Utilization of cassava peel waste (*Manihot esculenta* L.) with concentration variations of α-amylase and glucoamylase enzymes for liquid sugar

Pemanfaatan limbah kulit singkong (*Manihot esculenta* L.) melalui variasi konsentrasi enzim α-amilase dan glukoamilase dalam pembuatan gula

Rais Nur Latifah*, Nur Rahma Martiyana
Fakultas Sains dan Teknologi, Universitas Islam Negeri Walisongo Semarang
Jalan Prof. Dr. Hamka Km. 1, Semarang, Indonesia
* e-mail: rais.nurlatifah@walisongo.ac.id

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**ABSTRACT**

The production of liquid sugar utilizing cassava peel waste has been carried out in this study. The method used in the production of liquid sugar employs an enzymatic method. The stages in the enzymatic method are the liquefaction and saccharification processes. The liquefaction process involves the addition of α-amylase in varying amounts to break down starch into amylose and amylpectin. The saccharification stage aims to hydrolyze dextrins into liquid sugar using glucoamylase enzymes. The volumes of enzymes used were 0.2 mL and 0.6 mL. The research results indicate that the moisture content and ash content are in accordance with the Indonesian National Standard (SNI) 8779:2019, at 6.19% and 0.76%, respectively. Qualitative analysis of cassava peel starch shows positive results for amylum, dextrins, and glucose content. FTIR analysis of cassava peel starch reveals the presence of O-H groups, C-H groups, and C-O-C groups. Total Soluble Solid (TSS) testing for 0.2 mL and 0.6 mL enzyme volumes resulted in 12.56% and 17.37%, respectively. The optimum results for liquefaction and saccharification were observed with the addition of 0.2 mL enzyme volume. The functional groups of the liquid sugar were analyzed using FTIR, which showed the presence of O-H stretching, carbonyl groups (C=O), stretching vibration of C-OH, and vibration of glycosidic bonds C-O-C.

**Kata kunci:**
enzim a-amilase; limbah kulit singkong; glukoamilase; gula cair.

**ABSTRAK**

Pembuatan gula cair dengan memanfaatkan limbah kulit singkong telah dilakukan dalam penelitian ini. Metode yang digunakan dalam produksi gula cair menggunakan metode enzimatis. Tahapan dalam metode enzimatis yaitu proses liquifikasi dan sakarifikasi. Proses liquifikasi menggunakan variasi penambahan α-amilase untuk memecah pati menjadi amilosa dan amilopektin. Tahapan sakarifikasi untuk proses hidrolisis dekstrin menjadi gula cair dengan enzim glukoamilase. Volume enzim yang digunakan yaitu 0,2 mL dan 0,6 mL. Hasil penelitian menunjukkan bahwa kadar air dan kadar abu sudah sesuai dengan Standar Nasional Indonesia (SNI) 8779:2019 yaitu sebesar 6,19% dan 0,76%. Analisis kualitatif terhadap tepung pati kulit singkong menunjukkan hasil positif terhadap kandungan amilum, dekstrin dan glukosa. Hasil FTIR tepung pati kulit singkong menunjukkan adanya gugus O-H, gugus C-H dan gugus C-O-C. Pengujian TSS (Total Soluble Solid) pada 0,2 mL dan 0,6 mL volume enzim adalah 12,56% dan 17,37%. Hasil liquifikasi dan sakarifikasi menunjukkan hasil optimum pada penambahan volume enzim 0,2 mL. Gula cair dianalisis gugus fungsiya dengan FTIR. Hasil FTIR menunjukkan adanya gugus O-H stretching, gugus karbonil (C=O), vibrasri stretching dari C-OH dan vibrasri dari ikatan glikosidik C-O-C.

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1. Introduction

Sugar is one of the commodities in the food sector and source of calories. The level of sugar consumption has increased but is not matched by domestic sugar production which has implications for increasing sugar prices every year (Saviriti and Widyastuti, 2013). Sugar is a special commodity in agriculture that has been stipulated as a trade agreement of the World Trade Organization (WTO) (Srivastava and Rai, 2012). The world's need for sugar as a source of calories and energy sources continues to increase along with the increase in the world's population, but the increase in sugar production tends to be small or even unchanged, caused sugar prices to rise (Yani et al., 2012). Based on data from the Indonesian Sugar Association and the Indonesian Sugar Experts Association published by the Central Statistics Agency, white crystal sugar production fell 52% in 2020. Production fell from 2.2 million tons in 2019 to only 2.13 million tons in 2020. Indonesia's sugar production has fluctuated since 2017.

