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Physicochemical, Functional, and Macromolecular Properties of Waxy Yam Starches Discovered from "Mapuey" (*Dioscorea trifida*) Genotypes in the Venezuelan Amazon

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"Mapuey" tubers in Venezuela are staple food for indigenous peoples from the Caribbean coast and Amazon regions. Noticeable differences between genotypes of yam starches were observed. Granules were large, triangular, or shell-shaped with monomodal particle size distribution between 24.5 and 35.5 μm. Differential scanning calorimetry (DSC) analyses revealed onset gelatinization temperatures from 69.1 to 73.4 °C with high gelatinization enthalpy changes from 22.4 to 25.3 J g^{-1}. All X-ray diffractograms of starches exhibit B-type crystallinity. Crystallinity degrees varied from 24% to 40%. The highest crystallinity was found for the genotype having the highest amylose content. Iodo-colorimetric, amperometric, and DSC amylose determinations varied from 1.4 to 8.7%, 2.2 to 5.9%, and 1.4 to 3.5% for Amazonian genotypes, in comparison with commercial Mapuey starches: 12.0, 9.5, and 8.7%, respectively. Solubility and swelling power at 90 °C varied from 2.1 to 4.4% and 20.5 to 37.0%, respectively. Gel clarity fluctuated from 22.4 to 79.2%, and high rapid visco analyzer (RVA) viscosity was developed at 5% starch suspension (between 1430 and 2250 cP). Amylopectin weight average molar mass $\bar{M}_w$, radius of gyration $\bar{R}_G$, hydrodynamic coefficient ν_G, and apparent molecular density d_{Gapp} were determined using high-performance size exclusion chromatography (HPSEC) and asymmetrical flow field flow fractionation (A4F) techniques coupled with multiangle laser light scattering (MALLS) on the *Dioscorea trifida* genotypes exhibiting the lowest and highest amylose contents. Amylopectins showed very similar molecular conformations. $\bar{M}_w$ values were 1.15 $\times$ 10^8 and 9.06 $\times$ 10^7 g mol^{-1} using HPSEC and A4F, respectively, thus, 3−5 times lower than those reported with the same techniques for other yam species, and very close to those of potato and cassava amylopectins. This discovery of a new natural amylose-free starch in the neglected yam "Mapuey" could present some potential for the food industry.

KEYWORDS: *Dioscorea trifida*; cush-cush yam; physicochemical properties; waxy starch; amylopectin

INTRODUCTION

Yams (*Dioscorea* spp.) are important in household food security and income generation, especially in West and Central Africa where most of the world production occurs. Some root and tuber crops grown in the northern part of South America and the Caribbean Islands, Sri Lanka, and New Caledonia usually have low commercial value for direct consumption. Among those, some varieties such as *Dioscorea trifida* L (cush-cush yam) could be considered. The *Dioscorea trifida* L is believed to originate from the Guyana region of South America; it is by far the most important of the indigenous American yams (*1*−*3*). There are different colored *Dioscorea trifida* L varieties, ranging from white to purple to black. Very little information is available in the literature in relation to their composition and uses. Even though the information available on such an under-used crop is sparse, it has been demonstrated that their starch could be a good source for use in the food industry as a thickener and binder (*4*−*7*). Amylose has low water solubility. Contrary to amylopectin solution, an amylose solution is usually unstable with a high tendency to retrograde, to gel, and to turn opaque. Waxy roots and tubers starch (potato, sweet potato, and cassava) have improved paste clarity and viscosity and applications in both the food industry and in paper manufacture can be expected (*8*−*10*). An important property of starches that are used in food products is their freeze−thaw stability. Thus, due to their lack of amylose, waxy starches have improved freeze−thaw stability (*11*, *12*).

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Some reports on *D. trifida* (*5*) indicate amylose contents in the 34.7 to 43.3% range for starches being isolated from white and purple *D. trifida* varieties, respectively. Starch physicochemical and functional properties have been reviewed on yam crops, such as *D. alata*, *D. cayenensis*, *D. esculenta*, *D. rotundata*, *D. dumetorum*, and others with average amylose content above 7.0% (*13*−*15*). In Asia, a large diversity is observed between species in the 13.4−37.1% range (*16*−*20*), in Africa where the yam is a staple food with an amylose content in the 7.0−28.8% range (*7*, *21*−*23*), whereas in South America contents of 11.1−36.0% are reported (*24*−*30*).

It has been demonstrated that starches isolated from greater yam (*D. alata*), white yam (*D. rotundata*) and yellow yam (*D. cayenensis*) varieties show monomodal particle size distribution centered between 31.0 and 35.0 μm, while the bitter yam (*D. dumetorum*) exhibits a bimodal size distribution of starch granules centered in the 4.5 and 9.0 μm range (*21*,*22*). Lesser yam (*D. esculenta*) also presents very small granules 5.0−10.0 μm (*17*, *20*, *31*). The X-ray diffractogram of *D. alata*, *D. cayenensis*, *D. rotundata* is of B-type (*7*, *15*, *21*), bitter yam (*D. Dumetorum*) shows an A-pattern (*21*,*22*,*24*,*32*), whereas Chinese (*D. opposita*) and lesser yam are of C-type (*7*,*17*,*18*,*22*,*24*). The starches of the *D. cayenensis*−*rotundata* complex are a mixture of crystalline types, with a mean 70% of B-type. They are significantly different from the *D. alata* starches, which exhibit a mean B-type value of 93%, whereas starches from *D. esculenta* cultivars are intermediate (mean B-type value of 83%). The degrees of crystallinity range from 26 to 45%, with a mean value of 36% (*17*, *18*, *22*).

The gelatinization onset temperature and pasting temperature are measured by differential scanning calorimetry (DSC) and rapid visco analyzer (RVA) with temperatures of 69.4 and 75.0 °C for the yellow yam, 71.5 and 78.2 °C for the white yam, 76.5 and 79.8 °C for the water yam, and 78.1 and 83.1 °C for the bitter yam, respectively (*7*, *16*, *17*, *21*, *22*, *26*, *29*−*31*).

Starches are classified in three groups isolated from species of the *Dioscorea* genus according to their structural variability, physicochemical, and functional properties (*23*).

The first group (*D. alata* and the *D. cayenensis*−*rotundata* complex species) is characterized by a large granule diameter (approximately 25 μm), a high amylose content (around 25% on db), a high intrinsic viscosity (mean of 190 $cm^3\ g^{-1}$) and a high apparent viscosity, high paste clarity, higher amylopectin ν_G values (hydrodynamic coefficient), intermediate $\overline{M}_w$ (weight-average molar mass) and $\overline{R}_G$ (radius of gyration) values, and low paste gelatinization enthalpy change. The second group (*D. esculenta* varieties) is characterized by a small granule size (diameter 6 μm), a low intrinsic viscosity (121 $cm^3\ g^{-1}$), a high gelatinization enthalpy change (19 $J\ g^{-1}$), low $\overline{M}_w$, $\overline{R}_G$, and ν_G values, and a low paste viscosity. The third group (*D. dumetorum*) exhibits a pure A-type crystalline form with an opaque paste, with high $\overline{M}_w$ and $\overline{R}_G$ values and low amylopectin ν_G value.

Despite the high starch content of yams (70 to 80% db), this resource is not used for starch production at the industrial level. Therefore, the objective of this work is to characterize the physical attributes and the proximal composition of the edible portion, and to report for the first time on the compositional and functional characteristics and macromolecular features of starches isolated from three *Dioscorea trifida* (white, purple, and black) cultivated in the Venezuelan Amazon.

MATERIALS AND METHODS

Materials. Three independent batches of approximately 7.0 kg each of seven different *Dioscorea trifida* genotypes: three white, two light purple, and two dark purple tubers, locally known as "Mapuey blanco", "Mapuey morado rugoso", and "Mapuey morado liso", respectively, were collected in 2005 and 2009 and were provided by FUDECI (Fundación para el Desarrollo de las Ciencias Físicas, Matemáticas y Naturales, Venezuela). Six cultivars were selected from the "Piaroa" community garden of Puerto Ayacucho, Amazonas State, Venezuela. The starch isolated from white *D. trifida* sold on the market in Caracas and grown at Guiria, Sucre State (Caribbean coast of Venezuela) in 2009, designated in the study as "Commercial White CW2009", was also evaluated.

