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Research Article

PHYSICOCHEMICAL PROPERTIES OF STARCH FROM YOUNG GROWTHS OF *BORASSUS AETHIOPUM*

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ABSTRACT: The characterization of *Borassus aethiopum* starch showed that the crude protein (0.18 %), total lipid (0.21 %), ash (0.09 %) and the moisture (1 %) were typical of most starches The amylose content (26.31 %) falls within the apparent amylose range 17-30 %). The granular structure of *Borassus aethiopum* young growths starch showed significant variations in size and shape. Most of the granules are oval, although spherical, round, elliptical. The starch exhibited swelling power and solubility behaviors which were dependent on temperature. The maximum solubility and swelling power were obtained at highest temperature of 95 °C. The syneresis of starch paste was 78.58 % the first day and increased to 83.14 % at the 28 th day while the clarity decreased from 56.53 to 25.07 % during the same period. The optimum pH of enzymatic hydrolysis of *Borassus aethiopum* starch by the digestive juice of snail *Archachatina ventricosa* was pH 5 while the optimum temperature was 45 °C. The influence of gelatinization time on the enzymatic hydrolysis of gelatinized starch showed that the hydrolysis extent increases with the time of gelatinization up to 4 minutes then it does not vary enough whereas the duration of gelatinization is prolonged. The hydrolysis extent of gelatinized starch by the digestive juice of snail *Archachatina ventricosa* was 70.6 % after 2 hours of incubation.

Key words: *Borassus aethiopum*, *Archachatina ventricosa,* starch properties.

INTRODUCTION

Exploited for a long time, the palm tree pushes naturally in the areas where it rains less. In general, the palm tree (*Borassus aethiopum*) is used in the food (the fruit, sap, young growths) or like building material (the feather-grass, sheets) by the population. It is also useful in the pharmacopeia (roots and sheets). The sheets are used especially for manufacture of a multitude of objects of decoration (of sweep, the baskets, the fences). The palm wine plays a significant role in the food, the incomes and the social life of the population (Keay, 1989). The starchy young growths from 6 to 8 weeks are also consumed and the starch which they contain is not valorized. The native starch granule is mainly made up of two glucose polymers called amylose and amylopectine. Amylose is an essentially linear starch polymer of α-(1-4) linked D-glucopyranosyl units with very few α-(1-6) branch points (Hizukuri et al., 1981; Buléon et al., 1998). It has a degree of polymerisation (DP) in the range of 500–6000 glucose residues. Amylose can form inclusion complexes with lipids, alcohols and iodine (Zobel, 1988). With iodine, a coloured complex is formed, with colour intensity and wavelength for maximal extinction (λmax) depending on the concentration and average chain length of amylose (Banks et al., 1971). While Amylopectin is, in general, the major fraction of starch, with levels ranging from 75 to 85% for normal starch to even more than 99% for waxy varieties, which are essentially amylose free (Singh et al., 2003). The starch can be used to manufacture various products such as the foodstuffs, the textiles, the adhesives and in pharmaceutical and chemical industries (Olorunsola et al., 2012). The aim of the present study was to determine the physico-chemical properties of *Borassus aethiopum* starch extracted from young growths.

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MATERIAL AND METHODS

Enzymes

The enzymatic source was the digestive juice of snail *Archachatina ventricosa*. The snails are grown in the University of Nangui-Abrogoua (Côte d'Ivoire).

Young growths of Borassus aethiopum

They are cultivated in the center (Didievi) of the Côte d'Ivoire.

Chemical composition

Quantitative evaluation of moisture, crude protein, lipid and ash of the starch were determined using standard methods of AOAC (2000). The nitrogen content was determined by the Kjeldahl method and the crude protein was estimated by multiplying the nitrogen content by 6.25. The lipid was extracted with petroleum ether in a Soxhlet extractor.

Amylose content

The amylose content of starch was determined according to the method of William et al., (1970) using O.5 N KOH solution, 30 g of starch, 0.1 N HCL and 0.5 mL of iodine reagent. The absorbance value measured at 625 nm with spectrophotometer (spectra UV-Visible). The amylose content in the starch was determined using a derived standard formula:

$$\text{Amylose content (\%)} = (85.24 \times A) - 13.19$$

Where A = absorbance value.