In 2017, sugar production reached 2.12 million tons. That number fell 8 percent to 1.17 million tons in 2018. A year later in 2019, sugar production increased 89 percent to 2.22 million tons. Then the production of granulated sugar fell again in 2020. The Ministry of Industry in 2021, the national sugar production will be 2.35 million tons, while the demand for sugar in 2022 will reach around 6.28 million tons. The inability of the domestic sugar industry to meet the demand for sugar has caused Indonesia to import sugar. To reduce sugar imports, one has to look for alternative sweeteners as a sugar substance, one of which is by developing liquid sugar from Cassava peel waste.

Cassava peel has a high carbohydrate content so that it can be processed into raw materials for glucose production. The raw material in the manufacture of glucose is a material containing polysaccharides obtained from starch (Budiarti et al., 2017). Starch is a polymer with the chemical formula anhydrous monosaccharide (C6H12O5) with the main constituents amylose and amyllopectin (Djulardi and Sumarni, 2018). The high content of carbohydrates contained in Cassava peel waste can be developed for mass application to meet national food security. The sweetness of tubers can be obtained through the breakdown of carbohydrates (starch) by the enzyme amylase into sugar. The type of sugar can determine the sweetness of each type of tuber. Sweet taste in tubers correlates with the amount of sugar, especially reducing sugars such as fructose and glucose (Al-kayyis and Susanti, 2016). As many as 60% of carbohydrates in cassava are composed of starch.

The starch content is composed of glucose monomers consist of 15-30% amylose and 70-85% amylpectin (Maulida et al., 2016). Glucose is a material containing polysaccharides obtained from starch. Starch is a polymer with the chemical formula anhydrous monosaccharide (C6H12O5) with the main constituents amylose and amyllopectin (Wasishta et al., 2021). The content in cassava makes starch can be degraded into glucose which is useful for high-quality derivative products in the food industry. Universally, the sweetness of tubers is obtained process of breaking down carbohydrates or starch by the enzyme amylase into sugar (Souto et al., 2017).

Cassava peels are rich of starch content and competitive demand as industrial. The level of sugar quality produced is determined by the optimal conditions for hydrolysis of cassava peels and the level of sweetness for the resulting sugar. Cassava peels were obtained from Nusukan Market, Surakarta, Central Java. This study aims to utilize Cassava peel waste as a basic ingredient in the manufacture of liquid sugar by using the enzymatic hydrolysis method with α-amylase and glucoamylase enzymes. This study also aims to determine the effect of increasing the volume of α-amylase and glucoamylase enzymes in producing liquid sugar.

2. Method

2.1. Preparation of starch from cassava peel

Cassava peels were obtained from Nusukan Market, Surakarta, Central Java. Then the red outer skin of the cassava is separated from the impurities. Cassava skin washed with water until clean. 50 grams of Cassava peel is blended and added 500 mL water then filtered with a filter cloth. The filtrate and the filtered precipitate are separated with a filter funnel. The precipitate is obtained starch from filtration. The precipitate was washed with distilled water and dried in an oven until constant weight.

2.2. Testing water content and starch ash content of cassava peel waste

Water content is a factor that has a major influence on the durability of processed material. The principle of water content analysis is evaporate the free water molecules contained in the sample. The water content test was carried out with the oven method. 2 grams sample is weighed into a porcelain dish that has been dried. The sample is baked at 100-105°C for 6 hours and cooled in a desiccator for 30 minutes and weighed. The percentage of water content can be calculated with formula:

\[
\% \text{Water content} = \frac{B-C}{B-A} \times 100\%
\]

Information:

A is the weight of an empty porcelain cup (grams). B is the weight of the porcelain cup + the initial sample (grams). C is the weight of the porcelain cup + dry sample (grams).

The principle of ash content analysis is burned of organic materials which is broken down into water and carbon dioxide molecules, but inorganic substances do not burn. 2 grams starch sample was weighed with a porcelain dish that had been dried, after that it was put into a furnace at 550-600°C for 3 hours. The percentage value of ash content is calculated with the formula:

\[
\% \text{Ash Level} = \frac{C-A}{B-A} \times 100\%
\]

