

Optimisation of α -Amylase and Amyloglucosidase Enzyme Concentration on Glucose Syrup Characteristics from Purple Sweet Potato (*Ipomoea batatas* L var. Antin 2)

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ABSTRACT

This study investigated the impact of α -amylase and amyloglucosidase enzyme concentrations on purple sweet potato glucose syrup production. The results demonstrated that higher α -amylase levels enhanced total dissolved solids and reduced sugar content during liquefaction, while an optimal amyloglucosidase concentration of 0.8 mL/kg maximized yield and reduced sugar content during saccharification. The resulting syrup exhibited high antioxidant activity and adjustable viscosity. Enzymatic hydrolysis using amyloglucosidase proved superior to acid hydrolysis in producing high-quality glucose syrup. Purple sweet potato glucose syrup emerges as a promising functional natural sweetener with potential health benefits and commercial value.

Keywords: α -amylase, amyloglucosidase, liquid glucose, purple sweet potato.

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1. INTRODUCTION

The increasing population of Indonesia has fuelled the national sugar demand, which unfortunately cannot be fully met by domestic sugar production [1]. This causes Indonesia to still depend on sugar imports from other countries. This dependence on sugar imports needs to be overcome with various efforts, one of which is by diversifying the sources of sugar raw materials. In addition to sugar cane, various other carbohydrate sources, such as tubers and cereal crops, can be processed into alternative sugars. One potential that can be developed is purple sweet potato (*Ipomoea Batatas* L var. Antin 2) as a raw material for liquid glucose production [2].

Purple sweet potato is a potential raw material for the production of functional glucose syrup. It has a high anthocyanin content (138.15 mg/100 g) and strong antioxidant activity (86.68%) [3]. In addition, purple sweet potato is rich in starch (57.18%) which consists of amylose and amylopectin. The starch can be hydrolysed to produce glucose, which is a sweet component widely used in the food industry. The monomeric sugar components in the hemicellulose and pectin of purple sweet potato, such as galactose, arabinose, xylose, ramnose, and mannose, can

reach levels of 10.39%, 3.68%, 3.33%, 2.05%, and 1.27%, respectively. Therefore, liquid sugar production is one of the efforts that can be made to optimize the utilization of carbohydrate components in purple sweet potato [4].

Characteristics of purple sweet potato starch that need to be considered in the liquid glucose production process include granule size and shape, degree of swelling, gelatinization temperature, and solubility [3]. Liquid glucose, a product of enzymatic or acidic hydrolysis of starch, has a taste and sweetness that is almost the same as cane sugar, even though some types are sweeter [5]. Liquid glucose can be applied in various industries, such as food, beverage, and pharmaceutical industries. The utilization of purple sweet potato as a raw material for liquid glucose can not only help reduce dependence on sugar imports but also provide added value for farmers and can improve national food security. The clear and viscous liquid glucose product is rich in D-glucose, maltose, and D-glucose polymers, which are derived from the hydrolysis of starch from sweet potato. The advantages of glucose syrup over sucrose are that it is more heat stable, smoother in texture, and has the ability to prevent crystallization, making it an ideal



choice in food and beverage, pharmaceutical, and other industries.

This study aims to produce liquid glucose from purple sweet potato through optimal enzymatic hydrolysis and evaluate the physicochemical characteristics of the liquid glucose produced. This liquid glucose product is expected to be a natural functional sweetener with antioxidant benefits for health.

2. MATERIAL AND METHODS

This study used purple sweet potato var. Antin 2 is obtained from Balitkabi Malang as the main ingredient. The enzymes used for the hydrolysis process were α -amylase enzyme (Novo, Liquezyme Supra) and glucoamylase enzyme (Novo, Thermamyl).