Extraction of starch

The extraction of the starch was made according to the method of Banks and Greenwood (1975) modified by Amani (1993).

The young growths of *Borassus aethiopum* were washed, peeled and cut out in sections then soaked in a sodium bisulfite solution 0,4 % (p/v) to avoid the tanning. They were crushed in a mixor. The broyat obtained was included in a sodium chloride solution (4 %, p/v) to burst by osmosis the vegetable cells which imprison the last starch then through a series of sieve whose meshs are respectively 500, 250 and 100 µm diameter. The starch milk thus obtained alternatively was elutriated and washed. The deposit collected was spread out over aluminum foil to be dried at 40°C in a drying oven. The dried starch was crushed with the mixor then was preserved at the drying oven at 40°C.

Extraction of digestive juice of snail *Archachatina ventricosa*

The digestive juice of the snail *Archachatina ventricosa*, was extracted from three days unfed snails. The shell was broken and the digestive tube was isolated. The digestive juice was collected in the erlenmeyer by successive pressions on the digestive tube, centrifuged (10 000 g, 30 min, 4°C) and the supernatant was conserved at 4°C.

Microscopic analysis of the raw starch

All starch grains were washed two times with pure ethanol before microscopic analysis. After air dried, they were examined by scanning optical microscopy (mark Ceti) at an adequate magnification. The microscope is controlled by a computer via the kappa software that allows direct pictures taking.

Swelling power and solubility

Swelling power and solubility of the starches were determined by the method of Leach et al, (1959) using a 1% aqueous suspension of starch.

Light transmittance (Determination of clarity)

A suspension of 1% (w/v) starch is heated in boiling water bath for 30 minutes with gentle agitation. The gel obtained is distributed in 15 tubes and kept refrigerated but 3 tubes cooled to room temperature to determine the clarity zero day (Jo) by reading at 650 nm of gel starch transmittance (%T) against a white represented by distilled water (Craig and al,1989).

Syneresis

A suspension of 1% (w/v) starch was heated in boiling water bath during 30 minutes with gentle agitation. The gel was left in 15 centrifuge tubes and stored in the refrigerator but 3 tubes cooled to room temperature to calculate syneresis zero day (Jo). The syneresis is determined by the proportion (%) of the water collected after centrifugation (ALRESA) of the tubes at 3000 rev/min during 30 minutes on the mass of the gel according to the following formula (Zhen and al, 1998).

$$\text{Syneresis (\%)} = M_1 \times 100 / M$$

M_1: Mass of water collected after centrifugation

M: Mass of the gel

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Protein concentration assays

Protein concentration was measured with Lowry method (Lowry et al., 1951) using bovine serum albumin as standard.

Amylase activity

The amylase activity was assayed by measuring the reducing sugar released during the reaction by the dinitrosalicylate (DNS) method of Bernfeld (1955). The reaction mixture (200 µl) in 0.05 M acetate buffer (pH 5.0) contained 0.1 mL of 1% starch and 50 µl of enzyme solution. The mixture was incubated at 37°C for 30 min. The enzymatic reaction was then stopped by the addition of 300 µl of dinitrosalicylic acid solution. After 5 min heating at 100°C for the color development, the resulted samples were chilled to room temperature and then diluted with 3.0 mL distilled water. The absorbance at 540 nm was then measured. One unit of α-amylase activity was defined as the amount (µmol) of reducing sugar released by minute under standard assay conditions.

Effect of pH on enzyme activity

The pH optimum of the enzyme was determined by varying the pH of the assay reaction mixture using the following buffers: sodium acetate buffer 100 mM, (pH 3.6-5.50); phosphate buffer 100 mM, pH (5.6-8.0); citrate buffer 100 mM, pH (3.5-8.0). The residual activity was determined as described earlier.

Effect of temperature on enzyme activity

The temperature optimum of the enzyme was evaluated by measuring the amylase activity at different temperatures (30-60 °C) in 0.1 M sodium acetate buffer pH 5.0. The residual activity was determined as described earlier.

Effect of starch concentration on amylase activity

Effect of *Borassus aethiopum* starch concentration on hydrolysis was studied by varying its concentration from 1 % to 10 % (w/v) in the reaction mixture (total volume of 10.0 mL) containing the digestive juice of snail *Archachatina ventricosa* by shaking at 120 rpm at 37°C. To determine the extent of starch hydrolysis, end products were measured as described above.

