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Physicochemical characterization of starches from seven improved cassava varieties: Potentiality of industrial utilization.

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ABSTRACT

Objective: The present study, which is in line with tropical crops increased value, aimed to highlight the industrial potential of starches from seven newly improved cassava varieties through their morphological, physicochemical and functional properties.

Methodology and results: Starches from seven improved cassava varieties encoded respectively V_{54}, V_{55}, V_{64}, V_{66}, V_{69}, and V_{73} and V_4 .were extracted and their physicochemical and functional characteristics were determined. The whole starches granules were ovoid and conical bearing a hilum on the truncated side. They contained relatively high amount of small granules (<10 µm size) and amylopectin. In addition, all the starches showed very strong water absorption capacity and began gelatinizing early (55 – 60 °C). However, the physicochemical characteristics significantly varied from one starch variety to another. The starches of V_{54}, V_{64} and V_{73} varieties showed an exceptional low (0.01 %)??? and stable syneresis after four weeks at 4 °C and -15 °C for V_{54}. The highest clarity was topped by varieties V_{54} (78.54±3.30 %) and V_{64} (76.27±6.72 %). Three starch varieties encoded $V_{54,}$ V_{64} and V_{69} registered the higher amount of small granules (15.8±1.50, 16.60±0.90 and 14.40±0.90 % respectively). In contrast those encoded V_{55}, V_{66} and V_{73} with large granules, recorded the higher swelling power (30.10±3.00, 28.21±2.32 and 29.74±2.40 g/g respectively) and high amount of amylose (17.37±2.54, 20.86±2.36 and 19.03±2.45 %)

Conclusions and application of findings: The studied cassava starches varieties (particularly V_{54}, V_{73}, V_{64}, V_{55} and V_{66}) could find a wide range uses in food and non-food industries as pharmaceuticals, cosmetics, textiles thanks to their interesting physicochemical and functional properties.

Key words: Cassava, starch, functional properties, industrial utilization.

INTRODUCTION

Cassava (*Manihot esculenta Crantz*) is the third most important source of energy after rice and maize, for human and livestock in the world (Fauquet & Tohme, 2008; FAO, 2008). It is grown for its edible roots, which serves as a food security and income-generating crop for millions of people in the developing countries (Sanni & Ayinde, 2002; Amani *et al*., 2005). In spite of all these potentialities, cassava is faced with a crucial problem of post-harvest storage. An excessive glut and harvest losses (approximately 50 %) occur at the peak of harvesting time (CORAF/WECARD,

2011). Fresh cassava roots are highly perishables, with a shelf life of less than 3 days. Therefore, they are of poor market value. In this context, cassava roots are then processed into more storable forms (Phillips *et al*., 2004; FAO, 2008) such as starch following the Global Cassava Development Strategy advice in order to fulfil the global demand of cassava starch (FAO/IFAD, 2004). Starch is a multibillion-dollar business worldwide and is finding application in several industries. Indeed, outside the fact of being the main source of energy in the human diet, starch is also used in food industries and non-food industries such as manufacturing of adhesives, cosmetics, detergents, paper and textiles**.** In some foodstuffs, starch is used to influence or control such characteristics as aesthetics, moisture, consistency and shelf stability (De Cock, 1996; Ellis *et al*., 1998). This expansion of industrial uses justifies the incessantly increase in demand for starch (Davis *et al*., 2002). Potato, maize, wheat and cassava are the major sources of industrial starch but only around 10% of these starches derive from cassava (*Manihot esculenta Crantz*) roots. Although the uniqueness of starch physicochemical and functional characteristics, which is linked to its quality, varies according to its intended application (Svegmark & Hermansson, 1993), the significant differences among starch properties impose the search of new sources of starch with high and consistent qualities. In this respect, many research programs have implemented several strategies to develop high value cassava varieties (Ceballos *et al*., 2004*;* Ceballos *et al*., 2006). Some of these new cassava varieties obtained by bio fortification and introduced in Côte d'Ivoire by IITA (International Institute of Tropical Agriculture) showed a relatively high amount of starch (Koua *et al*., 2012). .

