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Granulometric structure, zeta potential and differential scanning calorimetry of native starch powders from *Dioscorea spp*.

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Abstract: Starch powders from two cultivars of Dioscorea rotundata (DR) were analysed on the physicochemical aspect. Granulometric structure, zeta potential and differential scanning calorimetry of starch powders showed the following properties. Various shapes with predominance of granule ranging from 10 to 40 µm were noted. The zeta potential of DR went from positive values to negative values as the pH was increasing from 2 to 8. From pH 2 to 4, the zeta potential was positive. A significant difference was obtained between each value ($p > 0.05$). The zeta potential took a negative value from pH 5 and above. The results of thermal analysis show that starches start swelling at 68.9±1.5 °C. Enthalpy of gelatinization was about 15 $J.g^{-1}$.

Keywords: Native starches; *Dioscorea spp*; characterization.

Introduction

Starch is after cellulose the main carbohydrate synthesized by higher plants from solar energy. It is an excellent source of energy for human feeding. Sources of important starches are represented by cereals, tubers and legumes. Some fruits can also be rich in starch[1]. *Dioscorea spp* are tubers naturally adapted to the tropical and subtropical climatic conditions, and grows in abundance without contribution of artificial inputs. In these developing areas, the starch-based plants are a success as basic food.

The global production of *Dioscorea spp* tubers was estimated at 38 million tons per year, which place them at the 4th rank in importance among the tuber plants[2]. 96% of these productions come from West Africa, in the area ranging between Côte d'Ivoire and Nigeria[3]. The white yam (*Discorea rotundata*) is generally considered to be the best edible yam in the area stretching from Côte d'Ivoire to Cameroon. The yellow yam (*Dioscorea cayenensis*) is also present in West Africa, whereas the water yam (*Dioscorea alata*) originated in South-East Asia[4].

In spite of this strong production observed in these countries, tubers are not used for the industrial production of starch. The starch industry has grown considerably in recent years and the global market of starches in 2008 according to the International Starch Institute covers

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66 million tons[5]. The developed countries (United States, France, Canada and Japan) share 77% of this global market. The food sector used 55% of this production in the fields of drinking and food ingredients. 45% of this starch went to the non-food industries (medicinal products, paper and textile etc). More than 70% of this starch comes from cereals (maize and wheat) and less than 30% comes from roots and tubers, mainly potatoes and cassava[6]. Starches of *Dioscorea spp* have received little attention from researchers and industrialists. They occupy less than 1% in the database of publications concerning the physicochemical and techno-functional properties of the starches in general.

The traditional cultivars of *Dioscorea spp* have been inventoried, described and classified, but rare are the studies focused on the physicochemical properties of the varieties cultivated in Cote d'Ivoire. Most studies conducted on these varieties are scattered and incomplete. This work aims to determine the granulometric structure, the zeta potential and the differential scanning calorimetry of two varieties of native starch powders from *Dioscorea rotundata***.**

Experimental Section

Samples

Starch powders were produced from tubers of two cultivars: *Dioscorea rotundata*, varieties ″kponan″ (DR-KP) and ″krenglê″ (DR-KR).

a : DR-KP **b : DR-KR**

Figure 1. Tubers of two varieties of *Dioscorea rotundata* (a: variety kponan DR-KP, b: variety krengle DR-KR)

Extraction of starch

Starch extraction was carried out according to the method previously described by Amani et al[7]. Briefly, the tubers were peeled, washed with distilled water and cut into pieces. 1 kg was weighed, soaked in 1 g/L of sodium bisulfite (VWR, Strasbourg, France), crushed in a blender (Moulinex Y45, Lisses, France). 1 L of sodium chloride (VWR) at 40 g/L (pH 8.0) was added to the mixture and then filtered on metal sieve 125 µm. The starch milk obtained underwent four decantations in 20 L of distilled water during 48 h.

The purified starch is finally spread out over aluminium paper, dried in an oven at 45°C and crushed in an oscillating granulator (Erweka, Heusenstamm, Germany). For yield calculation, tubers were weighed before crushing and the dry starch powder obtained was also weighing after spraying. The ratio of the two masses gave the extraction yield.