Effect of gelatinization time on starch hydrolysis

Starch was gelatinized at 100 °C during 1;2;4;6;8 and 10 minutes in the suitable buffer. After cooling, the reactional medium made up like was previously described.

Starch hydrolysis

The reaction mixture (total volume of 10.0 mL) containing 1 % of gelatinized starch and 5.0 mL of enzyme solution (4.5 U) in 0.1 M sodium acetate buffer pH 5.0 was incubated by shaking at 120 rpm at 37 °C. With interval of regular time of 5 min aliquots (200 µl) were taken and the reducing sugars were quantified as describe above.

RESULTS AND DISCUSSION

Chemical composition of young growths starch of Borassus aethiopum

The crude protein, total lipid, ash and the moisture of the *Borassus aethiopum* young growths starch were 0.18 %, 0.21 %, 0.09 % and 1 % respectively. The chemical composition of *Borassus aethiopum* starch is typical of most starches (Amani 2004; Barminas et al., 2006, sidibe et al., 2007). The amylose content (26.31 %) falls within the apparent amylose range 17-30 % (Spence and Jane 1999) and would indicate that *Borassus aethiopum* starch would be rich in amylopectine. The widely varying amylose contents might be due to different methods used for amylose determination, cultivar differences, climatic conditions during the growth, soil type and to the physiological state of the biological material (Hoover and Sosulski, 1991). It can also depend on the activities of the enzymes involved in the biosynthesis of linear and branched components within the starch granules during growth of plant.

Granule Size and Microscopic Appearance

The granular structure of *Borassus aethiopum* young growths starch showed significant variations in size and shape (Figure 1). Most of the granules are oval, although spherical, round, elliptical and irregularly shaped granules are also found. When observed under a scanning electron microscope, the surfaces of starch granules appear smooth with no evidence of any fissures. It was also observed few small granules.

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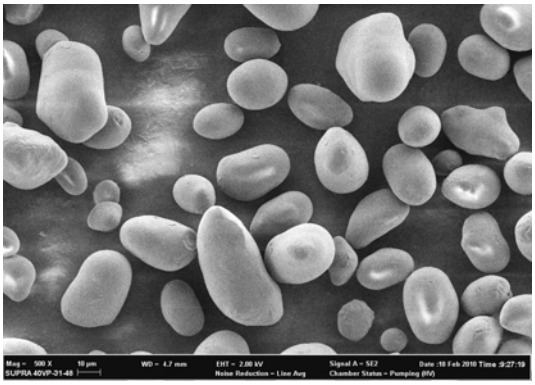

Figure 1: Scanning electron micrograph of starche of young growths of *Borassus*

Aethiopum

Adejumo et al., (2011) established a classification of the size of starch granule according to which the granules of small size have a diameter lower than 10 µm and that of large granules is higher than 25 µm. The granules of medium size have a diameter ranging from 10 to 25 µm. Thus, *Borassus aethiopum* starch has three classes of granules (figure 2).

The variations in size and shape of the starch granules may be due to their biological origin. The morphology of starch granules depends on the biochemistry of the chloroplast or amyloplast as well as on the physiology of the plant (Badenhuizen et al., 1969).

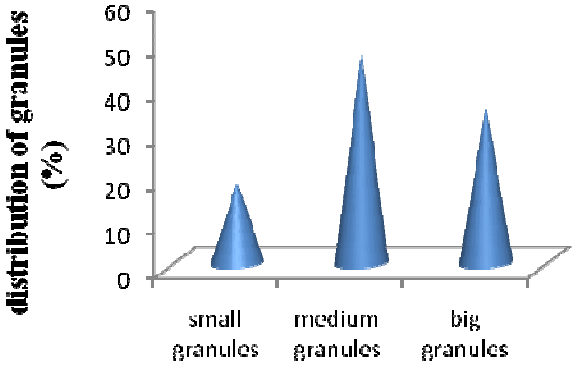

Figure 2: Percentage of distribution of starch granules of young growths of *Borassus aethiopum*.