Information:
2.3. Liquid sugar from cassava peel

The manufacture of liquid sugar used the enzymatic hydrolysis method that consists of two stages. The first stage is the liquefaction stage. In this stage, 30 grams of Cassava peel is dissolved in 200 mL of water and stirred until the solution becomes homogeneous, then the pH is adjusted to 5-7 with NaOH. The formed sample was heated at 90°C stirring evenly. This stage lasts for 60 minutes. Furthermore, the samples were analyzed for TSS (Total Soluble Solid). The results of the liquefaction stage proceed to the saccharification stage. The sample was cooled at 60°C, the pH was checked around 4-4.6, then 0.2 mL and 0.6 mL of glucoamylase were added. This stage lasts for 24 hours. The liquid sugar formed was neutralized with 0.2 N Na₂CO₃ and added 0.1 grams of activated charcoal then heated at 80°C and stirred for 30 minutes. The solution was allowed to stand for 1 hour and filtered. After that, it was heated again (80°C, 30 minutes) and the liquid sugar formed was checked for thickness with a refractometer. Liquid sugar with the highest TSS value was analyzed for functional groups using FTIR (Anita et al., 2013).

2.4. Liquid sugar qualitative analysis

Qualitative analysis were starch test, dextrin test and Benedict. Starch testing is dripped Lugol in the gelatinization process. Dextrin testing was carried out at the end of the liquefication process. The test was carried out by adding lugol and alcohol gradually. Benedict's test is used to determine the presence of glucose in the saccharification process. The test was carried out by dripping Benedict's reagent and heated on a bath for 5 minutes.

2.5. Analysis of TSS (Total Soluble Solid)

TSS (Total soluble solids) is solids that are dissolved within a substance. The measurement of the brix degree value was carried out with a refractometer. The measurement of the brix degree value serves to determine the level of sweetness of the liquid sugar sample. Refractive index measurements using a refractometer can use Snell's Law. This test procedure is carried out by dripping a sample of liquid sugar on a refractometer prism and read the scale. The TSS value obtained in % units uses the equation:

\[ \text{TSS} = \text{°Brix} \times \text{dilution factor} \]  

2.6. Functional group test with FTIR (Fourier Transform Infrared)

The FTIR test is carried out to identify the functional group or wave number of the compound formed from the liquid sugar sample. Based on information from infrared absorption reference data, the wave number of glucose is in the range of 900 cm⁻¹ – 1200 cm⁻¹ or in the wavelength of 8.3 m – 11 m.

3. Result and discussion

Cassava peel waste is the main raw material in the manufacture of liquid sugar. Cassava peel has a composition of 18-20% in each cassava fruit. Cassava consists of a brown outer cassava peel, pink and white skin layers and the cassava tuber itself. Cassava peel parts are considered as waste and discarded because they contain cyanide compounds and toxic. Cassava has three layers that provide protection against cassava tubers. The layer is the outer part of the brown skin, the red part and the white part which is rich in starch (Mohd-Asharuddin et al., 2017). The nutritional content in cassava is 1 grams of protein, 0.3 grams of fat, 154 kcal and 36.8 grams of carbohydrates (Bayata, 2019).

In this study, the Cassava peel was processed into starch by first removing the cyanide content in the cassava peel. Cassava peel starch flour was extracted by water and precipitated to obtain starch flour. The starch yield from this Cassava peel waste was 28.32%. The resulting Cassava peel starch is white. Starch has a smooth form. The size is 100 mesh.
The Figure 2 shows the highest water content at 75 minutes. That is 7.25%. The lowest water content is at the 100 minute with a level of 6.19%. The results of the water content from liquid sugar sample was obtained by the more enzymes added and the less water content. This is because more simple sugar molecules or reduce sugars. The more glycosidic bonds can be broken, the more simple molecules are produced. Each cleavage of the glycosidic bond will draw water into the resulting simple sugar molecule (Hasmadi et al., 2021). While the highest ash content is at minute 25 with an ash content of 1.89%. The lowest ash content is at minute 100 with an ash content of 1.26%. According to the Indonesian National Standard (SNI) 8779:2019, the ash content has a standard in the resulting liquid sugar syrup, which is a maximum of 1%. The less ash content in liquid sugar, the better the quality.

The resulting starch was analyzed qualitatively. Cassava peel starch was analyzed qualitatively by testing the content of starch, dextrin and benedict. Starch is a polysaccharide composed of amylose and amylopectin bound by glycosidic bonds. The starch test results showed a positive presence of starch content. This was indicated by a color change from white to dark blue and the presence of starch suspension in the test solution. Then the starch was tested for dextrin. In the dextrin test was dripped with Lugol, a suspension was not mix with Lugol, then it was dripped with alcohol and the resulting solution was mixed and changed color to yellow. This indicates the presence of dextrin in solution. The starch was carried out by Benedict’s test. The test results showed a color change to brick red. This indicates the presence of reducing sugar content in the starch hydrolysis process.

