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Production of Glucose Syrup Derived from Cassava Peel Starch Using Amylase Enzyme from Green Bean Seed Sprout Extract (*Phaseolus radiatus* L.)

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ABSTRACT

The production of glucose syrup derived from cassava peels starch using amylase enzyme from green bean seed sprout extract (*Phaseolus radiatus* L.) has been carried out by isolating the enzyme from the green bean seed sprouts extract then purified by precipitation using ammonium sulphate with the saturation of 60% (w/v). Amylase enzyme activity was determined using the Nelson Somogyi method. The research results obtained the activity of the amylase enzyme in the deposition of 60% ammonium sulphate at 12.22 U/mL. Amylase enzyme was applied as a hydrolyzed starch from cassava peels with a weight ratio of raw material with water 10% (w / v) to produce glucose syrup with the addition of 0.25% enzyme; 0.5%; 0.75%; 1% (v / v). The glucose syrup obtained was then tested for viscosity, reducing sugars, water content, ash content, and organoleptics. The best glucose syrup analysis results from adding 1% amylase enzyme with a viscosity value of 238.71cP, water content of 12.45%, reduction sugar content of 33.81%, ash content of 0.49%, and dextrose equivalent value of 50.16%.

Keywords: Amylase Enzyme, Cassava Peel Starch, Glucose Syrup, Green Bean Seed

ABSTRAK

Proses pembuatan sirup glukosa dari pati kulit singkong dilakukan dengan mengisolasi dan memurnikan enzim amilase dari ekstrak tauge kacang hijau (*Phaseolus radiatus L.*). Pemurnian dilakukan dengan pengendapan enzim menggunakan amonium sulfat dengan tingkat kejenuhan 60% (b/v). Aktivitas enzim amilase dinilai menggunakan metode Nelson Somogyi. Hasil penelitian menunjukkan bahwa enzim amilase mempunyai aktivitas sebesar 12,22 U/mL diendapkan menggunakan amonium sulfat 60%. Enzim amilase dimanfaatkan sebagai hidroliser pati yang berasal dari kulit singkong, dengan perbandingan berat 10% (b/v) bahan baku terhadap air. Proses ini bertujuan untuk menghasilkan sirup glukosa, dengan variasi penambahan enzim yang berbeda: 0,25% (v/v), 0,5%, 0,75%, dan 1% (v/v). Analisis viskositas, gula pereduksi, kadar air, kadar abu, dan organoleptik sirup glukosa yang dihasilkan selanjutnya dianalisis. Hasil analisis yang paling optimal untuk sirup glukosa diperoleh dengan penambahan enzim amilase 1% sehingga menghasilkan nilai viskositas sebesar 238,71 cP. Sirup tersebut mempunyai kadar air 12,45%, kadar gula reduksi 33,81%, kadar abu 0,49%, dan nilai setara dekstrosa 50,16%.

Kata Kunci: Enzim Amilase, Kecambah Biji Kacang Hijau, Sirup Glukosa, Pati Kulit Singkong

1. Introduction

Glucose syrup is a solution obtained through hydrolysis of starch, then neutralization and concentration to a certain level [1]. The hydrolysis method is a process for obtaining glucose syrup from starch, which is carried out by acid hydrolysis, enzymatic hydrolysis, and a combination of enzyme and acid hydrolysis [2]. The enzyme used to break down protein is the protease enzyme, the fat breakdown is the lipase enzyme, and the starch breakdown is the amylase enzyme. These enzymes are simultaneously produced by plants during the germination process [3].

Green beans are a type of legume that has the potential to produce the enzyme amylase. The composition of green beans shows that the contents of green beans include 11.91% water, 19.34% protein, 0.83% fat, 3.81% ash, 64.11% carbohydrates, and 43.46% starch [4]. Starch is a complex carbohydrate insoluble in water, in the form of a white powder, tasteless and odorless. Starch is the primary material produced by plants to store excess glucose (as a product of photosynthesis) in the long term [5]. Cassava is the second largest agricultural food product in Indonesia after rice, so the availability of cassava has the potential to be an important raw material in various food products. Cassava is a food ingredient commonly consumed by Indonesians [6-7].

Cassava peel is often considered useless waste by some cassava raw material industries. Cassava peel can be a highly economical product and can be processed into mocaf flour. The percentage of cassava skin is approximately 20% of the tubers, so per kg of cassava, tubers produce 0.2 kg of Salim cassava skin [8]. According to Richana (2013), the starch content in cassava skin ranges from 44-59%. So, it has the potential to be an alternative mixture that can replace ingredients that require the properties of starch [9].