Sample Preparation. *Fresh Root Samples*. Tubers were cleaned and rinsed with a large amount of tap water and manually dried for subsequent morphological description and starch isolation. The moisture content of the cleaned and peeled edible portion was immediately evaluated, whereas the remaining portions of the fresh peeled samples were frozen for subsequent composition analysis.

Starch Isolation. Starches of *Dioscorea trifida* cultivars were obtained from two different batches of tubers. The starches isolated in the years 2005 and 2009 from "Mapuey blanco", "Mapuey morado rugoso", "Mapuey morado liso", and the sample were designated as "Amazonian white AW2005 and AW2009″, "Amazonian light purple ALP2005 and ALP2009″, "Amazonian dark purple ADP2005 and ADP2009″, respectively. The cleaned tubers were peeled, and the edible portion was sliced. One kilogram portions were pounded for 2 min in a Waring blender with twice their volume of distilled water. The homogenate was passed through a 200 Mesh sieve. The grinding and screening operation was repeated four more times. The resulting slurry was centrifuged at 1500 rpm for 15 min for easy separation of the starch from the viscous mucilage. After removing the remaining mucilaginous layer, the sediment was washed several times by suspension in distilled water and centrifuged until it appeared to be free of nonstarch material. The sediment was then dried in a ventilated oven at 45 °C. Starches were blended, passed through a 60 Mesh sieve and stored at room temperature in sealed plastic bags inside hermetic glass containers until subsequent analysis.

Fresh Root Analysis. Only the tubers harvested in 2005 were analyzed for their physical attributes: morphology, size, and weight. Tubers harvested in both 2005 and 2009 were described in terms of color and external appearance. Pictures were taken of the whole external and transversal section of the tubers. Size was evaluated on a representative sample of stems by measuring length and width using a Vernier Caliper. The peeled material (edible portion) was weighed, and the yield was calculated using the following formula:

$$\%\ \text{Yield} = (\text{weight of edible portion}/\text{weight of whole tuber}) \times 100.$$

The difference between 100 and % Yield represents the peel fraction percentage.

Proximal analyses of the tubers from the 2005 genotypes were conducted according to approved analysis methods AACC and AOAC. The moisture content was analyzed by gravimetry (AACC 44-15A), crude protein (N × 6.25) was analyzed by micro-Kjeldahl (AACC 46-13), crude fat was analyzed by acid hydrolysis (AACC 30-10), whereas ash content was determined by gravimetry (AACC 08-01). Dietary fiber contents, with total (TDF), soluble (SDF) and insoluble fibers (IDF), were also evaluated by the enzymatic gravimetric, micro-Kjeldahl referenced methods (AOAC 985.29 and AOAC 960.52). The total carbohydrate was estimated on a dry basis by subtracting the amount of protein, fat, fiber, and ash. The mineral profile (phosphorus, potassium, calcium, magnesium, copper, and zinc) was also determined by the atomic absorption spectrophotometry technique (AOAC 975.03 and AOAC 965.09).

Starch Analysis. *Proximate Composition*. Starches were analyzed for moisture, ash, crude protein (N × 6.25), fatty material, and total sugar contents as a percentage (w/w), using the methods cited previously.

Degree of Purity. Purity was calculated from the difference between 100 and the percentage of moisture, crude protein, fatty material, and ash content using the following equation:

$$\%\ \text{Purity} = (100 - [\%\ \text{moisture content} + \%\ \text{crude protein} + \%\ \text{fatty materials} + \%\ \text{ashes} + \%\ \text{total sugars}])$$

Mineral Profile. The mineral profile (phosphorus, potassium, calcium, magnesium, copper and zinc) was also determined as previously described.

Granulometry. The determination of starch granule sizes were performed at room temperature using a Micrometrics International Co.

Saturn Digisizer 5200 V1-08. About 50 mg of native starch was suspended in 600 mL of water, and this suspension was fed directly into the measuring cell. Volume distribution was determined using the Fraunhofer scattering theory. Native starch granules were considered to be opaque. The granule size corresponded to the average granule diameter.

Microscopy. Granular shape and Maltese crosses were observed by optical microscopy using a polarized light filter. Starch was sprinkled onto a glass slide, 1−2 drops of distilled water were added and mixed with starch, 2−3 drops of Lugol solution were added, and the sample was left for 5 min. The slide was then covered with a slip cover glass, left for 2 more minutes, and then examined and photographed on a Nikon Optiphot-2 microscope. Starch granule diameter range was estimated by measuring 20−30 randomly selected granules from microphotographs in duplicate.

Granular shape, size, and distribution were also studied by scanning electron microscopy (SEM). Starch was sprinkled onto pieces of double-sided adhesive tape, attached to circular specimen stubs, coated with 50 nm of gold using a Denton vacuum Desk IV Ion Sputter examined at 20.0 kV, and photographed using a JEOL JSM-6490 scanning electron microscope.

Damaged Starch, Density, pH, Titrable Acidity, and Color. The damaged starch analysis was performed using the AACC protocol 76-30A. In addition to the density measurement, titratable acidity (expressed as meq g^{-1}) and pH were also measured (AACC 02-31 and AACC 02-52, respectively).

Color was determined using the method described by the Hunter Laboratory procedure using a Macbeth Color-eye colorimeter. The Euclidian distance (ΔE^*) and white index (WI) were computed as follows:

$$\Delta E* = \sqrt{(\Delta L*)^2 + (\Delta a*)^2 + (\Delta b*)^2}$$

where $\Delta L^* = (L^* - L)$; $\Delta a^* = (a^* - a)$; $\Delta b^* = (b^* - b)$; $L^* = 94.64$, $a^* = -1.24$, and $b^* = 2.27$ are the plate tile standard Hunter values; L, a, and b are the Hunter starch values of the samples.

$$\mathrm{WI} = \sqrt{(100 - L)^2 + a^2 + b^2}$$

Physicochemical Properties of Starch. *Onset Temperature and Gelatinization Enthalpy Change Determination*. DSC analyses were performed on a Perkin-Elmer DSC 7 device (Perkin-Elmer, Norwalk, CT, USA) using stainless steel sealed pans. The sample pan (10−11 mg of starch and 50 μL of lyso-phospholipid 2% w/v in water) and the empty reference pan were heated from 25 to 160 °C at a scanning rate of 10 °C min^{-1}, held for 2 min at 160 °C and cooled to 60 at 10 °C min^{-1}. The gelatinization enthalpy variation (ΔH) and the onset gelatinization temperature (GT) of each sample were determined on each thermogram within the 55−90 °C range of the linear baseline. The analysis was performed in duplicate, and mean values were calculated (*22*).

Colorimetric Amylose Determination. The amylose content was measured following the standard colorimetric procedures ISO6647. Starch granules were first dispersed with 95% ethanol and then gelatinized with sodium hydroxide during 24 h. An aliquot was then acidified with 1N acetic acid and treated with a 2% iodine solution, which produces blue-black stain coloration after 20 min in the dark. The color intensity, which is related to amylose content, was then measured with a spectrophotometer at 650 nm, and compared with standard curves, obtained using purified amylose extracted from potato tubers and amylopectin extracted from waxy cassava roots (variety AM 206-5) (*33*). Two replicates were carried out per starch sample, and mean values were calculated (*12*, *33*).

Amperometric Amylose Determination. Amylose content of the starches was measured by an amperometric method (*34*) at 25 °C, using amylose as a reference for iodine binding capacity. Native starches were first defatted using the following procedure: dissolution in a 5:95 water/dimethyl sulfoxide (DMSO) mixture, precipitation in ethyl alcohol 90% and dried. Under continuous stirring, starches were solubilized in 1 N potassium hydroxide at a concentration of 5 g L^{-1}, for 3 days at 4 °C. In a beaker were introduced 1 mL of solution, 2 mL of 1 N hydrochloric acid, 1 mL of 0.4 N potassium iodide solution, and 15 mL of distilled water. The beaker was maintained at 25 °C, and the mixture was stirred at a constant rate. An assembly carrying the electrode and the buret tip was then lowered into the solution. Under constant stirring, the mixture was then titrated with increments of 0.02 mL of standard 0.005 N potassium iodate solution every 10 s, until an amperometric potential of 10 μA or an added volume of 5 mL was reached. The titration curve shows a first linear part where iodine binds within the linear chains. When the polymer is saturated with iodine, an inflection point appears. Then, the second linear part corresponds to the increase of free iodine within the solution. The total iodine bound up to the inflection point can be calculated by determining the intersection point of these two linear equations. The iodine binding capacity (IBC) represents the amount of iodine bound per 100 mg of polymer (carbohydrate concentration was determined by the sulfuric acid-orcinol colorimetric method as per ref *23*). Finally, the amylose percentage can be calculated by the ratio of the starch IBC to the reference IBC amylose. The wavelength of maximum absorption of the amylose iodine complex (λ_{max}) was determined on the same solutions by the first derivative using the spectra recorded in the 450 to 750 nm range.

