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Longitudinal Distribution and Physico-Chemical Properties of Yam (*Dioscorea* spp.) Tuber Starches

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ABSTRACT

Granule size distribution of yam starches and tissues varies within the longitudinal axis of the tuber. In this study, starch was isolated from three parts of yam tubers (proximal, middle and distal) of two species: *D. alata* (*Bètè-bètè* and Tda 1176) and *D. cayenensis rotundata* (*Krenglè* and Tdr 95/19156), to investigate the effect of sectional variation on medullar parenchyma tissues and functional properties of starch. Cytoplasm air, cell wall thickness as well as intercellular space increased from the distal zone to the proximal part. Starch properties varied according to the species. The two varieties of *D. alata* had decreasing starch granule sizes from the proximal extremity (23.3 ± 6.5 µm) toward the distal zone (17.9 ± 6.2 µm), while those of *D. cayenensis* had smaller size granules in the proximal section than the middle and distal sections where the sizes were identical. Starch from the middle section of *Bètè-bètè* variety gave a clearer gel (35.1 ± 1.3%) than the proximal (28.5 ± 1.3%) and distal (29.2 ± 2.9%) extremities, and could be used to thicken fruit pie filling. Starch from the distal sections of the two varieties of *D. alata* (Tda 1176 and *Bètè-bètè*) were more resistant to syneresis at -20°C, and could be more useful for frozen food. Starch from the proximal part showed a general low breakdown viscosity. This stability is required for UHT products. These results suggest that not only does granule size distribution vary according to species and between the different parts of the tuber but their there are differences in some of some starch properties as well.

Keywords: granule size, medullar parenchyma, proximal, middle and distal parts
Abbreviations: Tda, Type *Dioscorea alata*; **Tdr**, Type *Dioscorea rotundata*

INTRODUCTION

Yam is a starchy plant belonging to the *Dioscorea* genus and is a monocotyledonous plant. It is essentially cultivated in inter- and subtropical areas where it constitutes an important food source for local populations. *Dioscorea* genus includes about 600 to 800 species (Kati *et al*. 2004). Some of these species are exploited for economic purposes including food and pharmaceutical industries where they are used for more than 2/3 of the world production in sex hormones and corticosteroids (Edison and Figueiredo 1991). World yam production is strongly dominated by West Africa producing about 95.72%. In the Ivory Coast (Côte d'Ivoire), yam constitutes the largest food crop with 58.67% of the production before cassava (33.25%) and taro (7.24%) (FAO 2003). However, a number of factors (diseases, climate change, and storage losses) hinder yam production making it laborious, expensive and unproductive. To face up this insufficiency, new yam varieties of the complex *D. cayenensis rotundata* (Tdr 3-89/02565 and Tdr 4-89/02677) and *D. alata* (Tda 4-95/00226 and Tda 3-95/00799) which have faired well in agronomic tests (output to the hectare, resistance to illnesses) and have been judged as being acceptable (Gondo *et al*. 2001) have been introduced.

The nutritional content of yam is comparable to potato with the major component being starch (60-80% of dry matter) (Emiola and Delarosa 1981). Physicochemical properties of yam starch have been investigated (Emiola and Delarosa 1981; Deang and Rosario 1993; Gebre and Schmidt 1998; Farhat *et al*. 1999; Amani *et al*. 2004), with most of the studies carried out on the whole tuber. According to the size, yam starch can be classified into two groups: large granule (*D. alata* and *D. cayenensis-rotundata*) and small granule (*D. esculenta* and *D. dumetorum*) with variable properties (Rolland-Sabaté *et al*. 2003). For the few studies consecrated to the proximal, middle and distal parts of the yam tuber, a difference on the granule size distribution (Miege 1957) and yam parenchyma along the longitudinal axis (Trouslot 1985) have been revealed. The firmness of yam tissue, dry matter contents and starch content was higher in the proximal and median parts than distal section. No significant difference was found for amylose content (Florido), Cohesiveness and elastic recovery in A5 from *D. alata*, Kponan and 156 from *D. cayenensis-rotundata* (Brunnschweiler 2004). In the current study, we hypothesized that starch properties could be influenced by the variations in the tuber above-mentioned. Therefore, the aim of this investigation was to study tissue variation and properties of starch isolated from the proximal, middle and distal sections of the yam tuber. Our findings could contribute to determining how the properties of starch from the different sections can be exploited for the production of satisfactory starchy products.