MATERIALS AND METHODS

Raw material: Seven improved varieties of cassava encoded respectively V_{54}, V_{55}, V_{64}, V_{66}, V_{69}, and V_{73} (yellow colored pulp) and V_4 (white colored pulp). These roots of eleven months old were kindly provided by the National Agricultural Research Center (CNRA) of Adiopodoumé (Côte d'Ivoire).

Starch isolation: Cassava starches were extracted within 24 hours after harvesting according to Banks and Greenwood (1975) method. Samples of two kilograms (2 kg) of each cassava roots were washed, peeled and cut into small slices (4x4 cm) with stainless steel knife. The slices were blended and the paste dispersed in a sodium chloride (4%) solution to separate proteins from the starch during 24 h at 25±1 °C. The slurry was sieved successively through 500 µm, 250 µm and 100 µm sieves. The starch obtained in a separating funnel was then dried at 45 °C for 48 hrs in an electric oven (Memmert 700, Germany) with fan-forced ventilation. The dry products were ground, quantified and then stored for analyses.

Starch granule morphology: The starch granules morphology was determined by electron microscope. Isolated starch granules were mounted on the surface of a brass disk using double-sided adhesive silver-tape, coated with gold/palladium (60/40) and viewed under a scanning electron microscope (FEG Supra 40 VP Zeiss 2008) with an acceleration of 1 kV. The diameter distribution was determined on 500 granules using a calibrated micrometer.

Physicochemical characteristics: The moisture and pH were carried out following the AOAC (1990) and Benesi *et al*. (2004) methods respectively. Amylose and amylopectin contents were carried out following the method described by Jarvis & Walker (1993): Defatted starch (0.1g) was dissolved in 5 ml of potassium hydroxide (1N) solution. The suspension was thoroughly mixed and 5 ml of HCl (1N) solution were added. The mixture was boiled in water bath for 15 min and the volume was adjusted to 10 ml. After centrifugation at 1000 rpm for 10 min, the supernatant was used for determination of amylose and amylopectin. For this, 0.05 ml of the supernatant was introduced in a test tube and 4.85 ml of distilled water, following by 0.1 ml of iodine reagent were added. The mixture obtained was left to stand for 10 min and the absorbance was read at 580 nm and 720 nm, respectively by using a spectrophotometer (Helios, Omega, UK). Standard curves of amylose and amylopectin were used as references.

Functional properties:

Swelling power and solubility of the starch were determined by the method described by Ly & Yeh (2001) and Sosulski (1962) used to evaluate the samples water absorption capacity. Stability of starches samples to freezing was determined according to

Schoch (1968). Ten (10) g of paste sample were conditioned in a plastic tube at -15 °C and 4 °C, respectively for 4 weeks. Freezing stabilities were performed every week by measuring the percentage of water expelled after centrifugation at 2700 rpm for 30 min. The paste clarity of starch samples was carried out as described by Graig *et al.* (1989). Aqueous dispersions (1%) of starch were boiled at 100°C and constantly shaken for 30 min. The paste was cooled at ambient temperature and stored at 4 °C for 4 weeks. The transmittance was measured at 650 nm every week by using a spectrophometer (Helios, Omega, UK).

Statistical analysis: All the experiences were performed in triplicate and the data were analyzed using the XLSTAT version 2007 Software and EXCELL. More precisely, in order to underline the differences between characteristics values means, Duncan's test of the ANOVA was used at 95 % of confidence.

