Characterization of cassava starch and its potential for fermentable sugar production

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Abstract. This study aimed to characterise starch properties of an improved cassava genotype generated from somaclonal variation, Revita RV1 and investigate its potential for fermentable sugar production. A total of six cassava genotypes including Revita RV1, Manggu, Adira 4, Menti, Kristal Merah and Roti were used for comparison analysis. Morphological properties of starch were observed using light microscope and electron microscope (SEM). Physico-chemical properties such as ratio of amylose and amylpectin, swelling power, syneresis, gelation capacity, and water holding capacity (WHC) and oil holding capacity (OHC) were measured. Glucose from starch fermentation was determined by HPLC. The results showed there was variation in shape (irregular, rounded and truncated) and diameter of starch granule among the six genotypes ranged between 19 and 147 \(\mu\)m. The highest swelling power (SP) was obtained from starch of Menti (7.02 ± 0.30) and the lowest SP was starch of Adira 4 (5.94 ± 0.54). The other properties such as ratio of amylose and amylpectin, syneresis, and gelation capacity also varied among tested cassava genotypes. HPLC quantification revealed that Roti and Revita RV1 produced fermentable sugar higher than other genotypes with glucose concentration of 110.77 g/L and 102.06 g/L, respectively. The lowest glucose concentration of 89.59 g/L was obtained from Menti. The efficiency of fermentable sugar/glucose production among cassava genotypes is more likely influenced by various properties of starch.

1. Introduction
Cassava (\textit{Manihot Esculenta} Crantz) is a multi-purpose crop that mainly cultivated for its starchy root. It provides a staple food particularly for people in the tropical areas \([1]\). Recently, cassava is widely used as raw material for various industrial purposes due to high content of starch, one of which for sugar production. Cassava starch can be used to produce malto-triose, maltose, and glucose as well as other modified sugars and organic acids\([2]\). The conversion of cassava starch to glucose can be done by either enzymatic hydrolysis or acid hydrolysis. Nowadays, enzymatic hydrolysis has been using more than acid hydrolysis because it can produce higher concentration of glucose compared to acid hydrolysis \([3]\).

Cassava starch has many remarkable characteristics such as high paste viscosity, high paste clarity, and high freeze-thaw stability, which are advantageous to many industries \([4]\). In sugar industry, starch from cassava show higher rate of glucose conversion up to 93.56\% compared to corn starch of 91.8\% \([5]\). Starch can be classified into two types; native and modified and its functional properties depending on the source of starch such as cassava, corn, sweet potato and sorghum \([6,7]\). In addition, starch characteristics including physicochemical properties may vary among cassava varieties that can influence fermentation process in the production of sugar. At Research Centre for Biotechnology, Indonesian Institute of Sciences (LIPI), we have successfully developed a variety with potential high starch content, namely Revita RV1. In this study, we studied the characteristic of cassava starch from six varieties from Indonesia including the improved genotype/variety and investigate their potential for sugar production.

2. Materials and Methods
2.1. Materials
About 11-12 month-old cassava roots from six cassava genotypes (Revita RV1, Roti, Manggu, Menti, Kristal Merah and Adira 4) were harvested from germplasm collection garden of Research Center for Biotechnology, Indonesian Institute of Sciences (LIPI), Bogor, West Java. Chemicals for analysis were purchased from Sigma Aldrich, unless otherwise stated.

2.2. Starch Extraction
Harvested cassava roots were peeled, washed, and ground using grinding machine. The pulp was suspended in ten times of water and filtered using double fold tofu cloth. The filtrate was allowed to stand for 12 hours or overnight at room temperature for the starch to settle down. On the next working day, the top liquid was decanted and discarded. The sediment (starch) was then dried under the sunlight for 2 days or until dry depending on the weather.

2.3. Morphological properties
2.3.1. Light Microscope
An aliquot of cassava starch was placed on glass slide and added one small drop of lugol solution. The shape and size of cassava starch granule were observed and measured at 1,000 X magnification using LEICA DFC 310 microscope.

2.3.2. Scanning Electron Microscope (SEM)
An aliquot of cassava starch was placed on sample holder and observed at 1,200 X magnification using TM3030 Tabletop Microscope from HITACHI.

2.4. Physico-chemical properties
2.4.1. Ratio of Amylose and Amylopectin
Determination of ratio amylose and amylopectin was done using assay kit from Megazyme. Ratio amylose/amylopectin was determined based on the specific formation of amylopectin complexes with the lectin concanavalin A (Con A).