MATERIALS AND METHODS

Materials

Two varieties of *Dioscorea cayenesis-rotundata* (*Krenglè* and Tdr95/19156) and *D. alata* (*Bètè-bètè* and Tda1176) were obtained from the experimental farm of Centre Suisse de Recherche Scientifique (CSRS, Abidjan, Ivory Coast). The two improved varieties (Tdr95/19156 and Tda 1176) were introduced from the International Institute of Tropical Agriculture (IITA) of Nigeria but *Krenglè* and *Bètè-bètè* are local varieties. Yam tubers with uniform size and shape and without any mechanical and pathological injuries were selected after a storage period of four months in traditional yam barns (31.57°C; 79.42 HR). For each variety, three tubers were

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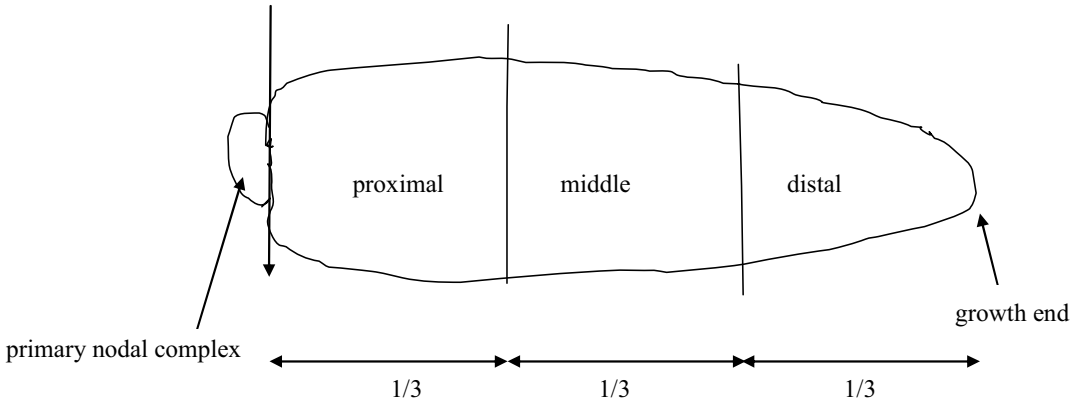

Fig. 1 Yam tuber sectioning into three parts of equal length for histological and starch granules studies. The primary nodal complex was eliminated.

randomly selected and used. In order to investigate the longitudenal behaviour of yam tissue and starch, the tubers were measured, and divided into three equal sections (**Fig. 1**) (proximal, middle and distal). Each of the sections of the tuber was subjected to a histological examination of the parenchyma tissue and to physicochemical analysis of the isolated starch. All tests were done in triplicate.

Analytical methods

Histological examination

Small cubes of yam tissue with an edge length of about 0.5 cm were fixed in formalin-calcium. Samples were placed into a Technicon waterbath (RH model-12 EP, N° 94010726, Tokyo, Japan), to undergo dehydration successively in seven baths of increasing degrees of alcohol (ONU 1170, Valdonne, France) (2 baths of 70^c, 2 baths of 80^c, 2 baths of 95^c and a bath of 100^c); lightened in three baths of toluene (ONU 1294, Valdonne, France) and impregnated with paraffin wax in two successive paraffin (Paraplast Kendall, Tyco/Healthcare, Ref: 8889) baths. Samples were allowed to remain for one hour per bath. The sample embedded in paraffin wax was cut into sections of 4 µm thickness with a microtome (Leica microtome RM 2025). These thin sections were transferred to a glass slide, and then oven-dried at 50°C for 15 min to eliminate the paraffin and improve the adherence. This process was completed by dipping the specimens in two baths of toluene. Before coloration, samples were hydrated in water after passage in three baths with a decreasing ethanol concentration (one absolute ethanol bath and 2 baths with 95^c ethanol). Samples were then stained in 2% of erythrosine (Merck KGaA, Germany) (cytoplasm stain), washed with water and stained in haematoxylin of HARRIS (Reactive RAL, Bordeaux, France) (nuclear stain), immersed in toluene and mounted in Canada balsam (Locquin and Langeron 1978). The stained sample was observed under a photonic (light) microscope (Olympus BX40, Tokyo, Japan) fitted with to a camera (Olympus DP12, Japan). The images were edited with Paint Shop Pro3 software.

