Enliven Archive
Intensifying Thoughts

Research Article www.enlivenarchive.org Enliven: Journal of Dietetics Research and Nutrition

Morphometric Variations of Banana Starches Issued from Various Genomic Groups and *In vitro* Starch Digestibility

Gibert O[1], Alemán S[2], Guzmán R[3], Raymúndez M.B[4], Laurentin A[4], Manzanilla E[5], Ricci J[1], and Pérez E[3*]

[1]*Centre for International Cooperation in Agronomic Research for Development (CIRAD), UMR QUALISUD, 73 Rue JF Breton, TA - B 95/15 F- 34398 Montpellier, France*

[2]*Instituto de Química y Tecnología, Facultad de Agronomía, Universidad Central de Venezuela, Maracay, Venezuela.*

[3]*Instituto de Ciencia y Tecnología de Alimentos (ICTA), Facultad de Ciencias, Universidad Central de Venezuela, Caracas, Venezuela.*

[4]*Instituto de Biología Experimental (IBE), Facultad de Ciencias, Universidad Central de Venezuela, Caracas, Venezuela.*

[5]*Instituto Nacional de Investigaciones Agrícolas (INIA-Aragua), Maracay, Venezuela.*

[*]**Corresponding author**: Dr. Elevina Pérez, Instituto de Ciencia y Tecnología de Alimentos (ICTA), Facultad de Ciencias, Universidad Central de Venezuela, Caracas, Venezuela, Apartado Postal 47097, Caracas 1041- A, Venezuela, Tel: +582127535684; Fax: +582127533871; E-mail: perezee@hotmail.com, elevina.perez@ciens.ucv.vce

Received Date: 31st July 2014
Accepted Date: 22nd September 2014
Published Date: 26th September 2014

Citation: Gibert et al., (2014) Morphometric Variations of Banana Starches Issued from Various Genomic Groups and In Vitro Starch Digestibility. Enliven: J Diet Res Nutr 1(1):003

Abstract

An analysis of shape and size of the granules of starches isolated from the edible portion of eight unripe banana genotypes was performed. The study aimed at investigating the potential of banana starches as ingredients for industrial purposes. It also aimed at investigating their *in vitro* digestibility in relation to their morphometric characteristics. The granular size distribution and granular morphometric characteristics were determined by diffraction Laser sizing (LDS), light polarized microscopy (LPM), and scanning electronic microscopy (SEM) techniques. Results reveal differences in the starch shape and size without any correlation between the granular profile and the genomic group. Granule shape includes symmetrical and asymmetrical spheres, symmetrical and asymmetrical shell-shapes, tubular-shapes, irregular and ellipsoidal-truncated shapes. Some granules exhibited smooth shapes, whereas others exhibit faceted surfaces. It was also observed some variation in the starch hydrolysis among samples, which was most pronounced in the BB banana starch genotype. The variation of the granular shape and size could be considered as a key for the differentiation of the banana genotypes. Such variation could affect the digestibility of the starches, even gelatinized. Thus, the granular distribution histogram can be used as an indicator of the potential uses of the starch from bananas. Among potential industrial uses, the isolated starch from the BB genotype can be used for paper and cosmetic industry according to its granule small size and uniformity. The other evaluated starches have granular size and shape variations that have to be sorted in relation to their functional properties and how they could affect the process within industrial formulations.

Keywords Bananas; Starch; Morphometry; Granular size; Laser diffraction (LDS) ; Scanning Electronic Microscopy (SEM); Polarized light microscopy

Introduction

There is a tremendous potential for the profitable commercial use of tropical starches. However, considerable research and product development is required to properly exploit starch materials [1]. Among others, in the tropical area, there are numerous varieties of banana with high potential for starch production. A solution for the diversification of the botanical sources of starches, including banana starches, could be to investigate their physical and functional properties [2-5]. If some physico-chemical features are helpful to differentiate the banana cultivars [6-7], some agro-morphological variations are often used to recognize genotypes among *Musa acuminata* and *Musa balbisiana*. Considered to be natural crossing of the two previous genomic groups, a combined group made of acuminata and balbisiana species usually exhibits characteristics that combine their morphological characters [8-9]. In consequence, diploïds (AA, BB or AB genomes), triploids (AAA, BBB, AAB, or ABB genomes), and tetraploids (AAAA, and ABBB genomes) are growing in nature.

Starch granules are formed in amyloplasts, which are, like chloroplasts, derived from proplastids [10], varying greatly in form, size and functionality, between and within botanical species. For instance, starch granules diameter can range from 1 to 100 µm, as a function of the botanical source [11]. Banana starch granules from the various hybrids differ in size, while being irregular in shape, and appear as elongated ovals with ridges by microscopy [3].

Starch morphometric differences, not only provide diverse properties, but also induce diverse behaviors during processing due to their inner inconsistency [12]. These morphometric variations could affect its bioavailability, since the granule size of starch can affect the rate of enzymatic-hydrolysis of starch granules [13-14].