RESULTS

Morphological and physicochemical characteristics: the cassava starches granules (small and large sizes) were ovoid and conical with a truncated side bearing an eccentric hilum. The surface of the whole granules was smooth except for some granules of the variety V_{64}, which was, crumpled (Figure 1). Concerning the granule size distribution reported on Table 1, variety V_{69} recorded the widest range (0.88 to 31.40 µm) when V_{54} got the narrowest (6.5 to 25.37 µm). Moreover, variety V_4 topped the highest amount of small granules (31±2.00 %) in opposition to V_{73} (03.40±0.65 %). The others varieties got intermediate values (7.40±0.6 % for V_{66} to 15.8±1.5 % for V_{54}). About the amylose and amylopectin content (Table 2); the test of Duncan revealed a significant difference between varieties compared with moisture and pH that presented no difference. Indeed, amylose contents were inversely correlated (r = -0.88) with amylopectin, and both ranged from 14.20±1.98 % (V_{54}) to 25.31±2.5 % (V_4), and from 85.86±2.23 % (V_{54}) to 74.69±3.56 % (V_4), respectively.

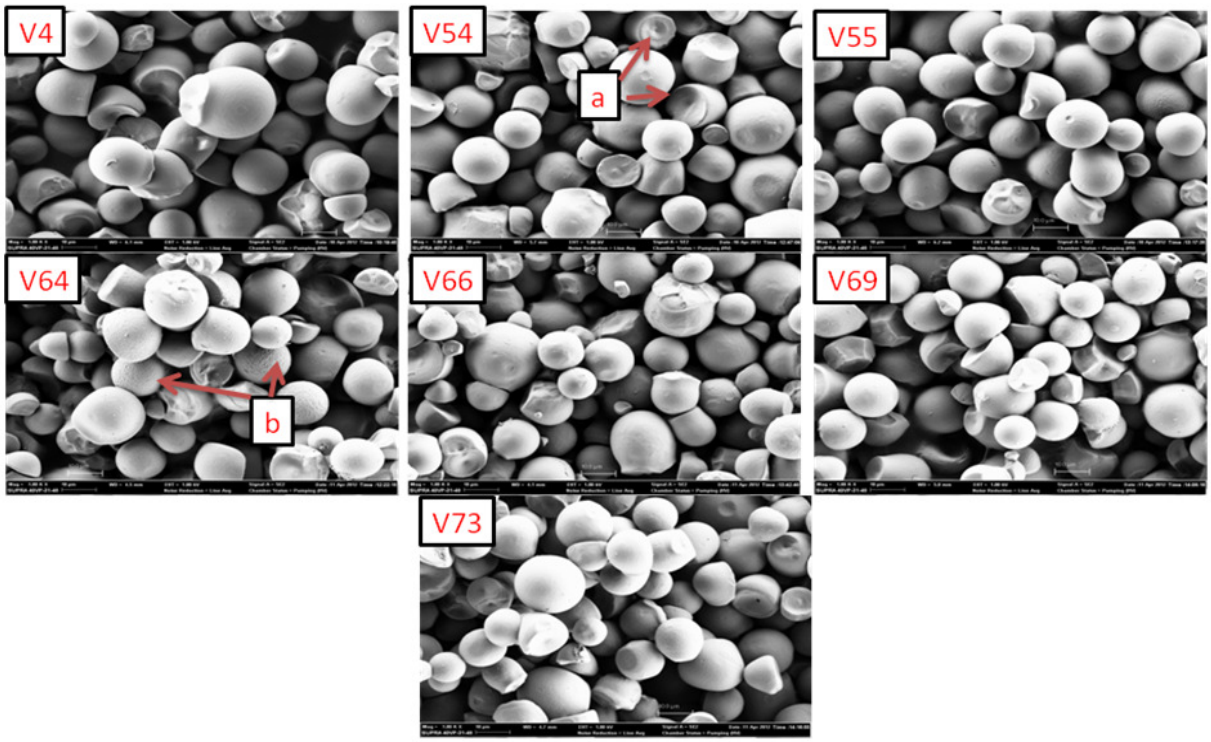

Figure 1: Scanning electron micrographs of starches (Gx 400) of cassava varieties.
Legend: **a** –hilum in the truncated side; **b** – granules with rough surface

Table 1: Starch granules distribution and proportion of small granules of cassava starches.