2.1. Research Design

The research consisted of two stages and used a completely randomised design (CRD), with each treatment repeated three times. The first stage of the research was the optimization of α -amylase enzyme concentration in the liquefaction process. At this stage, the concentration of α -amylase enzyme tried was 1.0 mL and 1.5 mL per kg of purple sweet potato substrate. The best parameter results were used for the recommendation of the second stage of research. The second stage of research was the optimization of glucoamylase enzyme concentration in the saccharification process. At this stage, three concentrations of glucoamylase enzyme were tried, namely 0.4 mL, 0.6 mL, and 0.8 mL per kg of liquefied purple sweet potato substrate. Each treatment was repeated three times. The data obtained were analyzed using one-way analysis of variance (ANOVA). If there was a significant difference, it was followed by the least significant difference test at the 5% real level.

2.2. Research Implementation

Preparation of main ingredients in the form of preparing purple sweet potatoes in good condition and free of rot, then peeled and washed with running water until completely clean.

2.2.1. Research Phase 1: Liquidation Process

- Purple sweet potato weighed as much as 200 g.
- Crushed using a blender until it becomes a paste/-porridge, and then put into Erlenmeyer.
- Added aquadest up to 1000 mL.
- Purple sweet potato pulp was conditioned at pH 5 using citrate buffer.
- The suspension was heated in a water bath until fully gelatinized.
- Then, the α -amylase enzyme was added according to the treatment: α -amylase enzyme concentration of 1 mL/kg sweet potato pulp (L1) and α -amylase enzyme concentration of 1.5 mL/kg sweet potato pulp (L2).
- The hydrolysis process was conditioned at 90°C for 15 minutes using an autoclave.
- After the process was completed, total soluble solids and reducing sugar were analyzed.

2.2.2. Phase 2 Research: Saccharification Process

The best liquefaction treatment results from stage 1 research were used for the saccharification process according to the glucoamylase enzyme concentration treatment. The steps of the experiment were as follows:

- The concentration of glucoamylase enzyme was made in various concentrations, namely 0.4 mL/kg (K1), 0.6 mL/kg (K2), and 0.8 mL/kg (K3).
- Hydrolysis was carried out at 60 °C for 72 hours.
- After hydrolysis, the undissolved solids were separated, and the suspension was neutralized to pH 7 using 0.1 M NaOH.
- Purification of glucose syrup was carried out by giving 0.5% activated charcoal.
- Subsequently filtered the resulting liquid glucose is then evaluated.

Evaluation of the quality of liquid glucose produced includes:

1. *Yield*: Liquid glucose yield was calculated as the percentage of liquid glucose syrup weight to the initial weight of purple sweet potato. The yield calculation used the following formula:

$$\text{Yield (\%)} = \frac{(\text{Final weight of liquid glucose syrup (g)})}{(\text{Weight of purple sweet potato (g)})} \times 100$$

2. *Anthocyanins*: Anthocyanin levels in liquid glucose syrup were measured using UV-Vis spectrophotometry at wavelengths of 530 nm and 700 nm. Calculation of total anthocyanin concentration was done with the following equation:

$$\text{Total Anthocyanins (\%w/w)} = \frac{A}{\varepsilon \times l} \times MW \times DF \times V / Wt \times 100$$

$$A = [(A_{520\text{nm}, \text{pH } 1} - A_{700\text{nm}, \text{pH } 1}) - (A_{520\text{nm}, \text{pH } 4, 5} - A_{700\text{nm}, \text{pH } 4, 5})]$$

where A is absorbance, ε is molecular absorbance of Cyanidin-3-glucoside (26900 L/(mol.cm)), l is width of cuvette (1 cm); MW is molecular weight of Cyanidin-3-glucoside (449.2 g/mol); DF is dilution factor, V is volume of pigment extract (l), and Wt is weight of starting material (g).

3. *Antioxidant Activity*: The antioxidant activity of liquid glucose syrup was measured using the DPPH (1,1-diphenyl-2-picrylhydrazyl) method. A standard curve was prepared by dissolving DPPH in methanol and measuring the absorbance at a wavelength of 518 nm. Antioxidant activity was calculated by the following formula:

$$\text{Antioxidant Activity (\%)} = \frac{((\text{initial DPPH}) - (\text{residual DPPH}))}{(\text{initial DPPH})} \times 100\%$$

where initial *DPPH* is absorbance of *DPPH* before reacting with the sample, residual *DPPH* is absorbance of *DPPH* after reacting with the sample.

