

## Study on The Starch Granules Morphology of Local Varieties of *Dioscorea hispida* and *Dioscorea alata*

Fauziah\*, Shofiyatul Mas'udah, Hendrian

Purwodadi Botanic Garden-Indonesian Institute of Sciences, Purwodadi, Indonesia

### ABSTRACT

Starch is regarded as vital in the food industry, where granule size and shape determine its function and uses. One plant taxon that is widely known as a starch source is *Dioscorea* (Dioscoreaceae), whose starch and protein content make it as healthy food. As the initial step to identify which varieties possess potential as starch sources, we conducted the study on starch granule morphology of some local varieties of *D. hispida* and *D. alata*. The aim of this study was to determine the shape and size variation of the starch granule of each variety of *D. hispida* and *D. alata*. The results showed that starch granule morphology does not only vary between species of *D. hispida* and *D. alata*, but also among varieties of those species. The method used was a simple preparation of starch granules with 10% iodine staining and transparent polish to prevent the preparations from shifting. The shape of starch granules in *D. hispida* is dominantly polygonal (polyhedral). It showed similar results as those reported by other authors in their studies, except that – in this study - we found there is also a triangular shape, which has never been reported before. The Starch granule size of *D. hispida* is found to be “Very small – Small” and only one variety of the species – that is Gadung Jahe (DC9) – is classified as “Small” type. According to previous studies, varieties with small type starch granule are recommended as potential sources for non-food industrial raw materials. The shape of starch granules in *D. alata* is similar to those reported in other studies, which is dominantly triangular and polygonal; the size is classified as “Medium” type, except for two varieties that are Uwi Ratu (DC4) and Uwi Ulo (DC5), which are classified as “Large”. According to previous studies, varieties with large type starch granule are recommended as potential sources of food. Thus, from this study three potential local varieties are identified, i.e. gadung jahe of *D. hispida*, and Uwi Ratu (DC4) and Uwi Ulo (DC5) of *D. alata*.

**Keywords:** *Dioscorea*, Starch granule morphology, varieties

### INTRODUCTION

Starch is the major carbohydrate of nutritional importance in the human diet and also an important industrial material. Starch performs various functions in food industry such as thickener, stabilizer and bulking agent [1].

Starch varies greatly in form and functionality between and within botanical species, and even within the same plant cultivar grown under different conditions [1, 2]. Granules from roots and tuber starches are relatively larger in size (2 – 100  $\mu\text{m}$ ) and have an oval shape although some varieties with round, polygonal,

and irregularly shaped granules can be found [3,4].

*Dioscorea alata* L. (Indonesian: *Uwi*) commonly referred to as ‘winged yam’, ‘water yam’ or ‘greater yam’ usually possesses tubers that are white, off white, to purple in colour. Tubers of *D. alata* are also known for their high nutritional content, with crude protein content of 7.4%, starch content of 75-84 %. Due to the high starch content, *D. alata* tubers provide a good source of dietary carbohydrates in the tropical and subtropical regions [5].

Bitter yam (Indonesian: *Gadung*) is common name of *Dioscorea hispida* Dennst. This tuber crop is an important source of carbohydrates and has been used as a staple food, especially by people in the tropical and subtropical regions but is rarely eaten by natives these days because it requires a lot of time and effort to prepare [6]. This tuber’s digestive property has suggested

\*Corresponding author:

Fauziah

Purwodadi Botanic Garden-Indonesian Institute of Sciences,  
Jl. Raya Surabaya-Malang KM 65 Purwodadi, Pasuruan 67163,  
Indonesia

E-mail: fauziahkrp@gmail.com

the utilization of *gadung* tuber in reducing the risk of obesity, diabetes and other related diseases [7].

A food alternatives development program based on minor tubers such as *Dioscorea* is currently being promoted by the government to support national food security. As an initial step to determine the potentials of *Dioscorea*, a study of starch granule characterization on some local varieties of *D. alata* and *D. hispida* has been conducted.

