

## Article

# Diversity of the Starch Granule Morphology of Several Types Cassava (*Manihot esculenta* Crantz)

### Article Info

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**Abstract.** Cassava (*Manihot esculenta* Crantz), which is commonly called singkong is multipurpose crop that can be processed as food, feed and other cassava-based bioproduct. Potential application of cassava starch is determined by the properties of starch as the main component of cassava tuber biomass. The size of the cassava starch granules is different for each variety due to genetic and the environment factors in which it grows. The size of the starch granules affects the application of the starch. This study aimed to determine the shape and diameter of the starch granule in several types of cassava through microscopic analysis. The shape and the size of the 62 starch samples tested was varied. The starch granules shape was dominated by spheres form. The diameter of cassava starch granules ranged from  $2.016 \pm 0.015 \mu\text{m}$  –  $3.318 \pm 0.045 \mu\text{m}$ . The highest diameter was Tidung1.2 and the lowest was Tidung 2.2.

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## 1. Introduction

Cassava (*Manihot esculenta* Crantz), also known as Singkong, is a plant originated from the American continent, to be precise in Brazil, and spread to almost all over the world, including Indonesia [1]. This cassava plant is often found growing on community land because it does not require special land and care. In Indonesia, cassava is a very popular commodity and has become an alternative food substitute for rice [2]. In fact, cassava is the third foodstuff after rice and corn for Indonesian people. According to data reported by the Indonesian Ministry of Agriculture, the harvested area for cassava in Indonesia in 2018 reached 792,952 ha, higher than in 2017, which was 772,975 ha [3].

The diversity of cassava varieties in Indonesia is quite high. Gene Bank ICABIOGRAD Bogor recorded 600 accessions of germplasm, 452 of which are in the data base [4]. This condition causes various varieties of cassava in the field. Some of the cassava varieties found growing in Indonesia include Valenca, Mangi, Betawi, Basiorao, Bogor, SPP, Muara, Mentega, Adira 1, Adira 2, Adira 4, Malang 1, Malang 2 [5], Gading, W-1705, W-1548, Singapore, Tapikuru [6], Darul Hidayah, UJ-5, and UJ-3 [7].

Characterization of the physical and chemical properties of cassava was determined by the properties of starch as the main component of cassava [8]. Naturally, starch is small granules which are often called granules [9]. Starch granules come in various shapes (round, oval, lenticular, and polygonal) and sizes (2-100  $\mu\text{m}$  diameter) that are specific species. Generally, the granules of cereal starch are smaller than the starch of tubers and beans. The structure and type of material between each starch source differ depending on the botanical properties of the starch source [10].

The application of starch in a product is influenced by its ability to form the desired characteristics of the final product [11]. The factors that cause differences in these applications include granule shape, amylose / amylopectin ratio, starch molecular characteristics and the presence of other components that also affect starch application [12].

Based on the information above, characterization and comparative studies of the physicochemical and functional properties of starch in a variety need to be carried out to predict the similarities and differences in behavior at the application stage [11]. This study aims to determine the shape of the starch granules and the diameter of the starch granules of various varieties of cassava microscopically.

## 2. Experimental Section

### 2.1 Research Materials

The materials used were 62 samples of cassava starch consisting of 51 different types of cassava. Starch samples were extracted from several types of cassava collections belonging to the Indonesian Center for Biotechnology Research, which are maintained in the germplasm collection garden. The types of starch tested were Tasik 2, Tasik 3, Tasik 5, Merauke 1, Tidung 2, Tidung 1, Ubi Gedi, Sweet Potato 3, Adira 4, East Java Yellow Cassava 2, White Crystals 2, White Rose 1 Crystals, Caster 1, Kastal 2, Kastal 3, Carvita 25, Lombok 1, Lombok 2, Tali 1, Taon 1, Taon 3, Puring 2, Puring 3, Malang 2, Malang 6, Kasetsart 3, Manggala 1, Manggala 2, Palenca 2, Palenca 3, Gatot Kaca 1, Kandora Sumarorong 1, Kandora Sumarorong 3, Lampung 1, Budin Kuning 2, Andora Malia 2, Sweet Potatoes 1, Sweet Potatoes 2, Sweet Potatoes 3, Malra 24, Sentul 1, Sentul 2, Budin Ketan 1, Budin Ketan 3, Batak Siliuang 1, Yatim Yatim 1 Cassava, Crystal White Rose2, Crystal White Rose 3, Ubi Kuning 3, and Tabalong 2.