DSC Amylose Determination. Amylose content was measured from the energy of amylose/lyso-phospholipid complex formation using DSC from the cooling stage of the thermogram previously used for the onset temperature determination. The analysis was performed in duplicate, and the mean values were calculated (*22*, *33*).

Swelling Power, Solubility, and Dispersed Volume Fraction Measurement. Swelling power and solubility patterns (*33*) were slightly modified with a 2.5% db (w/w) starch dispersion (0.7 g db dispersed in 27.3 g of distilled water) at 75 and 90 °C. Paste was prepared in an RVA starting at 35 °C for 1 min, increasing temperatures at a rate of 6 °C min^{-1}. Two different and independent analyses were made maintaining final temperatures at 75 or 90 °C for 2.5 min. Stirring was performed at 960 rpm for the first minute and then maintained at 160 rpm for the entire analysis. The paste was immediately transferred onto a 50 cm^3 centrifuge tube. After centrifugation at 6000*g* for 10 min at 25 °C, the supernatant and sediment were collected and weighed (W_{su} and W_{se}, respectively) then dried at 100 °C for 24 and 48 h, and weighed (D_{su} and D_{se}, respectively). The values thus obtained were used to calculate three parameters: concentration of soluble material in the supernatant (solubility), the swelling power, and the volume fraction of the dispersed phase (Φ) as follows:

$$\text{Solubility (\%db)} = 100 \times D_{su}/0.70$$

$$\text{Swelling Power (g water/g starch)} = (W_{se} - D_{se})/D_{se}$$

$$(\Phi) = (27.77 - (W_{su} - D_{su}))/27.77$$

where 27.7 is the volume (cm^3) of the paste calculated with the starch specific density (1.5 g cm^{-3}).

Functional Properties of Starch. *Gel Clarity*. Clarity was analyzed as follows. A 1% (db) aqueous starch dispersion was boiled at 96.5 °C (1000 m above sea level) with continuous mixing every 5 min for 30 min. Transmittance was measured at 650 nm after cooling to room temperature (*12*, *22*, *23*, *33*).

Starch Pasting Properties. Hot starch dispersion viscosity profiles were obtained with an RVA model RVA-4 Series (Newport Scientific, Australia). Starch (1.25 g db) was dispersed in distilled water (about 23 cm^3) to 5% suspension. Viscosity was recorded using the temperature profile: holding at 50 °C for 1 min, heating from 50 to 90 °C at 6 °C min^{-1}, holding at 90 °C for 5 min, and then cooling down to 50 at 6 °C min^{-1}. The gel was then maintained for 2 min at 50 °C with continuous stirring at 160 rpm. Four parameters were measured: pasting temperature (PT), peak viscosity (PV), hot paste viscosity at the end of the plateau at 90 °C (HPV) and the cool paste viscosity (CPV) at 50 °C. Three additional parameters were calculated: breakdown (BD) estimated as PV − HPV, setback (SB) estimated as CPV − PV, and consistency (CS), estimated as CPV − HPV (*29*).

Supramolecular and Molecular Structure of Starch. *X-ray Diffractometry*. X-ray diffraction was performed on native starches after adjustment of the water content at 90% relative humidity for 20 days under partial vacuum in the presence of a saturated barium chloride solution. The samples (20 mg) were then sealed between two tape foils to prevent any significant change in water content during the measurement. The diffraction diagrams were recorded using a BRUKER (Karlsruhe, Germany) D8 Discover spectrometer. The X-ray radiation Cu $K\alpha_1$ (λCu $K\alpha_1$ = 1.5405 Å), produced in a sealed tube at 40 kV and 40 mA was selected and parallelized using a double Gobël mirror parallel optics system, and collimated to produce a 500 μm beam diameter.

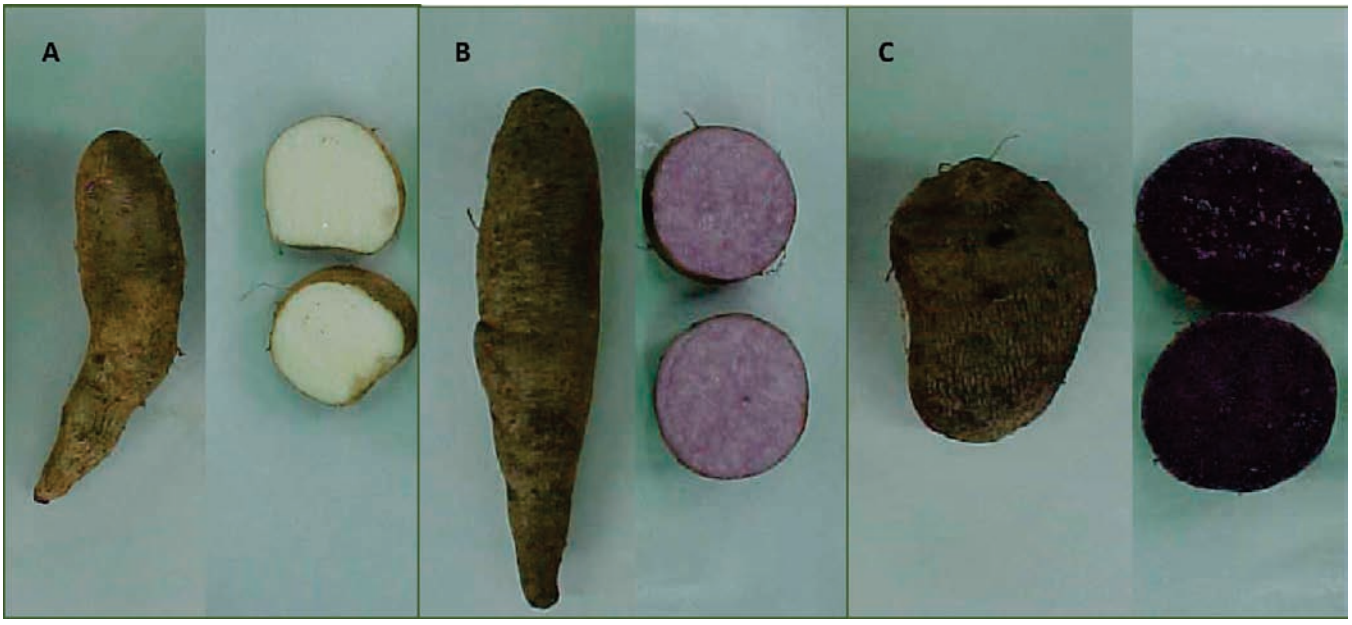


Figure 1. External and internal appearances (transversal section) of *Dioscorea trifida* tubers: (**A**) Amazonian white, (**B**) Amazonian light purple, and (**C**) Amazonian dark purple harvested in 2005.

Diffraction diagrams were collected with a two-dimensional GADDS detector, and recording time was set to 600 s. The distance from the sample to the detector was 100 mm and with an angle of 25° (2θ). Relative crystallinity was determined after normalization of all recorded diagrams at the same integrated scattering between 2θ values of 3° and 30°. A- and B-type recrystallized amyloses were used as crystalline standards after scaled subtraction of an experimental amorphous curve, in order to obtain null intensity in the regions without diffraction peaks. Dry extruded potato starch was used as the amorphous standard. The degree of crystallinity of the structures was determined using the method initially developed for cellulose (*35*). The percentage of crystallinity was taken as the slope of the line $(I_{sample} - I_{amor})2\theta = f(I_{crys} - I_{amor})2\theta$, where I_{sample}, I_{amor}, and I_{crys} are the diffracted intensities of the sample, the amorphous, and the crystalline standards, respectively.