The aim of this study is to obtain more information regarding variation on shape, size and type of starch granules of some *D. alata* and *D. hispida* local varieties; so that their potentials – as prospective sources for food and non-food material – can be determined, studied and developed.

## MATERIALS AND METHODS

Starch granule characterization was conducted in the Purwodadi Botanic Garden laboratory, during the period of June – July, 2014, when *Dioscorea* plants are dormant.

The method used was a simple preparation of starch granules with 10% iodine staining and transparent polish to prevent the preparations from shifting. The materials in this study were the tubers of each variety of *D. alata* and *D. hispida* of the Purwodadi Botanic Garden collections which were collected in the District of Pasuruan. Tools used included cutter, object glass, cover glass, soft brush, light microscope, micrometer and digital cameras. Iodine dye was used because starch-iodine inclusion complexes have been studied extensively by many researchers over the years and are widely used for the determination of amylose content of starches in solution systems. The color of the starch-iodine complex can range from brown to red, red-violet, blue-violet, and finally to blue, depending on starch polymer length [8].

In the first stage, tubers were cut into two halves, and scraped the center to sample the starch and the scraped material was placed on an object glass to which was added a drop of iodine solution, and then covered with a cover glass. This was followed by squashing the starch using a pencil to avoid clumping of starch granules and to ensure an even spread. In the next stage, the stained glass was observed under a microscope that was equipped with a micrometer and photographed using a digital camera. Starch granules observed with a magnification of 100x for *D. alata* and 400x for *D. hispida* because *D. alata* starch granule size is larger than *D. hispida*.

Shape and size were measured under the light mi-

croscope. We used analysis of variance single factor and Tukey test ( $\alpha = 5\%$ ) to determined the significant size of starch granule.

## RESULTS AND DISCUSSION

*Dioscorea* has variations in starch granules between and within species based on morphological shape and size. Starch granules of *D. hispida* were different with *D. alata*, as well as within its varieties. Varieties of *D. hispida* display polygonal (polyhedral) and triangular shapes (Figure 1-a, b), but were predominantly polygonal/polyhedral (Table 1). Based on diameter measurement, *D. hispida* has a  $3.1 \pm 1.1 - 6.6 \pm 1.5 \mu\text{m}$  diameter average, so it classified as “very small to small” (<5-10  $\mu\text{m}$ ) granule type. It also varies in granule size distribution (Table 1). *D. hispida* tend to be smaller than *D. alata* in granule size distribution, about 2.5-10  $\mu\text{m}$  (Figure 1).

Statistical analysis on diameter size of *D. hispida* starch granules showed there was significant difference between varieties. *Gadung* (DC1, DC2 and DC3), *Gadung Kuning* (DC8 and DC12), *Gadung Mentega* (DC19) and *Gadung Kripik* (DC25) were not significantly different in starch granule diameter size compared to *Gadung Kuning* (DC8). *Gadung Jahe* was variety of *D. hispida* had the largest starch granule diameter and was significantly different to the others. This statistical analysis supports the classification of *Gadung Jahe* as “Small” type which is different from the other *D. hispida* varieties.

Based on this research, starch granules of *D. hispida* display polygonal/polyhedral and triangular shapes (Figure 1-a, b). Starch granule shape in varieties of *D. hispida* were found to be predominantly polygonal /polyhedral [9].

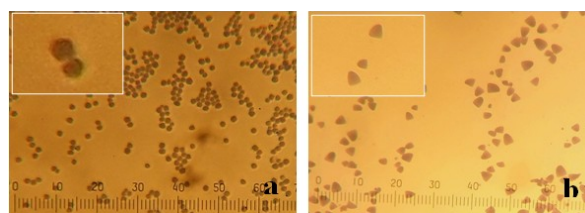


Figure 1. Morphology of starch granules from *D. hispida* (a) polygonal and (b) triangular

