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Physicochemical properties and digestibility of starch from bulbils of two cultivars of *Dioscorea bulbifera* during the growth

Libra Michel Archange, Dabonné Soumaila*, Binaté Siaka, Gonnety Jean Tia, Kouame, Lucien Patrice

Laboratory of Biochemistry and Food Science and Technology, University of Abobo-Adjamé, 02 BP 801 Abidjan 02, Côte D'Ivoire

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Abstract

Starches from bulbils old from to 3 to 6 months of two cultivars of *Dioscorea bulbifera* were extracted and characterized in order to establish physicochemical changes during bulbils development. The results showed that sharp of starch granules was regular and ovo-triangular; the particles size analysis revealed an increasing of granules size with a unimodal distribution; granules size varied between (30 µm – 47.70 µm) for mauve cultivar and between (7.31 µm – 56.38 µm) for yellow cultivar. The chemical analysis of both cultivars showed that the starches contained low levels of lipids and ash; the protein content was nil. The functional properties such as swelling capacity and solubility increased when starches were heated in presence of water over 65°C for mauve cultivar and 70°C for yellow cultivar. Gelatinized starches from less developed bulbils were more digestible by α-amylase.

*Corresponding Author: Dabonné Soumaila ✉ sdabonne@yahoo.fr

Introduction

Starch is an important polysaccharide reserve in higher plants. It's abundant in cereals, roots and crops (Christensen, et *al.*, 1976). Starch is consisted primarily of branched and linear chain of glucose molecules named amylopectin and amylose respectively (Syahariza et *al.*, 2010; Mali et *al.*, 2006; Garcia et *al.*, 2005). Amylose is an α(1→4)-D glucopyranosyl polymer. Whereas residues in amylopectin are α(1→4) D glucopyranosyl units with α(1→6) linkages of approximately 20-25 units, depending on the plant source (Kong et *al.*, 2010, Atichokudomchai, et *al.*, 2003; Navdeep et *al.*, 2003; Meera, et *al.*, 2002). Starch is essential in many formulated foodstuffs. It constitutes an important source of energy and contributes to the structure and the texture of food, as thinckming or gelling agent (Loisel et *al.*, 2006); it is used in paper-making, fine chemicals, packing materials, industry etc. Although starches have the same chemical structure, their botanic origin, the maturity of biological material, the size distribution of starch granules and amylose and amylopectin ratio strongly influences the functional properties (Yong et *al.*, 1998; Cheetam et *al.*, 1997; Jenkins et *al.*, 1995; Sievert et *al.*, 1993) and give to starches a unique property. Moreover, previous studies reported that within the same cultivar starch's properties vary as the development of starch source progress. As bulbils are aerial tubers, it's easier to follow their development stage and study changes in physicochemical properties of starches.

Discorea bulbifera is a vigorous climber plant cultivated in western Côte d'Ivoire for its bulbils and also for its leaves used for traditional medicine and food (Coursey, 1967). Two cultivars as used for plantation (Libra *et al.,* 2011). Bulbils weighing up one kilogram are not exceptional but those of 200-300 g are usual (Degras, 1986). They produce aerial starchy bulbils.

The purpose of the present study was to provide information about sharp, size, thermal properties and enzymatic sensibility of starch of bulbils from two cultivars of *D. bulbifera* during the growth period.

Material and Methods

Plant materials

Bulbils used for the study were collected from the Experiment Station and Research Flora of University of Abobo-Adjamé (Côte d'Ivoire). Bulbils of two cultivars of *Dioscorea bulbifera* (cultivar yellow and cultivar mauve) were harvested each month according their age from three to six months.

Starch isolation

Starch from bulbils of each cultivar, was extracted using the method of Bank and Greenwood (1975). Bulbils were washed, peeled, sliced and steeped in 0.1% sodium bisulphite water solution. The slices were grounded in a Moulinex 2000 blender and the paste recovered in 4% sodium chloride solution and then sieved successively through 500 µm, 250 µm and 100 µm. The filtrate was allowed to settle about one hour for decantation. The operations settling/decantation were repeated until the supernatant became clear. Sediment starch was rinsed with 70% ethanol and the deposit obtained was spread on an aluminium paper and oven dried at 47°C for 48 hours. The starch was ground in porcelain mortar and then stared.