Chemical composition of starch

For starch's identification, 1 g of starch powder was dissolved into 50 mL of distilled and heated to boiling. 1 mL of this suspension was added to 1 mL of 0.01 N iodine solution. The observation of a dark blue colour after cooling is a characteristic of starch. Moisture was determined by drying 1 g of powder at 130 °C during 3 h. The result is given by the following mathematical formula:

$$\text{Moisture (\%)} = \frac{M - Me}{M - Mo} \text{x } 100$$

Mo = mass of the empty crucible
M = mass of the crucible containing the sample (1 g of starch powder)
Me = mass of the crucible after drying

The dry matter was obtained by difference. Ashes were obtained by burning in a muffle furnace at 550°C during 8 hours according to the method previously described[8]. Total proteins were evaluated by the method of Kjeldhal[9]. The conventional factor 6.25 for the conversion was used. Lipids were determined according to the method described elsewhere[9]. Sugars were also quantified[9].

Granulometric structure

Microscopic analysis of starch powders (DR-KP and DR-KR) was carried out using an optic microscope equipped with a digital camera (Ceti, Medline Scientific, Oxfordshire, UK). A pinch of each starch powder was added on a slide and diluted in a drop of cedar oil to have a microscopic clarity. A coverslip was applied and the images were observed and controlled by computer via the Kappa software which also measures the granule size.

The size distribution of starch granules was carried out with an apparatus capable of measuring the dynamic light scattering (Zetasizer Nano-ZS, Malvern®, Worcestershire, UK) using a helium-neon laser of output power of 4 mW operating at the fixed wavelength of 633 nm (wavelength of laser emission in the red).

Zeta potential determination

Zeta potential of DR-KP and DR-KR were performed using the Zetasizer Nano-ZS. The measurement was carried out on the diluted solutions at various pH values (pH 2, pH 3, pH 4, pH 5, pH 6, pH 7 and pH 8). Using a syringe, 1 mL of the appropriate solution of starch was introduced in the measurement vessel (special tank U-shaped). Vessel was positioned in the optical drive of the apparatus. Temperature was set at 25 °C and voltage applied was 200 mV. Duration of analysis was approximately 10 min.

Differential scanning calorimetry

Differential scanning calorimetry (DSC) was performed with a Perkin Elmer DSC 7 calorimeter (Norwalk, CT, USA). For this analysis, 10 mg of starch and 50 mL of 2% lyso-phospholipid were placed in a stainless-steel capsule and sealed. Lyso-phospholipid solution was used because it forms a stable complex with starch and showed less change during storage and heating[10]. The samples were prepared 30 min before measurement. A capsule containing 50 mL of ultra-pure water was used as control. The two capsules (control and sample) were placed in two identical compartments of the calorimeter oven. The oven was set to a linear kinetic heating (10 °C per min from 25 °C to 160 °C, kept constant at 160 °C

for 2 min and finally cooled to 60 °C at 10 °C per min). The different temperatures of gelatinization were determined and enthalpy of gelatinization (ΔH) was calculated.

Results and Discussion

Preliminary assays

Table 1. Diagnosis and chemical composition of starch powders of DR-KP and DR-KR

starch powders	Extraction yield (%)	Starch's identification (Blue color)	Moisture (%)	Ashes (%)	Proteins (%)	Lipids (%)	Sugars (%)
DR-KP	98.2	+++	11.7	0.10	0.18	0.10	5.9
DR-KR	97.9	+++	17.8	0.08	0.22	0.10	5.4

Chemical analysis of starch powders reveals a dark blue color which disappears by heating and appears again after cooling as indicated in the pharmacopoeia. Moisture, ashes and sugars values are within the limit set by the pharmacopoeia[11] (moisture ≤ 20 %, ashes ≤ 0.6 %, sugars < 10 %). The presence of protein may influence the physical stability of the native starches by the formation of protein-carbohydrate combinations responsible for the Maillard reaction after a few months of storage. The presence of lipids could explain the starch-lipid bonds that justify the emulsifying character of native starch powders.