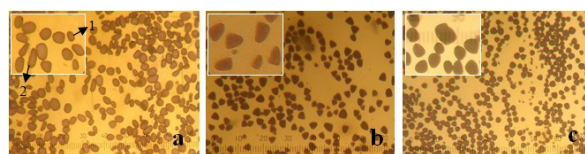


Figure 2. Morphology of starch granules from *D. alata*. (a) 1. oval, 2. ellipsoid, (b) triangular, and (c) polygonal

Regarding granule size, the average diameter of starch granule of *D. hispida* was about 3.1-6.6  $\mu\text{m}$ , larger than those that was reported by Ashri *et al.* [9], which is 1.3-4.3  $\mu\text{m}$ . According to Lindeboom *et al.* [10], the size was classified into “Very small” to “small”.

It can be assumed that there is a variation both in size and shape of starch granule in varieties level of *D. hispida*. Further studies are needed with more samples so that complete morphological variation of size and shape of starch granules of *D. hispida* can be identified.

Other studies on *Dioscorea dumetorum* showed small starch granule size. Small type of starch granule could be used in food and non-food industries. Based on this study, *Gadung Jahe* has “Small” type of granule, about  $6.6 \pm 1.5 \mu\text{m}$ ; it is smaller than *D. dumetorum* which has  $7.25 \pm 0.32 \mu\text{m}$  granule size and used as fat replacers (carbohydrate-based fat replacers) [11]. The “Small” starch granules likes *D. dumetorum* are also suited to use as stabilizers in baking powder, aerosols, face powder, or dusting powder in the cosmetic industry, and as laundry-stiffening agents because they can penetrate fabric and give a high gloss and stiffness in textile industries). *D. dumetorum* has potential to be utilized as a substitute for rice starch in such applications [11]. As a carbohydrate source, tubers of *Dioscorea* do not contain any gluten, which makes them an important material in reducing the incidence of Celiac Disease (CD) or other allergic reactions [12]. Due to the same size of starch granule, *Gadung Jahe* cultivar could be recommended for use as a carbohydrate source and industrial raw material much like *D. dumetorum*.

Starch granule shapes of *D. alata* observed were oval, ellipsoid, triangular and polygonal/polyhedral (Figure 2-a, b, c) but it was dominantly triangular and polygonal for each variety (Table 2). Average of *D. alata* starch granule size was about  $13.3 \pm 3.8 - 26.0 \pm 6.4 \mu\text{m}$ ; it is classified as “Medium-Large” type, ranging in size from 10- >25  $\mu\text{m}$  (Table 2). Based on granule distribution, *D. alata* has larger distribution than *D. hispida*, about 10-40  $\mu\text{m}$ . The results of the study shows starch granule between varieties tend to be the same shape, although some showed variation in shape between varieties.

Granule shape of *D. alata* found in this study were oval, ellipsoid, triangular and polygonal/polyhedral (Figure 2-a, b, c). However, the triangular and polygonal shapes are predominant. Others reported that granules of *D. alata* from Indonesia and China are round, oval, ellipsoid and spherical [13, 14]. According to Ri-

ley *et al.* [15], granules of some Jamaican *D. alata* are ellipsoid, polyhedral, triangular and rod-like, but predominantly ellipsoid and triangular. While the study by Otegbayo *et al.* [11] revealed that starch granule shape of some varieties of Nigerian *D. alata* is dominantly triangular and oval. This study showed that there are some differences in shape of *D. alata* local varieties compared to that reported by Nadia *et al.* [14]. The result of this study is closer to that of Riley *et al.* [15] and Otegbayo *et al.* [11]. However, there is still a difference in the dominant shape of the granules.

Statistical analysis of *D. alata* varieties showed significant difference in diameter size of starch granule. The most significant difference was in *Uwi Jaran* (DC13) with smallest diameter size. *Uwi Ratu* (DC4), *Uwi Ulo* (DC5) and *Uwi Putih* (DC23) showed no significant difference in diameter size among these three varieties, but there was significant difference with other *D. alata* varieties. These 3 varieties of *D. alata* showed the largest size diameter among the 12 varieties of *D. alata* tested. This analysis supports classification of *Uwi Ratu* and *Uwi Ulo* (*D. alata*) into “Large” type based on starch granule size.

