produce liquid sugar from cassava flour

Combination	Substrate concentration in 150 mL (%)	Liquification time (min)	Saccharification time (min)
1	40	20	40
2	40	60	40
3	20	60	40
4	20	20	40
5	40	40	60
6	30	40	40
7	30	60	20
8	30	40	40
9	30	20	20
10	20	40	20
11	30	20	60
12	30	60	60
13	20	40	60
14	40	40	20
15	30	40	40

Preparation of cassava flour to produce liquid sugar using gelatinization, liquification, and saccharification process.

The raw cassava flour was collected from Bandar Agung Village and was processed into an even 80 mesh size before being used as a raw material. After adjusting to SNI 01-2997-1996, the raw material analysis aims to determine the sample's starch, fiber, ash, and moisture contents. This is necessary for the raw materials to fulfill the cassava quality standards. In addition to estimating the impact and causes of the liquid sugar that will be produced, the raw material analysis also seeks to identify the properties of cassava flour.

Three steps are involved in the hydrolysis of starch into glucose syrup: gelatinization, liquefaction, and saccharification (**Figure 1**) (Muhammad *et al.*, 2023; Wahyuni *et al.*, 2022). The first step (gelatinization) involves heating a solution of cassava flour and moisture (suspension) until the consistency thickens. A 150 mL cassava flour suspension substrate was prepared with three predefined concentration variations (i.e., 20%, 30%, and 40% (w/v). After this, the suspension was heated to 60°C until the gelatinization process started or the suspension thickened. A recent study conducted by Xiao *et al.* (2020) has reported that the starch and tapioca will gelatinize at 75°C. Hydrolyzing cassava flour into smaller molecules, like maltose, glucose, and dextrin, at 90 to 95°C, employing 0.3 mL of the enzyme α-amylase, is known as liquefaction. The periods employed in the liquefaction process are 20, 40, and 60 min. Then, 0.3 mL of amyl glucosidase enzyme was added to the solution, and the saccharification process started at 60°C. The substrate solution was stirred continuously for 20, 40, and 60 min when the temperature was maintained at the predetermined levels. The cake and liquid were separated by centrifuging to remove any leftover saccharification material (sugar). The modified somogy test was used to quantitatively assess the reduction of sugar content in the resultant product.

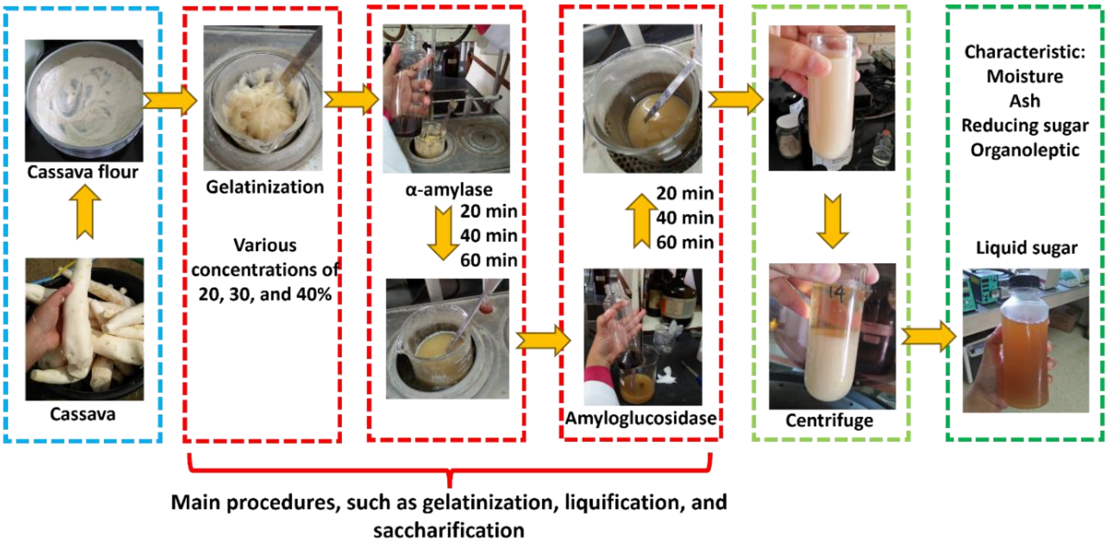

Figure 1. The procedure of production of liquid sugar from cassava flour uses three steps such as gelatinization, liquification, and saccharification

Analyzing the properties of sugar and liquid starch

The moisture, ash, starch, and fiber content test procedures are according to SNI 01-2891-1992, while the organoleptic test adheres to SNI 01-2346-2006.