High-Performance Size Exclusion Chromatography Coupled with Multiangle Laser Light Scattering and Differential Refractometric Index Detection (*HPSEC-MALLS-DRI*). As previously described, starches were DMSO-pretreated and then solubilized in water by microwave heating under pressure (*23*, *36*). Samples were then filtrated directly into the autosampler cell through 5 μm Durapore membranes (Waters, Bedford, MA, USA). Carbohydrate concentrations were determined using the sulfuric acid−orcinol colorimetric method (*23*, *36*, *37*). Sample recovery rates were calculated from the ratio of the initial mass to the mass after filtration. Solutions were immediately injected into the HPSEC-MALLS system. The equipment was the same as that described previously (*23*) except for the Dionex high-performance liquid chromatography pump. The HPSEC column used was a KW 802.5 from Shodex (8 mm i.d. × 30 cm) from Showa Denko K.K. (Tokyo, Japan) together with a Shodex KW guard column (6 mm i.d. × 5 cm). The column and precolumn were maintained at 30 °C using a Crococil temperature control system from Cluzeau (Bordeaux, France). The dual detection of solutes was carried out using a Dawn Heleos MALLS system fitted with a K5 flow cell and a GaAs laser, (λ = 658 nm), supplied by Wyatt Technology Corporation (Santa Barbara, CA, USA) and an ERC-7515A refractometer from Erma (Tokyo, Japan). Before use, the mobile phase (Millipore water containing 0.2 g L^{-1} sodium azide) was carefully degassed and filtered through a Durapore GV (0.2 μm) membrane from Millipore, and eluted at a flow rate of 0.3 mL min^{-1}. Sample recovery rates were calculated from the ratio of the mass eluted from the column (integration of the DRI signal) and the mass injected. Injected masses were also determined using the sulfuric acid−orcinol colorimetric method (*23*,*36*,*37*).

Asymmetrical Flow Field Flow Fractionation (*A4F*) *Coupled with MALLS and DRI* (*A4F-MALLS-DRI*). A4F equipment, including the asymmetrical channel, Control-Box V3, Flow box P2.1, and the valve box, were obtained from Consenxus (Ober-Hilbersheim, Germany). The device, its configuration, the membrane, and the flow method used for A4F were exactly the same as previously described (*36*). Sample recoveries were calculated from the ratio of the mass eluted from the channel (integration of the DRI signal) and the injected mass. The injected masses were determined using the same method as described above.

Data Processing. $\overline{M}_n$ (the number average molar mass in g mol^{-1}), $\overline{M}_w$ (g mol^{-1}) the weight average molar mass, $\overline{M}_w/\overline{M}_n$ the polydispersity index, and $\overline{R}_G$ (nm) the root-mean-square radius were established using ASTRA software from Wyatt Technology Corp. (version 5.3.2.13 for PC) as previously described (*23*, *36*, *37*). A value of 0.145 mL g^{-1} was used as the refractive index increment (dn/dc) for glucans. M_i and R_{Gi} (molar mass and radius of gyration of the ith slice, respectively) were obtained at each slice of the chromatogram peak using the Berry extrapolation (with a first-order polynomial fit) of the light scattered to zero angle:

$$\sqrt{\left(\frac{Kc}{R_\theta}\right)_i} = \sqrt{\frac{1}{M_i}\left(1 + \frac{16\pi^2 n^2}{3\lambda^2} R_{Gi}^2 \sin^2(\theta/2)\right)}$$

where c is the concentration, K is the optical constant, R_θ is the excess Rayleigh ratio of the solute, λ is the wavelength of the incident laser beam, and θ is the angle of observation.

The normalization of photodiodes was achieved using a low molar mass P20 pullulan standard.

RESULTS AND DISCUSSION

Fresh Root Analysis. *Physical Attributes of Fresh Roots*. There are noticeable differences in morphology, overall external appearance, color, shape, and size between the three tubers (**Figure 1**). In spite of different external appearance, the three varieties of *Dioscorea trifida* exhibited a similar extended shape, wider and obtuse toward one end. The white and light purple tubers have a larger size than the dark purple one with a downy peel (**Figure 1A**,**B**,**C**, respectively). The latter tuber has a rough superficial appearance. The peel of the purple and dark purple varieties have a dark brown skin color, exhibiting an edible portion with purple and intense purple color, respectively (**Figure 1B**,**C**). These characteristics are in agreement with those described in the literature for purple varieties (*3*, *27*, *38*). The white varieties were characterized by a brown crust color with a

white edible portion (**Figure 1A**). The physical features of the three varieties were different with regard to their weights, widths, and lengths, whereas a similar peel percentage was obtained, with an edible portion yield in the 80.7−83.2% range (**Table 1**).

Proximate Composition and Mineral Profile of Fresh Roots. **Table 2** summarizes the proximate composition of the edible portion of the three tuber genotypes harvested in 2005. The moisture content of the *Dioscorea trifida* varieties varied from 69.4 to 75.3%. On the basis of nitrogen analyses, protein contents in cush-cush yams were reported between 6.0 and 8.0% (*3*, *34*), whereas our study reported a higher protein content for the white one (6.8%) than for the light and dark purple ones (4.7% and 4.9%, respectively). The ash and fat contents were higher in the white variety than in the purple and black ones, but the total carbohydrates exhibited the opposite trend. The dietary fiber (total, soluble, and insoluble) content was higher in the black variety than in the two others.

With regard to mineral profile, the potassium content of the three varieties is quite high (0.83−1.35% db). This result can be of considerable nutritional interest, as also being reported by others (*27*). According to the U. S. Department of Agriculture (USDA) nutrient national database (http://www.nal.usda.gov/) and another recent source (*39*), bananas and yams are the starchy staples with the highest potassium and low sodium content. No differences in calcium and magnesium contents were observed between varieties. However, phosphorus, copper, and zinc contents were lower when comparing the white genotype with the purple genotype.

Table 1. Physical Attributes of the Three Genotypes of *Dioscorea trifida* Tubers Harvested in 2005[a]

attribute	Amazonian white	Amazonian light purple	Amazonian dark purple
weight (g)	243.00 ± 58.51	216.27 ± 35.50	112.37 ± 31.66
length (cm)	9.87 ± 4.63	19.23 ± 16.46	9.86 ± 4.63
width (cm)	7.08 ± 0.87	16.43 ± 0.55	4.63 ± 0.54
edible portion yield (%)	82.17 ± 0.80	80.73 ± 0.66	83.16 ± 0.75
peel fraction (%)	17.83 ± 0.35	19.27 ± 0.27	16.84 ± 0.28

[a] Results are the means of two determinations.

Table 2. Proximate Composition and Mineral Profile of the Three Genotypes of *Dioscorea trifida* Harvested in 2005[a]

	Amazonian white	Amazonian light purple	Amazonian dark purple
moisture (%)	69.39 ± 0.34	72.65 ± 0.04	75.28 ± 0.35
crude protein (%)	6.79 ± 0.02	4.72 ± 0.05	4.87 ± 0.54
fatty material (%)	0.30 ± 0.02	0.28 ± 0.11	0.03 ± 0.01
ash (%)	3.37 ± 0.22	3.15 ± 0.03	1.89 ± 0.13
total carbohydrates (%)	89.54	91.58	93.21
starch (%)	64.00 ± 0.12	58.00 ± 0.03	63.00 ± 0.03
TDF (%)	4.34 ± 0.00	3.44 ± 0.00	12.65 ± 0.00
SDF (%)	0.39 ± 0.20	0.99 ± 0.50	1.29 ± 0.40
IDF (%)	3.95 ± 0.02	2.45 ± 0.35	11.36 ± 0.12
phosphorus (%)	0.10 ± 0.00	0.12 ± 0.00	0.05 ± 0.00
potassium (%)	1.19 ± 0.00	1.35 ± 0.00	0.83 ± 0.00
calcium (%)	0.04 ± 0.00	0.04 ± 0.00	0.04 ± 0.00
magnesium (%)	0.05 ± 0.00	0.04 ± 0.00	0.05 ± 0.00
copper (ppm)	11.94 ± 0.00	6.65 ± 0.00	8.97 ± 0.00
zinc (ppm)	13.93 ± 0.00	6.24 ± 0.00	17.94 ± 0.00

[a] Except for the moisture content, results are given on dry basis (db). Results are the means of two determinations.

Starches Analysis. *Proximate Composition*. The moisture contents of the three starches are similar to those of most dry products (**Table 3**), to obtain a desirable shelf life similar to other conventional starches. Usually, the recovered starch contains a minor amount of proteins. Together with traces of other components such as glycerids, usually less than 0.1%, most other starches also contained about 0.5−0.6% free fatty acids that appeared to complex with molecular compounds of the granular native starch. Proteins, fatty material (as ether extractable lipids) and ash contents were similar for the three genotypes with insignificant amounts. However, except for moisture content, all the other compounds showed higher amounts than those obtained from the commercial yam starch isolated (**Table 3**).

Degree of Purity. As can be seen, the purity percentage values in the four isolated starches were quite high, corroborating that the isolation method was simple and efficient.