2. Materials and Methods

2.1. Equipment

In this study, the tools used were a set of glassware, blender, analytical balance, hot plate, pH meter, refrigerator, cuvette, filter, magnetic bar, UV-visible equipment, Hitachi brand centrifuge, FT-IR equipment

2.2. Materials

Materials used in this study were Mung Bean Sprouts$_{(s)}$, Cassava Peel$_{(s)}$, $NaCl_{(s)}$, Aquadest$_{(l)}$, $BaCl2_{(s)}$, $(NH_4)_2SO_{4(s)}$, Phosphate buffer$_{(aq)}$, $NaH_2PO_4.H_2O_{(s)}$, $Na_2HPO_{4(s)}$, $NaOH_{(s)}$, $H2SO_{4(p)}$, $CuSO_4.5H_2O_{(s)}$, $KNaC_4H_4O_6.4H_2O_{(s)}$, $Na_2HAsO_4.7H_2O_{(s)}$, Iodine$_{(s)}$, Starch Indicator$_{(s)}$.

2.3. Research Procedure

2.3.1 Isolation and Purification of Amylase Enzyme from Green Bean Seed Sprouts with Ammonium Sulfate

In cold conditions, 150 g of green bean sprouts were homogenized with 250 mL of 1% isotonic NaCl solution. Then, blend until smooth and filter until the filtrate and dregs are separated. The filtrate was centrifuged at 10.000 rpm at 20ºC for 10 minutes. The supernatant obtained was added with ammonium sulfate with a saturation of 60% (w/v). Leave it overnight in the refrigerator. The mixture was then centrifuged at 10.000 rpm at 20ºC for 30 minutes. The residue was inserted into a cellophane membrane soaked in a phosphate buffer solution and dialyzed while stirring for 8 h. The solution was changed every 2 h. The dialysis solution was tested with $BaCl_2$ to ensure that the ammonium sulphate salt and other impurities had been removed from the crude enzyme solution. The enzyme that has been dialyzed is then tested for activity again [10].

2.3.2 Preparation of Starch from Cassava Skin

Starch was prepared by cleaning 1 kg of cassava skin with running water until it was clean and white. Next, water is added to the cassava skin in a 1:1 ratio. Next, grind it using a blender and leave it for 30 minutes until it forms two layers in the form of sediment and is filtrated and filtered. The precipitate obtained was washed with distilled water and precipitated again for 30 minutes. After that, the precipitate was placed on a baking sheet, dried at 70ºC for 30 minutes, and sieved with a 60 mesh sieve to obtain refined starch [10].

2.3.3 Making Glucose Syrup

The manufacture of glucose syrup in this research was carried out through several stages of treatment until cassava peel pulp was produced and ready to be processed. Cassava peel starch was dissolved in water to form a starch solution with a ratio of 10% (w/v). Next, the starch was gelatinized for 1 h at 60ºC. The starch solution was subjected to a liquefaction process, the process of hydrolysis of starch into dextrin by the amylase enzyme with varying concentrations of 0.25%, 0.5%, 0.75%, and 1% (w/v) of green bean seeds for ± 120 minutes at 60ºC. The starch that has become dextrin is cooled to 40ºC with continuous stirring for 72 hours. The bleaching process was then done by adding 0.5% activated carbon by weight of starch. Next, filtering and evaporation are used to obtain the desired glucose syrup.

2.3.4 Reducing Sugar Content Test Luff Schoorl Method

2 g of glucose syrup was put into a 50 mL measuring flask and diluted to the upper limit of the measuring flask. Then, pipette 10 mL using a volume pipette and put it in an Erlenmeyer flask. Add 25 ml of luff schrool solution and 15 ml of distilled water. Heat the mixture and leave for 10 minutes. Then, cool the sample with water containing ice. After cooling, slowly add 15 mL of 20% KI and 25 mL of 25% H_2SO_4. Titrate with 0.1 N $Na_2S_2O_3$ solution until the solution is pale yellow, then add 0.5% starch indicator and titrate again until the solution is milky white. Then note the volume of 0.1N $Na_2S_2O_3$ used. The same treatment was carried out for the blank volume.