4. *Total Dissolved Solids (TSS)*: TSS in liquid glucose syrup was measured using a handheld refractometer. The refractometer prism was rinsed with distilled water and dried with a soft cloth. The sample was dripped onto the prism, and then the degree of Brix was measured.
 5. *Reducing Sugar*: Reduced sugar content in liquid glucose syrup was measured using the Nelson-Somogyi method. A standard curve was prepared by dissolving glucose and measuring the absorbance at a wavelength of 540 nm. Reduced sugar content was calculated using the following formula:
- $$\text{Reduced Sugar Content (\%)} = \frac{(\text{Abs of sample} / \text{Abs of standard}) \times \text{Concentration of standard} \times 100\%}{1}$$
6. *Fibre Content*: Fibre content was analyzed using the AOAC 2005 method [6].
 7. *Viscosity*: The viscosity of liquid glucose was measured using a rheometer. The spindle is selected according to the concentration of the material or the degree of viscosity of the material. The scale shown on the rheometer was recorded and converted into units of dPas (centiPoise).
 8. *Sweetness Level*: The sweetness level of liquid glucose syrup was measured using sensory testing with trained panelists. Panelists were asked to rate the sweetness level of the samples on a hedonic scale of 1–5 (1 = not sweet, 5 = very sweet).

3. RESULTS AND DISCUSSION

3.1. Liquidation Result

The liquefaction stage is the first step in the conversion of purple sweet potato starch into glucose syrup. The results showed that the use of higher α -amylase enzyme concentration (L2) in the hydrolysis of purple sweet potato pulp resulted in higher total soluble solids (TSS) and reduced sugar content compared to the L1 treatment. The TSS value in L2 ($12.7^\circ\text{Brix} \pm 0.0058^\circ\text{Brix}$) was higher compared to L1 ($10.67^\circ\text{Brix} \pm 0.0058^\circ\text{Brix}$). Similarly, the reducing sugar content of L2 ($19.19\% \pm 0.0026\%$) was higher than that of L1 ($18.07\% \pm 0.0069\%$). Other studies have shown that bananas and Cilembu sweet potatoes can also be utilized as raw materials for glucose syrup production [3], [7]. Increasing the concentration of α -amylase enzyme is thought to increase the enzyme activity in breaking down starch molecules into intermediate products such as dextrin, glucose, maltose, and maltotriose. As a result, the content of soluble solids and reducing sugars in the liquefied yield increased.

3.2. Saccharification Result

The saccharification process plays an important role in breaking down the complex sugars contained in the

liquefied purple sweet potato starch slurry into simple sugars such as glucose. The saccharification results of the amyloglucosidase enzyme treatment are shown in Fig. 1.

Based on the results of the analysis of diversity in the saccharification process showed that the combination of α -amylase enzyme concentration of 1.5 ml with three variations of amyloglucosidase enzyme concentration of 0.4 ml (K1), 0.6 ml (K2), and 0.8 ml (K3) had a significant effect on yield, water content, total soluble solids, reducing sugar content, and anthocyanin content in syrup. Increasing the concentration of amyloglucosidase enzyme generally increased the yield and reduced the sugar content of the syrup but had the opposite effect on anthocyanins. The complete data is shown in Table I.