Determination of Chemical composition of starch

Lipid content was determined by soxhlet extraction with hexane (AOAC, 1995). Ash was determined by measurement of residues left after combustion in a furnace at 550°C for 8 hours (AOAC, 1995). Crude protein by determination of nitrogen using a factor of 6.25 (AOAC, 1995). Starch content was determined following the B.I.P.E.A (1976) method.

Microscopy examination

Starch sample was oven dried at 40°C for 24 hours. A pinch of dehydrated starch was placed in a glass slide and covered with a cover slip. Then the preparation was observed under 40 x magnification using a photonic microscope (CETI, Japan) mounted a camera (JVC) connected to a monitory which is

connected also to a computer equipped with Kappa Image Base and Kappa Control software which respectively captured the images and determined the dimensions (length and wide) of starch granule. The diameter was indicated is the average of the large and short axes of the granules Rasper (1971). Experiment described above was carried out of using 1000 seeds.

Table 1. Granule size distribution evolution of starch from bulbils of two cultivars of *Dioscorea bulbifera* during the growth time.

Age	Yellow cultivar		Mauve cultivar		Distribution
	Diameter (µm)	Modal class (µm)	Diameter (µm)	Modal class (µm)	
3 month	7.31 - 52.08	[15.45 – 19.52]	9.88 – 30.81	[17.77 – 20.40]	unimodal
4 month	9.44 - 55.53	[22.01 – 26.20]	8.30 - 43,39	[17.87 – 21.06]	unimodal
5 month	11.50 - 56.38	[23.74 – 27.82]	10.74 - 47.70	[17.46 – 20.82]	unimodal
6 month	13.6 - 56.06	[29.04 – 39.90]	12.24 - 46.78	[24.81 – 27.94]	unimodal

Table 2. Chemical composition in starches from bulbils of *Dioscorea bulbifera* of different growth times.

	Growth time	% Proteins	% Fats	% Ash	% Starch	Energy
Yellow cultivar	3 Month	0	0.04 ± 0.02	0.25 ± 0.01	98.20 ± 0.03	393.16
	4 Month	0	0.07 ± 0.02	0.23 ± 0.02	98.43 ± 0.01	394.35
	5 Month	0	0.05 ± 0.01	0.24 ± 0.01	98.61 ± 0.02	394.89
	6 Month	0	0.07 ± 0.03	0.26 ± 0.03	98.81 ± 0.04	395.87
Mauve cultivar	3 Month	0	0.03 ± 0.01	0.21 ± 0.04	97.09 ± 0.02	388.63
	4 Month	0	0.06 ± 0.02	0.23 ± 0.07	97.33 ± 0.03	389.86
	5 Month	0	0.09 ± 0.02	0.26 ± 0.05	97.41 ± 0.05	390.45
	6 Month	0	0.1 ± 0.03	0.39 ± 0.04	97.52 ± 0.02	390.98

Values are means of percentage on dry weight basis Mean ± SD (n = 3). Means within a column with the same superscript are not significantly different at α = 0.05 confidence level.

Determination of swelling capacity and solubility of starches

Swelling capacity and solubility determinations were based on method of Leach et *al.*, (1959). 0.1 g of starch was weighted in to a 15 ml graduated centrifuge tube. Distilled water was added to give a total of 10 ml. The suspension was stirred sufficiently and heated at desired temperature from 60°C to 95°C in a bath water for 30 min with a constant stirring. The tubes were cooled to room temperature and centrifuged for 15 min at the rate of 3000 rounds/min. The weight of the sediment in the supernatant was determined.

Determination of iodine spectra absorption of starches

The iodine spectral of starches was measured according the method proposed by Robin (1976). Starches were heated at 75°C and 95°C for 15 min in water bath. The mixture was centrifuged at 3000 rounds / min. 10 ml of supernatant was removed and iodine solution (0.2 ml) was added. The mixture was brought to 2 ml with distilled water and mixed immediately. The λmax was determined after 15 min, by measuring the wavelength of maximum absorbance at 5 nm intervals from 400 to 700 nm in spectrophotometer Genesys. The proportion of amylose to amylopectin was determined as the ratio of the optical density of amylose at 630 nm to that of amylopectin at 540 nm (Garcia et *al.*, 1988).