Table 1. Qualitative analysis of Cassava peel waste starch

<table>
<thead>
<tr>
<th>Number</th>
<th>Test type</th>
<th>Observation result</th>
<th>Perubahan warna</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Starch test</td>
<td>There is a color change to dark blue</td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>Dextrin test</td>
<td>There is a change in color to yellow</td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td>Benedict's test</td>
<td>There is a color change to brick red</td>
<td></td>
</tr>
</tbody>
</table>

3.1. FTIR analysis of cassava starch

Analysis of the Fourier Transform Infrared (FTIR) instrument was carried out to determine the functional groups contained in Cassava peel starch flour.

The FTIR spectrum of cassava peel starch gave absorption peaks in the wave region of 3249.00 cm\(^{-1}\); 2931.45 cm\(^{-1}\); 1640.00 cm\(^{-1}\); 1336.48 cm\(^{-1}\); 1149.82 cm\(^{-1}\); 1077.06 cm\(^{-1}\); 999.39 cm\(^{-1}\); 928.83 cm\(^{-1}\); 860.59 cm\(^{-1}\); 763.45 cm\(^{-1}\); 704.35 cm\(^{-1}\).

Table 2. Results of functional groups and wave number of cassava peel starch flour

<table>
<thead>
<tr>
<th>Functional groups</th>
<th>Wave number (cm(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Standard Starch</strong> (Castillo et al., 2019)</td>
<td><strong>Cassava peel starch</strong></td>
</tr>
<tr>
<td>O-H stretching</td>
<td>3800 – 3020</td>
</tr>
<tr>
<td>C-H stretching</td>
<td>3020 – 2830</td>
</tr>
<tr>
<td>-OH bending</td>
<td>1240</td>
</tr>
<tr>
<td>C-O-C</td>
<td>1080 – 1010</td>
</tr>
<tr>
<td>Glycosidic bond</td>
<td>930</td>
</tr>
<tr>
<td>C-O-C carbohydrate</td>
<td>856</td>
</tr>
</tbody>
</table>

Based on Table 2 the FTIR results of Cassava peel starch flour have an O-H group at a wave number of 3249.00 cm\(^{-1}\). The O-H vibrational bond appears in the range of the wave number region of 3700-3100 cm\(^{-1}\). This indicates the presence of a water absorption area in the sample (Anita et al., 2013). The C-H group is wavelength of 2931.45 cm\(^{-1}\), the -OH group at a wavelength of 1336.48 cm\(^{-1}\) and the C-O-C group at a wave number of 1077.06 cm\(^{-1}\). According to (Prameswari et al., 2022) at a wavelength in the range of 1240.28 cm\(^{-1}\), there is an -OH group in the plane and there is a C-O-C group outside the plane in the wave range of 1080.18 cm\(^{-1}\). The FTIR spectrum contained glycosidic polysaccharide bonds at a length of 928.83 cm\(^{-1}\) and bonds without C-O-C carbohydrates at a wave number of 860.59 cm\(^{-1}\). Based on research (Prameswari et al., 2022) glycosidic bonds of polysaccharides appear at a wave number of 928.76 cm\(^{-1}\) and bonds without C-O-C carbohydrates appear at a wavelength of 860.29 cm\(^{-1}\) (Budiarini et al., 2018).
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![Chemical structure of starch](image1.png)

**Figure 4. Chemical structure of starch**

### 3.2. Liquification

The initial stage in the process of making liquid sugar is the hydrolysis process. This hydrolysis process occurs two stages, namely liquification and saccharification. Liquification process is the initial stage in the process of breaking down starch into maltose, dextrin and glucose (Simpson et al., 2022). The result of the initial stages in this process is the shortening of starch chains which is not easily soluble in water to become dextrins. Dextrin is easily soluble in water and has low solubility. The process of hydrolysis tapioca starch by enzymes aims to make liquid sugar from starch as raw material. Two main processes occur, namely liquefaction and saccharification. During thawing, starch will be broken down into dextrin, maltose and glucose. Dextrin is the result of incomplete hydrolysis of starch. This process also involves alkalis and oxidizing agents. Reducing the chain length will result in a change properties where in water-insoluble starch that converted to soluble dextrin. Dextrins are highly soluble in hot or cold water, with relatively low viscosity (Ukwuru et al., 2013). Cassava peel waste starch flour obtained from the liquefaction process with variations in the amount of alpha amylase enzyme as Figure 5 and 6.