In this study, the average granule diameter of *D. alata* was about 13.3-26.0  $\mu\text{m}$ , and local varieties of *D. alata* had a smaller diameter than Nadia *et al.* [14] and Otegbayo *et al.* [11], and closest to Riley *et al.* [15]. Granule of local varieties of *D. alata* could be classified as “Medium-Large” type and only two varieties had “Large” type of starch granule; the others were mostly “medium”.

Nutritional value of tubers varied between and within species. It can be affected by factors such as cultivation method, climate and soil characteristics, harvest time, storage time and post-harvest processing [5]. The variation of shape and size was a character of the starch source, growth condition and harvest time. Granule shape and function varied between species, and even within cultivars, due to different environmental condition [2]. It assumed that the difference in starch granules between varieties in the same species was affected by environmental factors. On the other hand, variation in size was affected by chloroplast biochemistry condition, amyloplast, including plant physiology.

The variation size and shape effected functional characteristic of starch and it industrial used. Starch granule size contributed to increase starch gelatinization, gelatinization temperature, swelling power and viscosity [16]. Starch composition, gelatinization and sticky properties, enzyme susceptibility, crystal structure

Table 1. Granule size and shape of *D. hispida* variety starches

No.	Varieties	Accession Number	Interval Distribution of Size ( $\mu\text{m}$ )	Average Diameter of Granules Size ( $\mu\text{m}$ )	Granules Form	Type Classification
1	Gadung	DC1	2.5 – 5	3.3 $\pm$ 1.2 <sup>a</sup>	Polygonal	Very Small
2	Gadung	DC2	2.5 – 5	3.7 $\pm$ 1.3 <sup>a</sup>	Polygonal	Very Small
3	Gadung	DC3	2.5 – 5	3.7 $\pm$ 1.3 <sup>a</sup>	Triangular-polygonal	Very Small
4	Gadung Kuning	DC8	2.5 – 5	4.0 $\pm$ 1.2 <sup>ab</sup>	Polygonal	Very Small
5	Gadung Kuning	DC12	2.5 – 5	3.1 $\pm$ 1.1 <sup>a</sup>	Polygonal	Very Small
6	Gadung Jahe	DC9	5 – 10	6.6 $\pm$ 1.5 <sup>c</sup>	Polygonal	Small
7	Gadung Mentega	DC19	2.5 – 5	3.6 $\pm$ 1.3 <sup>a</sup>	Polygonal	Very Small
8	Gadung Kebo	DC22	2.5 – 7.5	4.8 $\pm$ 1.5 <sup>b</sup>	Polygonal	Very Small
9	Gadung Kripik	DC25	2.5 – 5	3.4 $\pm$ 1.2 <sup>a</sup>	Polygonal	Very Small
10	<i>D. hispida</i>	Ashri et al. (2014) [9]	1.3 – 4.3	-	Polyhedral	Very Small
11	<i>D. dumetorum</i>	Otegbayo et al. (2013) [11]	-	7.25 $\pm$ 0.32	Polygonal	Small

\*Note: The different letters indicate a significant difference in average diameter of granule size values