Granule morphology and particle size distribution of starches has a direct influence on material properties such as reactivity or dissolution rate, stability in suspension, delivery efficiency, texture and feeling, appearance, ability to flow and handling, viscosity, packing density, and porosity. It is of consensus that the average particle size on the food systems is known to influence properties related to processing (e.g. gelatinization, water and reagents absorption and solubility) [15-17], and nutritional value (e.g. starch digestion rate) [18-21] of human foods and animal feeds. Since the size and shape of the granules of starch are among important factors in the determination of the potential starch uses, its granule size and particle size distribution must be measured.

Optimizing the particle size distribution in suspensions can produce up to 50-fold reductions in shear viscosity, facilitating pumping, mixing and transportation in the fuel, concrete, paint and food industries [22]. For example, in food industry, as reported by Campbell et al. [23], small granules (< 2.0 µm) can be used as fat substitutes due to its similar size with the lipid beads; the size also influence the wet-milling extraction. Same authors [23] have also pointed out that other applications in which the size of the granules is important is on the production of biodegradable plastic films and carbonless fax papers. Moreover, the smallest granules can be used as a vehicle in cosmetics. The starch suitability for the above-mentioned applications is often determined by granule size.

Several techniques to determine particle size distribution can be used, including laser light scattering, light microscopy and SEM, sieving, sedimentation analysis, permeability of a powder column, and electrical sensing zone technique. Those techniques measure different parameters and each one have its advantages and disadvantages; therefore, the choice of the technique will largely depend on the application. Laser diffraction particle size analyzer has proved to be an effective tool for providing accurate and precise particle size measurement, because its requires little time for analysis, cover a wide size range, and require small sample amount, facilitating detailed studies of particle size populations. Laser diffraction particle size analyzers provide indirect size measurements of spherically equivalent particles, based on the principle that particles of a given size diffract light through a given angle that increases logarithmically with decreasing size [24].

On the other hand, a researcher dedicated to microscope techniques is able to identify starches from different botanical sources [25-29]. Among others, the scanning electron microscope (SEM) is a microscope that produces images from the starch by scanning it with a focused beam of electrons. Both sources of electrons, those ones from the equipment and from the starch, interact and produce signals that contain information about the surface of the sample topography and composition. It helps to describe parameters as dimension, forms and porous presence of the granular structure of the starch. Indeed, as postulated before, each one defines the functional properties of the starch. With the help of these tools, scientists are slowly beginning to build up a picture of how starch is constructed and how it is behaving at the food and other systems.

The goal of this study was then to determine morphologies and granular size distribution of isolated starches from different bananas genotypes, relating them to their *in vitro* digestibility in order to suggest some food applications.

Material and Methods

Materials

Eight isolated and purified starches from different genotypes of bananas belonging from the field collection at the Instituto Nacional de Investigaciones Agrícolas (INIA), estado Aragua, Venezuela were studied. Three samples were from acuminata ascendance (AA, AAA, AAAA genomes), three from *acuminata* and *balbisiana* ascendance (AAB, ABB, and AAAB genomes), and just two from *balbisiana* (BB, BBB genomes).

Methods

Sample preparation

Fresh unripe bananas at full green stage of maturity equivalent to a Cavendish-like Grade 1 [30] were cleaned and rinsed with a large amount of tapwater and wiped for starch isolation. The edible portion was cleaned, peeled and sliced into 2.5 inch pieces. The sliced portions were immediately immersed in 1% of a citric acid solution in order to avoid enzymatic browning prior to further processing.

Starch isolation and purification

The starch isolation was performed on independent batches of approximately 1-2 kg of each The starches were isolated and purified using the procedure described by Pérez et al. [31] with some modifications. After slicing, pieces were milled during 3 min at high speed using a Waring blender with small volumes of distilled water. This grinding and screening operation were repeated four times. The resulting slurry was sieved consecutively through a 200-mesh muslin cloth sieve, and centrifuged at at 500xg during 20 min. After removing the mucilaginous layer, the sediment was washed several times by suspension in distilled water and centrifuged until it appeared to be free of non-starch material. The sediment was then oven-dried at 45°C during 24 hours. The dried starch was blended, passed through a 60-mesh sieve, and stored at room temperature in sealed plastic bags.

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 [5,32]. Aggregated starches were also sonicated in a 2% solution of sodium dodecil sulfate (SDS).

Granule morphology: Scanning Electron Microscopy (SEM) and Polarized Light Microscopy (PLM)

Starch samples were sprayed on a metal plate previously covered with double sided adhesive tape and shadowed under vacuum with gold-palladium. starch granules were examined with a scanning electron microscope (Hitachi S-2400) at 20kV accelerating voltage [25]. Those starches former presumed as aggregate were also measured using polarized light microscopy. This analysis was performed on a NIKON Optiphot 2 microscope with a polarized filter, and with a Nikon FX – 35DX camera attached, according to methodology originally described by Sivoli et al. [26]. Starches could be seen under the microscope by placing a small amount of the starch powder on the slides, adding a drop of distilled water and covering it with a glass in order to increase the refractive index of the sample and to obtain better images.