## 2.2 Making Starch

The manufacture of cassava starch begins with separating the cassava tubers and their skins by peeling them. Wash the peeled tubers thoroughly with water to separate the dirt. Clean peeled tubers are weighed. Furthermore, the tubers are grated or mashed with a machine until smooth. The shredded tubers are added with water (3 liters of water for every kg of tubers) to form pulp and knead so that more starch is released. The tuber pulp is then filtered with a cloth so that the starch escapes from the filter as a suspension of starch and fibers are left on the filter cloth. The starch suspension obtained is then collected in a settling vessel and precipitated for 12 hours. The liquid above the sediment is removed and the sediment is dried under sunlight to dry and then used for histochemical test of starch granules.

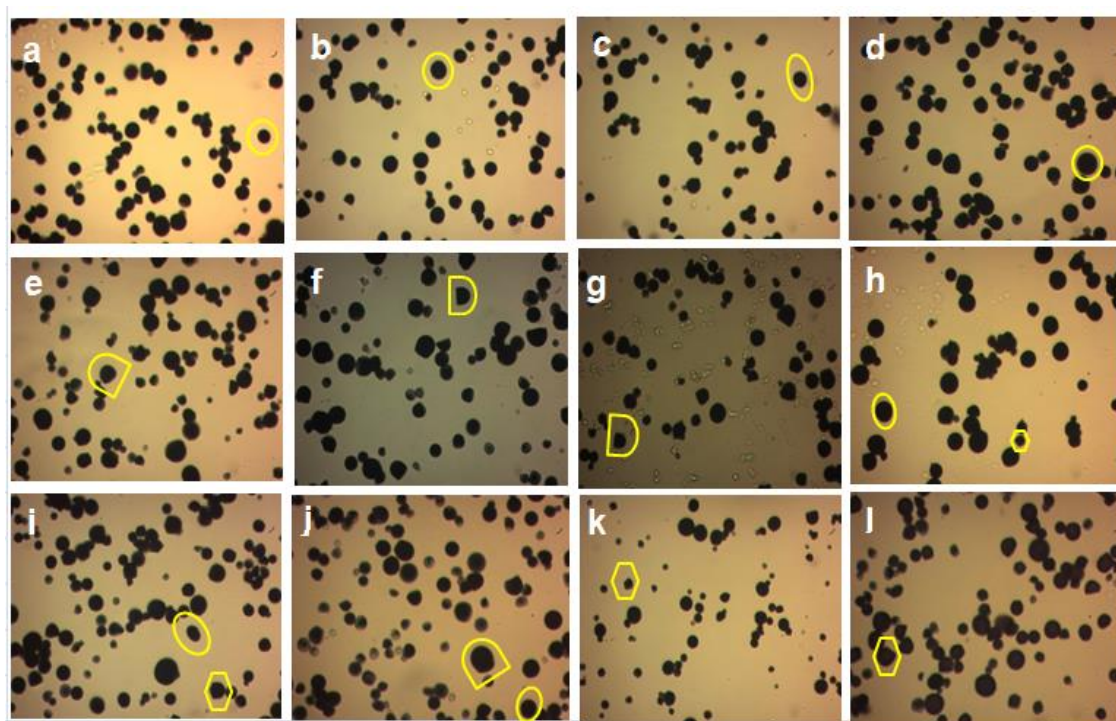
## 2.3 Histochemical Test of Starch with Lugol to Determine the Shape and Diameter of Starch Granules