Mineral Profile. Some phosphorus, calcium, copper, and zinc contents were also detected in these starches. These minor chemicals and minerals mainly depend on the extraction method rather than on the botanical source. In spite of the obvious importance of the presence of minerals in starch, this aspect has been barely investigated. According to the general consensus, minerals change the functional properties of starch; therefore, it will be interesting to study the relationship between mineral composition and functional properties. Phosphorus content is an important parameter used to define the functional properties of starch. The phosphorus content of tropical tuber starch varied from 0.01 to 0.05% (*15*). *Dioscorea trifida* starch from the white and purple varieties exhibited lower phosphorus content than potato starch (**Table 3**).

Physical and Physicochemical Properties. *Microscopy*. Optical and scanning electron micrographs (SEM) show the granular structures of the starches (**Figure 2**). The irregular shapes are quite similar between the three starches. The starch granules are large, oval or shell-shaped, truncated and with an obtuse end, with smooth surfaces. Optical microscopy showed sizes of 42.9 ± 9.1, 48.5 ± 9.0, and 50.1 ± 8.1 μm for white AW2005, purple

Table 3. Proximate Composition, Purity, and Mineral Profile of the Three Waxy Starches of *Dioscorea trifida* Harvested in 2005, and for the White Commercial Yam from 2009[a]

parameters	commercial white	Amazonian white	Amazonian light purple	Amazonian dark purple
moisture (%)	11.75 ± 0.05	9.40 ± 0.15	9.16 ± 0.14	8.29 ± 0.13
crude protein (%)	0.020 ± 0.0010	0.090 ± 0.001	0.090 ± 0.001	0.140 ± 0,030
fatty materials (%)	0.050 ± 0.001	0.100 ± 0.040	0.070 ± 0.010	0.080 ± 0.007
ash (%)	0.020 ± 0.001	0.030 ± 0.001	0.050 ± 0.005	0.080 ± 0.001
purity (%)	99.91	99.78	99.79	99.70
phosphorus (%)	ND	0.03 ± 0.001	0.04 ± 0.001	0.07 ± 0.001
calcium (%)	ND	0.01 ± 0.001	0.01 ± 0.001	0.09 ± 0.001
copper (ppm)	ND	4.590 ± 0.000	3.670 ± 0.001	2.720 ± 0.000
zinc (ppm)	ND	5.240 ± 0.000	6.870 ± 0.001	4.080 ± 0.000

[a] Except for the moisture content, results are given on dry basis (db). Results are means of two determinations. ND = not determined.

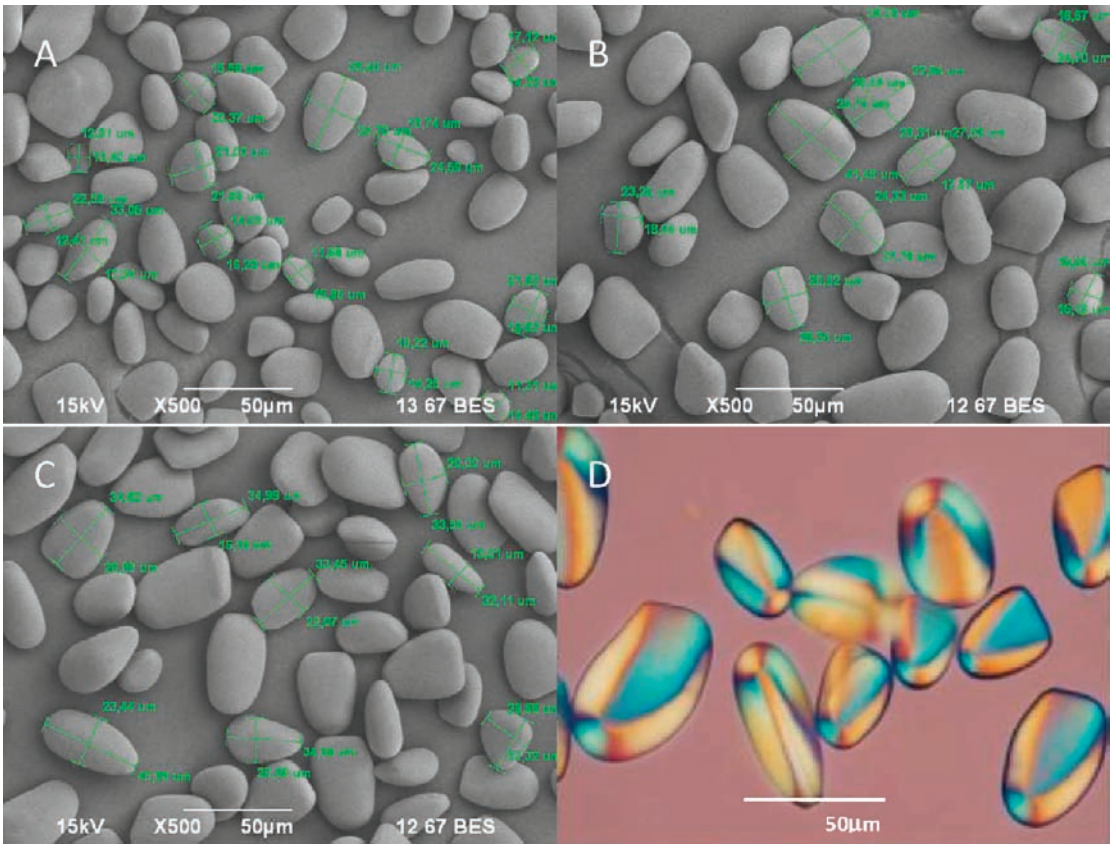


Figure 2. SEM (500×) micrographs of the starches isolated from *Dioscorea trifida* harvested in 2009: (A) AW2009; (B) ADP2009; (C) ALP2009. (D) Polarized optical light microscopy (100×) micrograph of starch isolated from *Dioscorea trifida* harvested in 2005.

Table 4. Damaged Starch, Density, pH, Titrable Acidity, and Color of the Three Waxy Starches of *Dioscorea trifida* Harvested in 2005[a]

parameters		Amazonian white	Amazonian light purple	Amazonian dark purple
damaged starch (%)		2.95 ± 0.01	0.41 ± 0.00	0.41 ± 0.00
titratable acidity (meq g^{-1})		0.0004 ± 0.0000	0.0003 ± 0.0000	0.0004 ± 0.0000
pH		6.31 ± 0.11	6.36 ± 0.03	6.32 ± 0.12
density (g mL^{-1})		1.49 ± 0.20	1.38 ± 0.22	1.46 ± 0.02
color	*L*	95.06	96.68	94.03
	a	1.11	0.57	1.52
	b	3.68	1.86	2.56
	Δ*E*	1.47	2.18	2.39
	white index	93.73	96.15	93.32

[a] Results are the means of two determinations.

ALP2005, and black starches ADP2005, respectively ($n = 30$). This result is similar to data reported in the literature (*5*). Under the light microscope, the starch granules stained with iodine and illuminated with polarized light showed distinctive Maltese cross diffractions and an eccentric position of the *hilum* (**Figure 2**D).

SEM photographs (**Figure 2A**,**B**,**C**; AW2009, ADP2009, and ALP2009, respectively) showed lower granule size when being directly obtained from SEM software.

Damage Starch, Density, pH, Titrable Acidity, and Color. **Table 4** summarizes some physical characteristics of the starches isolated from the three *Dioscorea trifida* varieties. The proportion of damaged starch was quite low, suggesting that the isolation process was successful. There were no significant statistical differences ($p \leq 0.05$) between the three starches with regard to titratable acidity, pH (6.31 to 6.36), and density (1.38 to 1.49). The white index was higher in the starches isolated from the purple tubers than in starches from the other two. Nevertheless, it can be concluded that the three starches are quite white, when compared with the white standard plate (Δ*E*). However, a slightly yellow and red hue can be noted on the starches isolated from white and black varieties (**Table 4**).

Starch Granule Size Distribution. Granule sizes determined using laser granulometry ranged from 24.5 to 35.5 µm for AW2005 and ADP2005, respectively (**Table 5**). All samples showed similar granule sizes, and the distributions were monomodal; however, the granule sizes were slightly smaller for Amazonian white starches. These results were of the same order of magnitude as those previously obtained by several authors for yam starches of different origins (*7*, *15*, *17*, *22*, *24*, *37*). Nevertheless, the granule size of the starches studied here were larger when being compared with values previously reported for *D. esculenta* and *dumetorum* (*15*, *24*, *31*).