For the quantitative analysis of granular sizes, a random sample of 100 granules on 50x magnification surface area were observed and measured for major and minor diameters. The larger and smaller diameter (average) ratio was also measured in order to compare the elongation of the granules.

In vitro starch digestibility

In vitro starch digestibility of the gelatinized starch suspension was analyzed [33]. A α-amylase (1200 UI/mg and 27 mg of proteins/ml) from porcine pancreas preparation were used (A3176, Sigma Chemical Co., St. Louis, USA). About 700mg of dry starch were suspended into 50 ml of a sodium and potassium phosphate buffer (0.5 M pH 6.9) and homogenized. The starch suspension was gelatinized during 20 min into a boiling water bath under continuous stirring. The suspension was then cooled to 37°C. About 4 mg/ml of the α-amylase solution diluted in the phosphate buffer were mixed to gelatinized starch suspensions and incubated at 37°C for an hour. Sampling were carried out in triplicate at various time (5, 15, 30 and 60 min) in addition to the initial condition where no enzyme was used. A standard curve was prepared using pure dry maltose from the 0-2 mg/ml range, in addition to the use of a control made of pure potato starch. The 3,5-dinitrosalicylic acid method (DNS) was used, while adding 0.2 ml of sample, plus 0.8 ml of distilled water and 1 ml DNS solution in boiling water bath for 10 min, prior to cooling at room temperature and reading at 540 nm against a DNS blank. The extent of the hydrolysis was computed as the percentage of dry hydrolyzed starch (mg of maltose/100 mg of pure starch).

Statistical analysis

The Results were analyzed using statistical standard parameters such as mean and standard deviation from replicates of the samples.

Results and Discussion

The results of the analysis of size and shape of the starch granules from the different banana genotype are shown in Table 1 Figures 1 to 4. Results showed differences in sizes and shapes among all genotype sources. As can be seen, in the starches granules measured by SEM exhibited a wide range of sizes, ranging from 3 to over 80 µm, differently shaped (Table 1), which are in agreement with values reported by several authors [2,34-41] for green banana starch isolated from different genotypes. The frequent shape was those shell-like. The largest range of size was observed in starch from the AAB genome (10-80 µm), and the smallest ones were at the starches from AAAA genome (2-14 µm), and the BB genome (4-16 µm) [2,40,41]. Some author has pointed out that the granular size and shape varied with the ripening stage [35]. However, as it is revealed here, diferences must be due to the botanical source.

Table 1. Morphometry of isolated starches from banana genotypes measured by polarized light microscopy

Starch from Banana clone	Range of size (µm)	Shape
AA genome Dessert banana (Titiaro)	8-36	Small round, and ellipsoidal, and large shell, and ellipsoidal shaped.
AAA genome Dessert banana (Grande Naine)	6-28	Small tubular-like and largest ellipsoidal-truncated and shell-like.
AAAA genome Dessert banana (FHIA 17)	2-14	Small round and oval-truncated, and largest oval truncated shaped
BB genome Dessert banana (Balbisiana)	4-16	Small round and medium shell-shaped.
BBB genome Dessert banana (Let chan kut)	10-48	Small round, medium like-shell, and largest triangular-shaped.
AAB genome Cooking banana (Hartón)	10-80	Small round, and tubular-like, and largest shell-like.
ABB genome Dessert banana (Topocho)	12-56	Small round and tubular and largest ellipsoidal-truncated and shell shaped.
AAAB genome Dessert banana (FHIA 1)	4-46	Small shell-shaped and largest tubular