Varieties	Granules size distribution (µm)	Proportion of small granules (%)
V_4	3.51 – 24.35	**31.00±2.00a**
V_{54}	5.45 – 30.59	15.80±1.50bc
V_{55}	4.95 – 24.67	07.80±1.05d
V_{64}	6.50 – 25.37	16.60±0.90b
V_{66}	1.58 – **31.71**	07.40±0.60d
V_{69}	0.88 – **31.40**	14.40±0.90bc
V_{73}	4.99 – 23.77	03.40±0.65e

Data are represented as means±SEM (n=3). Mean with different letters in the same row are statistically different ($p < 0.05$) according to Duncan's test. *Legend:* In bold, highest values; underlined, the weakest.

Table 2: Physicochemical characteristics of cassava starches

Varieties	Moisture (%)	pH	Amylose (%)	Amylopectin (%)
V_4	12.95±0.30a	5.69± 0.21a	**25.31±2.50a**	74.69±3.56e
V_{54}	13.55 ±0.26a	4.83± 0.10a	14.20±1.98f	**85.80±2.23a**
V_{55}	13.30±0.53a	5.64 ±0.04a	17.37±2.54d	82.63±2.44bc
V_{64}	13.20±0.62a	5.80±0.10a	16.90±2.30d	83.10±1.76b
V_{66}	13.75±0.33a	5.57±0.11a	**20.86±2.36b**	79.14±2.09d
V_{69}	13.50±0.33a	5.66±0.13a	16.62±2.09e	82.38±2.45bc
V_{73}	12.80±0.31a	5.43±0.31a	19.03±2.45c	80.97±2.78c

Data are represented as means±SEM (n=3). Mean with different letters in the same row are statistically different ($p < 0.05$) according to Duncan's test.. *Legend:* In bold, highest values; underlined, the weakest. Amylose and Amylopectin content are based on dry mater.

Functional properties : Concerning the swelling power (SP) as function of temperature, all the starches showed the same profile of Gaussian curve, with a peak at 80 °C for variety V_{66} and at 85 °C for all the other varieties (Figure 2A). However, the Duncan's test revealed significant (F=76659.714; p=0.0001) differences concerning the maximum of SP of the seven varieties of cassava starches (Figure 2B). In fact, variety V_{55} recorded the most important SP (30.19±3.00) in opposition to V_4 (24.53±2.50 g/g). The others varieties registered intermediary values ranging from 25.98±2.63 g/g (V_{54}) to 28.26±2.30 g/g (V_{64}).