In vitro digestibility

In vitro digestibility was carried according the modified method of Jancy (1999). 2 % of starch was gelatinised in distilled water at 75 °C for 15 min and cooled to ambient temperature. 0.8 ml gelatinised

sample was removed in a test tube. 0.8 ml of 2mM sodium acetate buffer pH 5.0 and 0.2 ml of enzymatic solution α-amylase from *Aspergillus niger* were successively added. The whole was homogenous and put in a constant temperature of 37°C in a shaking bain-marie for 120 min. At 0 nm then every 10 min, 2 ml of supernatant were removed and the reaction was terminated by adding 2 ml of DNS. Soluble reducing sugars were measured and quantified against glucose as standard. The red-orange coloured intensity of the colouring was red at 540 nm with a spectrophotometer Genesys.

Statistical analysis

All determinations were carried in triplicate and standard deviation (SD) was calculated using Statistical 7.0 software method. Duncan's multiple range tests was used to determine significant differences ($P < 0.05$).

Results and discussion

Morphology and size

The morphology of bulbils starch is illustrated in the Fig. 1 (a, b, c, d) for yellow cultivar and Fig. 1 (e, f, g, h) for mauve cultivar. The granular structure of bulbils starches from yellow and mauve cultivar of *D. bulbifera* show significant difference in morphology and size when observed by microscope. However, starch granules of bulbils from both cultivars are regular and have ovo-triangular shape as shown by Sahoré (2005). The granular size distribution of the starch of bulbils during their development is illustrated in Table 1. Granules size starch distribution of bulbils from both cultivars increased with the growth time from 3 to 6 months and showed a unimodal distribution. For yellow cultivar, diameters of starch granules from bulbils old of 3 month varied from 7.31 µm to 52.08 µm with a modal class equal to (15.45 µm – 19.52 µm) , 4 month from 9.44 µm to 55.53 µm with a modal class equal to (22.01 µm – 26.20 µm), 5 month from 11.50 µm to 56.38 µm with a modal class equal to (23.74 µm – 27.82 µm) and 6 month from 13.6 µm to 56.06 µm with a modal class equal to (29.04 µm – 39.90 µm). For mauve cultivar granules diameters were ranged at the 3 month from 9,88 µm to 30,81 µm with a modal class equal to (17,77 µm – 20,40 µm), 4 month from 8,30 µm to 43,39 µm with a modal class equal to (17.87 µm – 21.06 µm), 5 month from 10,74 µm to 47,70 µm with a modal class equal to (17.46 µm – 20.82 µm) and 6 month from 12,24 µm to 46,78 µm with a modal equal to (24,81 µm – 27,94 µm)]. The increasing of starch granules size with tubers development and their variability were noted in previous studies (Liu et *al.*, 2003; Amani et *al.*, 2004; Noda et *al.*, 1995). At the same stage of growth, mauve cultivar starch granules are smaller than those of yellow cultivar.

Proximate composition

Proximate composition of the starch is presented in Table 2. Whatever the growth time and the cultivar was, no protein was detected in starch. Fats were ranged 0.04 ± 0.02 % - 0.07 ± 0.03 % and 0.03 ± 0.01 % - 0.1 ± 0.03 % and ash 0.25 ± 0.01 % - 0.26 ± 0.03 % and 0.21 ± 0.04 % - 0.39 ± 0.04 % in yellow and mauve cultivar respectively. The composition is linked to the growing conditions and methods used for starch and fats isolation.

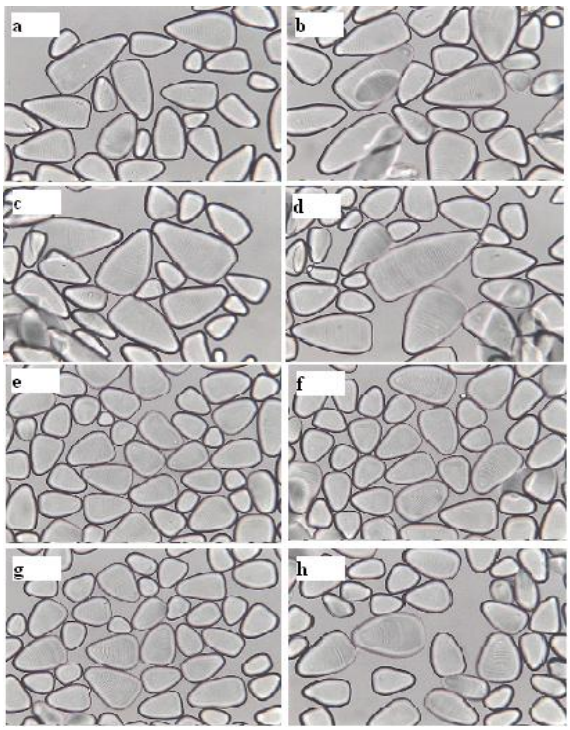

Fig. 1. Photomicrographs of starch of yellow and mauve cultivars of *Dioscorea bulbifera* during growth. Yellow cultivar (a : 3 months, b : 4 months, c

: 5 moths, d : 6 months); Mauve cultivar (e : 3 months, f : 4 months, g : 5 months, 6 : months).