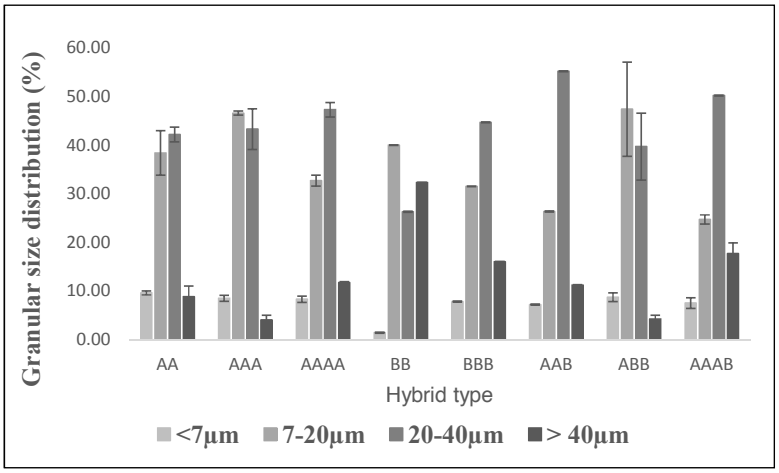

Figure 1. Histogram of the granular distribution of the banana starches

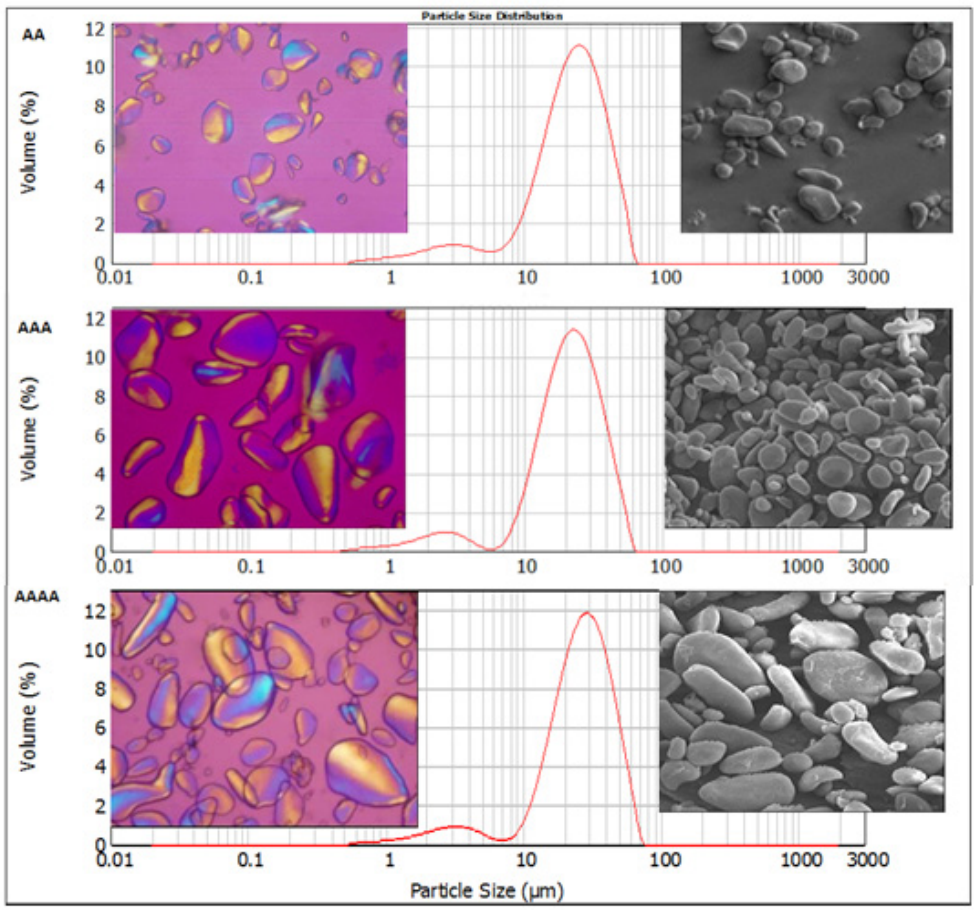

Figure 2. Photomicrographs (PLM: left side, and SEM: right side) of isolated starches from banana (diploid , triploid and tetraploid from *acuminata* genome) : granules and particle size distribution (laser).