The highest yield increase (76.28%) and the highest reducing sugar content (48.6%) were obtained in the treatment with amyloglucosidase enzyme concentration of 0.8 mL/kg. An increase in amyloglucosidase enzyme concentration is thought to increase the efficiency of polysaccharide breakdown into glucose, resulting in an increase in yield and reduced sugar content. Conversely, increasing the concentration of amyloglucosidase enzyme decreased the water content and anthocyanins in the syrup. This is likely due to the increased enzyme activity that breaks down polysaccharides into more soluble glucose molecules, thus increasing the bound water content, which is more difficult to evaporate. Research results of Sunrixon *et al.* [4] explained that the conventional hydrolysis process using α -amylase and amyloglucosidase enzymes was able to convert purple sweet potato starch into simple sugars. Meanwhile, the fiber component in purple sweet potato is not hydrolyzed because the enzyme only works on α -1.4 and α -1.6 glycosidic bonds in starch molecules. Research Arif *et al.* [8] showed that the combination of α -amylase and xylanase enzymes at the liquefaction stage produced the highest total sugar yield (90.83 g/L) compared to the treatment without xylanase enzyme. The effect of saccharification temperature on glucose syrup characteristics is also associated with amyloglucosidase enzyme activity. The optimal temperature for amyloglucosidase enzyme activity generally ranges from 50°C – 60°C . The interaction between amyloglucosidase enzyme concentration and saccharification temperature shows that the right combination of enzyme concentration and temperature produces optimal glucose syrup characteristics [5]. Saccharification is the process of hydrolysis or breakdown of complex sugars contained in the starch slurry from the liquefaction results of stage 1 research into simpler sugars glucose in stage 2 research.

3.3. Anthocyanins

The results showed that the concentration of glucoamylase enzyme (0.4 mL/kg, 0.6 mL/kg, and 0.8 mL/kg) did not show a significant effect on anthocyanin levels in purple sweet potato glucose syrup. This is in line with research by El Husna *et al.* [9] and Munirayati *et al.* [10] which shows that the decrease in anthocyanin levels in processed purple sweet potato products is not influenced by the type of processed product. The results of the study of anthocyanin levels in glucose syrup from purple sweet potato ranged from 51.49 mg/g to 63.46 mg/g. Sweet potato varieties



Fig. 1. Liquid glucose from saccharification.

TABLE I: RESULTS OF SACCHARIFICATION ON YIELD AND CHEMICAL COMPONENTS

Amyloglucosidase concentration (ml/kg)	Yield (%)	Water content (%)	Dissolved total solids (⁰ Brix)	Reduced sugar (%)	Crude fibre (%)	Anthocyanins (mg/g)
0.4 (K1)	75.34 ^{ab}	19.04 ^b	21.33 ^a	34.12 ^a	4.87	63.46 ^c
0.6 (K2)	75.03 ^a	18.86 ^{ab}	24.67 ^b	44.69 ^b	3.41	51.49 ^a
0.8 (K3)	76.28 ^c	16.77 ^a	25.67 ^{bc}	48.6 ^c	3.37	57.89 ^b

Note: a, b, c, d = Values with different letters in a column have significant differences.

TABLE II: ANTIOXIDANT ACTIVITY OF LIQUID GLUCOSE SYRUP FROM PURPLE SWEET POTATO

Amyloglucosidase concentration (ml/kg)	Antioxidant activity (%)		
	5 ppm DPPH	10 ppm DPPH	20 ppm DPPH
0.4 (K1)	10.06 ^a	6.883 ^a	4.247 ^a
0.6 (K2)	11.35 ^b	7.289 ^b	4.345 ^b
0.8 (K3)	12.02 ^c	7.498 ^c	4.396 ^c

Note: a, b, c, d = Values with different letters in a column have significant differences.

also affect anthocyanin levels; the ‘Churakanasa’ variety, with higher cyanidin content, produces glucose syrup with higher anthocyanin levels compared to other varieties [11]. The difference in anthocyanin content is thought to be due to the effect of heating temperature during the hydrolysis process at the liquefaction and saccharification stages, which can result in anthocyanin damage.

3.4. Antioxidant Activity

Measurement of antioxidant activity was carried out by reacting the sample solution with DPPH. The sample solution containing a compound that can function as an antioxidant when reacted with DPPH, the DPPH solution, which was originally purple, turns yellow; this identifies that DPPH has been reduced so that DPPH turns into DPPH-H (diphenylpicryl hydrazine). The results of the antioxidant activity test of glucose syrup from purple sweet potato with DPPH are shown in Table II.