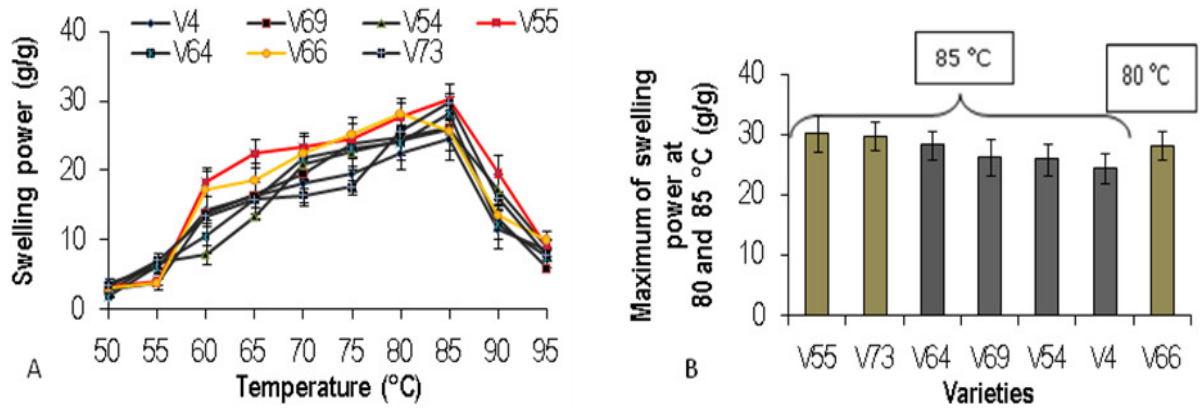

Figure 2: Cassava starch swelling power evolution as function to the temperature and to the varieties. *Legend:* **A** – Swelling power evolution, **B** – Maximum of swelling power

The solubility profile (continuous increasing) was the same for all the varieties (Figure 3A). Starch solubility went increasing from 50 to 95 °C with two phases. From 50 to 70 °C, starches solubility rose more slowly than it did from 70 to 95 °C, for all the varieties. However, the maximum of solubility (at 95 °C) significantly (F=1473.085; p=0.0001) varied from a starch to another (Figure3B). Hence, variety V_{55} topped the highest value (8.44±0.65 %) while V_{73} had the lowest (6.04±0.83 %) at the same temperature (95°C).

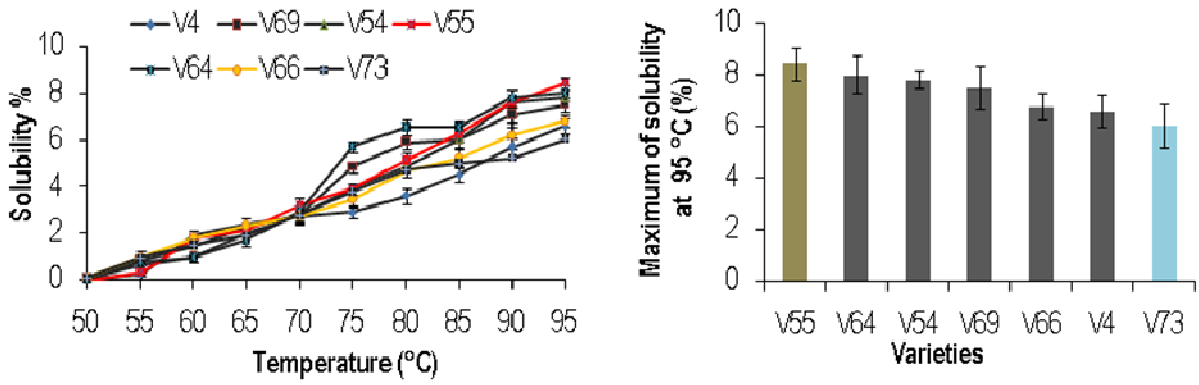

Figure 3: Cassava starch solubility evolution as function to the temperature .Legend: **A** – Solubility evolution, **B**- Maximum of solubility

The water absorption capacity (WAC) also showed a significant (F=4.281; p=0.012) difference among starches varieties (Figure 4). Indeed, the highest value was registered by V_{55} (119.94±0.57 %) and the slightest by V_4 (107.08± 0.50 %) followed by V_{54} (118.24±0.46 %). The others varieties obtained intermediary values ranging from 108.04±1.40 % (V_{66}) to 118.24±0.46 % (V_{54}).