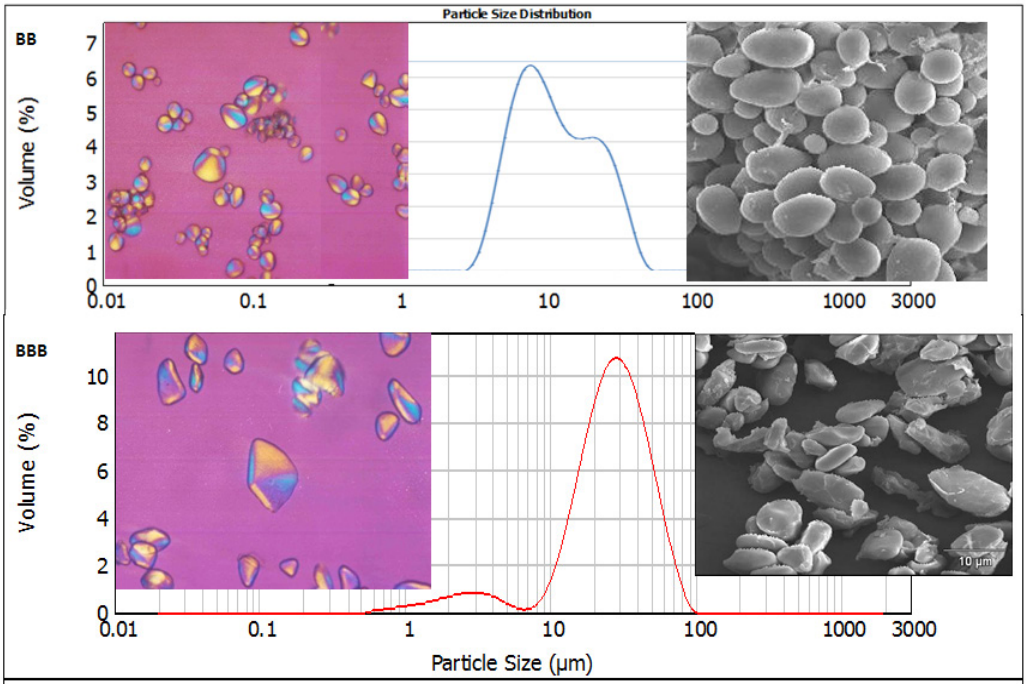

Figure 3. Photomicrographs (PLM: left side, and SEM: right side) of isolated starches from banana (diploid and triploid of *balbisiana* genome): granules and particle size distribution (laser).

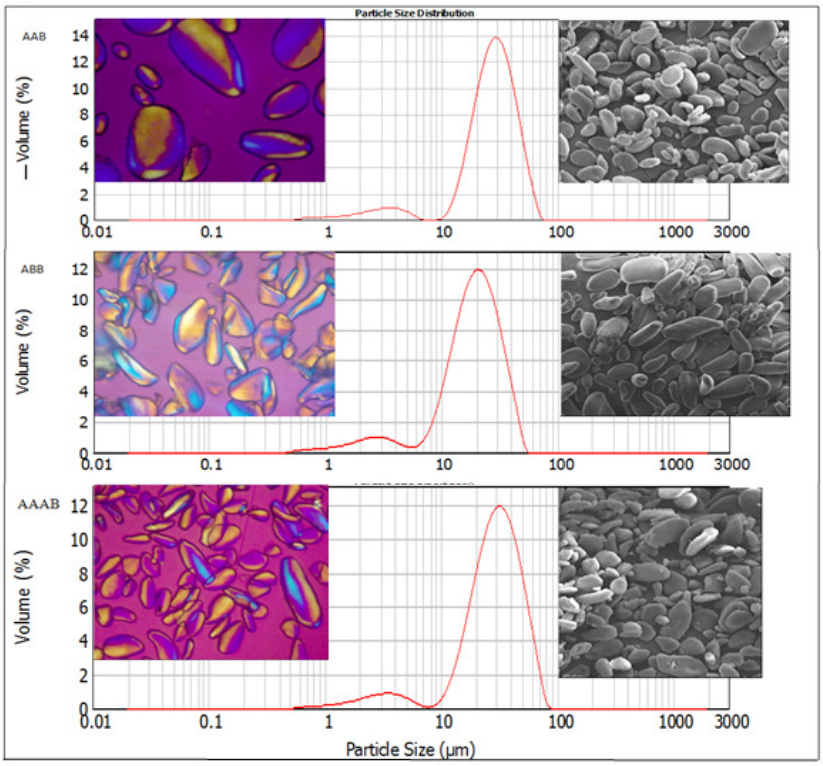

Figure 4. Photomicrographs (PLM: left side, and SEM: right side) of isolated starches from banana (triploid and tetraploid genotypes from *acuminata and balbisiana*): granules and particle size distribution (laser).

Table 2 combine the granule size distribution (%) of isolated starches from banana genotype measured by laser light diffraction technique, and Figure 1 shown the histogram of the granular distribution of each genotype. As expected, the largest population of granules was located at 7-40 µm range [2,36,38,39,41]. AA and ABB genomes exhibited the highest fraction of small granules with sizes below 7 µm, whereas the BB starch granule exhibited the lowest fraction of granule below 7 µm, despite that BB genome starch granule was revealed as the smallest granule average by SEM. All banana starches showed the characteristic eccentric Maltese crosses, indicating that the isolation method used yielded intact native starch granules (Figures 2, 3 and 4; left side).

Table 2. Granular size distribution (%) of isolated starches from banana genotype measured by laser diffraction technique.

Genome/ Size ranging	AA	AAA	AAAA	BB	BBB	AAB	ABB	AAAB
<7µm	9.6±0.4	8.5±0.6	8.3±0.6	1.4±0.1	7.8±0.1	7.2±0.1	8.7±0.9	7.5±1.1
7-20µm	38.4±4.6	46.6±0.4	32.7±1.1	40.0±0.1	31.5±0.1	26.36±0.1	47.4±9.7	24.7±1.0
20-40µm	42.2±1.5	43.3±4.2	47.3±1.5	26.3±0.1	44.7±0.1	55.2±0.1	39.7±6.9	50.2±0.1
> 40µm	8.8±2.2	4.0±1.0	11.7±0.2	32.3±0.1	16.0±0.1	11.2±0.1	4.2±0.8	17.7±2.2

Figure 2 is comparing the morphometric characteristic of starches isolated from banana with *acuminata* genotype (diploid, triploid and tetraploid genomes) by microphotographs of PLM and SEM, and curves of the granular distribution sizes. As can be seen, there are differences in shapes (Table 1) and sizes, which is plotted by the curve.

