# IDENTIFICATION BY OPTICAL, ELECTRON MICROSCOPY AND DIFFRACTION LASER OF THE STARCH ISOLATED FROM ÑAME CONGO (*Dioscorea bulbifera* L.)

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## ABSTRACT

Granular structure of several wild-type of starches is not well known and the starch isolated from *Dioscorea bulbifera* ("ñame congo") is among them. Through its characterization new products could be prepared. Morphometric characteristics, and presence and position of the *hilum* are signs of starch organization and indicative of the way that they could be used in the industry. The goals were to isolate starch from the edible portion of bulbils of *Dioscorea bulbifera* L. gathered at the region of Anzoátegui, Venezuela, as well as to characterize its morphometry, *hilum* and granular distribution. Starch was isolated and purified and its shape, size, distribution granular, and Maltese cross presence were studied by optic polarized light microscopy, scanning electron microscopy (SEM) and laser diffraction. Results show a starch with an eccentric Maltese cross, and a nearly triangular shape, with smooth and polished surfaces, which vary in size in a range of 1-40 microns and agglomerated as a cluster. The granular distribution of the larger population of starch granules with sizes ranging from 7 to 60 µm represent the 93.04 ± 0.25% of the overall population, and the rest represent the 6.96 ± 0.25%, with granule sizes ranging from 1 - 7 µm. The particle size distributions were Dv 10 = 12.67; Dv 50 =24.12, and Dv 90 = 39.86, indicating that 50% of the population is concentrated in a size of ≈25 µm. Those results show that *Dioscorea bulbifera*´s starch is fully characterized for its possible uses in food and other industries.

**Keywords:** Starch, *Dioscorea bulbifera*, SEM, laser diffraction, polarized light optical microscopy, yam starch.

## IDENTIFICACION DEL ALMIDÓN DE ÑAME CONGO (*Dioscorea bulbifera* L.) MEDIANTE EL USO DE MICROSCOPIA ÓPTICA Y ELECTRÓNICA Y DIFRACCIÓN LASER

## RESUMEN

La estructura de almidones provenientes de plantas silvestres no ha sido bien estudiada, siendo el de *Dioscorea bulbifera* L. ("ñame congo") uno de ellos, por lo que a través de su caracterización podrían desarrollarse nuevos productos de uso industrial. Las características morfométricas del gránulo así como la presencia y posición del *hilum* son los índices que muestran preservación de su organización granular, e indicativos para su uso en diferentes industrias. Los objetivos del estudio fueron por lo tanto, aislar el almidón de cormelos de *Dioscorea bulbifera* que crecen en el estado Anzoátegui, Venezuela, así como caracterizar su morfometría, *hilum* y distribución granular. El almidón fue aislado y purificado y fueron estudiadas a través de microscopía óptica con luz polarizada, microscopía electrónica de barrido (SEM) y difractometría láser su forma granular, la presencia de cruz de Malta, además de su tamaño y distribución granular. Los resultados muestran una estructura granular con cruz de Malta excéntrica, de forma casi triangular, con superficies lisas y pulidas, que varían de tamaño en un rango de 1-60 micras, así como la formación de aglomerados. La distribución granular de la población más representativa, con tamaños que van desde 7 a 20 µm corresponden al 93,04 ± 0,25% de la población total, mientras el resto representa el 6,96 ± 0,25%, con tamaños de gránulos variando entre 1 y 7 micras. Las distribuciones de tamaño de partículas fueron Dv 10 = 12,67; Dv 50 = 24,12, y 90 Dv = 39,86, lo que indica que 50% de la población se concentra en un tamaño de ≈25 µm. Estos resultados muestran que algunas importantes características micromorfológicas del almidón de *Dioscorea bulbifera* están plenamente identificadas para su uso potencial en la industria.

**Palabras clave**: almidón, *Dioscorea bulbifera*, microscopía de luz polarizada , SEM, difractometría láser, almidón de ñame.