2.4.2. Swelling Power
Swelling power determined using the method of Sasaki & Matsuki (1998). 5 ml of 0.1 % AgNO₃ was added to 0.16 g of starch in corning tube of 15 ml. The tubes were placed in a shaking water bath at 70°C for 10 min and transferred into a boiling water bath f. After boiling for 10 min, the tubes were cooled in cold water for 5 min and centrifuged at 3000 rpm for 10 min. The supernatant was then removed carefully and swelling power was determined as sediment weight (g/g).

2.4.3. Syneresis
The determination of syneresis of the starches was followed the method described by Singh et al. (2004). Starch suspension (2%, w/v) was heated at 85°C for 30 min in a water bath, followed by rapid cooling in an ice-water bath to room temperature. The starch samples were stored for 24, 48, and 120 h at 4 °C. Syneresis was measured as the percentage of water released after centrifugation at 3000g for 15 min.

2.4.4. Gelation Capacity
Gelation capacity was determined using the method described by Mo (2017). Starch Suspensions of were prepared using 2 –20% (W/V) of the samples in 5 ml of distilled water in corning tubes. The samples were heated for 1 hour in a boiling water followed by rapid cooling for 2 hours in cold water. Then, the gelation capacity was determined for each sample as the least gelation concentration when the sample from the inverted tube will not slip.

2.5. Glucose production
About 30% of cassava starch was heated at boiling water until completely gelatinized. 10% of cassava peel-isolated amylase enzyme was added into the gelatinized starch. The sample was incubated at
60°C for 3 h and it was then centrifuged at 10,000 g and 4 C for 15 minutes. The supernatant was filtered and used for glucose analysis.

2.6. Glucose Analysis
Glucose from fermentation was quantified by HPLC (High Performance Liquid Chromatography) using an AMINEX PHX 87H column at 60°C, flow rate 0.6 ml/min, refractive index detector and eluent 0.05M of H$_2$SO$_4$ (Sulphuric acid). Glucose concentration was calculated based on glucose standard.

2.7. Statistical Analysis
The experiments were carried out in triplicate and the data was expressed as standard deviation. Student T-Test was used for statistical analysis of data.

3. Results and Discussions
3.1 Cassava starch granules morphology
The morphology properties of starch did not show significant different (P > 0.05) for all the six varieties observed (Figure 1). However, the variation in shape and size of starch were detected among samples as shown in table 1, which indicate that different varieties may have different functional properties such as swelling power, syneresis, ratio of amylose and amyllopectin and water and oil binding capacity that were also measured in this study.

![Figure 1](image)

**Figure 1.** Variation on shape and size of starch granules from six cassava genotypes observed by Light Microscope under 200x magnification.

The shape of starch granules varies among varieties from irregular shape to round shape, while the diameter size of starch granules ranged between 19 and 147 µm (Table 1). This results are relatively bigger than Taggart (2009) and Murtiningrum et al. (2012) thatshow there measured smaller size of cassava granules diameter between 4 – 35 µm and Rolland-Sabate et al (2012) that observed the range size of starch between 7 and 20 µm. In addition, compared with other crops (potato, sago, corn, wheat and rice), the range of cassava starch granules diameter size is smaller than potato and sago, similar to corn and bigger than wheat and rice (Table 2).
### Table 1. Cassava starch granule properties from different genotypes

| No | Genotype     | Diameter Size (µm) |
|----|--------------|--------------------|
| 1  | Revita RV1   | 36 – 90            |
| 2  | Manggu       | 23 – 106           |
| 3  | Roti         | 45 – 131           |
| 4  | Adira 4      | 25 – 136           |
| 5  | Kristal Merah| 19 – 80            |
| 6  | Menti        | 28 – 147           |

### Table 2. Starch granule properties from different sources [5]

| Starch  | Type      | Diameter (µm) |
|---------|-----------|---------------|
| Corn    | Cereal    | 5 – 30        |
| Potato  | Tuber     | 5 – 100       |
| Cassava | Root      | 4 – 35        |
| Wheat   | Cereal    | 1 – 45        |
| Rice    | Cereal    | 3 – 8         |
| Sago    | Pith      | 15 – 65       |

The variation of shape and size of starch granules can be seen more clearly (irregular shape, rounded and truncated granules) from morphological surface appearance observed by scanning electron microscope (SEM) as shown in figure 2.
Figure 2. Scanning Electron Microscope of selected 3 cassava genotypes; Revita RV1, Roti and Menti