Table 3. Absorbance and ʎmax (nm) of KI-I stained solution containing bulbil-solubilized starch.

	75°C				95°C			
	Do at 540 nm	DO at 630 nm	DO630 / DO540	ʎmax	Do at 540 nm	DO at 630 nm	DO630 / DO540	ʎmax
				Yellow cultivar				
3 Month	0.137 ± 0.02^{a}	0.215 ±0.01^{a}	1.57 ± 0.0^{d}	605 - 610	0.491 ± 0.02^{a}	0.822 ± 0.01^{a}	1.61 ± 0.01^{b}	610
4 Month	0.542 ± 0.03^{g}	0.837 ±0.02^{g}	1.54 ± 0.02^{c}	605 - 610	0.494 ± 0.02^{a}	0.82 ± 0.02^{a}	1.60 ± 0.01^{b}	615 - 620
5 Month	0.514 ± 0.02^{e}	0.728 ±0.02^{e}	1.50 ± 0.01^{b}	600	0.499 ± 0.02^{b}	0.844 ± 0.02^{c}	1.57 ± 0.02^{a}	620
6 Month	0.335 ± 0.02^{c}	0.509 ± 0.0^{b}	1.42 ± 0.03^{a}	600 - 605	0.504 ± 0.02^{c}	0.864 ± 0.02^{d}	1.56 ± 0.01^{a}	615
				Mauve cultivar				
3 Month	0.323 ± 0.02^{b}	0.518 ± 0.02c	1.60 ± 0.02^{e}	610	0.491 ± 0.02^{a}	0.837 ± 0.02 ^{b}	1.60 ± 0.01^{b}	615
4 Month	0.351 ± 0.02^{d}	0.583 ± 0.02^{d}	1.66 ± 0.01^{f}	610	0.503 ± 0.02^{c}	0.837 ± 0.02 ^{b}	1.64 ± 0.01^{c}	615
5 Month	0.563 ± 0.02^{h}	0.969 ± 0.02^{h}	1.72 ± 0.01^{g}	615 - 620	0.506 ± 0.02^{c}	0.876 ± 0.02^{e}	1.70 ± 0.02^{d}	620
6 Month	0.524 ± 0.02^{f}	0.82 ± 0.02^{f}	1.56 ± 0.01^{d}	605	0.51 ± 0.02^{d}	0.885 ± 0.02^{f}	1.73 ± 0.01^{e}	615

Values are means of percentage on dry weight basis Mean ± SD (n = 3). Means within a column with the same superscript are not significantly different at α = 0.05 confidence level.

Iodine spectra

The absorbance (ʎ) (nm) of KI-I stained solution containing bulbil-solubilized starch at 75°C and 95°C is illustrated in table 3. When starch is heated in water above its gelatinization temperature, lixiviation occurs, with amylose diffusing preferentially out of the starch granule (Banks and Greenwood 1975). Adding iodine give to solution a blue color which confirmed the presence of amylose (Yamamory et *al.*, 2006). The ʎ value varies from 600 nm to 610 nm at 75°C and from 610°C to 620 at 95°C for the starches from yellow cultivar and between 605 nm and 620 nm at 75°C and 615 nm and 620 nm for starches from mauve cultivar. These values are different from those reported by Dadié *et al.* (1998) on potato and Amani et *al.* (1993) respectively on potato and cocoyam. The amylose/amylopectin ratios of starch present a significant difference (5%). Values are ranged in interval (1.42 ± 0.03 – 1.57 ± 0.0) at 75°C and (1.56 ± 0.01 – 1.61 ± 0.01) at 95°C for starches from yellow cultivar and (1.56 ± 0.01 – 1.72 ± 0.01) at 75°C and (1.60 ± 0.01– 1.73 ± 0.01) at 95°C for starches from mauve cultivar. These values were higher than those reported by Sahoré (2006). Values obtained at 95°C were higher than those obtained at 75°C; moreover, at the same temperature, values of starches from mauve cultivar are higher than those of yellow cultivar. These results suggest that lixiviation of amylose increases with the increasing of the temperature and lixiviation is important in mauve cultivar starches because of the smallest granules size.