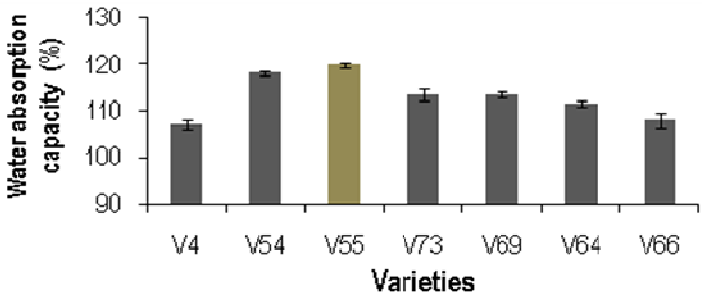

Figure 4: Cassava starches water absorption capacity the varieties

About gel syneresis at -15 and +4 °C, the starches registered 0.00 % the first day (J_0), then the syneresis increases continuously from the first week to the fourth one (Figure 5A and 5B). Nevertheless, varieties V_{54}, V_{64} and V_{73} kept their very low syneresis (0.01 %) constant during the same period at +4 °C. V_{54} showed the same behaviour at -15 °C. Concerning variety V_{55}, comparatively to varieties V_4, V_{66} and V_{69} which syneresis values increased more rapidly from J_0 to the fourth week, its syneresis rose very slightly (0.00 to 2.10 %) at +4 °C during the same period and from J_0 to the first week (0.00 to 1.39±0.05 %) at -15 °C.

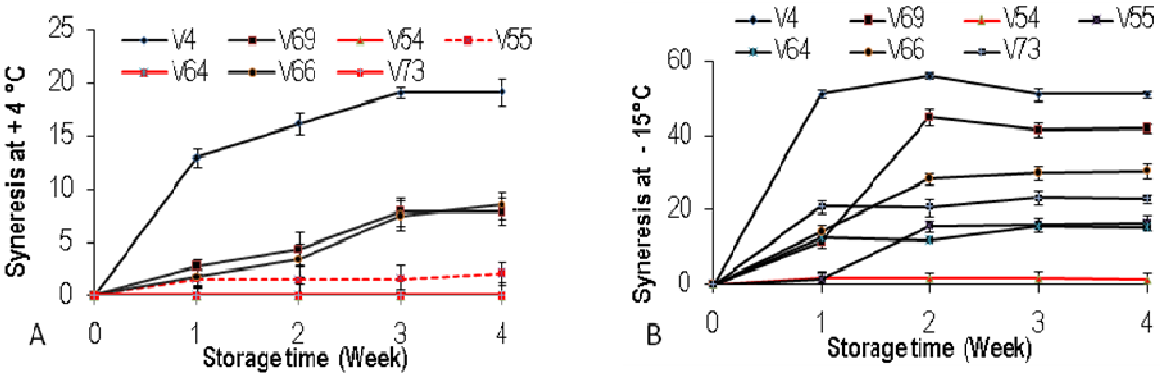

Figure 5: Evolution of the cassava starches gel syneresis during storage (A): =4°C, (B):-15˚C

As far as gels clarity, all the varieties recorded their highest values at J_0, and then decreased continuously until the fourth week (Figure 6A). It is worth underlining the varieties V_{54} and V_{64}, which presented the most important value of clarity at J_0 and kept this same importance from the first to the fourth week. Moreover, the Duncan's test showed a significant (F=789.258; p=0.0001) difference between varieties clarity at J_0 (Figure 6B). In fact, variety V_{54} topped 78.54±3.30 % when V_{55} registered 38.51±3.05 %. The other values ranged from 41.73±4.70 % (V_{69}) to 76.27±6.72 % (V_{64}).