Starch isolation and granules measurements

Yam starch was isolated by a method previously described by Amani *et al*. (2004). Starch granules of each tuber section were examined with a photonic microscope (Olympus U-SPT, BX 40F, Tokyo, Japan) (magnification X400) equipped with a drawtube (Locquin and Langeron 1978) that superimposes image of the specimen and the pencil image. This technique permits reproduction of the shape observed in the ocular accurately. Squared paper was used to estimate the granule size by counting the square millimetres contained inside the granule shape. The diameters of each granule measured were corrected by a calibration factor (15.1 µm).

A pinch of starch sample in water was prepared, and the slide was placed on the stage of a compound microscope and observed. The length and the width of granules were determined as described above. One thousand counts were performed with 200 granules counted per slide for each tuber section.

Swelling power and solubility

The starch suspension of 1% (w/w) was heated for 30 min over the pasting range 60 to 95°C by immersion in a water bath with gentle stirring. After cooling for 15 min at room temperature it was centrifuged at 5000 rpm for 30 min. the supernatant was immediately separated from the sediment, both were oven-dried (130°C for 2 h) weighed and the swelling power and solubility determined (Leach *et al*. 1959).

Pasting characteristic

The Pasting Characteristic of 6% starch paste (27 g of starch on a dry weight basis in 450 ml of water) was obtained determined with a Brabender viscoamylograph (OHG Duisburg, Germany) by heating an aqueous starch suspension from 50 to 95°C, maintaining it at this temperature for 15 min and then cooling it down to 50°C. The speed of the rotor was fixed at 75 rpm and the rate of heating and cooling was fixed at 1.5 min^{-1} throughout the range of gelatinization, holding and cooling steps. The outset temperature (T_o temperature at which the viscosity begins to increase), the peak viscosity (P_v), the hot paste viscosity (H_v i.e. the viscosity after 15 min stirring at 95°C) and the cold paste viscosity (C_v. i.e. the viscosity after cooling to 50°C) were recorded. The breakdown (BD) or thermostability and the setback (SB) were calculated and expressed as Brabender Units.

Paste clarity

Paste clarity was determined according to the method of Craig *et al*. (1989) Transmittance was determined for 1% (w/w) of yam starch dispersion, employing a unico spectrophometer (N°1100).

Gels syneresis

A 4% (w/w, dry weight basis) gel was heated with gentle stirring (Agimatic-M Staufen, Germany) for 15 min (Pingault 1995). The gel was immediately distributed into centrifuge tubes at a rate of 6 ± 0.5 g per tube. Three tubes of every sample were cooled to room temperature and then centrifuged (J. P. SELECTA, THIS 95 N° 289886, Barcelona, Spain) at 5000 rpm for 30 min. This constituted the initial syneresis (week 0). The remaining tubes were frozen at -20°C (4 weeks). Each week, three tubes of each sample were thawed at 50°C for 90 ± 5 min, and then centrifuged in the same conditions previously described. After centrifugation, the syneresis rate was calculated as the weight of leaked-out water divided by the weight of the original gel.

Statistical methods

The software StatSoft (Statistica, 99th Edn, Paris,France) was used for the statistical analyses. The differences between the three sections of tuber were calculated with the test of Newman-Keuls at $P \leq 5\%$.