Swelling capacity

The dependence of swelling capacity on temperature is tested 5 intervals between 55°C and 95°C as shown fig. 2. The rapid increase of swelling capacity was detected between 65°C and 95°C and between 70°C and 95°C for mauve and yellow cultivar respectively. Between 55°C and 65°C and 55°C and 70°C swelling capacity for mauve and yellow cultivar respectively increased slightly. The increasing of swelling capacity with the temperature was in the range expected for starch (Ma et *al.*, 2010). Swelling capacity of starches from bulbils at different growth time of yellow and mauve cultivar showed a significant difference ($p < 0.05$) when heating in water. Within the same cultivar, swelling capacity of starches increased with the growth time. Onset

gelatinization temperatures were 65°C and 70°C for starch from mauve and cultivar yellow respectively.

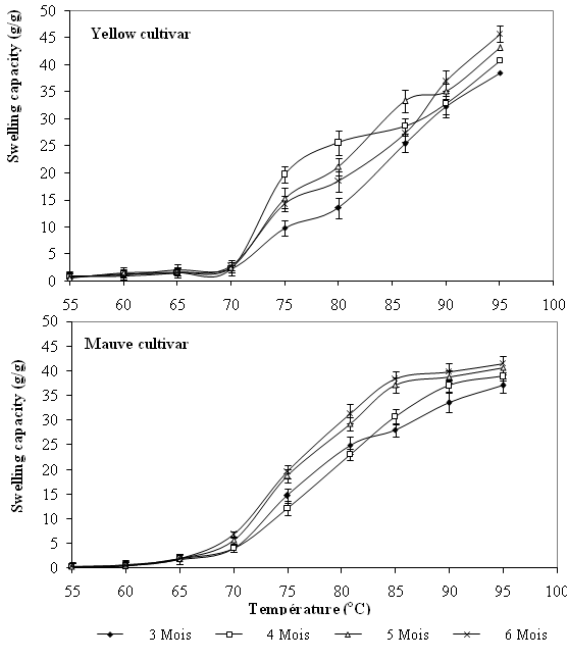

Fig. 2. Swelling capacity of starches of yellow and mauve cultivars bulbils of *Dioscorea bulbifera* during the growth time.

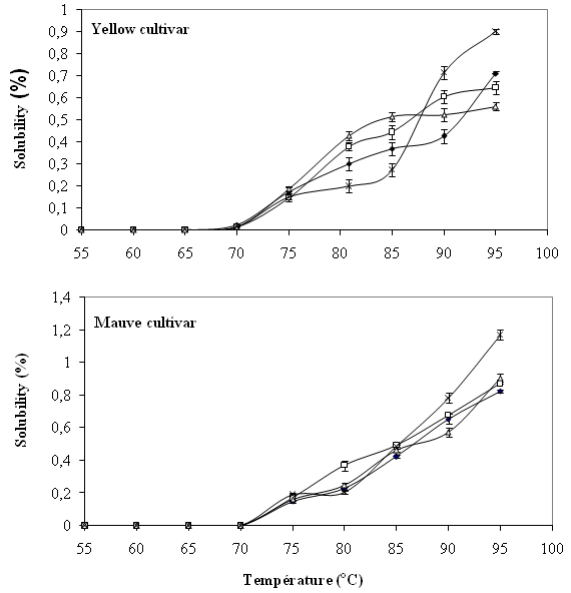

Fig. 3. Solubility of starch from yellow and mauve cultivars bulbils of *Dioscorea bulbifera* during the growth time.