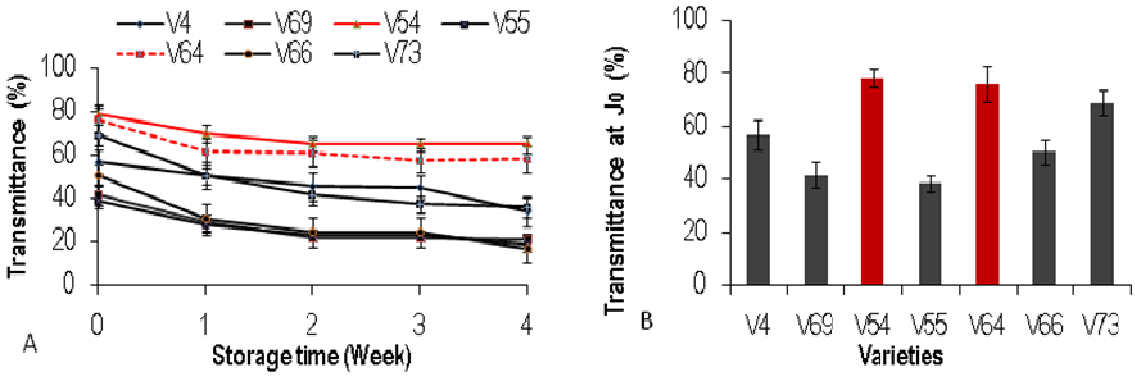

Figure 6: Evolution of gels clarity during storage at +4°C (A) and Gel clarity of the cassava starch varieties at J_0 (B)

DISCUSSION

Nowadays the food and non-food industry is faced with the challenge of providing quality foods and non-food products. For a product like starch, which is used as a basic material in many industries, quality can only be defined with reference to the end use. This exigency is generally referred to its functional and physicochemical properties (Aviara *et al.*, 2010; Garsetti *et al.*, 2005). Hence, higher starch swelling power is better for its involvement in food as thickeners and binding agent and in non-food industries as adhesive and pastes glues (Sanni *et al.*, 2005). Indeed the loss of free water due to enormously swollen granules occupying more space contributes to improve the texture and the consistency of the starch heating system (Berry, 1997; Aviara *et al.*, 2010). Cassava starch varieties V_{55}, V_{66} and V_{73} had strong swelling power at relatively low temperatures (80 °C and 85 °C) comparatively to corn starch usually employed as a raw material for manufacturing glues or pastes adhesives at higher temperature (more than 100 °C), could then be recommended for that purpose (Sanni *et al.*, 2005). It is important to note that these starch varieties contained not only the highest amount of large granules but also the most important amylose content. These physicochemical properties would justify their behaviour in water, comparatively to the other varieties (Massaux *et al.*, 2006; Nuwamanya *et al.*, 2011). Furthermore, these cassava varieties could be suitable for preparations, which require precise temperatures of cooking. For instance, the variety V_{66} could be