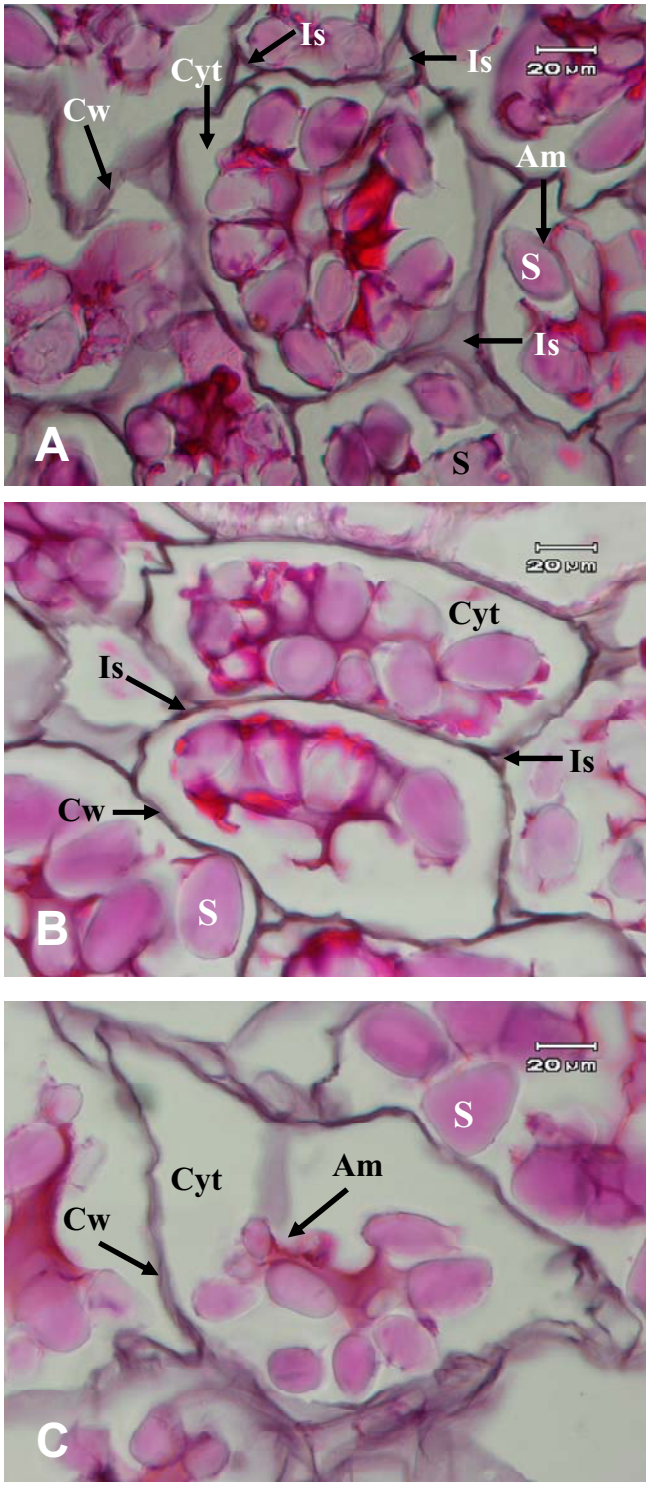

Fig. 2 Starch cells of three sections of the tuber (Tda 1176 of *D. alata*). (**A**) Proximal part, (**B**) the middle and (**C**) distal section. Starch granules present three shapes: the spherical or ovoid, ova-triangular and elliptical on each part of the tuber. Starch granules (S) were surrounded individually with amyloplast (Am). Cell wall (Cw) does not rigid, due to water loss during the conservation. Intercellular spaces (Is), and starch density by cell are more important on the proximal part toward the distal extremity contrary to the cytoplasm area (Cyt).

RESULTS

Yam parenchyma structure

A comparative study of yam tissue was carried out on the starch-rich cells of medullar parenchyma (**Fig. 2**). The cells differed in shape and size between the three sections of the tuber; rounded (**Fig. 2A**), elongated (**Fig. 2B**) and larger cell (**Fig. 2C**). All cells exhibited irregular cell walls in terms of thickness and the shape, showing an aspect of dehydrated cells. Cells from the proximal section appeared limited by a thicker-wall (Cw) with intercellular spaces (Is), which decreased from the proximal section toward the distal extremity. Starch granule (S) density was higher in the proximal and middle sections than the distal part. Inversely, the granule size increased from the distal to the proximal section for variety Tda 1176. Each granule was surrounded by a membrane (Am), and bounded to the neighbouring granules; all making a membrane network inside of which starch granule appeared. Unlike granule size, granule shape did not show any pattern of variation between the three sections of a tuber. Three forms were observed at all the sections: ovoid, ovo-triangular and elliptical. In spite of the post-harvest period and the maturity of all samples, small granules were observed in each part of the tuber.