Figure 3 representes the morphometric characteristics of two starches isolated from *balbisiana* genotype (diploid and triploid genomes). 90% of granular size distribution fluctuated in the 0.1-40 µm range for both populations. On the one hand, the diploid BB starch exhibited the lowest starch fraction of the smallest size (0.1-7µm) and the highest fraction of the starch (16%) above 40 µm (Figures 2 to 4). The highest fraction of the starch population above 40 µm revealed by laser diffraction analysis was most probably due to its agglomeration. Such hard agglomeration was so strongly bound that neither the reagent nor the mechanical force used for conditioning the sample prior to laser diffraction analysis were effective for dis-agglomeration. In the other hand, the BBB starch exhibited small rounded and medium shell-shaped granules, and the largest triangular-shapes. The low granule range (from 4 to 16 µm) of the BB starch genotype and regularity of its size rendered the resource attractive with desirable feature for chemical papers as used for copying and fax, and as well as for cosmetic industry.

Some other starches exhibited polymodal granular distribution, suggesting that the granules can be sorted into more than one size range, and shape (Figure 3). Banana starch, as found in this study, exhibited a distribution of both large and small granules, and with variations in granular shapes. Granule shapes included symmetrical and asymmetrical spheres, symmetrical and asymmetrical shell-shapes, tubular-shapes, and irregular and ellipsoidal-truncated shapes. Besides that, some granules exhibited smooth shapes, whereas others showed faceted surfaces.

Figure 4 compares the granular population of the isolated starches from acuminata and *balbisiana* genomic groups through the representation of the AAB, ABB and AAAB genotypes.

The AAB and ABB genome`s starches exhibited small rounded and tubular granules, as well as the largest shell-like ones with size variations. In fact, AAB genotype exhibited the largest variation by SEM as illustrated in Table 1 with a highest fraction of granules with sizes above 40 µm (Table 2). Contrary to AAB genotype, the ABB triploid exhibited the lowest fraction of granules above 40 µm (Figure 4) and highest fraction of granules of the smallest granular size average in the 0.1 to 7 µm range. The AAAB starch exhibited the smallest granular size average with small shell-shapes and large tubular granules varying from 4 to 46 µm.

Digestibility of native starches has been attributed to the interplay of many factors, and the Being the granular size is one of them [3]. The extent of the hydrolysis of the cooked banana starches studied here fluctuated from 0 to 68% (Figure 5) and all of them exhibited lower digestibility extent to that of the standard curve. Except for *balbisiana* ancestry (BB and BBB), starches showed slight differences in their rates of hydrolysis up to nearly 50 minutes of incubation. The diploid and triploid clones from *balbisiana* (BB and BBB) exhibited atypical starch hydrolysis behavior, when compared with the six other starches.

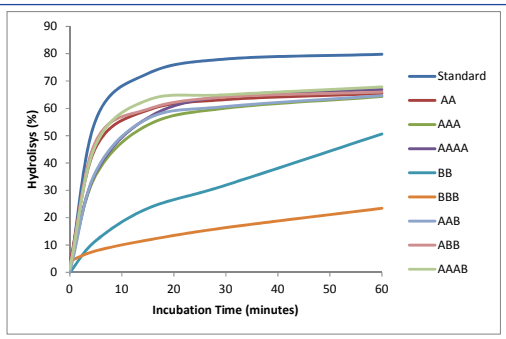

Figure 5. Starch hydrolysis of the banana starches samples gelatinized during 20 min at 98±1°C

It has been reported that dessert banana starch was appreciably more resistant to α-amylase and glucoamylase attack than corn starch [36]. Cooking banana starches had the least resistance to α-amylase digestion, which could be due to the resistant starch (RS) content [36]. It has been shown that banana starch has important content of resistant starch (RS) [41-42]. Among other contributions, according to the morphometric variations in starch granules observed, an effective contribution of the shape and granular size could be suggested as earlier illustrated in Figure 1 and 3. Some investigations may later confirm the variation of the morphometric characteristics here observed and specific potential for industrial uses, based on a larger number of accessions belonging to various genomic groups [36, 39-41].

Conclusion

There exist wide differences in size and shape as a function of the banana genotype, but the granular profile was not correlated with the genotype. However, the granular shape variation of the starches can be typified as a functional property for various uses in the industry. The histogram of the distribution of the population seemed a potential indicator for some uses of the starch from banana, including the BB isolated starch for paper and cosmetic industry according to its small size and size and shape uniformity. The other evaluated starches exhibited significant variations in their granular sizes and shapes, which may be considered for specific functional properties within manufactured items.