The results showed that the highest antioxidant activity of glucose syrup from purple sweet potato was obtained in the addition of 5 ppm DPPH in the α -amylase enzyme treatment sample 1.5 ml/kg (L2) and amyloglucosidase enzyme concentration 0.8 ml (K3). Research by Prasetyo and Winardi [12] shows that antioxidant activity in purple sweet potatoes is influenced by the anthocyanin content. The concentration of amyloglucosidase enzyme 0.8 ml in

the K3 treatment still produces relatively high anthocyanin levels. According to Laga *et al.* [13], the heating process can reduce antioxidant activity in purple sweet potatoes. This is thought to be because the heating process can cause the degradation of anthocyanin compounds into ketone products, thus having a lower antioxidant ability. The optimal concentration of amyloglucosidase enzyme can increase the efficiency of the breakdown of purple sweet potato starch polysaccharides into glucose, thus facilitating the liberation of anthocyanins. This condition results in higher antioxidant activity.

3.5. Viscosity and Sweetness Level

The results showed that the viscosity of the purple sweet potato glucose syrup ranged from 3.656 cp–4.872 cp. The lowest viscosity was obtained in the treatment of glucoamylase enzyme 0.4 ml/kg purple sweet potato pulp substrate (K1), while the highest viscosity was in the concentration of glucoamylase enzyme 0.8 ml/kg purple sweet potato pulp substrate (K3). The results of the variance analysis showed that the viscosity value was significantly different ($p = 0.05$). The increase in glucoamylase enzyme concentration from 0.4 ml/kg (K1) to 0.8 ml/kg (K3) caused the viscosity of purple sweet potato glucose syrup to become thicker. This is in line with research by Hidayah *et al.* [14] which shows that the viscosity of liquid sugar

increases with increasing enzyme and saccharification time. The increase in viscosity is thought to be due to the addition of the enzyme glucoamylase, which increases the efficiency of the breakdown of purple sweet potato starch polysaccharides into glucose, resulting in more glucose molecules. These more glucose molecules cause the glucose syrup to become thicker. The use of glucoamylase enzyme to hydrolyze purple sweet potato starch into glucose syrup. The use of glucoamylase enzyme is more effective than acid hydrolysis [15]. Therefore, the enzyme glucoamylase can break glycosidic bonds specifically without leaving residues and minimize color damage. The results of sensory testing on the level of sweetness obtained the highest score of 3.9 (sweet) from the results of the treatment of α -amylase enzyme concentration of 1.5 ml and glucoamylase enzyme concentration of 0.8 ml (K3) with a score of 3.9 (sweet). Increasing the concentration of amyloglucosidase enzyme can increase the efficiency of the breakdown of purple sweet potato starch polysaccharides into glucose, thus having an impact on increasing the sweetness level of the resulting liquid glucose.

4. CONCLUSION

The effect of α -amylase and amyloglucosidase enzyme concentration on the hydrolysis of purple sweet potato pulp substrate affects the characteristics of purple sweet potato liquid glucose. Higher α -amylase enzyme concentration (1.5 ml/kg substrate) increased the total soluble solids and reduced sugar content at the liquefaction stage.

At the saccharification stage, the optimal amyloglucosidase enzyme concentration (0.8 mL/kg substrate) produced the highest yield and reduced sugar content but resulted in a decrease in water and anthocyanin content. The highest antioxidant activity of purple sweet potato glucose syrup was obtained by adding 5 ppm DPPH to the sample with amyloglucosidase enzyme concentration of 0.8 mL/kg substrate.

The viscosity of purple sweet potato glucose syrup increased with increasing concentration of glucoamylase enzyme and produced a sweet taste. The use of glucoamylase enzyme is more effective than acid hydrolysis to produce purple sweet potato glucose syrup with better quality.

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CONFLICT OF INTEREST

The authors declare that they do not have any conflict of interest.

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