recommended as a composite additive to potato flour or starch as well as texturing and binding agent in the preparation of sausages with giblets whose precooking is made at 80 °C (Hendrick *et al*., 1994). The high swelling power of varieties starches V_{55}, V_{66} and V_{73} could be explained, according to Singh *et al*. (2003) and Wickramashinge *et al.* (2009) by the amount of large (> 10 µm) granules (for the whole varieties) and amylose. Indeed, water swelling (in heating condition) would be as higher as amylose content would be higher. This situation is confirmed by the results of the present study though the previous varieties registered the highest values of water swelling and amylose. Moreover, the two phases of solubility of starch varieties would suggest and underline the relative strongest of granules intra molecular structure due to the high stability of starch amylopectin structure (Graffham *et al*., 2000) mostly in the starch varieties V_{64}, V_{54} and V_{69}. It is not worth précising here that these varieties contained more amylopectin and small granules than the other did, what would suppose an important crystalline structure, and would confirm that small granules are more robust than large ones (Massaux *et al*., 2006). About starch granules size, it should be essential for the cosmetic and pharmaceutical industries since the starch with higher small granule amount, could be used as a dusting agent in cosmetic and pharmaceutical industries (Massaux *et al*. 2006). According to the same authors, large granules would be better in plywood industry for plastic film manufacturing, so that the films would be biodegradable. Hence, based on their granules sizes, starch varieties of V_4, V_{54} and V_{64} could be used in the cosmetic and pharmaceutical industries as dusting agent, while V_{55}, V_{66} and V_{73} could be involved in plastic films. It would be worth recalling that all the cassava starch varieties also present interesting (more than 100%) swelling capacity at ??? ambient temperature. In fact, the increase in swelling capacity is indicative of suitability of a starch being used as a disintegrant agent in pharmaceutical industry (Chowdary, 2011). This property is generally exploited in potatoes (72.2 to 89.6 %) and corn (82.1 % to 97.7 %) starches (Kim *et al*., 1995; Zuluaga *et al*., 2007). Hence, it would be better to exploit the whole cassava starch in the tropical pharmaceutical industries, to reduce importations costs of potato and corn starch. Starch gel Syneresis is a parameter that represents the volume of water separated from the formed gel under storage at room temperature, refrigeration and freezing. This parameter characterizes the starch stability to these temperatures (Salwa *et al*., 2010). The lowest syneresis (highest resistance to freeze-thawing cycle) is obtained by gels of varieties V_{54}, V_{55}, V_{64} and V_{73} with negligible water exuded under refrigeration and freezing. This shows a very stable behaviour of these gels under storage quite similar to those obtained with waxy starches (maize, rice and cassava) which improved freeze–thaw stability compared to normal starches (Sanchez *et al*., 2010). So, these cassava starches varieties with such performance could be proposed as a substituent to waxy starches or other modified starches, which generally result from genetics manipulations and are expensive. However, it is important to notice that, contrary to the results of Schmitz *et al*. (2006) and Davies *et al*. (2008), no significant relation appeared between amylose content and syneresis, since varieties V_{54} and V_{64} got at far less amylose than varieties V_{55} and V_{73} but all of them adopted the same syneresis profile. This may be due to the higher weight-average molecular weight of the amylose fraction in cassava starches (Susuki *et al*., 1985) delaying the retrogradation. The latest phenomena would also be evaluated by gel clarity which would denote the starch purity (Masamba *et. al*., 2001; Nuwamanya *et al*., 2011), allowing its utilization in food and non-food products without interfering with their color and shelf life. The high clarity of the whole starch and the relative stability (for four weeks) of varieties V_{54} and V_{64} gels at +4 °C would confirm the aptitude of cassava starch to being utilized for its bright clarity (Masamba et. al., 2001). This aptitude of clarity stability (V_{54} and V_{64}) could be due to their lowest amylose contents. Hence, if all the starch varieties could be exploited in the textile industry, varieties V_{54} and V_{64}, might be used in food industry in creams, syrup and other preparations where high clarity is required (Sanni *et al*., 2005; Jyothi *et al*., 2007). In addition to their functional properties, other properties such as moisture, pH and amylose content are also importants as they dispose starch to typical utilization under specific exigencies. Hence, the moisture content of the seven cassava starch varieties, which was within recommended range (10 to 20 %) for starches (Soni *et al*., 1993), might allow them for commercialization. Moreover, the pH values obtained by these cassava varieties might permit their use in the pharmacy, cosmetic and food industry (Nnemeka *et al*., 2009) where required pH is 3 to 9.

CONCLUSION

The whole cassava starch varieties would be apt to being exploited in pharmaceutical industry as disintegrant for tablets because of their strong water absorption capacity which was at far superior to those of potato and corn starch.. However and preferentially, varieties V_4, V_{54} and V_{64} might be chosen for their highest amount of small granules which would permit their employment as dusting agent not only for pills but also for some cosmetics. Varieties V_{55}, V_{66} and V_{73} could interest the chemical industrial for glue and detergent manufacturing due to their high swelling power/solubility induced by their high amount of both amylose and large granule. Considering the high proportion of large granules, they could also be exploited in plywood industry for biodegradable plastic films confection. Varieties V_{54}, V_{55}, V_{64}, V_{66}, and at least V_{73} could be involved in preparation which might be stored at fridge temperature 4 °C for the whole starches and in freeze (-15 °C) for V_{54}, due to their low and relatively stable syneresis and their high clarity. These varieties could then occur as raw material in the food industry as, thickeners texturing and binding agents, they could be exploited in textile industry due to the high clarity.

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