2.3.5 DE (Dextrose Equivalent) Value Analysis

The DE value is calculated based on the ratio of glucose syrup reducing sugar to total DE glucose syrup sugar

$$\text{DE} = \frac{\text{Reducing Sugar}}{\text{Total Sugar}} \text{ x } 100\%$$

2.3.6 Organoleptic Test

Organoleptic assessment of aroma, taste, and texture was carried out using hedonic tests. The method is that glucose syrup that has been coded is tested randomly by panellists. Testing is carried out sensory (organoleptic), which is determined based on a numerical scale. Organoleptic tests were carried out on untrained panellists on 25 USU FMIPA students (15 women and 10 men).

2.3.7 Water Content Test (SNI 01-2891-1992) Glucose Syrup

2 g glucose syrup was placed in a porcelain cup of known weight and dried in an oven at 105ºC for 3 hours. Next, it was cooled in a desiccator and weighed again until a constant weight was obtained [11].

2.3.8 Ash Content Test (SNI 01-2891-1992) Glucose Syrup

2 g of glucose syrup was placed in a porcelain cup of known weight and dried in the oven at 600ºC for 6 h. The obtained ash was cooled in a desiccator and then weighed until a fixed weight was obtained [11].

2.3.9 Viscosity Test

10 mL of distilled water was put into the Oswald viscometer. Inhale the distilled water solution until the line appears, then stop suction. The time it takes for distilled water to fall from the upper to the lower limit is measured. Then 10 mL of glucose syrup was added, using the same steps as above, and repeated 3 times. The same thing was done for glucose syrup with varying concentrations of the amylase enzyme, and the viscosity was calculated.

3. Results and Discussion

3. 1 Results of Isolation and Partial Purification of Crude Amylase Enzymes from Mung Bean Seed Sprouts Using Ammonium Sulfate

The isolation and purification of crude amylase enzyme from green bean sprouts using 60% ammonium sulphate and a dialysis membrane resulted in 65 mL of amylase enzyme in 300 mL of green bean sprout extract.

$$\% \text{ Yield} = \frac{\text{Weight of crude amylase enzyme}}{\text{Weight of green bean sprouts}} \text{ x } 100\%$$
$$= \frac{65\ mL}{300\ mL} \text{ x } 100\%$$
$$= 0.21\%$$

3. 2 Results of Making Starch from Cassava Skin

The starch used in this research resulted from isolating cassava peel starch, where 1 kg of cassava peel 125 g (12.5%) was obtained. From FT-IR spectroscopy data, the isolated starch gives a spectrum with vibration peaks in the wave number area of 3426.58 cm^{-1}, 2931.80 cm^{-1}, 1635.64 cm^{-1}, and 1018.41 cm^{-1}. The FT-IR spectra of commercial starch and cassava peel starch shown in Figure 1.

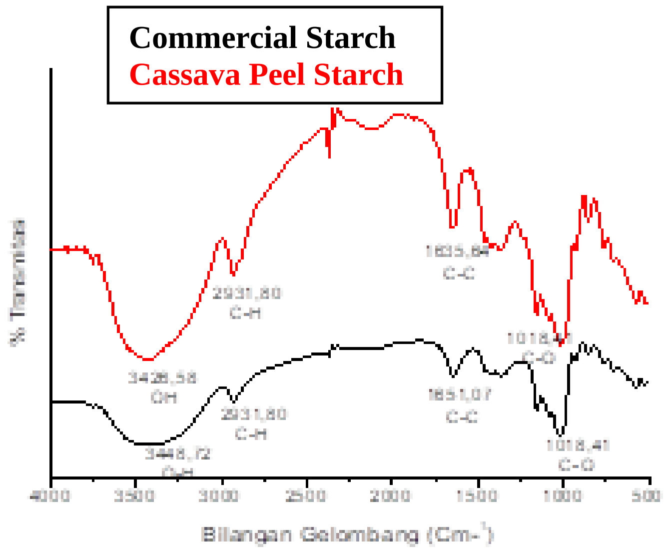


Figure 1. FT-IR spectra of commercial starch and cassava peel starch

3. 3 Making Glucose Syrup

The concentration of the amylase enzyme is in a high variation, namely 1%, whether isolated from green bean sprouts or commercial amylase enzyme. The greater the starch hydrolyzed by the enzyme, and there is a tendency that the more starch is hydrolyzed, the higher the glucose level, which occurs during incubation. Further degradation by the amylase enzyme.