## INTRODUCTION

Several species of the genus *Dioscorea* L. serve as staple crops in many regions of the world, among them *Dioscorea bulbifera* L. (air potato), which is called in Venezuela as "ñame congo", "ñame de mata" or "ñame criollo" [1]. Native from Africa, southern Asia and northern Australia, *Dioscorea bulbifera* is a perennial vine with broad leaves and two types of storage organs. The plant forms bulbils on the leaf axils of the twining stems, and tubers beneath the ground [2]. Its tubers mainly consist of starch-bearing tissue covered by a suberin layer which ultimately forms a "skin" or bark [3]. Starch is a carbohydrate extracted from agricultural raw materials which is widely present in literally thousands of everyday food and non-food applications. The morphology, granule size and particle size distribution of the starches is quite important in food systems and other industries for the control of functional properties (e.g. water absorption and solubility), palatability and nutritional value (e.g. starch digestibility rate) of human foods and feeds [4]. During the starch synthesis, the point of initiation of the granular formation, where the layers of carbohydrates are deposited, is called *hilum* [5]. The *hilum* can be found near the center of elliptical granules or positioned on the axis of symmetry, at the end of pear-shaped granules. Shape, size and the presence and position of the *hilum* on the starch is a sign of its organization and of a non altered granular structure, which at the same time is indicative of the way that they could be used in the industries [6]. *Dioscorea bulbifera*´s starch granules are of irregular shape, similar to a pyramid with rounded vertices, and with a smooth surface [7]. However, details of the granular structure of *Dioscorea bulbifera*´s starch are not well known and through its characterization many new products for industrial uses could be prepared. The goals of the study were to isolate the starch from the edible portion of *Dioscorea bulbifera* bulbils and to characterize its morphometry and granular distribution.

## MATERIAL AND METHODS

*Materials*

Bulbils of "ñame congo" (*Dioscorea bulbifera* L.) growing in Santa Ana estado Anzoátegui, Venezuela were used for the starch extraction. .

*Methods*

Starch isolation and purification

The starch isolation was performed on three independent batches of approximately 1–2 kg of the dibble portion of the bulbils. Starch was isolated and purified using the method described in literature [8], extracting starch by blending (with a Waring blender using twice the volume of water to edible tuber portion), sieving (200 mesh sieve) and centrifuging (1500 rpm for 15 min), prior to suspension in distilled water and air drying at 45°C and doing it in triplicate.

The degree of purity of the starch was analyzed [9] after determining the moisture, crude protein (N × 6.25%), ash and fatty material content. Color, as white index (WI) as well as damaged starch proportion was also determined following the official methods [10].

Optical polarized light microscopy (OPM)

Granular shape and Maltese crosses were observed by optical microscopy (Nikon Optiphot 2, Nikon Corp., Chiyoda-Tokio, Japan), at a 150x and 300x magnification levels with a resolution of 0.2 microns, using a polarized light filter [8, 11]. The microscope slices with a starch sprinkle spread over one or two drops of distilled water were covered with a slip cover glass, hold for 2 minutes and then examined and photographed using a Sony Cybershot digital camera (Sony Corp., Tokyo, Japan).

Scanning electron microscopy (SEM)

Shape, size and granular distribution granular were studied by scanning electron microscopy (SEM). Starch was sprinkled onto double-sided adhesive tapes, attached

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to circular specimen stubs, coated with 200 Å of pt/Pd using a Hitachi E 102 Ion Sputter, examined at 20.0 kV, and photographed in a Hitachi S 2400 scanning electron microscope [8].

Particle Size Distribution (PSD): Laser Diffraction Sizing (LDS)

Particle size distribution was studied at room temperature by laser diffraction by means of a laser light scattering Malvern Mastersizer 2000. Few milligrams of native starch powder were fed directly into the measuring cell where was submitted to ultrasound (LADD Mod. Sonicor SC-T56 60kHz) during 5-10 min. Volume distribution of the diffraction equivalent sizes was determined using the Fraunhofer scattering theory, while considering that native starch granules were opaque [12]. Aggregated starches were also sonicated in a 2% solution of sodium dodecil sulfate (SDS) [8]

## RESULTS AND DISCUSSION

Granular materials are ubiquitous in nature and at the industrial processes. They have been studied long time go, both because of their practical importance and considerable scientific interested. In more recent years, the interesting and often counter-intuitive properties of granular materials have attracted the attention of processors where its collective behavior must be treated as a matter of study branch . Starch from different source is a very interesting granular material to be studied, because to its multifaceted applications at the industries. The granular sizes and form of the starches should define its uses, machineries for processing it, and quality of the final products. Then it is imperative before apply a starch for any use, to be conscious which one is its form and size. Results shown a *Dioscorea bulbifera* L. starch with an eccentric *Maltese cross* [Figure 1], and mostly triangular shaped, with smooth superficies [Figure 2] as was pointed out in literature [7].