Swelling power and solubility

The swelling power and solubility of *Borassus aethiopum* starch as a function of heating temperatures ranging between 50 and 95 °C (Figure 3). The result indicated that the starch exhibited practically no swelling between 50 and 65 °C, while the maximum swelling was obtained between 80 and 95 °C. The relatively high swelling power of the young growths *Borassus aethiopum* starch is most probably due to the low percentage of lipid in the starch and by extension, the near absence of amylase lipid complex (Galliard and Bowler, 1987). Phospholipids are known to form water-insoluble complexes with amylose during heating. These complexes severely restrict granular swelling and maintain the integrity of swollen granules thereby limiting the swelling behavior of the starch (Galliard and Bowler, 1987). Hence, the decrease in the rate of swelling power suggests a loss of starch granule integrity after successive swelling (Srichuwong et al., 2005). In general, when starch is heated in excess water, the crystalline structure is disrupted (due to breakage of hydrogen bonds) and water molecules form hydrogen bonds to the exposed hydroxyl groups of amylose and amylopectin. This causes an increase in granule swelling and solubility. Swelling power and solubility provide evidence of the magnitude of interaction between starch chains within the amorphous and crystalline domains. The extent of this interaction is influenced by the amylose/amylopectin ratio, and by the characteristics of amylose and amylopectin in terms of molecular weight distribution, degree of branching, length of branches and conformation of the molecules (Hoover and Hadziyev, 1981).

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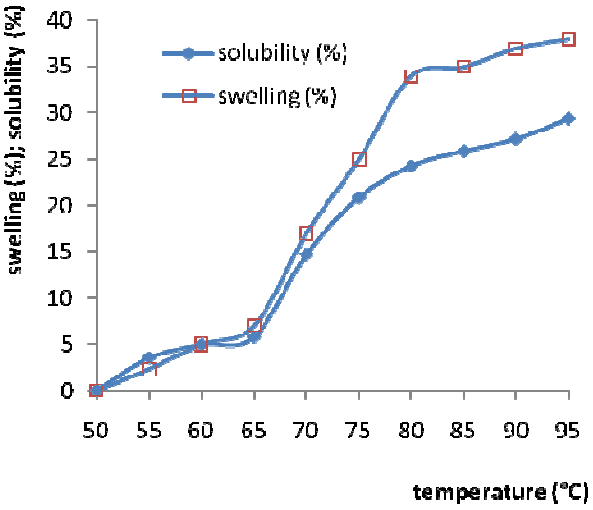

Figure 3: Swelling and solubility of *Borassus aethiopum* starch at different temperatures.

The retrogradation

The syneresis of starch paste was 78.58 % the first day and increased to 83.14 % at the 28th day (Figure 4) while the clarity decreased from 56.53 to 25.07 % during the same period (Figure 4). The lower syneresis exhibited by the starch paste of young growths of Borassus can be attributed to its lower amylose content. Amylose aggregation and crystallization have been reported to complete within the first few hours of storage while amylopectine aggregation and crystallization occur at later stages (Ortega-Ojeda and Eliasson, 2001). The retrogradation properties of starches are also indirectly influenced by the structural arrangement of starch chains within the amorphous and crystalline regions of the ungelatinized granule, because this structural arrangement influences the extent of granule breakdown during gel storage (Perera and Hoover, 1999).