Moisture content

Weighing two grammes, it was baked in an oven (in Kern, Germany) for three hours at 105°C (Herlambang *et al*., 2023; Laresha *et al*., 2024). After that, the sample was left to cool in a desiccator (Duran, Germany) for 15 to 30 min. Equation 1 can be used to determine the moisture content.

$$Moisture\ content\ (\%) = \frac{(W1-W2)}{(W1-W)} X\ 100 \quad (1)$$

When the empty cup's mass is W, the cup plus sample before the oven is W1, and the cup plus sample after the oven is W2, respectively.

Ash content

A Pyrex porcelain cup with a known mass was used to weigh two grams of the substance (Herlambang *et al*., 2023; Laresha *et al*., 2024)The samples were placed in a furnace (B-One, China) and exposed to ash for three hours at a maximum temperature of 550°C. After cooling for 15 to 30 minutes in a desiccator (Duran, Germany), the sample was weighed until a consistent weight was reached to determine the amount of ash contained in Equation 2.

$$Ash\ content\ (\%) = \frac{(W1-W2)}{W} x100\% \quad (2)$$

In contrast, W, W1, and W2 displayed the mass of the sample after the furnace, the mass of the empty crucible, and the sample itself.

Reducing sugar content and starch content

The modified Somogyi method was used to determine the starch content. One gram of the material should be weighed in an Erlenmeyer flask. Once the mixture is homogenized, add 50 mL of distilled moisture to the flask. Slowly homogenize the mixture after adding 0.1 mL of Novozymes' alpha-amylase enzyme. Use aluminum foil to cover the flask. The flask should be incubated for 30 min at 95 to 100 °C in a water bath. Let it cool to 60°C after that. In a moisture bath (Prio DWB-6H-22L), incubate the flask for 60 min at 60°C after adding 0.1 mL of glucoamylase enzyme (Liquozyme Supra, Denmark). After incubation, use a 100 mL measuring flask to add distilled moisture to the flask until it reaches a capacity of 100 mL. Using filter paper, strain the mixture and gather 10 mL of the filtrate. In another container, pipette 10 mL of filtrate and add 10 mL of distilled moisture. After adding 10 mL of Sol A to the container, heat it for three min on a Thermo hot plate. Take the container off the hot griddle and splash some moisture to cool it down. Add 10 mL each of Sol B and Sol C to the blank solution and sample solution. To ensure even mixing, give the solutions a shake. Using a 0.05 N $Na_2S_2O_3$ solution, titrate the solutions until the color turns a faint shade of green. Titrate with a 0.05 N $Na_2S_2O_3$ solution after adding 5 drops of the 1% starch solution indicator. When the color turns blue (light) for the first time, stop the titration and note how much $Na_2S_2O_3$ solution was used. Using Equations 3 and 4, respectively, determine the decreasing sugar and starch content.

$$Reducing\ sugar\ content = \frac{(B-S)x\ 1{,}449\ x\ fp}{10.000} x\ 100\% \qquad (3)$$

$$Starch\ content = 0{,}9\ x\ kadar\ gula\ pereduksi \qquad (4)$$

In this case, B, S, and fp represent the reference solution, sample solution, and dilution factor, respectively.

Fiber content

Weigh approximately 2 grams of the sample, then transfer it into an Erlenmeyer flask. After adding 50 mL of H_2SO_4 (Merck) to the flask, let it cool vertically. After that, submerge the flask in boiling moisture for half an hour. Take the flask out of the boiling moisture and boil it for thirty minutes. Then, add 50 mL of NaOH solution. While the sample is still hot, remove the flask and filter the mixture through filter paper. Use 50 mL of hot H2SO4, 250 mL of hot distilled moisture, and 50 mL of 95% ethanol to rinse the precipitate on the filter paper. Dry the filter paper and residue for 3 hours at 105°C. Finally, let the filter paper cool in a desiccator for thirty min or until its weight stabilizes. To find the fixed weight, weigh the filter paper. To determine the crude fiber content, utilize Equation 5.

$$Fibre\ content = \frac{W-W1}{W2} x\ 100\% \qquad (5)$$

Where W, W1, and W2 displayed the mass of the sample, the mass of ash after the furnace, and the mass of precipitate on the filter paper after the oven.