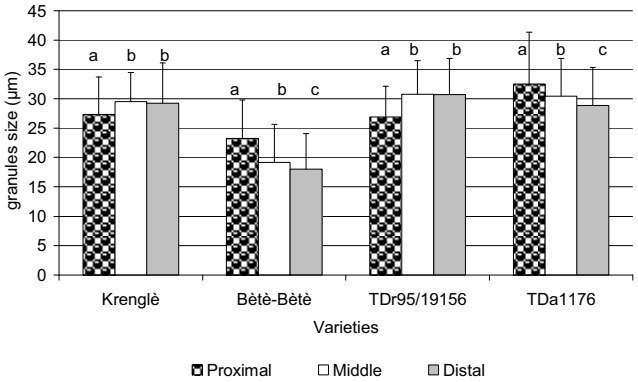

Fig. 3 Granules size of starches from the three sections of the tuber. Histogram bars with different letter are not significantly different at $p<0.005$ for each variety.

Starch granule size

Granule size distribution (**Fig. 3**) varies between the different tuber sections and by species. The granule sizes in the two varieties of *D. alata* (*Bètè-bètè* and Tda 1176) decreased from the proximal section toward the distal zone. Variance analysis of the granule sizes from the different sections showed a significant difference ($P < 0.001$). In both varieties of *D. cayenensis* (*Krenglè* and Tdr 95/19156), a significant difference ($P < 0.002$) was noted between the proximal section of the tuber and the two other sections (middle and distal). The average of the diameters increased from the proximal section towards the middle and distal sections of the tuber of both *D. cayenensis* varieties. The improved varieties had similar average sizes (29.5 µm for Tdr 95/19156 and 30.6 µm for Tda 1176) which were significantly higher than ($P < 0.001$) those of *Bètè-bètè* (20.1 µm) and *Krenglè* (28.7 µm).

Paste viscosity

The pasting properties of starch from the three sections of the tuber are given in **Table 1**. The gelatinization temperature varied only slightly between the three sections. Generally, the starch from middle section had relatively lower gelatinization temperatures than the proximal and distal section starches. The break down (BD) in viscosity during the isothermal period was higher in the middle and distal sections of the tuber. During the cooling period (setback), the gel showed stability; except the proximal extremities of vars. *Bètè-bètè* and Tdr 95/19156. This stability was more pronounced in the middle section than the distal part of the tubers of both varieties.

Swelling and solubility

For the improved varieties, Tdr95 and 19156 as well as *Bètè-bètè*, the swelling power of starch (**Fig. 4**) increased

Table 1 Pasting properties (Brabender) of three sections of yam tuber

Varieties	Parts	To (°C)	Pv (UB)	Hv (UB)	Cv (UB)	BD (UB)	SB (UB)
Bètè-bètè	Proximal	76	765	760	840	5	75
	Middle	76	750	635	600	115	-150
	Distal	76	780	710	780	70	0
	Proximal	74	830	750	880	80	50
Tdr95/19156	Middle	73	835	620	810	315	-125
	Distal	74	925	635	800	290	-125
Krenglè	Proximal	76	850	750	840	100	-10
	Middle	74	835	565	690	270	-145
	Distal	76	820	580	760	240	-60

T_o: temperature that the viscosity begins to increase; Pv: peak viscosity; Hv: hot paste viscosity; Cv: cold paste viscosity; breakdown (BD) and the setback (SB); UB Brabender Units; $BD=P_v-H_v$ after a holding period of 15 min at 95°C, $SB= C_v-P_v$

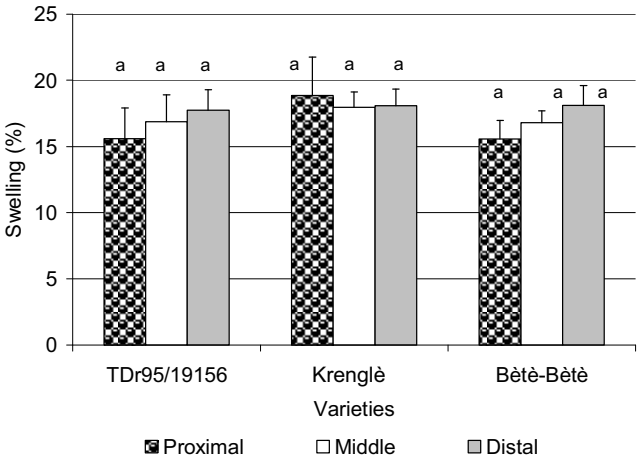

Fig. 4 Swelling power of three sections of the tuber. Histogram bars with different letters are significantly different at p<0.005 for each variety.