Amylose Content. The literature points out a controversy related to the amylose determination (*36*). The most frequently used method is colorimetric (iodine binding with amylose). However, such a procedure does not appear to be consistently

Table 5. Granular Structure, Amylose Content, and Physicochemical Properties of *Dioscorea trifida* Starches Harvested in 2005 and 2009

	commercial white	Amazonian white		Amazonian light purple		Amazonian dark purple	
parameters	CW2009	AW2005	AW2009	ALP2005	ALP2009	ADP2005	ADP2009
granule size distribution (μm)[a,b]	ND	29.9	24.5	ND	33.5	35.5	33.5
colorimetric amylose (%)[c]	11.99 ± 1.00	1.85 ± 0.53	2.04 ± 0.13	5.58 ± 1.40	1.42 ± 0.47	8.65 ± 1.06	3.03 ± 0.07
DSC amylose (%)[c]	8.66 ± 0.71	1.37 ± 0.88	2.71 ± 0.20	3.55 ± 0.26	2.02 ± 0.07	2.60 ± 0.07	1.51 ± 0.22
amperometric amylose (%)[a]	9.5	2.4	2.2	3.7	3.4	5.9	2.2
λ_{max} (*n*m) [a]	593	550	565	558	550	575	547
onset temperature (°C) [c]	71.9 ± 0.2	71.4 ± 0.4	73.4 ± 0.4	69.1 ± 0.6	72.3. ± 0.4	72.8 ± 0.1	71.0 ± 0.0
enthalpy change (J g^{-1}) [c]	22.4 ± 1.7	22.5 ± 0.2	24.8 ± 0.9	23.3 ± 1.2	25.0 ± 0.9	25.1 ± 1.1	25.3 ± 0.1
peak gel (°C) [c]	76.90 ± 0.19	75.2 ± 0.4	75.2 ± 2.3	73.7 ± 1.2	73.0 ± 2.8	77.3 ± 0.1	73.8 ± 1.7
end gel (°C) [c]	83.20 ± 0.02	81.4 ± 1.9	83.6 ± 1.1	79.80 ± 1.33	81.9 ± 0.1	83.4 ± 0.1	81.9 ± 0.9
gel clarity (%) [c]	22.40 ± 1.12	50.8 ± 1.0	55.6 ± 0.8	48.3 ± 1.3	79.2 ± 0.2	24.3 ± 1.0	62.1 ± 0.4
crystallinity (%) [a]	33	29	28	29	26	24	40
moisture content (%)	*22.9*	*22.4*	*23.1*	*22.5*	*22.6*	*21.5*	*23.3*

[a] The experimental uncertainties were about 5% for amylose contents, crystallinities, and granule sizes, and less than 1% for λ_{max} values. [b] The granule size corresponded to the average granule diameter. [c] Results are the means of two determinations. ND = not determined.

accurate because of the complex formed between iodine and the long chain of the amylopectin, which absorbs light at a wavelength similar to that of the amylose iodine complex. Moreover, the existence of intermediate size polymers, the difference in lengths of amylose chain and branched amylose, and the variations in the fine structure when the amylopectin macromolecule is the only starch component seem to affect amylopectin reactivity and the iodine-binding properties.

Starch amylose contents determined by the iodo-colorimetric method ranged from 1.4 to 12.0%. The iodo-colorimetric method requires a calibration standard curve using pure amylose, whereas a standard curve has to be produced for each source when measuring different botanical sources. Thereby, the selection and availability of a standard curve is usually difficult (**Table 5**). All *Dioscorea trifida* starches analyzed had developed a brown staining color with iodine as pure amylopectin. Normal yam starch develops a blue staining pattern (*11*). This suggested that these Amazonian starches were waxy.

The six Amazonian genotypes studied by DSC at different periods exhibited a low amylose variability between 2005 and 2009 (about 1.4−3.6%), whereas the starch isolated from commercial "mapuey" CW2009 cultivated in Guiria on the Caribbean coast exhibited about 8.7% amylose.

Starch amylose contents determined by amperometric method IBC ranged from 2.2 (ADP2009 and AW2009) to 9.5% (CW2009). The colorimetric amylose contents were higher than the amperometric ones, especially for the CW2009 sample with the highest amylose content (12.0 vs 9.5%), for ADP2005 (8.7 vs 5.9%) and for ALP2005 (5.6 vs 3.7%). Contrary to our results, the amylose content of the samples may be overestimated with the DSC procedure if the amylopectin linear chains are long enough (at least 30 glucosyl units) to form complexes with lipids in the samples (*40*). If this was the case, the IBC procedure would also have had led to an overestimation of amylose content. The IBC measurements were made on defatted samples and took into account the total amount of amylose, contrary to the DSC measurements where the measurements were carried out without lipid extraction. Even if the amylose content estimation was different by DSC, IBC, or iodo-colorimetric methods, the commercial sample showed the highest amylose content (8.7, 9.5, and 12.0%, respectively), whereas the other starches exhibited low amylose contents (close to the detection limit).

In **Table 5**, λ_{max} values ranged from 547 to 593 nm for ADP2009, and CW2009, respectively. λ_{max} values depend on the amylose content and more generally on the degree of polymerization between two α-(1,6) links (*40*). The higher the λ_{max} is, the greater the amylose content, and/or the higher the chain length in the sample are. These λ_{max} values were in accordance with the literature (*12*, *37*) and thus confirm that ADP2009, ALP2009, and AW2005 starches contained the lowest amount of amylose or chains with shorter length.

Unlike root and tuber starches, yam gel is well-known for its high resistance to large cooking or sterilization (*12*, *41*). The first natural waxy mutation for roots and tubers was discovered in cassava (*33*), unlike the genetically modified waxy starch properties of sweet potato, potato, and cassava reported previously (*8*, *10*). Hence, *Dioscorea trifida* starch is the second natural roots and tubers waxy resource discovered, whereas the amylose contents of starches from different cultivars of *Dioscorea* are usually reported being in the 7.0−36.2% range (*13*−*15*, *17*−*20*, *24*, *26*, *32*).

Functional Properties. *Starch Gelatinization*. The starch gelatinization profiles measured by DSC showed an onset temperature range between 69.1 and 73.4 °C (**Table 5**). Considering the characterization of various West African yams, a large onset variation was observed in the range 69.9−76.5 °C (*22*), whereas some other authors found between 69.0 and 78.8 °C (*21*). Peak gel and end gel (°C) were related to the onset temperature and showed values from 73.0 to 77.3 °C and 81.4 to 83.6 °C, respectively. Among roots and tubers, yam starch has the highest acidic resistance in addition to the thermal resistance (*41*). *Dioscorea trifida* waxy starch could cumulate both properties against technological stresses inherent in industrial processing. Hence the "Mapuey", cush-cush yam *Dioscorea trifida* starch could become a natural ingredient for future food product development.

The gelatinization enthalpy variation (ΔH) was in the 22.4−25.3 J g^{-1} range, with the highest enthalpy change for the dark purple starch genotype. These values are the highest reported in the literature for *Dioscorea* species. With the same method, a great diversity of yam starch from Ivory Coast was earlier screened, and the authors reported variation from 13.7 to 20.3 J g^{-1} (*22*).

Gel Clarity. Gel clarity is one of the most important functional properties of pastes, with high clear gels favored for food uses. Clear gels from waxy starches are used for refrigerated and frozen products due to their low syneresis under stressed conditions (*12*). Gels from roots and tubers usually exhibit higher clarity than cereal starches. Gel clarity from *Dioscorea trifida* starches varied considerably (from 22.4 to 79.2%), as shown in **Table 5**. The amylose-free *Discorea trifida* clone (light purple harvested in 2009) has the clearest yam gel, with an intermediate clarity between cassava (50%), waxy cassava starch (61%), potato (88%), and waxy potato starch (92%) (*12*).