3. 4 Analysis of Glucose Syrup Reducing Sugar Content

Based on research data, glucose syrup has various commercial enzymes and enzymes isolated from green bean sprout extract with variations of 1%, 0.75%, and 0.5%. Still meets SNI standards, while the 0.25% variation no longer meets SNI standards. According to SNI 01-3544-1994, the reduced sugar content is at least 30%.

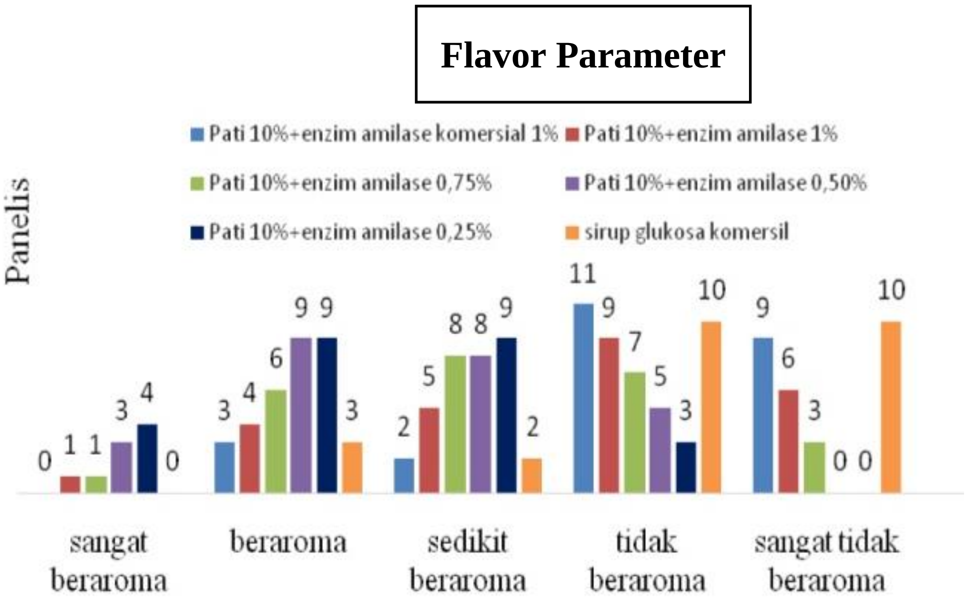
Table 1. Analysis of glucose syrup reducing sugar content

No	Variation	Reduced Sugar Levels (Glucose)	Dextrose Equivalent Levels
1	Starch 10 % + commercial amylase enzyme 1 %	34.56 %	51.27 %
2	Starch 10 % + amylase enzyme 1 %	33.81 %	50.16 %
3	Starch 10 % + amylase enzyme 0.75 %	32.89 %	48.79 %
4	Starch 10 % + amylase enzyme 0.5 %	30.18 %	44.77 %
5	Starch 10 % + amylase enzyme 0.25 %	28.67 %	42.54 %

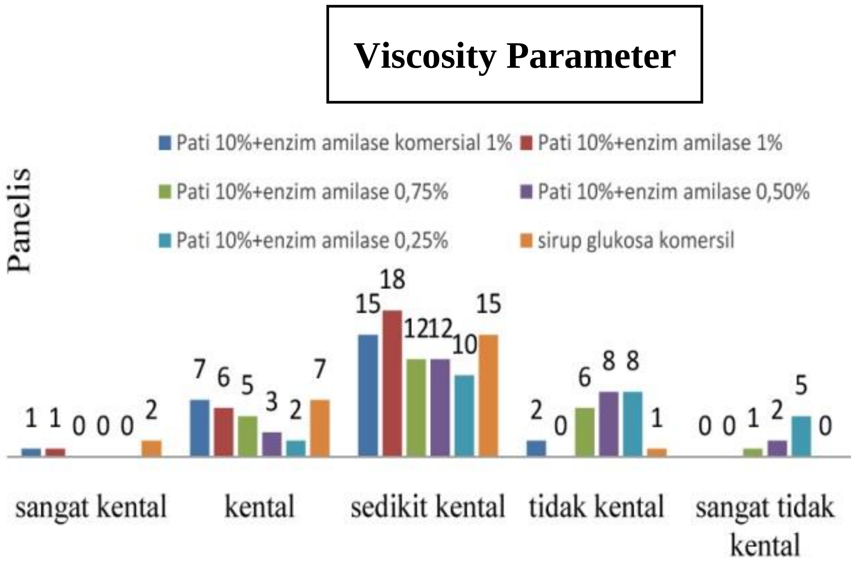
3. 5 Organoleptic Tests

Organoleptic testing was tested based on the sensing process. Organoleptic tests on food products are important because they relate to the panellists' acceptance of the products produced [12]. The parameters tested in this research include aroma, texture, sweetness, and preference using the hedonic method.