Granulometric structure

The analysis of micrographs of starch granules observed with an optical microscope (GX40) show various shapes (polyhedral, spherical, ovoïde or ellipsoïde) with predominance of large granule (Figure 2).

a: DR-KP **b: DR-KR**

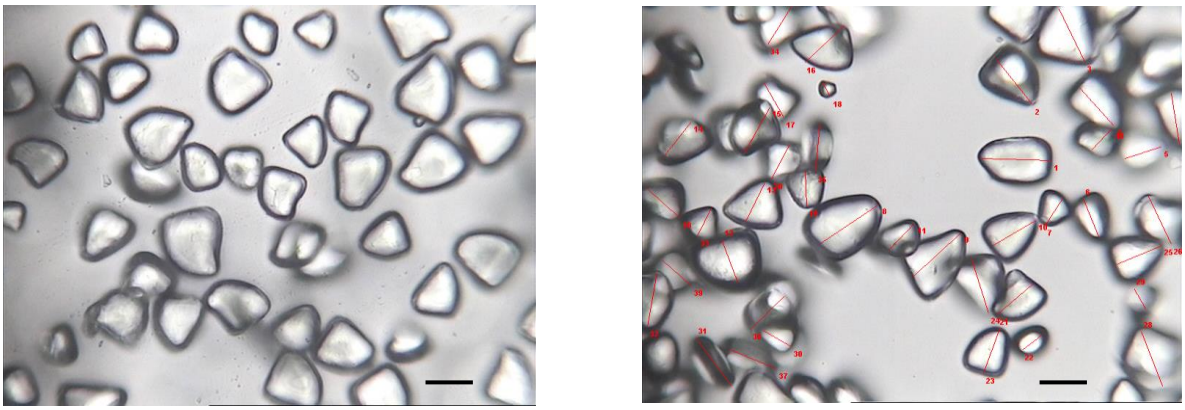

Figure 2. Micrographs of starch granules observed with an optical microscope at a magnification of G×40 (a: DR-KP, b: DR-KR, reference bars equal to 20 µm)

Measuring the granules size showed a size ranging from 10 to 40 µm for the two cultivars, with a mean of 27.2 µm for DR-KP and 29.3 µm for DR-KR. In comparison with other starch granules described in the Pharmacopoeia, starch granules of *Dioscorea rotundata* (10-40 µm) are between wheat starches (2-38 µm) and potato starches (15-100 µm). They are similar to granules of wheat starches in size and potato starches in shape. Previous authors such as

Farhat et al[12] and Amani et al[13] have found sizes between 20 and 35 µm for *Dioscorea rotundata*. The distribution frequency of starch granules of DR-KP and DR-KR was shown in table 2. Most of grains of both cultivars have a size between 26 and 28 µm (35 % for DR-KP and 34 % for DR-KR).

Table 2. Distribution frequency of starch granules of DR-KP and DR-KR

		Granules size (µm)							
		10-12	14-16	18-20	22-24	26-28	30-32	34-36	38-40
Granules number (%)	DR-KP	1	2	6.5	15	35	27	9	4.5
	DR-KR	3.5	6	10	13	34	21	7	5.5

Zeta potential determination

The electrical charges of DR-KP and DR-KR were determined from their movement in an electrical field using an apparatus capable of microelectrophoresis. The results are reported in Figure 3.