The starch histochemical test was carried out using cassava starch samples. Then the sample was dripped with Lugol's solution (1: 2) and observed using a digital light microscope with a magnification of 40X. Observations were made in three fields of view for each sample. Microscopic profiles of starch were documented for analysis of granule shape and diameter. Images were analyzed using the ZEN application with a 2.5X zoom to determine the size and diameter in  $\mu\text{m}$  units. Starch diameter was calculated by repeating 20 granules in each of the three fields of view. The color of the starch granules as a reaction between the starch molecules and the solution was visually observed.

## 3. Results and Discussion

### 3.1 Forms of Starch Granules

Starch is a complex carbohydrate that is insoluble in water, colored white like a paste or powder and has no smell. Starch obtained from the extraction of cassava roots is white. According to [13] starch grains can be observed through a microscope so that it can be seen from the shape, size and form of starch grains, which solitary or assembled (compound starch grains). Starch in tubers is generally oval, or round. The original shape of the starch grains and the size distribution of the starch grains play a role in identifying the source of these starch grains. Based on the results of microscopic observations, in general cassava starch granules are round. The color variations of starch granules are categorized as blackish blue, bluish purple, and light purple (Figure 1).



Information: ○ round, ◌ oval, ⬡ polygonal, ◐ truncated oval

**Figure 1.** Microscopic Profile of Starch Granules of Various Types of Cassava with Various Sizes and Shapes a. Budin Ketan 1, b. Yellow Budin, c. Tidung 2, d. Tidung 1, e. Budin Kuning 2, f. Sentul 1, g. Tabalong 2, p. Sweet Potatoes 3, i. Carvita 25, j. Lombok 2, k. Gatot Kaca 1, l. Manggala 2.

Based on Figure 1, the starch forms found in the sample are round, oval, polygonal and truncated oval. The calculation of the ratio in the form of the percentage of each granule shape in all tested samples showed that the starch granule shape observed was predominantly round and the low percentage shapes (rarely) were oval and polygonal (Table 1). The starch of all the samples tested after being dripped with Lugol's solution has a blackish color as shown in Figure 1 and visually. The color change occurs because there is a reaction between starch and Lugol's solution which indicates the presence of carbohydrate compounds. This is in accordance with that stated by [14] starch is purplish blue or black with lugol or iodine.

**Table 1.** The Ratio Based on the form of Cassava Starch Granules in All Tested Samples

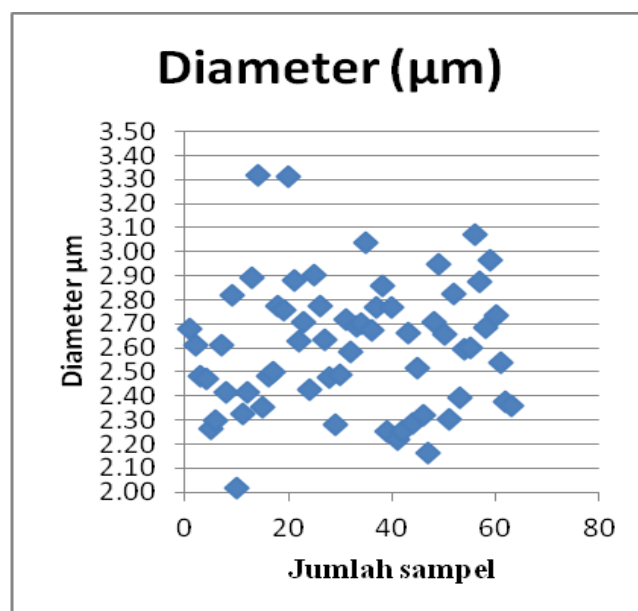
Granule Shape	Percentage range (%) over the entire sample
Round	10.48 ± 1.72 - 65.09 ± 3.4
Oval	1.52 ± 0.48 - 19.68 ± 2.41
Polygonal	1.95 ± 0.57 - 21.49 ± 1.63
Truncated oval	17.10 ± 1.6 - 41.84 ± 0.09