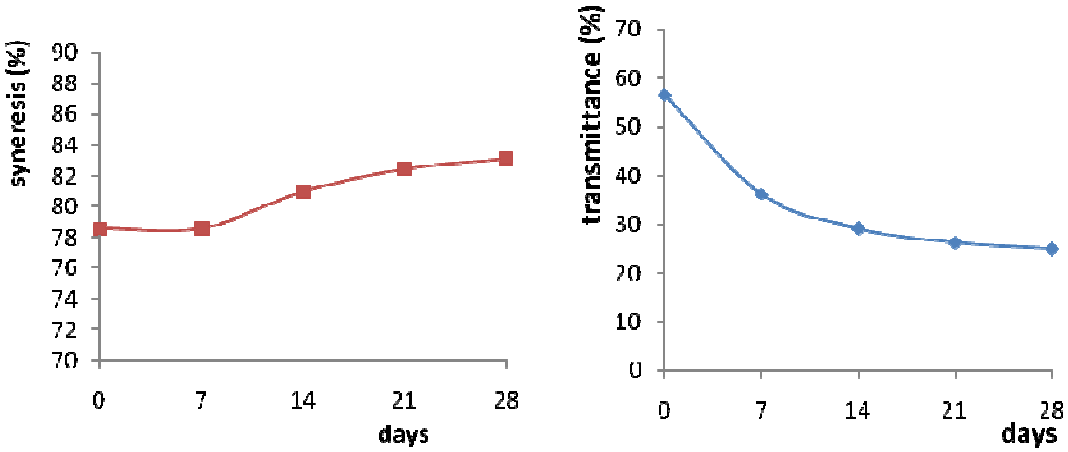

Figure 4: Syneresis (%) and transmittance (%) pattern of starch paste of young growths of *Borassus aethiopum*

The initial gel firmness during retrogradation can be due to the formation of an amylose matrix gel and the subsequent slow increase in gel firmness to reversible crystallization of amylopectine (Ring et al., 1987). During retrogradation, amylose forms double helical associations of 40-70 glucose units whereas amylopectine crystallization occurs by association of the outermost short branches (DP=15) (Leloup et al., 1992).

Young growths starch showed high transmittance values initially. This may be due to the presence of fewer granule remnants in the starch paste which in turn depends on the morphological structure of starch granules (Craig et al., 1989).

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Effect of pH on the enzymatic hydrolysis of gelatinized starch

Optimum pH of enzymatic hydrolysis of the starch of the young growths of *Borassus aethiopum* was pH 5 (Figure 5). The optimum pH of starch hydrolysis was in the range (pH 4-7) reported for most of amylases (Leveque et al. 2000).

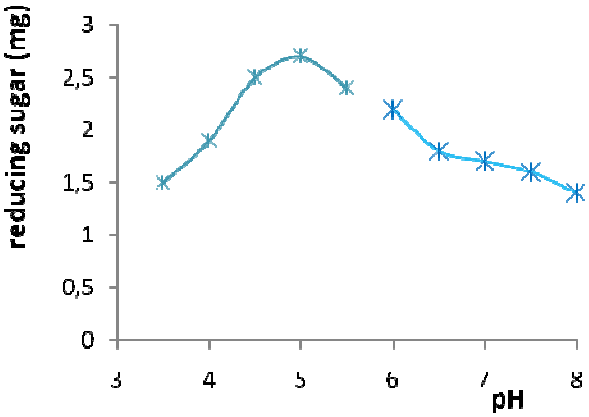

Figure 5: Effect of pH on the enzymatic hydrolysis of gelatinized starch of young growths of *Borassus aethiopum* by digestive juice of snail *Archachatina ventricosa*

Effect of temperature on the enzymatic hydrolysis of gelatinized starch

The optimum temperature of enzymatic hydrolysis of the starch of the young growths of *Borassus aethiopum* by the digestive juice of snail was 45 °C (Figure 6). This optimal temperature was much lower than those of amylases of *Pyrodyctium abyssi* (100°C) (Siquiera et al., 1997). However, it optimal temperature was higher than that of *Nocardiopsis sp.7326* (35°C) (Zhang and Zeng, 2008).

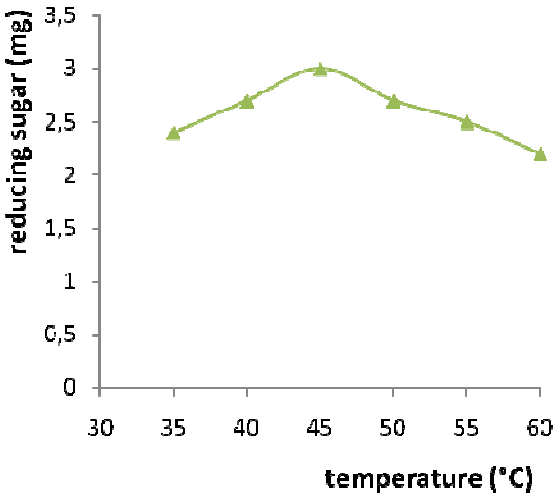

Figure 6: Effect of temperature on the enzymatic hydrolysis of gelatinized starch of young growths of *Borassus aethiopum* by digestive juice of snail *Archachatina ventricosa.*

Effect of gelatinization time on the enzymatic hydrolysis of gelatinized starch

The influence of gelatinization time on the enzymatic hydrolysis of gelatinized starch was represented by figure 7. The hydrolysis extent increases with the time of gelatinization up to 4 minutes then it does not vary enough whereas the duration of gelatinization is prolonged. This time of gelatinization is the same one as that of the starch waxy of barley but lower than those of the starches known as normal of the barley which are 7 and 12 minutes respectively (Marc Gregor et al., 2002).