![Figure 5](image2.png)

**Figure 5. The results of the liquefaction process with the addition of 0.2% alpha amylase**

The amylase enzymes used in this study that two volume variations of the enzyme. The amount of amylase enzyme is 0.2 mL and 0.6 mL. The concentration of the substrate used in this study 23% as much as 30 mL. The operating temperature is at 60°C and pH of 6. Based on the Figure 5, it shows addition of 0.2 mL alpha amylase enzyme, the highest Brix value is 29% at 28 minutes. This indicates under these conditions the concentration of the working substrate that is optimum at 0.2 mL. The increase of the value of brix reached its peak and experienced a high increase at 0-12 minutes during the liquefaction process. Meanwhile, the addition of 0.6 mL alpha amylase enzyme increased brix every 4-7 minutes gradually. The addition of 0.6 mL of enzyme increased the brix slowly. This is influenced by the gelatinase process that occurs at a temperature of 50-65°C. Besides, there was a decrease in pH when the amount of alpha amylase enzyme was added, thus affecting the speed of the liquefaction process.

![Figure 6](image3.png)

**Figure 6. The results of the liquefaction process on the addition of 0.6% alpha amylase**

### 3.3. Saccharification

Saccharification is the process of converting dextins into simple sugars. At this stage, the temperature was lowered to 60°C and the pH was adjusted from 4.0 to 4.8, then 0.2 mL and 0.6 mL of glucoamylase were added. Glucoamylase enzyme functions to catalyze the hydrolysis reaction of α-1,4-glycosidic and α-1,6 glycosidic bonds of non-reducing starch, starch and oligosaccharides to form α-D-glucose.

![Figure 7](image4.png)

**Figure 7. Brix value in the scarification process with variations in the addition of the glucoamylase enzyme**

The Figure 7 shows addition of 0.2 mL of glucoamylase that takes the least amount of time compared to 0.6 mL of glucoamylase. At 10 minutes, the addition of 0.2 mL of glucoamylase has reached the Brix value of 34%. Meanwhile, the addition of 0.6 mL of glucoamylase was increased the Brix value slowly. In the 30 minute the resulting Brix value is 27% and the
Brix value at 70 minute has only reached 70%. This is influenced by the initial liquefaction process which is less than optimum and has an effect on the amount of dextrin.

3.4. Total soluble solid in liquid sugar

The addition of enzyme volume affects the percentage value of total solids. Variations in the addition of 5 mL of enzyme resulted in the highest total dissolved solids of 13.77% because the sample contained a lot of glucose in it. The large amount of glucose content is due to the use of enzymes as catalysts that cause collisions between water and starch molecules.

The collision causes the activation energy of the reaction to decrease, thereby making the reaction rate faster (Efendi et al., 2019). The results of the determination liquid sugar solids can be seen in the Figure 8.

The increase of total dissolved solids in liquid sugar is due to the severance of long chains from carbohydrate compounds into soluble sugar compounds. The increase in the value of total dissolved solids is in line with the increase in the volume of enzymes which causes the breaking of long chains from carbohydrate compounds into sugar compounds that have dissolved in water. Carbohydrates are counted as total dissolved solids that dissolve in water so that carbohydrates will form simpler groups such as glucose and sucrose (Efendi et al., 2019).

Figure 8. Result of determination total solids in liquid sugar

3.5. Liquid sugar functional group analysis

Based on the picture, the FTIR spectrum of the liquid sugar sample gives absorption peaks in the wave region of 3285.25 cm⁻¹; 1637.48 cm⁻¹ and 1036.79 cm⁻¹. The absorption at a wavelength of 3285.25 cm⁻¹ is the absorption of the hydroxyl group, namely O-H stretching, the absorption at a wavelength of 1637.48 cm⁻¹ is the carbonyl group, namely C=O stretching vibrations of mannose or galactose (Kavita et al., 2014). The wave number of 1036.79 cm⁻¹ is an absorption band dominated by overlapping ring vibrations or stretching vibrations of C-OH and vibrations of C-O-C glycosidic bonds of carbohydrates.

Figure 9. Liquid sugar functional group

4. Conclusion

Liquid sugar was successfully produced from Cassava peel waste by enzymatic hydrolysis. The optimum volume of enzyme addition is 0.2 mL with a TSS value of 12.56%. The results of the FTIR analysis of liquid sugar showed the presence of O-H stretching groups, carbonyl groups (C=O), stretching vibrations of C-OH and vibrations of C-O-C glycosidic bonds.

Daftar pustaka


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