### 3.2 Diameter of Starch Granules

Microscopic observation of starch granules showed that the cassava starch varied in size. The largest diameter of starch was found in Tidung 1,2, which was  $3,318 \pm 0.045 \mu\text{m}$  and the lowest was found in Tidung 2,2, namely  $2,016 \pm 0.015 \mu\text{m}$  (Table 2). The difference in size is the character of each type of starch [15]. Wang et al. (2009) stated that the characteristics of starch are different for each variety [16]. In Figure 2, analysis of the dots plot distribution of samples based on the diameter size category, it can be seen that the observed starch granule diameter is dominated by the size with an interval of 2-3  $\mu\text{m}$ , only two samples have a diameter of more than 3, namely Tidung 1,2 and Crystal White Rose.

**Table 2.** Size of the Diameter of Cassava Starch Granules (*M. esculenta* Cranz)

No.	Varieties	Diameter ( $\mu\text{m}$ )		No.	Varieties	Diameter ( $\mu\text{m}$ )	
1	Tasik 2	2.68	$\pm 0.01$	32	Malang 6	2.69	$\pm 0.02$
2	Tasik 3,1	2.61	$\pm 0.02$	33	Kaporo 2	2.7	$\pm 0.02$
3	Tasik 3,2	2.48	$\pm 0.02$	34	Kaporo 3	3.04	$\pm 0.01$
4	Tasik 3,3	2.47	$\pm 0.02$	35	Kasetsart 3	2.67	$\pm 0.02$
5	Tasik 5,2	2.26	$\pm 0.02$	36	Manggala 1	2.77	$\pm 0.02$
6	Tasik 5	2.3	$\pm 0.01$	37	Manggala 2	2.86	$\pm 0.02$
7	Merauke 1.4	2.61	$\pm 0.01$	38	Palenca 2	2.25	$\pm 0.02$
8	Merauke 1.1	2.41	$\pm 0.01$	39	Palenca 3	2.77	$\pm 0.02$
9	Tidung 2,2	2.02	$\pm 0.02$	40	Gatot Kaca 1	2.22	$\pm 0.02$
10	Tidung 2,3	2.33	$\pm 0.02$	41	Kandora Sumarorong 1	2.25	$\pm 0.02$
11	Tidung 2,1	2.41	$\pm 0.02$	42	Kandora Sumarorong 3	2.66	$\pm 0.01$
12	Tidung 1.1	2.89	$\pm 0.03$	43	Lampung 1	2.28	$\pm 0.02$
13	Tidung 1,2	3.32	$\pm 0.04$	44	Yellow Budin 2	2.52	$\pm 0.02$
14	Ubi Gedi	2.35	$\pm 0.02$	45	Andora Malia 2	2.32	$\pm 0.02$
15	Ubi Gedi 3	2.48	$\pm 0.01$	46	White Sweet Potato 1	2.16	$\pm 0.01$
16	Adira 4	2.5	$\pm 0.02$	47	White Sweet Potato 2	2.71	$\pm 0.01$
17	East Java Yellow Cassava 2	2.77	$\pm 0.02$	48	White Potatoes 3	2.95	$\pm 0.02$
18	White Crystal 2	2.76	$\pm 0.02$	49	Malra 24.3	2.66	$\pm 0.02$
19	White Rose Crystal	3.31	$\pm 0.11$	50	Malra 24.2	2.3	$\pm 0.01$
20	Castal 1	2.88	$\pm 0.02$	51	Sentul 1,1	2.82	$\pm 0.01$
21	Castor Cassava 2	2.63	$\pm 0.13$	52	Sentul 2,1	2.39	$\pm 0.02$
22	Oven Caster 3	2.71	$\pm 0.02$	53	Budin Sticky 1	2.59	$\pm 0.01$
23	Carvita 25.1	2.43	$\pm 0.02$	54	Budin Sticky 3	2.6	$\pm 0.02$
24	Lombok 1.1	2.9	$\pm 0.01$	55	Batak Siliuang 1	3.07	$\pm 0.02$
25	Lombok 2,3	2.78	$\pm 0.02$	56	White Crystal 1	2.87	$\pm 0.01$
26	Cassava Tali 1 Oven	2.63	$\pm 0.02$	57	East Java Yellow Cassava 1	2.68	$\pm 0.02$
27	Taon 1	2.48	$\pm 0.01$	58	White Rose Crystal 2	2.97	$\pm 0.01$
28	Taon 3	2.28	$\pm 0.02$	59	White Rose Crystal 3	2.74	$\pm 0.02$
29	Cassava Puring 2	2.49	$\pm 0.02$	60	Tabalong 2,3	2.54	$\pm 0.02$
30	Cracked cassava 3	2.72	$\pm 0.02$	61	Tabalong 2.1	2.37	$\pm 0.02$
31	Malang 2,3	2.59	$\pm 0.02$	62	Yellow Sweet Potato 3	2.36	$\pm 0.02$