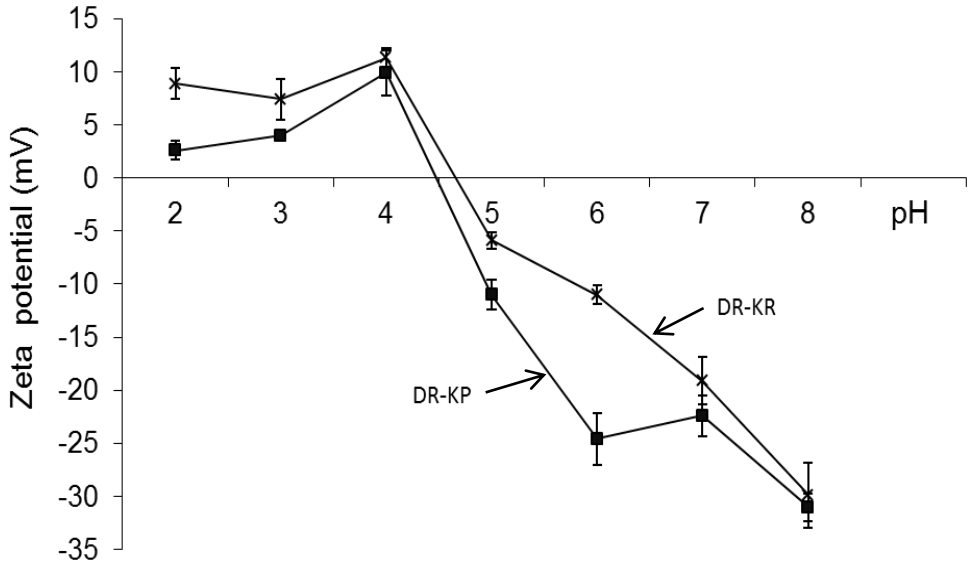

Figure 3. Zeta potential of DR-KP and DR-KR as a function of pH. Data are means of three measurements. The error bars represent standard deviations.

The zeta potential of DR-KP went from positive values (+2.6±0.9 mV) to negative values (-34.0±1.3 mV) as the pH was increasing from 2 to 8. From pH 2 to 4, the zeta potential was positive. A significant difference was obtained between each value ($p > 0.05$). The zeta potential took a negative value from pH 5. The zeta potential of DR-KR was positive at pH 2, 3 and 4 and negative at pH 5, 6, 7 and 8. With regard to the results of Figure 3, it is clear that the zeta potential is influenced by the pH of the solution. A high zeta potential value reflects the stability of a solution[14,15]. Moreover, it is accepted that a value above ± 30 mV means a high zeta potential[16]. Theoretically the higher the zeta potential the better the interactions with oppositely charged compounds. To our knowledge, this is the first study that determines electrokinetic charge of starch particles in an aqueous solution. The zeta potential measurement allows to predict the behavior of suspensions and emulsions (stability, creaming, coalescence, etc.).

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Differential scanning calorimetry

This technique of thermal analysis was used to determine the value of ΔH, which is the energy provided to the system to change liquid starch in gelled starch. This energy was provided in the form of heat by introducing the sample into the calorimeter. Temperature of gelatinization and enthalpy of gelatinization (ΔH) are summarized in table 3.

Table 3. Gelatinization temperatures of starch powders of DR-KP and DR-KR

starch powders	Initial temperature of gelatinization (°C)	Peak temperature of gelatinization (°C)	End temperature of gelatinization (°C)	Enthalpy of gelatinization (*ΔH*, J.g-1)
DR-KP	70.4±0.3	73.4±0.5	75.0±0.1	14.2±0.9
DR-KR	67.4±0.2	69.9±0.3	72.3±0.4	15.9±1.1

The results of this study show that starches start swelling at the initial temperature. The highest peak gelatinization temperature was recorded in DR-KP starches (73.4 °C). Gelatinization is an endothermic phenomenon *(*ΔH>0) occurring during the heat treatment of starches[17, 18]. It is a process that breaks down the intermolecular bonds of starch molecules in the presence of water and heat, allowing the hydrogen bonding sites to engage more water. Penetration of water increases randomness in the general starch granule structure and decreases the number and size of crystalline regions. Crystalline regions do not allow water entry. Heat causes such regions to become diffuse, so that the chains begin to separate into an amorphous form. Under the microscope in polarized light, starch loses its birefringence. The gelatinization temperature of starch depends upon plant type and the amount of water present, pH, types and concentration of salt, sugar, lipid and protein.

Conclusion

Starch powders from *Dioscorea rotundata* present a morphology characterized by variability in size and shape. When diluted in aqueous solution, the starches have an electrophoretic mobility dependent of the pH of the medium. When heated, the granules undergo gelatinization phenomena. These characteristics seem to be relating to the granules size and the nature of the surrounding environment granules.

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