One of the easiest tests to identify particles as starch grains is their appearance in polarizing light. With their high degree of internal organization and relatively round shape, starch grains are almost crystalline in structure and exhibit birefringence under cross-polarized light. The starch grains appear bright against the dark field and each starch grain shows a Maltese cross when viewed with the light microscope. The presence of the telltale Maltese cross can be used to determine if starch is present and has many practical applications [13].

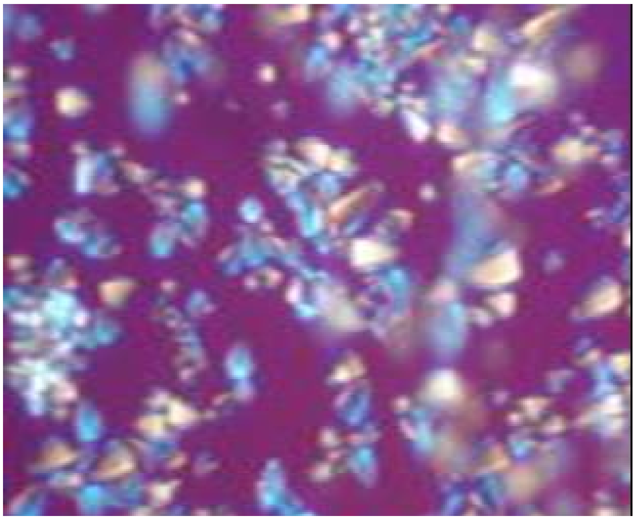

**Fig. 1.** Micrograph of the *Dioscorea bulbifera* starch granules using optic microscopy (OPM) 10x

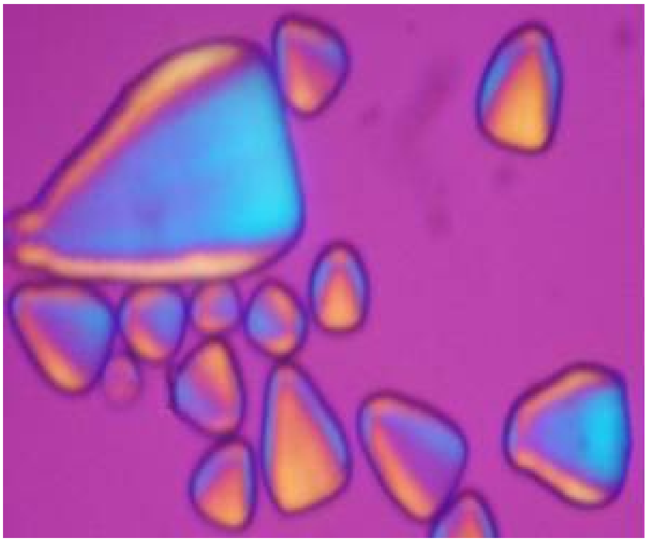

**Fig. 2.** Micrograph of the *Dioscorea bulbifera* starch granules using optic microscopy (OPM) 100x.

The magnificent effect of birefringence to shown the beauty of nature on the starch granules expressed in the bottle-shaped multicolored granules, is also revealing that the starch granules isolated and purified from *Dioscorea bulbifera* L., corresponding to a native starch (Figures 1 and 2). Figure 2 also shows at least four different triangular shaped sizes in the granular population with the remarkable eccentric Maltese cross always on their vertices, as an index that this starch has not been modified by gelatinization.