Acknowledgments

Special thanks are due to Instituto Nacional de Investigaciones Agrícolas (INIA-Aragua), Maracay, Venezuela. for supplying the fresh material, and to the local stakeholders in Ocumare, Aragua state, Venezuela for the identification and the delivery of fresh material of genotypes, donated by the INIA, available in their crops. The authors would like also to thank to the Consejo de Desarrollo Científico y Humanístico (CDCH) de la Universidad Central de Venezuela for the financial support for this study, through the projects N°(s): 0376072009/1-2 and 03.32.3873.97-00-04. Authors gratefully acknowledge the fund provided by the Programa de Cooperación de Postgrado (PCP) France-Venezuela, between the CIRAD at Montpellier, France and Instituto de Ciencia y Tecnología de Alimentos (ICTA), Facultad de Ciencias, Universidad Central de Venezuela, Caracas, Venezuela

References

1. Satin M (2000) Functional properties of starches. FAO Agricultural and Food Engineering Technologies Service.
2. Pérez E (1997) Characterization of starch isolated from plantain (Musa paradisiaca Normalis). Starch\Stärke 49: 45-49.
3. Zhang P, Whistler RL, BeMiller JN, Hamaker BR (2005) Banana starch: production, physicochemical properties, and digestibility - A review. Carbohyd Polym 59: 443-458.
4. Alemán S, Pacheco-Delahaye E, Pérez E, Schroeder M (2011) Elaboration of blackberrie (*Rubus glaucus* Benth) jelly with native and modified banana starches (*Musa* ABB). Afr J Food Sci 5: 181-187.
5. Pérez E, Rolland-Sabaté A, Dufour D, Guzmán R, Tapia M,et al. (2013) Isolated starches from yams (*Dioscorea* sp) grown at the Venezuelan Amazons: Structure and functional properties. Carbohyd Polym 98: 650-658.
6. Gibert O, Dufour D, Giraldo A, Sánchez T, Reynes M, et al. (2009) Differentiation between cooking bananas and dessert bananas. 1. Morphological and Compositional characterization of cultivated Colombian *Musaceae* (*Musa* sp.) in relation to consumer preferences. J Agr Food Chem 57: 7857-7869. Erratum: (2010). J Agr Food Chem 5: 9290.
7. Dufour D, Gibert O, Giraldo A, Sánchez T, Reynes M, et al. (2009) Differentiation between cooking bananas and dessert bananas. 2. Thermal and functional characterization of cultivated Colombian Musaceae (*Musa* sp.). J Agr Food Chem 57: 7870-7876.
8. Valmayor RV, Jamaluddin SH, Silayoi B, Kusumo S, Danh LD, et al. (2000) Banana Cultivar Names and Synonyms in Southeast Asia. International Network for the Improvement of Banana and Plantain-Asia and the Pacific Office. Filipinas.
9. González J (2004) Transformación genética de plátano (*Musa sp. Cv. "Hartón"*) mediante *Agrobacterium tumefaciens*. Proyecto de Tesis Doctoral, no publicado. Biblioteca de la Universidad Central de Venezuela, Caracas Venezuela.
10. Briarty LG, Hughes CE, Evers AD (1979) The developing endosperm of wheat.-A stereological analysis. Ann Bot 44: 641-658.
11. Ellis RP, Cochrane MP, Dale MFB, Duffus CM, Lynn A, et al. (1998) Starch production and industrial use. J Sci Food Agr 77: 289-311.
12. Copeland L, Blazek J, Salman H, Tang MC (2009) Form and functionality of starch. Food Hydrocliloid 23: 1527-1534.
13. Kimura A, Robyt JF (1995) Reaction of enzymes with starch granules - Kinetics and products of the reaction with glucoamylase. Carbohydrate Research 277: 87-107.
14. Ai Y (2013) Structures, properties, and digestibility of resistant starch. Paper 13558. Graduate Theses and Dissertations. Graduate College. Iowa State University, USA.
15. Lin JH, Kao WT, Tsai TC, Chang YH (2013) Effect of granular characteristics on pasting properties of starch. Carbohyd Polym 98: 1553-1560.
16. Hasjim J, Li E, Dhital S (2013) Milling of rice grains: Effects of starch/flour structures on gelatinization and pasting properties. Carbohyd Polym 92: 682-690.
17. Iwuoha CI, Nwakanma MI (1998) Density and viscosity of cold flour pastes of cassava (*Manihot esculenta Crantz*), sweet potato (*Ipomoea batatas* Lam) and white yam (*Dioscorea rotundata* Poir) tubers as affected by concentration and particle size. Carbohyd Polym 37: 97-101.
18. De la Hera E, Gómez M, Rosell CM (2013) Particle size distribution of rice flour affecting the starch enzymatic hydrolysis and hydration properties. Carbohyd Polym 98: 421-427.
19. Al-Rabadi GJ, Torley PJ, Williams BA, Bryden WL, Gidley MJ (2012) Particle size heterogeneity in milled barley and sorghum grains: Effects on physico-chemical properties and starch digestibility. J Cereal Sci 56: 396-403.
20. Mahasukhonthachat K, Sopade PA, Gidley MJ (2010) Kinetics of starch digestion in sorghum as affected by particle size. J Food Eng 96: 18-28.