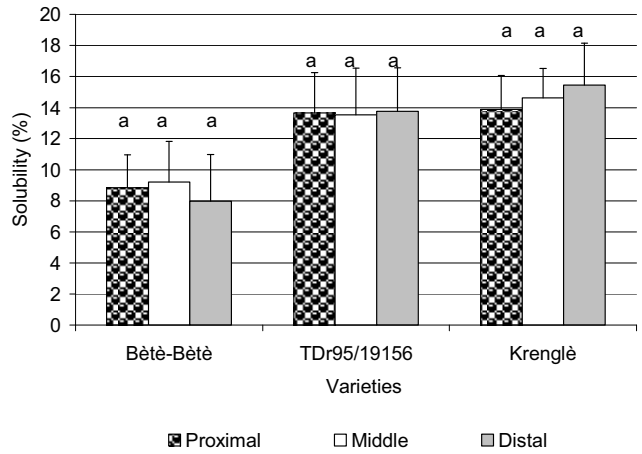

Fig. 5 Solubility of three sections of the tuber. Histogram bars with different letters are significantly different at p<0.005 for each variety.

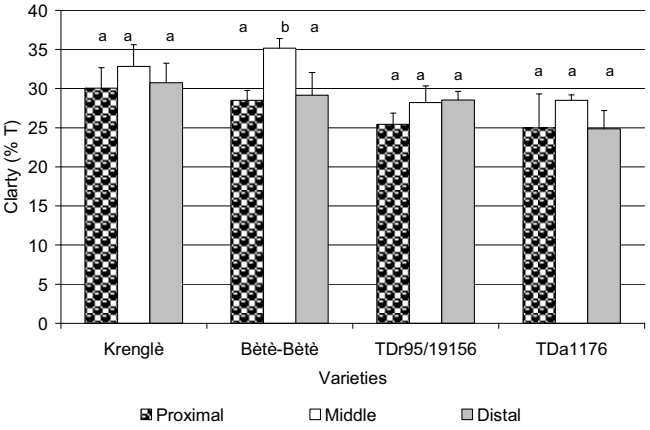

Fig. 6 Paste clarity of the three sections of the tuber. Histogram bar with different letters are significantly different at p<0.005 for each variety.

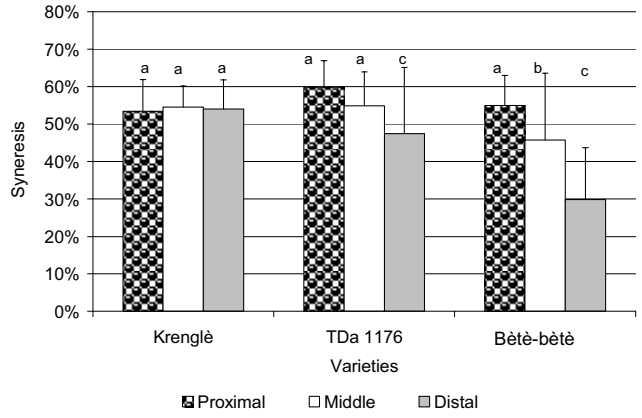

Fig. 7 Gel syneresis of the three sections of the tuber. Histogram bars with different letters are significantly different at p<0.005 for each variety.

from the proximal section toward the distal extremity. For var. *Krenglè*, it decreased from the proximal section toward the middle and distal sections with no significance difference (P = 0.6784) between the three sections of the tuber. The starch solubility (**Fig. 5**) of the three sections increased from the proximal to the distal sections for var. *Krenglè*. However these differences were not statistically (P = 0.764) significant.