Table 6. Solubility, Swelling Power, and Dispersed Volume Fraction of *Dioscorea trifida* Starches Obtained in 2005 and 2009[a]

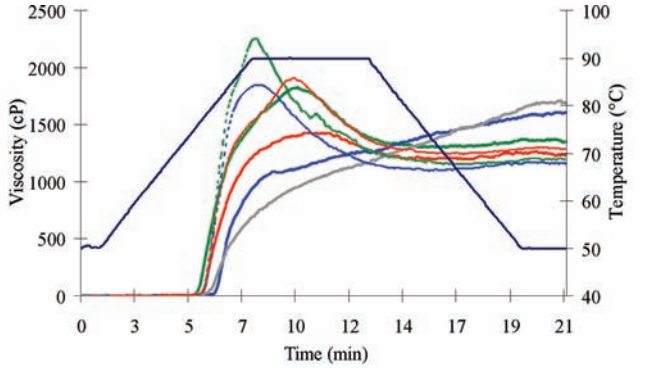
	solubility (%)		swelling power		dispersed phase (Φ)	
variety	75 °C	90 °C	75 °C	90 °C	75 °C	90 °C
CW2009	3.4 ± 0.3	4.4 ± 0.2	13.8 ± 1.0	20.5 ± 0.7	0.4 ± 0.00	0.6 ± 0.0
AW2005	2.4 ± 0.1	2.4 ± 0.0	19.2 ± 0.4	20.8 ± 2.0	0.5 ± 0.0	0.6 ± 0.0
AW2009	2.7 ± 0.4	2.1 ± 0.3	22.8 ± 0.7	31.4 ± 1.3	0.6 ± 0.0	0.8 ± 0.0
ALP2005	4.3 ± 0.4	4.4 ± 0.0	20.1 ± 0.2	28.1 ± 0.2	0.6 ± 0.0	0.7 ± 0.0
ALP2009	3.9 ± 0.3	2.8 ± 0.2	26.5 ± 0.8	37.0 ± 0.4	0.7 ± 0.0	0.9 ± 0.0
ADP2005	2.8 ± 0.2	3.7 ± 0.0	8.6 ± 0.4	21.4 ± 0.1	0.3 ± 0.0	0.6 ± 0.0
ADP2009	2.1 ± 0.1	2.5 ± 0.2	22.7 ± 0.7	32.2 ± 1.4	0.6 ± 0.0	0.8 ± 0.0

[a] Results are the means of two determinations.

Swelling Power, Solubility, and Dispersed Volume Fraction. Swelling and solubility patterns provide some information on the nature of the associative bonding within starch granules. The packing arrangement of the amylose and amylopectin molecules in the granular structure and their concentration affect the swelling and solubility of the starch. These waxy starches exhibited substantially different solubility and swelling powers (**Table 6**) when compared with nonwaxy starches isolated from other *Dioscorea* varieties (*22*, *25*, *28*), or with the same species but grown at different locations (*5*). Solubility was relatively lower, but a higher swelling power was obtained than those reported above. Indeed, 10.8−16.4% swelling and 7.2−13.5% solubility are reported (*22*) in varieties of *D. alata* and *D. cayenensis*−*rotundata*, while another author (*20*) reports values from 18.8 to 25% for swelling power and from 10.8 to 12.4% for solubility at 75 °C for *D. alata* and *D. rotundata*, respectively by means of steam pressure treatments. Moreover, swelling power values from 5 to 8% were reported at 60 °C, whereas at 90 °C variation from 40 to 65% was found (*17*).

Pasting Properties. The rheological profiles of the three starch suspensions (5%) are shown in the RVA curves (**Figure 3**). Except for the Amazonian white 2005 and commercial Guira, the overall profiles are different from each other with the presence of a peak viscosity for all Amazonian starches. The maximum viscosities developed at 5% suspension were 1800−2250 cP. Starches isolated from 2009 harvest exhibited the highest viscosity, up to 1900 cP, with the highest viscosity for the Amazonian light purple (ALP2009 with 2252 cP). In comparison with another study on normal and waxy starches conducted using the same RVA condition, the *Dioscorea trifida* "Mapuey" starches presented a lower viscosity than normal and waxy potatoes (2550 and 2441 cP, respectively), but significantly higher viscosities than those of maize and waxy maize (176 and 973 cP), rice (343 and 498 cP), and cassava starches (954 and 1119 cP). Similarly to potato starch, *Dioscorea* starch exhibited high viscosities but with higher pasting temperatures (75.5−80 °C vs 65.2 °C for potato). The starches from the five genotypes that exhibited a peak viscosity also exhibited a high breakdown during the holding stage at 90 °C, between 220 and 966 cP when potato starch showed 1200 cP and cassava starch was around 500 cP (*12*). The pasting behavior of a 4% starch suspension of different yam varieties from the Ivory Coast had also been reported (*22*). The authors highlighted that the highest pasting temperature (87 °C) was measured on *D. dumetorum* starch and the lowest pasting temperature was measured on *D. esculenta* starch (78.7 °C), whereas starches from *D. alata* and from *D. cayenensis*−*rotundata* complex exhibited an intermediate pasting temperature (around 83 °C).

Crystallinity. All starches investigated exhibited a B-type crystallinity. Crystallinity degrees varied from 24% for ADP2005 to 33% for CW2009 (**Table 5**). If the 40% obtained for ADP2009 could not be explained by the high water content of the sample, it could be interesting to later confirm the finding by additional investigations on crystallinity. Amazonian white and Amazonian light purple starches exhibited the same crystallinity, irrespective to the year of harvest. The crystallinity of CW2009, was often the highest, with the highest amylose content. The B-type crystallinities agreed well with those reported by many other authors (*7*, *15*, *17*, *22*, *24*, *31*). Contrary to some earlier reports, the absence of A-type crystals was here reported with varieties exhibiting low amylose contents.



genotype		PT (°C)	PV (cP)	BD (cP)	SB (cP)	CS (cP)
commercial	2009	78	*	*	*	-1002
amazonian white	2005	77	1428	220	-175	-32
	2009	76.8	1906	531	-620	91
amazonian light purple	2005	75.5	1822	425	-456	48
	2009	76.9	2252	966	-1071	91
amazonian dark purple	2005	80	*	*	*	-666
	2009	77	1848	683	-694	5

Figure 3. RVA profile at 5% suspension and functional properties of *Dioscorea trifida* starches. Commercial yam cultivated at Guiria (Sucre State) in 2009 with gray continuous line. White, purple, and black harvested in 2005 (solid lines) and 2009 (dashed lines).

Macromolecular Characteristics. ALP2009 and CW2009 starches were microwaved and injected in the A4F-MALLS-DRI and HPSEC-MALLS-DRI set ups. The solubilization rates were 100% for ALP2009 and 95% for CW2009. The elution recoveries were 100% using A4F and 62% using HPSEC. Elution recovery represents the percentage of macromolecules percolated through a defined setup. These recovery rates indicated that the fractionation response was more quantitative with A4F than with HPSEC. This mode of analysis was thus considered as enabling a representative structural characterization of starches. Nevertheless, the results obtained with both methods were analyzed. For both samples, A4F and HPSEC elugrams of starches revealed one DRI and one LS peak (**Figure 4**). With A4F, the first peak at a retention time of 12.8 mL was attributed to the amylose fraction and the second at a retention time of 17.5 mL to the amylopectin fraction (*36*). With HPSEC, the first peak at an elution volume V_e of 5.9 mL was attributed to the amylopectin fraction and the second at a V_e of 6.6 mL to the amylose fraction (*23*, *36*). In addition, with HPSEC, CW2009 displayed a shoulder in the DRI peak at a V_e of 6.6 mL. This shoulder corresponded to the amylose population and confirmed that it was present in a very small quantity in this sample. For ALP2009, no additional peak was observed. With both techniques, the light scattering (LS) signal consisted of a single peak, corresponding to the amylopectin fraction.

Amylopectins were characterized by integrating the A4F and HPSEC elugrams at elution volumes including the LS and DRI amylopectin peak. Amylopectin $\overline{M}_w$ and $\overline{R}_G$ values obtained using HPSEC were 1.22×10^8 and 1.10×10^8 g mol^{-1}; 164 and 157 nm, for ALP2009 and CW2009 amylopectins, respectively. Amylopectin $\overline{M}_w$ and $\overline{R}_G$ values obtained using A4F were

9.46×10^7 and 8.66×10^7 g mol^{-1}; 151 and 147 nm, for ALP2009 and CW2009 amylopectins, respectively (**Table 7**). Then, with both techniques, CW2009 appeared to have a slightly smaller amylopectin than ALP2009.