Results and discussion

Characteristic cassava flour to produce liquid sugar

Table 2 presents the moisture content, ash content, fiber content, and starch content of cassava flour, all of which follow the quality standards for cassava flour (SNI 01-2905-199

Table 2. Characteristics of cassava flour as compared to SNI 01-2905-1992

Parameter	Results (%)	SNI 01-2905-1992 (%)
Moisture content	9.208 ± 0.068	Max. 14
Ash content	0.987 ± 0.001	Max. 1
Fiber content	2.187 ± 0.033	Max. 4
Starch content	79.876 ± 0.000	Min. 70

Based on the testing findings, cassava flour's moisture content is 9.208 ± 0.068 %, although it has been found that the flour satisfies the quality criteria with a maximum moisture content of 14%. Less than 14% moisture content in food goods makes them safe enough to stop mound growth (Kabak *et al.*, 2006). The moisture content found in this research is less than 13%, but the moisture content of cassava flour is between 13.48% and 13.65% (Hartiati & Yoga, 2015). Besides, cassava flour's ash content indicates that its content satisfies quality standards. The test findings indicate that the ash content is

0.987 ± 0.001%, while the maximum is 3%. Increased amounts of contaminants or minerals in the samples are assumed to cause the increased ash content test results in this investigation.

According to SNI 01-2905-1992, the crude fiber content of the cassava flour tested was 2.187 ± 0.033 %, meaning that the cassava flour produced complied with quality standards. While cassava flour has a greater crude fiber level, tapioca flour only has a 0.09% crude fiber content (Folake O *et al*., 2012; Widiarto *et al*., 2019). This is likely because cassava undergoes a drying phase before being ground to form flour. Other than that, the resulting starch content satisfies SNI requirements. According to SNI 01-2905-1992, cassava flour must satisfy the minimum starch content 70 to satisfy the super quality criterion. The starch content test result was 79.876 ± 0.000 %, indicating that the cassava flour satisfies this requirement. Climate, planting site, variety, and harvest age all have a significant impact on cassava starch levels (Hartiati & Yoga, 2015). Elevated quantities of starch signify elevated glucose levels. A recent study conducted by Bertoft (2018) has reported that glucose is the smallest unit in the starch chain and that starch is mainly composed of a polymer of either glucose or maltose. According to Widiarto *et al*. (2019) has reported that tapioca flour's starch content is 83.70%. Although cassava flour has a lower starch content, it is believed to still contain a significant amount of fiber. This is why the starch content of the flour was relatively high during the tapioca processing process.

Reducing sugar of liquid sugar products from cassava flour

This study has investigated the method of producing liquid sugar using cassava flour. In the process of producing liquid sugar, three variables have been optimized: substrate concentrations (20%, 30%, and 40%) with liquefaction process times (20, 40, and 60 minutes), as well as saccharification process periods (20, 40, and 60 minutes). Using Minitab software version 18, the Response Surface Methodology approach produces conditions with a lower sugar content. These conditions determine the best treatment combination. There are fifteen different experimental combinations in the steps of producing liquid sugar. **Figure 2** displays the results of 15 combinations of process optimization for lowering sugar.

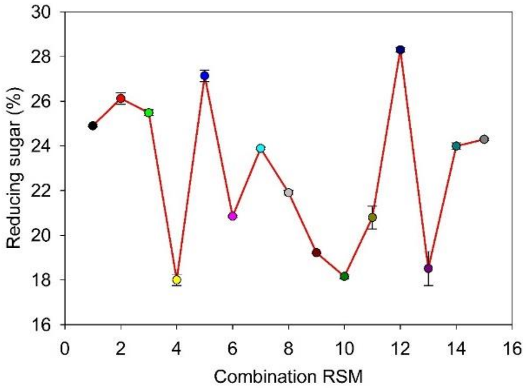

Figure 2. Reducing sugar content with various concentrations (i.e., substrate concentrations, liquification time, and saccharification time) using RSM approach

Figure 2 shows that the study's reduced sugar content fell between 18.004 ± 0.254% and 28.299 ± 0.101%. The lowest reducing sugar content was achieved with a substrate amount of 20%, a liquefaction time of 20 minutes, a saccharification time of 40 minutes, and a total sugar content of 18.004 ± 0.254%. Using 30% substrate, liquefaction, and saccharification times of 60 minutes each resulted in the greatest reducing sugar concentration of 28.299 ± 0.101%.