Paste clarity

Clarity of the middle section starches was higher than both the distal and proximal sections except for var. Tdr 95/19156 (**Fig. 6**) where the middle and distal sections had a similar clarity (28.2 ± 2.1%) (P = 0.148). A significant difference (P<0.001) was observed between clarity of starches from the middle section and the distal and proximal extremities of the tuber of var. *Bètè-bètè*.

Gel syneresis

Gel syneresis (**Fig. 7**) decreased from the proximal section towards the distal extremity for the two varieties of *D. alata* (Tda 1176 and *Bètè-bètè*). Identical values were noted (P = 0.8803) for the three parts of var. *Krenglè*. For var. Tda 1176, syneresis increased from 59.84 ± 7.06% in the proximal section to 54.8 ± 9.0% and 47.5 ± 17.6%, respectively for the middle and distal sections of the tuber with a significant difference between the distal section and the middle section (P = 0.039). Significant differences (P < 0.001) were observed between the three parts of the tuber for var. *Bètè-bètè*.

DISCUSSION

The yam tuber has two growth axes: longitudinal and radial and these assure the development of the plant both in length and in width. The longitudinal axis of the tuber presents a gradient evolution in which the oldest tissues are in the parts that move away from the apical meristem. Increasing dry matter and firmness of yam tissue in the middle and proximal parts confirmed this gradient maturity (Brunnschweiler 2004). This evolution could be responsible for the tissues variations and granule size distribution. Small starch granules appeared in each part of the tuber, indicating that the distal tip is not the only section responsible for granule formation in yam tuber which can partly explain starch size distribution in the tuber. This phenomenon (granule formation) was more pronounced in the distal section as observed after 8 weeks in the floury and at 10 weeks in the horny endosperm of maize (Gallant and Bouchet 1986).

The order of size evolution and the differences in granule size distribution between yam species as well as between the different sections of the tuber have also been noted in previous studies (Miege 1957; Ketiku and Oyenuga 1973; Farhat *et al*. 1999; Amani *et al*. 2004). These variations in size distribution could be attributed to the tissue environment in which the granules were grown (i.e., the three sections of the tuber) and the species (the variations are specific to each species). The size of the starch granules is an important factor to consider when determining and subsequently interpreting the physicochemical properties of starch, as it exerts important effects on them (Deang and Rosario 1993). Therefore, size variation could partly be responsible for properties of starch.

The slightly higher clarity in the middle section generally and significantly in var. *Bètè-bètè* indicated the possible relation between tuber section and starch clarity. However, some of starch properties (Swelling power and solubility) were not bound to the tuber portion.

Gelatinization temperature values (73-76°C) of the three sections of the tuber were generally in agreement with previous studies on yam starch (Farhat *et al*. 1999; Amani *et al*. 2004; Erica *et al*. 2005). The low breakdown viscosity of the starch extracted from the proximal and distal extremities during the isothermal holding period was an indication of the shear and heat resistance of yam starch. During the cooling period, the gel instability was more pronounced in the proximal and distal extremities than the middle section, except in var. Tdr 95/19156 where the middle and distal parts had the same values. These variations indicated the possible differences in the starch structure in the three parts of the yam tuber.

The rate of *Krenglè* syneresis (54 ± 7%) was in the stability interval (35-70%) of *D. cayenensis* starch after one week of storage at -20°C (Amani *et al*. 2005). The distal extremity of *Bètè-bètè* was the most resistant to freezing (29.4 ± 14.4%). For this variety, the percentage of syneresis may be bound to the tuber section. Gel formation may be attributed to the ability of the granules to gelatinize during heating, and its stability during storage, to the retrogradation of the linear fraction and amylopectin (Deang and Rosario 1993; Tetchi 2006). For the same amylose content (Brunnschweiler 2005), the percentage of syneresis variation may be due to the amylopectin. The short chains of amylopectin with a degree of polymerization (DP) 6-9, have been said to inhibit retrogradation (Fredriksson 1998). However, structure of starch macromolecules along the tuber axis was not determined. Variation observed along the tuber axis and starch granule influence some of starch properties. Oldest tissues in the tuber in general could be explained this. To understand the influence of tuber maturity on starch properties, this study will be done during the tuberization.

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