From 65°C to 95°C, values of starch from mauve cultivar old to 3, 4, 5 and 6 month were ranged between 0.30 ± 0.2 g/g and 37.45 ± 1.5 g/g; between 0.33 ± 0.11 g/g and 38.90 ± 0.8 g/g; between 0.35 ± 0.2 g/g and 40.05 ± 1.4 g/g and between 0.36 ± 0.17 g/g and 41.50 ± 1.4 g/g respectively. Those of yellow cultivar were ranged in the same order, between 0.84 ± 0.8 g/g and 38.45 ± 1.5 g/g; between 0.7 ± 0.6 g/g and 40.66 ± 1.8 g/g; between 0.8 ± 0.4 g/g and 43.05 ± 1.3 g/g and between 0.81 ± 0.5 g/g and 45.66 ± 1.6 g/g. At the same growth time, starches from yellow cultivar had the highest swelling capacity. Furthermore, for high temperatures (90°C - 95°C) starches from older bulbils presented the highest swelling capacity. Swelling capacity may be attributed to starch granules and also to amylopectin (Tester et *al.*, 1990).

Solubility

The solubility profiles of starches from bulbils of both cultivars at different growth time are shown by fig. 3. From 55°C to 70°C, the solubility is nil. However, with continued increasing of temperature from70°C to 95°C, the solubility increased gradually for all starches. Values were ranged from 0.01 % to 0.9 ± 0.01 % for starches from bulbils of yellow cultivar and from 0.01 % to 1.17 ± 0.01% for starches from bulbils of mauve cultivar. There's a significant difference ($P < 0.05$) between solubility of each cultivar. Starch from mauve cultivar showed higher solubility and starch from older bulbils had the highest solubility. When applying higher temperature, starch granules imbibed water and swelled considerably. Over their swelling capacity, granules break off (Fig.4) and released amylose in solution.

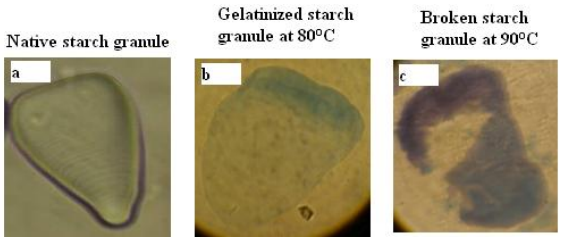

Fig. 4. Solubility of starch from yellow and mauve cultivars bulbils of *Dioscorea bulbifera* during the growth time.

Digestibility

Figure 5 shows the digestibility of gelatinized starch of bulbils from yellow and mauve cultivars during the growth. The reducing sugars released increase as

the time progress. The rate of digestible starch of both cultivars by α-amylase followed the order : starch from bulbils old of 3 month > 4 month > 5 month > 6 month. Moreover at the same growth stage, starch from mauve showed high digestibility than those from yellow cultivar. The rapid digestibility of starch is due to the size distribution of starch granules. The smaller are starch granules size the higher is they digestibility. The results suggested that small granules are more accessible to hydrolytic enzymes than large granules. These observations are in agreement with those reported by kulp (1976) and Vasanthan et *al.*, (1996) on wheat and barley starch respectively.

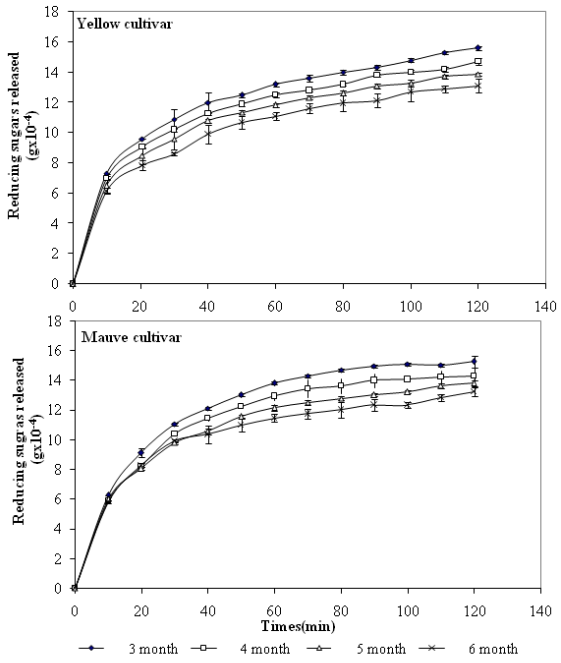

Fig. 5. enzyme digestibility of starches from bulbils of yellow cultivar of *Dioscorea bulbifera* during the growth time.

Conclusion

The particle size of *Dioscorea bulbifera* increased during the growth time. The difference in the granules morphology, size distribution and swelling-solubility depend on the stage of maturity of bulbils and botanical origin. These characteristics high light functional properties of bulbils starch. Thus, the present study provides useful informations for subsequent applications.

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