21. Singh J, Kaur L, McCarthy OJ (2007) Factors influencing the physico-chemical, morphological, thermal and rheological properties of some chemically modified starches for food applications—A review. Food Hydrocolloid 21: 1-22.
22. Servais C, Jones R, Roberts I (2002) The influence of particle size distribution on the processing of food. J Food Eng 51: 201-208.
23. Campbell MR, Li J, Berke TG, Glover DV (1996) Variation of starch granule size in tropical maize germplasm. Cereal Chem 73: 536-538.
24. Beuselinck L, Govers G, Poesen J, Degraer G, Froyen L (1998) Grain-size analysis by laser diffractometry: comparison with the sieve-pipette method. Catena 32: 193-208.
25. Matos ME, Pérez E (2003) Characterization of native and modified cassava starches. I. Ultrastructural study by scanning electron microscopy and X-ray diffraction techniques. Cereal Food World 48: 78-81.
26. Sívoli L, Pérez E, Rodríguez P, De Abrisqueta A, Raymúndez MB (2005) Cambios en la estructura granular durante el proceso de modificación química (fosfatado-acetilado) del almidón de yuca analizado por microscopía óptica y electrónica. Acta Microsc 14: 5-9.
27. Sívoli L, Pérez E, Lares M, Leal E (2008) Studies of conformational changes, crystalline and granular structure and in vitro digestibility of the cross-linked and methylated corn starches. Interciencia 34: 52-56.
28. Sívoli L, Pérez E, Rodríguez P, Maria B. Raymúndez, Ayesta C (2009) Técnicas microscópicas y de dispersión de luz empleadas en la evaluación de la estructura del almidón de yuca (*Manihot esculenta C*. Acta Microsc 18: 195-203.
29. Matos ME, Pérez E, Pacheco E (2009).Effect of the heating treatment on the ultra structure of sweet potato (*Ipomoea batatas* L.) starch. Efecto del calentamiento sobre la ultra estructura del almidón de batata (*Ipomoea batatas* L.). Rev Fagro-UCV 35: 15-20.
30. Von Loesecke HW (1950) Bananas. (2ed.) Interscience, New York USA.
31. Pérez E, Bahnassey Y, Breene W (1993) A simple laboratory scale method for isolation of *Amaranthus* starch. Starch/Stärke 45: s211-214.
32. Webb PA (2000) Technical Workshop Series: Introduction to the Latest ANSI/ISO Standard for Laser Particle Size Analysis. A Primer on Particle Sizing by Static Laser Light Scattering.
33. Holm J, Björck I, Asp NG, Sjöberg Lb , Lundquist I (1985) Starch availability in vitro and in vivo after flaking, steam-cooking and popping of wheat. J Cereal Sci 3: 193-206.
34. Kayisu K. Hooo LF, Vansoest PJ (1981) Characterization of starch and fiber of banana fruit. J Food Sci 46: 1885-1890.
35. Lii CY, Chang SM, Young YL (1982) Investigation of the physical and chemical properties of banana starches. J Food Sci 47: 1493-1497.
36. Eggleston G, Swennen R, Akoni S (1992) Physicochemical studies on starches isolated from plantain cultivars, plantain hybrids and cooking bananas. Starch/Stärke 44: S121 -S128.
37. Millán-Testa CE, Mendez-Montealvo M G, Ottenhof M-A, Farhat I A, Bello-Perez LA (2005) Determination of the molecular and structural characteristics of okana mango and banana starches. J Agric Food Chem 53: 495-501.
38. Bello-Pérez LA, Francïsco AD, Agama-Acevedo E, Gutierrez-Meraz F, García-Suarez FJL (2005) Morphological and molecular studies of banana starch. Food Sci Tech Int 11: 367-372.
39. Carmona-García R, Sánchez-Rivera MM, Méndez-Montealvo G, Garza-Montoya B, Bello-Pérez LA (2009) Effect of the cross-linked reagent type on some morphological, physicochemical and functional characteristics of banana starch (*Musa paradisiaca*). Carbohydr Polym 76: 117-122.
40. Otegbayo B, Lana O, Ibitoye W (2010) Isolation and physicochemical characterization of starches isolated from plantain (*Musa paradisiaca*) and cooking banana (*Musa sapientum*). J Food Biochem 34 :1303-1318.
41. Agama-Acevedo E, Rodriguez-Ambriz SL, Garcia-Suarez FJ, Gutierrez-Meraz F, Paheco-Vargas G, et al. (2014) Starch isolation and partial characterization of commercial cooking and dessert banana cultivars growing in Mexico. Starch/Stärke 66: 337-344.
42. Faisant N, Buleon A, Colonna P, Molis C, Lartique S, et al. (1995) Digestion of raw banana starch in the small intestine of healthy humans: Structural features of resistant starch. Br J Nutr 73: 111-123.