The relationship between substrate concentration, liquefaction, and saccharification in the optimization of liquid sugar products

The relationship between the liquefaction time and the amount of substrate is shown in **Figure 3. (a)**. It is established from the contour plot that the amount of total reducing sugar generated increases with increasing substrate concentration and liquefaction time. The high concentration of substrate that will be employed constitutes a few of the variables that affect the enzyme-catalytic reaction of liquid sugar products. A recent study conducted by Hartwell (2023) has reported that not all enzymes will bind to the substrate at a low substrate concentration, which prevents the enzyme from working at its fastest. However, all enzyme molecules can form complicated relationships at high substrate concentrations, resulting in longer catalytic reaction times. The ideal temperature range for the liquefaction process is between 80 and 95°C. According to Li *et al.* (2023), starch will not completely hydrolyze enzymatically or break down if liquefaction is performed below the gelatinization temperature. The above research results suggest that the total yield of sugar that is produced is primarily determined by the substrate concentration and liquefaction time, but it is also influenced by a few other factors, including the ideal conditions for the enzyme to function, the ideal conditions for the liquefaction time, and the substrate concentration.

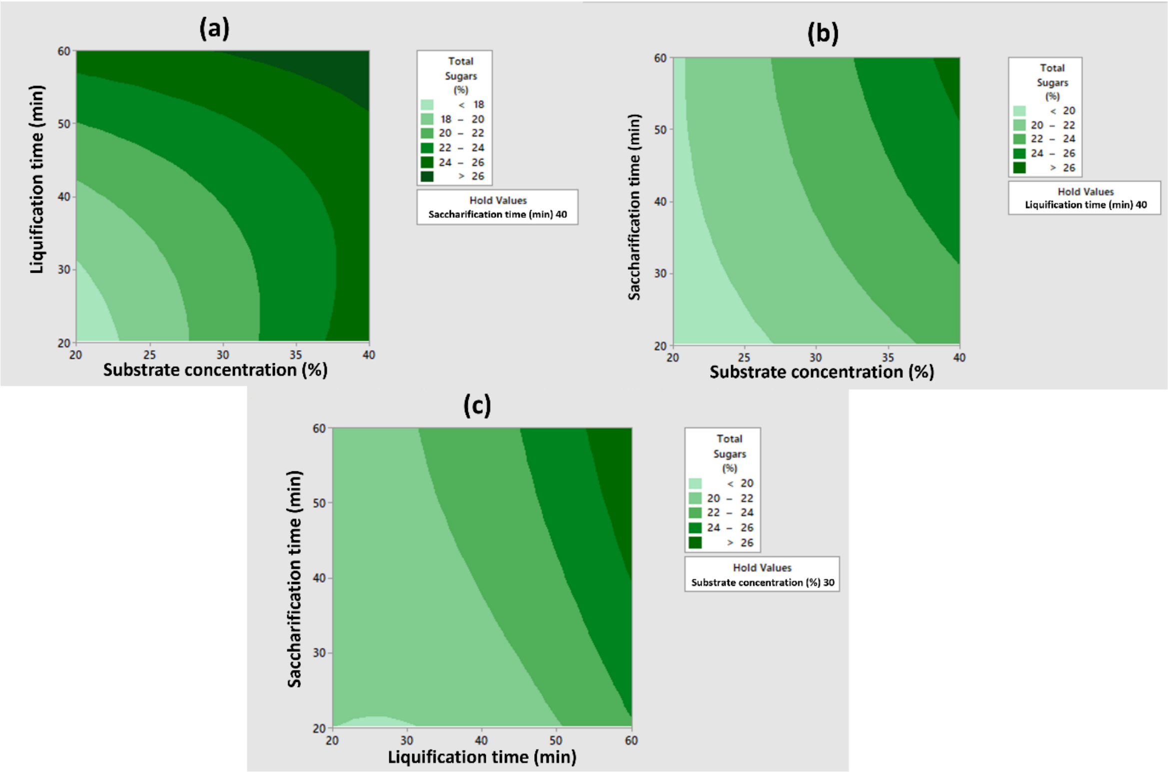

Figure 3. The effect of (a) substrate concentrations and liquification time, (b) substrate concentrations and saccharification time, and (c) liquification time and saccharification time during the production of liquid sugar from cassava flour

Figure 3. (b). indicates that the production of reduced sugar increases with longer saccharification times and greater substrate concentrations. A previous study by Amândio *et al.* (2023) states that the hydrolysis process can be influenced by the raw materials' cellulose content, pH levels, the length of the liquefaction and saccharification processes, temperature, pressure, and enzyme concentration. The substrate concentration and the saccharification duration mainly determine the amounts of reducing sugar generated. However, there are ideal conditions for both parameters. Based on the research conducted by Kusmiyati *et al.* (2023) has reported that saccharification times increase so does the benefit of sugar reduction. Enzyme reactions are assumed to be the cause of this.