3.2 Ratio Amylose and Amylopectin
The structure of starch compromise of the two components/polysaccharides; amylose and amylopectin. Amylose is a linear polymer consisting of α-1,4 linked D-glucose backbones [14]. The amylose content is a major quality of starch and determines diverse functional properties, which has been attractive to many users [15]. The amylose content of most starches ranges from 15.2 to 26.5% of total starch [16], which is similar to the starch content of six cassava varieties measured from this study (Table 3).

| Cassava varieties          | Amylose % |
|---------------------------|-----------|
| Adira-4                   | 22.31     |
| Menti                     | 25.28     |
| Roti                      | 24.99     |
| Revita RV-1               | 25.69     |
| Kristal merah             | 27.530    |
| Manggu                    | 26.100    |
| Wheat                     | 31.753    |
| Reference strach (RS)     | 59.683    |

Amylopectin is a major component of starch. This compound is a highly branched polymer with 5-6% branches of glucose which consists of α-1,6 linkages [17,11]. Amylopectin is located in the crystalline region, while amylose in the amorphous one. The ratio of amylose and amylopectin can be one of the key factors affecting glucose production because the latter region is more susceptible to attack by acids and enzymes and water initially enters this region prior to gelatinisation [7].

3.3 Swelling power
In hot water or when starch is heated at certain temperature, the granule swells. In water, granule swelling increases and the level of starch granule swelling is measured as swelling power [18]. The swelling power (SP) of six cassava starch at 90 °C is described in Table 4. Overall, there was no statistically significant differences in swelling power among six cassava starches observed in this study. However, the results showed variation among varieties where Menti had the highest swelling power of 7.02 ± 0.30, while Adira 4 showed the lowest swelling power of 5.94 ± 0.54. It is noticed
that Menti showed greatest swelling power may be due to its larger size of granule among others. The similar result was obtained from potato [10]. The lower swelling power could be attributed to the presence of lipids which may reduce the swelling power of the individual granule of the starch by forming a complex with amylose [20]. Hence, this result indicates that Adira 4 may have the lowest lipid content compared with other genotypes although it is not analysed in this study.

Table 4. Swelling power of different cassava genotypes

| Sample            | Swelling power | Standard deviation |
|-------------------|----------------|--------------------|
| Menti             | 7.02 ± 0.30    |                    |
| Kristal merah     | 6.85 ± 0.36    |                    |
| Manggu            | 6.58 ± 1.23    |                    |
| Adira 4           | 5.94 ± 0.54    |                    |
| Roti              | 6.35 ± 0.65    |                    |
| Revita RV1        | 6.46 ± 0.47    |                    |
| Terigu            | 6.96 ± 0.56    |                    |
| Potato starch     | 6.21 ± 1.58    |                    |

In addition to lipid content, variation in swelling power among cassava varieties is affected by various factors such as crystallinity, amylose-lipid complex content, and interaction among starch chain in the amorphous region where amylose is localised, temperature for heating, genetic modification, harvesting time and protein content. The swelling power increase with the increase of heating temperature treatment [21]. Some studies showed a waxy genotype from natural mutation had increased swelling power and an induced mutant with high amylose content (30%) and smaller granules had much lower SP compared with commercially starch [22,23]. Swelling power can also be influenced by the protein content in starch. During heating treatment, the granule-associated protein was redistributed and the protein envelope encasing the starch content which could retain the granular content during swelling was formed [24,25].

3.4. Syneresis

In this study, the syneresis of starch gels was measured as amount of water released from gels during storage at 4°C for up to 11 days as shown in figure 3. The results presented that syneresis of all type of starches increased with the increase of storage time. Among cassava starches, Menti had the highest of syneresis percentage up to 4.5% while the lowest syneresis of 3% was obtained from Kristal Merah.

Figure 3. Syneresis of cassava starches, wheat and potato starch at two different storage time: 6 and 11 days.
Syneresis of pasted gels also reflects starch retrogradation properties. Upon cooling, the gelatinized starch and water undergo molecular interactions by hydrogen-bonding or re-association of starch chain. This led to re-ordering system and partial re-crystallization of molecules with the water expelled [19]. Syneresis properties is greatly influenced by amylose and amylopectin composition in the starch. Amylose re-association is most responsible for the initial hardening of the gel, whereas amylopectin re-crystallization determines the long-term gelling and retrogradation [26]. The low amylose content might have caused lowering syneresis of the starch during the gel formation and resulted in weaker gel structure [19]. However, this study found the opposite result, where starch with the highest amylose from Kristal Merah had the lowest syneresis properties.