3.5.1 Flavor



Regarding aroma parameters, the one with the most aroma in glucose syrup is 10% starch with the addition of 0.25% amylase enzyme.



3.5.2 Texture

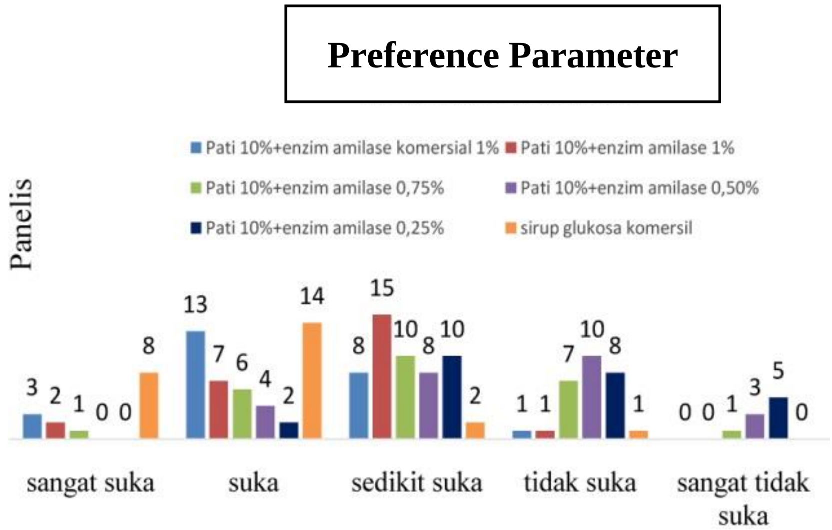
In terms of viscosity parameters, glucose syrup from 10% starch with the addition of 1% commercial amylase enzyme and 10% starch with the addition of 1% amylase enzyme from green bean sprouts has the thickest texture.

3.5.3 Sweetness

In terms of sweetness parameters, the ones that taste sweeter are commercial glucose syrup, glucose syrup with 10% starch with the addition of 1% commercial amylase enzyme, and 10% starch with the addition of 1% amylase enzyme.

3.5.4 Preference

In terms of preference parameters, what is preferred is glucose syrup from 10% (w/v) starch with the



addition of 1% commercial amylase enzyme and 10% (w/v) starch with the addition of 1% amylase enzyme from green bean sprouts.

3. 6 Water Content

Based on tests, the water content of the glucose syrup produced in this study ranged from 9.31-23.74%. Based on research data, the amount of water content of glucose syrup when adding a variety of commercial enzymes of 1% and isolated enzymes with variations of 1%, 0.75%, and 0.5% is still within the SNI standard limits, whereas when adding 0.25% enzyme the results are obtained has passed the SNI threshold. According to SNI 01-3544-1994, the maximum water content is 20% [11]. The histogram of water content can be displayed in Figure 2.

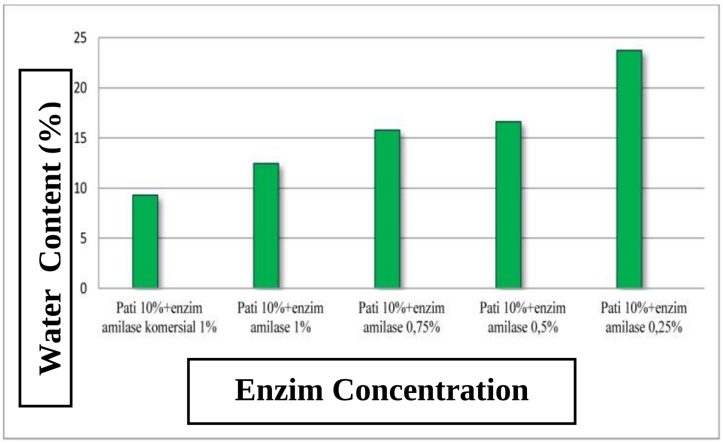


Figure 2. Histogram of water content

3. 7 Ash Content

The ash content of the glucose syrup produced in this was around 0.45%-0.95%. Based on research that has been carried out, the ash in all variations of the addition of enzyme concentration is still within the SNI standard limits. According to SNI 01-3544-1994, the maximum ash content is 1%. The histogram of ash content can be displayed in Figure 3.