Figure 3. (c). illustrates the straight proportionality between the liquefaction and scarification times in the reducing sugar production process. More total sugar will be generated the longer the saccharification and liquefaction times. However, if one continues using it too long, the suspension will get more saturated, and the amount of sugar produced will either stay the same or decrease. 48 minutes is the ideal duration for starch hydrolysis utilizing the α-amylase enzyme, but 54 hours is the ideal duration when employing the glucoamylase enzyme (Olosunde *et al.*, 2023). According to (Abdrabuo *et al.*, 2024), the temperature may significantly impact the synthesis of maltodextrin and glucose syrup products, even though the duration of saccharification generally impacts the outcomes, which frequently decline. To summarize, **Table 3** shows the reduction in sugar levels derived from the data validation procedure utilizing the Response Surface Methodology (RSM) approach.

Table 3. Prediction and validation process using RSM approach based on reducing sugar

Substrate concentration (%)	Liquification time (min)	Saccharification time (min)	Reducing sugar	
			Prediction RSM (%)	Validation research (%)
40	60	60	29,6735	29.668 ± 0.761

According to **Table 3**, an estimated reducing sugar content of 29.668 ± 0.761 % will be produced by the estimated RSM at a substrate concentration of 40%, liquefaction time of 60 minutes, and saccharification time of 60 minutes. The reduced sugar content obtained from the validation was 29.6682%, indicating little difference between the reduced sugar content obtained from the RSM estimate and the reduced sugar content obtained from the validation data. Based on the optimization, this study's liquefaction and saccharification procedures seek to hydrolyze or break down starch into simple sugars or glucose enzymatically, resulting in a more optimal lowering of sugar levels. In this investigation, the reduced sugar content was attained at 29.668 ± 0.761% with a liquefaction and saccharification duration of 60 minutes. The raw ingredients utilized, various plant sources, plant conditions, soil quality, planting, care, and other factors can all contribute to this variation.

Characteristic of liquid sugar using the validation process

Table 4 presents the sugar properties gained by optimization, including moisture content, ash content, decreasing sugar, and organoleptic (taste, color, and odor).

Table 4. Characteristics of the validation process using optimum conditions, such as concentration substrate, liquification time, and saccharification time of 40%, 60%, and 60%, respectively

Parameter	Validation results	Standard SNI 01-2978-1992
Moisture content (%)	5.194 ± 0.003	Max. 20
Ash content (%)	0.996 ± 0.000	Max. 1
Reducing sugar (%)	29.668 ± 0.761	Min. 30
Smell	Slightly Smelly	Odorless
Taste	Sweet	Sweet
Color	Colored	Colorless

The analysis findings indicate that the proportions of moisture, ash, and reducing sugar were 5.194 ± 0.003 %, 0.996 ± 0.000 %, and 29.668 ± 0.761 %, respectively. A higher moisture content leads to a shorter storage duration due to increased microbial activity. Microbes find it easier to assault food products during storage if they contain more moisture. Aside from that, the liquid sugar's ash concentration came in at 0.85%. SNI 01-2978-1992 criteria have been satisfied by the moisture, ash, and reducing sugar content. This study has examined organoleptic outcomes (taste, smell, and color) in addition to these tests. Table 4 indicates that the produced liquid sugar is brownish, tastes sweet, and retains an odor. While the taste and scent measurements do not match standards, some preceding parameters follow SNI 01-2978-1992. A previous study by Hartiati and Yoga (2015) shows that the color analysis of liquid sugar made from sweet potato starch does not match the standards either, as it is still brown, although according to the SNI 01-3743-1995 standard, it should be colorless to yellowish.

Conclusion

Response Surface Methodology (RSM) optimization employing factors including substrate concentration, liquefaction time, and saccharification time of 30%, 60%, and 60%, respectively, reduced sugar by 29.668 ± 0.761%. Therefore, it is essential to carefully monitor the saccharification process to ensure the optimal duration is reached without exceeding it. By doing so, the maximum benefit of sugar reduction can be achieved while avoiding unnecessary production delays. The liquefaction process can be optimized by maintaining the temperature within the ideal range and closely monitoring the liquefaction time to achieve maximum efficiency. Additionally, ensuring that the substrate concentration is optimal will also improve the overall sugar yield during the process. The differences in reducing sugar content and optimal substrate concentration observed in this investigation compared to previous research may be attributed to the variability in raw ingredients and plant conditions. Further studies should consider these factors to optimize liquefaction. To meet industry standards, further research is needed to improve the organoleptic properties of liquid sugar produced from sweet potato starch. Additionally, exploring different processing methods may help achieve the product's desired color, taste, and smell.

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