Table 2. Granule size and shape of *D. alata* variety starches

No.	Varieties	Accession Number	Interval Distribution of Size ( $\mu\text{m}$ )	Average Diameter of Granules Size ( $\mu\text{m}$ )	Granules Form	Type Classification
1	<i>Uwi Ratu</i>	DC4	15 – 40	26.0 $\pm$ 6.4 <sup>d</sup>	Oval, elliptic, polygonal	Large
2	<i>Uwi Ulo</i>	DC5	20 – 40	25.3 $\pm$ 6.3 <sup>d</sup>	Triangular, polygonal	Large
3	<i>Uwi Ulo</i>	DC14	10 – 30	21.7 $\pm$ 6.1 <sup>cd</sup>	Oval, triangular, polygonal	Medium
4	<i>Uwi Ulo</i>	DC16	10 – 30	20.3 $\pm$ 5.9 <sup>cd</sup>	Triangular, polygonal	Medium
5	<i>Uwi Tanduk Rusa</i>	DC7	10 – 30	20.0 $\pm$ 4.4 <sup>bcd</sup>	Triangular, polygonal	Medium
6	<i>Uwi Talas</i>	DC10	10 – 25	17.3 $\pm$ 4.5 <sup>abc</sup>	Triangular	Medium
7	<i>Uwi Talas</i>	DC11	10 – 25	13.5 $\pm$ 4.4 <sup>ab</sup>	Triangular	Medium
8	<i>Uwi Jaran</i>	DC13	10 – 20	13.3 $\pm$ 3.8 <sup>a</sup>	Triangular, polygonal	Medium
9	<i>Uwi Ireng</i>	DC17	10 – 30	20.7 $\pm$ 5.2 <sup>cd</sup>	Triangular, polygonal	Medium
10	<i>Uwi Cemeng</i>	DC21	10 – 40	22.3 $\pm$ 6.9 <sup>cd</sup>	Triangular, polygonal	Medium
11	<i>Uwi Putih</i>	DC23	10 – 40	24.8 $\pm$ 7.8 <sup>d</sup>	Polygonal	Medium
12	<i>Uwi Alang-alang</i>	DC24	10 – 40	22.0 $\pm$ 7.0 <sup>cd</sup>	Triangular, polygonal	Medium
13	<i>D. alata</i>	Indonesia -> Nadia et al. 2014 [14]	6 – 100	21.5 – 32.0	Round, oval, ellipsoid, and spherical	Medium - Large
14	<i>D. alata</i>	Jamaica -> Riley et al. 2006 [15]	-	22.8 – 28.0	Ellipsoid, polyhedral, triangular, rod-like	Medium - Large
15	<i>D. alata</i>	Nigeria -> Otegbayo et al. 2013 [11]	29 - 41	-	Circular, triangular, oval	Large
16	<i>D. alata</i>	China -> Jiang et al. 2012 [13]	-	-	Spherical or oval	-

\*Note: The different letters indicate significant difference in average diameter of granule size values

re, swelling and solubility affected by starch granule size [10]. Starch granule shape and size was important characteristic of starch extraction industry because of shape and size of starch granule determined the size of mesh for application and purification filter [17].

Starch granule with large size usually has a high extractability and sedimented faster during the extraction compared with small granule [18]. Due to the small size of the granule are often wedged in protein and fine fiber sedimented during the purification process, it could be the reason why the starch granule *D. hispida* with small size is difficult to be extracted or isolated. Therefore, the granule with a large size could be one of the important components for food products and applications in the starch industry. The potential use of starch in industrial scale based on the size of the starch, *Uwi Ratu* and *Uwi Ulo* (DC5) has the potential to be developed as a functional food product. Both of these varieties have large diameter starch granules and are classified as large type, so they could be recommended as potential food for further development.

## CONCLUSIONS

This study showed that starch granules of *D. hispida* and *D. alata* can be distinguished based on their shape and size. The shape and size of the starch granules of the two species even varies noticeably at the varieties level.

As reported in previous studies by other authors, the shape of starch granules in *D. hispida* are dominantly polygonal (polyhedral). Here, besides the polygonal shape we also found a triangular shape, which has never been reported before. The size of starch granules in *D. hispida* are categorised as “Very small–small”, and only *gadung jahe* has “Small” type. Because of this type *gadung jahe* could be recommended as a source of non-food industrial raw material.

Starch granule shape of *D. alata* observed in this study was similar to those that were reported in other studies. Starch granule size of *D. alata* varieties is dominantly “Medium” type and smaller than those that was reported by previous studies. There were two varieties of *D. alata* that have “Large” type starch granule size: *uwi ratu* and *uwi ulo* (DC5), and these could be recommended as potential food sources.

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