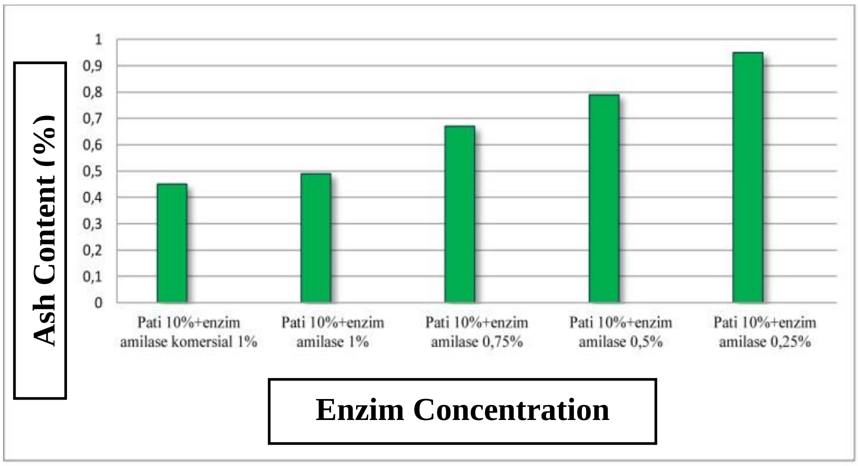


Figure 3. Histogram of ash content

3. 8 Viscosity Test Results

Based on the viscosity test results, the highest viscosity of glucose syrup was shown in 10% starch with 1% commercial amylase enzyme and 10% starch with 1% amylase enzyme from green bean sprouts. The data shows that glucose syrup with the addition of the amylase enzyme from mung bean sprout extract at 1% is closest to the viscosity value of the commercial enzyme, namely 238.71cP. The viscosity test result is illustrated in Figure 4.

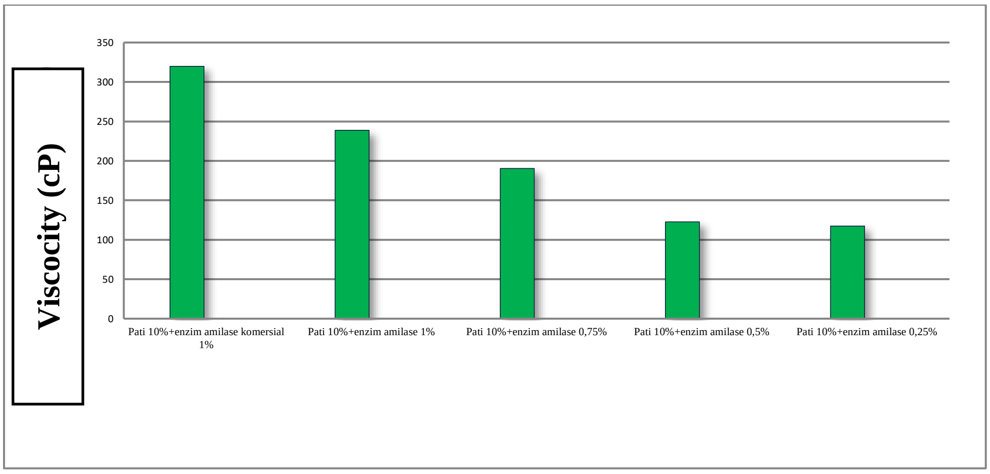


Figure 4. Histogram of viscosity test result

4. Conclusion

The amylase enzyme from green bean sprout extract has been isolated by precipitation fractionation using ammonium sulphate and dialysis. So 65 mL of crude amylase enzyme was obtained from 300 mL of green bean sprout extract. Furthermore, the amylase enzyme has been purified using ammonium sulphate with a saturation of 60%, increasing enzyme activity. Where previously the enzyme activity had an average of 9.3 U/mL, there was an increase in activity of 12.22 U/mL. In addition, glucose syrup produced from the hydrolysis of cassava peel starch using the amylase enzyme isolated from the extract of green bean sprouts (*Phaseolus radiatus* L.) is the best under optimum conditions, namely a concentration variation of 1% with a viscosity value of 238.71cP, reducing sugar content of 33.81 %, dextrose equivalent content 50.16 %, water content 12.4521%, ash content 0.49%.

5. Acknowledgements

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6. Conflict of Interest

Authors declare no conflicts of interest

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