3.5. Gelation Capacity
The least gelation concentration (LGC) is defined as the lowest protein concentration at which gel remained in the inverted tube was used as index of gelation capacity. This study revealed that starches among six cassava varieties had different LGC [27]. The gelation capacities of different cassava starches are depicted in Table 5.

| Sample         | Concentration of Starch and Gelation |
|----------------|-------------------------------------|
|                | 2%  | 4%  | 6%  | 8%  | 10% | 12% | 14% | 16% | 18% | 20% |
| Menti          | Gel | +   | Firm | +   | Firm | +   | Firm | +   | Firm | +   | Firm |
| Kristal Merah  | -   | Viscous | -   | Viscous | -   | Viscous | +   | Firm | +   | Firm | +   | Firm |
| Manau          | -   | Viscous | -   | Viscous | +   | Firm | +   | Firm | +   | Firm | +   | Firm |
| Adira 4        | -   | Viscous | -   | Viscous | -   | Viscous | +   | Firm | +   | Firm | +   | Firm |
| Roti           | -   | Viscous | -   | Viscous | +   | Firm | +   | Firm | +   | Firm | +   | Firm |
| Revita RV1     | +   | Firm | +   | Firm | +   | Firm | +   | Firm | +   | Firm | +   | Firm |
| Wheat          | -   | Viscous | -   | Viscous | -   | Viscous | +   | soft | +   | soft | +   | soft |
| Potato         | +   | Firm | +   | Firm | +   | Firm | +   | Firm | +   | Firm | +   | Firm |

Gel. = Gelation, App. = Appearance

Starch from Menti and Revita RV1 were able to form gel quickly at the lowest concentration of starch (2%), while Adira 4 formed firm gel at the significantly higher concentration of starch (12%). Compared to other crops, potato had low LGC of 2% and wheat showed even higher LGC than that obtained of Adira 4. It is noticed that wheat still formed soft gel at the highest concentration of 20%. This study agrees with Chandra et al (2015) where the gelling properties decreased with the increase of starch concentration.

Gelation capacity is affected by physical competition for water between protein gelation and starch gelatinization [28]. The lower the LGC, the better the gelating ability of the protein ingredient [29] and the swelling ability was enhanced [28]. The variation of gelling properties of starches may be due to the ratios of different components such as protein, carbohydrates and lipids in starches indicating that interaction between those components may also play important role in functional characters of starch [30].

3.6. Glucose production and analysis
The potential of cassava starch from different genotypes for glucose production was investigated. Table 5 shows the variation of the cassava starch fermentation product, glucose concentration and sweetness after fermentation process at 60°C for 3 hours. The highest concentration of glucose was obtained from genotype Roti, followed by Revita RV1 and Adira 4 with the concentration of 110.77
g/L (0.37% w/w), 102.06 g/L (0.34% w/w) and 100.57 g/L (0.33% w/w), respectively. While, genotype Menti had the lowest concentration of 89.59 g/L (0.29% w/w).

Table 6. HPLC quantification of glucose and its sweetness analysis resulted from fermentation of 30% different cassava starches using 10% α-amylase enzyme at 60°C for 3 hours.

| No | Genotype       | Glucose concentration (g/L) | Glucose concentration (% w/w) | Glucose conversion (%) | Sweetness level (% Brix) |
|----|----------------|-----------------------------|-------------------------------|------------------------|--------------------------|
| 1  | Revita RV1     | 102.06                      | 0.34                          | 1.13                   | 24                       |
| 2  | Manggu         | 90.73                       | 0.30                          | 1.00                   | 21                       |
| 3  | Roti           | 110.77                      | 0.37                          | 1.23                   | 24                       |
| 4  | Adira 4        | 100.57                      | 0.33                          | 1.11                   | 24                       |
| 5  | Kristal Merah  | 97.78                       | 0.32                          | 1.08                   | 22                       |
| 6  | Menti          | 89.59                       | 0.29                          | 0.99                   | 17                       |

The concentration and percentage of glucose conversion were actually still very low. Bear in mind that this results are still preliminary test in order to investigate the potential of different cassava starch for glucose production. It should be noted that the fermentation process was conducted at short time (3 hours). Optimisation of fermentation process including fermentation condition, substrate concentration and enzyme is required. The correlation analysis between amylose content or other starch properties and glucose production also could not be performed yet until optimisation study conducted.

4. Conclusion
We conclude that starch properties are genotype-dependent and three out of six genotypes including an improved cassava genotype Revita RV1, Roti and Adira 4 showed promising result for glucose production. The starch conversion to glucose is likely to be affected by specific starch characteristic but more study on optimization process is still needed.

5. Acknowledgment
This research was fully funded by PN Biorefineri and Biokatalis 2019, Research Center of Biotechnology LIPI. We would like to thank you everyone who contributed to this research.

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