**Figure 2.** Diagram of Dots Plot of Starch Granule Diameter of all Types of Cassava

In Figure 2 it can be seen that the observed starch granule diameter size is dominated by size with 2-3µm intervals. The largest diameter size of starch was found in the hood 1.2 variety, namely  $3.318 \pm 0.045 \mu\text{m}$  and the lowest was found in Tidung 2, 2 varieties, namely  $2.016 \pm 0.015 \mu\text{m}$  (Table 2). The difference in size is the character of each type of starch [15]. Wang et al. (2009) stated that the characteristics of starch are different in each variety [16].

The diameter size of the starch observed in the cassava starch samples tested was categorized as very small or small, with a diameter of 2.02 - 3.32 µm. Unlike the Royang type of cassava starch from Thailand, which is planted by irrigation and rainfed, it has a small to large diameter range of  $7.21 \pm 33.78 \mu\text{m}$  which is influenced by irrigation and rainfed irrigation systems [17] [18]. This measure is in accordance with previous research by Lindeboom et al. (2004) who reported that starch grain size was classified into four groups, namely, small (<5 µm), small (5-10 µm), medium (10-25 µm), and large (> 25 µm). Meanwhile, according to Jading et al. (2011) there are two types of starch grains, namely small (5-10 µm) and large starch grain sizes, namely (25-40 µm).

The size of the starch granules has a very important role in the application of the food industry. Granule size is one of the functional properties that determines starch utilization [14]. Starch with small granule size has the ability to absorb water better and is easily digested by enzymes. It is important in the manufacture of liquid sugar (glucose, fructose and dextrans) from starch. Likewise for the manufacture of vermicelli, small starch is preferred because it can increase water absorption and the strength of the vermicelli before cooking, so that they are not easily broken/brittle [19]. In addition, the small starch granule size (<10 mm) and relatively the same as the potential fat globule is used as a fat replacer in the food industry [20].

Large granules are more resistant to hydrolysis, and the enzyme will break down small granules compared to large ones, and the enzymatic digestion pattern of starch granules at small sizes will be different from larger granule sizes [21]. In addition, cassava starch with large granule size has a higher gelatinization temperature than starch with small granule size [22].

#### 4. Conclusion

The starch samples that were dripped with Lugol's solution were black in color and had various forms of starch granules, namely round, oval, polygonal and cut oval, of the 62 starch samples consisting 51 different types of cassava showing different granule diameter sizes with a range of  $2.016 \pm 0.015 \mu\text{m}$  -  $3,318 \pm 0.045 \mu\text{m}$ . Based on the analysis of the distribution of samples according to diameter, there are 2 types of starch that have a larger diameter than the other samples, namely Tidung 1,2 and Crystal White rose.

#### 5. Acknowledgement

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