Figure 3 is the micrograph of the *Dioscorea bulbifera* L. starch granules, obtained with SEM. It can be easily observed the smooth and polished surfaces without erosion, but with some slightly slots in some of the granules (see arrows in Figure 3), that are not visualized in OMP.

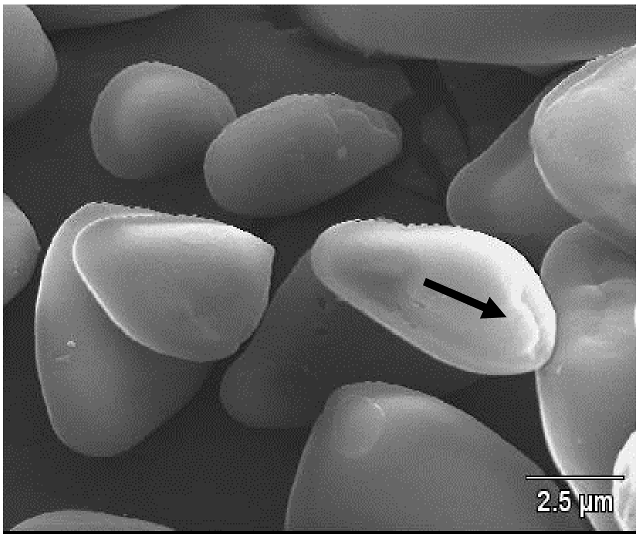


**Fig. 3.** Micrograph of the *Dioscorea bulbifera L.* starch granules using a Hitachi S 2400 scanning electron microscope (SEM)

All of these Figures (1, 2, and 3) also are revealing that the morphology of the granules of *Dioscorea bulbifera* starch would be not quite adequate for some uses; for example filtration or for spreading as powder. The granules of these starches are not round- shaped, and it shall produce a spatial effect. The effect occurs when the volume occupied by the bottle-shaped granule prevents its moving (spread) or go through the filter (filtration). As result of the study part, Dioscorea bulbifera´s starch shape is fully characterized, being these data useful for its possible applications.

In the other hands, the granular size distribution of population of starch granules is shown in Table 1. The granular distribution of the larger population of starch granules with sizes ranging from 7 to 60µm represents the 93.04 ± 0.25% of the overall population, and the rest represent the 6.96 ± 0.25%, with granule sizes ranging from 1-7 µm

**Table 1.** Granular size distribution of the *Dioscorea bulbifera L.* starch.

| Granule size | |
|---|---|
| Dv10 | 12.67±0.05 |
| Dv50 | 24.12±0.02 |
| Dv90 | 39.86±0.15 |
| Range [0,1-7] µm (%) | 6.96±0.25 |
| Range [7-20] µm (%) | 93.04±0.25 |

The particle size distributions were Dv 10 = 12.67; Dv 50 =24.12, and Dv 90 = 39.86, indicating that 50% of the population is concentrated in a size of ≈25µm (Figure 4).

As its shape, the size of the starch and its distribution in the population (bi or polimodal) need to be known before apply it in the different industrial processes. The results of this study made available to the processor, the granular morphology (size and shape) of the granules isolated from Dioscorea bulbifera starch, for its possible uses in food and other industries.

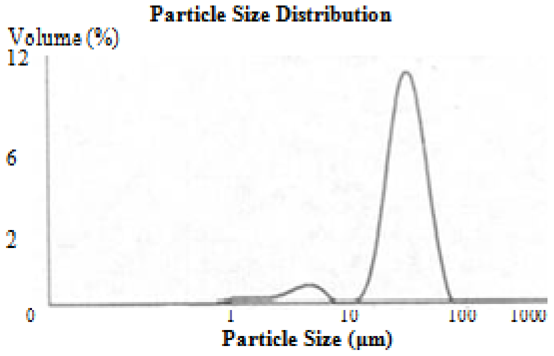


**Fig. 4.** Particle size distribution of the granular population of *Dioscorea bulbifera* starch.

## CONCLUSIONS

All of those starch micrographs, taken by using the polarized light optical microscope and the scanning electron microscope, besides the characterization of the granule´s sizes and forms provide valuable information for the processor about the way as the Dioscorea *bulbifera starch* can be used in industry

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