By plotting the gyration radius and molar masses of the same fraction from the elugrams (**Figure 4A**,**B**), structural data could be determined from the exponent ν_G using the equation $R_{Gi} = K_G M_i^{\nu G}$. The values for ν_G depend on temperature, polymer−solvent interactions, and polymer shape (0.33, 0.5−0.6, and 1, for a sphere, a linear random coil, and a rod, respectively). The experimental ν_G values decrease when density increase; then for branched polymers, ν_G values were believed to decrease when the degree of branching increase. By plotting the log−log plot of R_{Gi} versus M_i for amylopectins, ν_G values for amylopectins were obtained from the corresponding slope. ν_G values obtained for ALP2009 and CW2009 starches were 0.34 and 0.38 and 0.45 and 0.43, using HPSEC and A4F, respectively (**Table 7**). According to these ν_G values, ALP2009 and CW2009 amylopectins seemed to have the same density (i.e., probably the same branching degree).

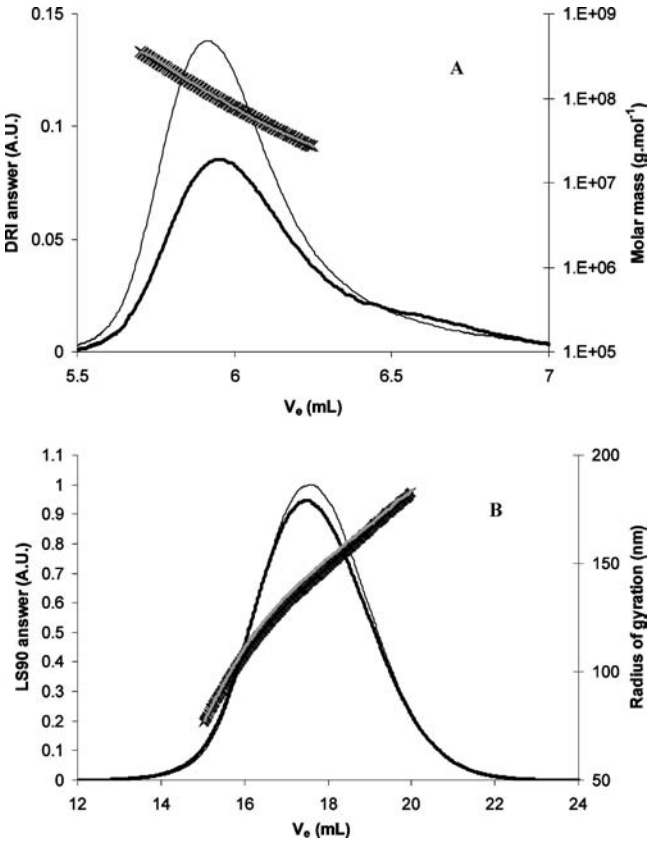


Figure 4. (**A**) HPSEC: starches DRI answers and molar masses of ALP2009 (thin line and gray dots, respectively) and CW2009 (thick lines and black crosses), respectively. (**B**) A4F: starches LS and radii of gyration of ALP2009 (thin line and gray dots, respectively) and CW2009 (thick lines and black crosses), respectively.

The ν_G values were in the same range compared to some other data reported in the literature (*23*, *37*). Even if the values obtained using A4F and HPSEC were different in absolute, they were all near 0.4 (between the sphere and the random coil) showing similar results for both samples.

The determination of apparent particle density could provide another means to approach the conformation of the molecule and could then give additional indication on branching. Apparent particle density was calculated on the basis of a smeared uniform density in the particle and based on the following equation for equivalent homogeneous spheres: $d_{Gapp} = M_w/(4\pi/3)R_G^3$. The values reported in **Table 7** showed similar densities for both amylopectins (about 6.5 g·mol^{-1} nm^{-3}) and using both methods, confirming that these two samples showed very similar molecular amylopectin structures. The results obtained with HPSEC and A4F were matchable for both starches studied.

The $\overline{M}_w$ and $\overline{R}_G$ values, ν_G, and densities were in agreement with the values reported by some authors (*37*), but the $\overline{M}_w$ and $\overline{R}_G$ values were very low compared to those reported previously by other authors (*23*) for yam starches containing higher amounts of amylose. The differences were probably linked to biological variations. It should be noted that yam starches studied here had particularly low amylose contents compared to others starches of the literature. The $\overline{M}_w$ and $\overline{R}_G$ values and densities obtained for these yam starches were close to those obtained for potato and cassava amylopectins (*23*, *36*, *37*).

In conclusion, the waxy *Dioscorea trifida* yam starch represents a new industrial opportunity for the development of a supply chain, and of job opportunities based on the natural resource. As a staple food, yam starch is traditionally sold on the local market and consumed in the Venezuelan Amazon (*5*). After the recent discovery of the first natural mutation in cassava (*33*), the waxy yam starch discovery is therefore an additional promising ingredient to formulate refrigerated or frozen products (*12*). Some investigations will be later carried out at the Central Venezuelan University of Caracas on the technological behavior of yam starch and flour, targeting long-time sterilized weaning-foods, products being stored under acidic conditions (*41*), as well as frozen and refrigerated foods. Since the domestication of the *trifida* species originates from the American continent, other investigations should be conducted subsequently in Southern America and in the Caribbean (French West Indies, Jamaica, Cuba, and Trinidad and Tobago) for the characterization of the cush-cush variety. Among others, the production of starches from the well-known collection of the Guadeloupian INRA station (West Indies) should be interesting for postharvest screening of *Dioscorea trifida* diversity (*1*). According to the unique starch functional properties highlighted, the production of *Dioscorea trifida* may soon become industrial with the combined efforts of breeders, geneticists, and food technologists.

ABBREVIATIONS USED

ICTA, Instituto de Ciencia y Tecnología de Alimentos; CIRAD, Centre de Coopération Internationale en Recherche Agronomique

Table 7. Macromolecular Characteristics for the Amylopectin Population of Two Yam Starches Determined by HPSEC-MALLS and A4F-MALLS[a,b,c]

		$\overline{M}_w \times 10^{-6}$ (g mol^{-1})	$\overline{R}_{Gz}$ (nm)	ν_G	d_{Gapp}[d] (g mol^{-1} nm^{-3})
ALP2009	HPSEC-MALLS	122.4	164.0	0.34	6.6
	4F-MALLS	94.6	150.9	0.45	6.5
CW2009	HPSEC-MALLS	109.7	157.0	0.38	6.8
	4F-MALLS	86.6	147.0	0.43	6.2

[a] These values were taken over the whole amylopectin peak. [b] The experimental uncertainty was 5%. [c] Weight average molar mass ($\overline{M}_w$), *z*-average radius of gyration ($\overline{R}_{Gz}$), hydrodynamic coefficient (ν_G), and apparent molecular density (d_{Gapp}). [d] $d_{Gapp} = M_w/(4\pi/3)R_G^3$.

pour le Développement; INRA, Institut National de la Recherche Agronomique; CIAT, International Center for tropical Agriculture; DSC, differential scanning calorimetry ; RVA, rapid visco analyzer; $\overline{M}_w$, weight average molar mass; $\overline{R}_G$ radius of gyration; ν_G, hydrodynamic coefficient; d_{Gapp}, apparent molecular density; HPSEC, high-performance size exclusion chromatography; A4F, asymmetrical flow field flow fractionation; MALLS, multiangle laser light scattering; FUDECI, Fundación para el Desarrollo de las Ciencias Físicas, Matemáticas y Naturales of Venezuela; CW2009, commercial white harvested in 2009; AW2005, Amazonian white harvested in 2005; AW2009, Amazonian white harvested in 2009; ALP2005, Amazonian light purple harvested in 2005; ALP2009, Amazonian light purple harvested in 2009; ADP2005, Amazonian dark purple 2005; ADP2009, Amazonian dark purple 2009; AACC, American Association of Cereal Chemists; AOAC, Association of Official Agricultural Chemists; TDF, total dietary fiber content ; SDF, soluble dietary fiber content; IDF, insoluble dietary fiber content; SEM, scanning electron microscopy; WI, white index; ΔH, gelatinization enthalpy variation; GT, gelatinization onset temperature; DMSO, dimethyl sulfoxide; IBC, iodine binding capacity; db, dry basis; W_{su}, supernatant weight; W_{se}, sediment weight; D_{su}, dried supernatant weight; D_{se}, dried sediment weight; PT, pasting temperature; PV, peak viscosity; HPV, hot paste viscosity; CPV, cool paste viscosity; BD, breakdown; SB, setback; CS, consistency; DRI, differential refractometric index detection; $\overline{M}_n$, the number average molar mass; dn/dc, refractive index increment; M_i, molar mass of the ith slice; R_{Gi}, radius of gyration of the ith slice; ANOVA, analysis of variance; USDA, U.S. Department of Agriculture; V_e, elution volume; LS, light scattering.

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