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Starch, when heated in the presence of excess water, undergoes an order to disorder phase transition called gelatinization over a temperature range characteristic of the starch source. This phase transition is associated with the diffusion of water into the granule, water uptake by the amorphous background region, hydration and radial swelling of the starch granules, loss of optical birefringence, uptake of heat, loss of crystalline order, uncoiling and dissociation of double helices (in the crystalline regions) and amylose leaching (Jenkins , 1994). According to Jenkins, (1994), gelatinization is primarily a swelling driven process. This swelling acts to destabilize the amylopectin crystallites within the crystalline lamellae, which are ripped apart (smaller crystallites are destroyed first) during the process. This process occurs rapidly for an individual crystallite, but over a wide range for the whole granule. The gelatinization and swelling properties are controlled in part by the molecular structure of amylopectin (unit chain length, extent of branching, molecular weight, and poly-dispersity), starch composition (amylose/amylopectin ratio, lipid complexed amylose chains, and phosphorus content), and granule architecture (crystalline/amorphous ratio) (Tester,1997).

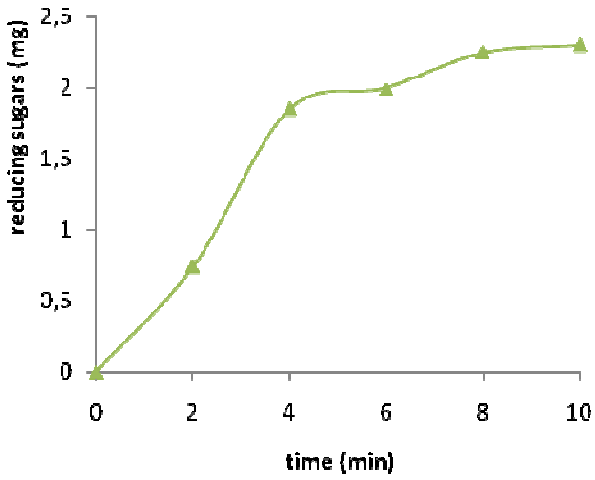

Figure 7: Effect of gelatinization time on the enzymatic hydrolysis of gelatinized starch of young growths of *Borassus aethiopum* by digestive juice of snail *Archachatina ventricosa.*

Effect of starch concentration on the enzymatic hydrolysis

The rate of hydrolysis grows as the starch concentration increases. From the starch concentration of 2.5 %, this percentage seems constant (Figure 8).

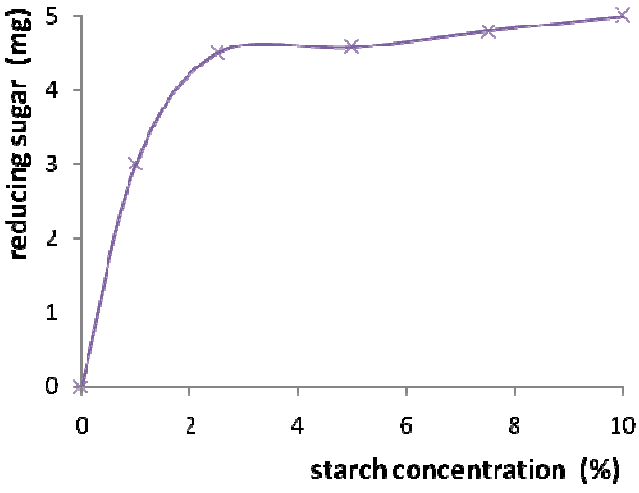

Figure 8: Effect of *Borassus* starch concentration on the activity of the digestive juice of snail *Archachatina ventricosa*

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Enzymatic hydrolysis of *Borassus aethiopum* starch

The hydrolysis extent of gelatinized starch by the digestive juice of snail *Archachatina ventricosa* was 70.6 % after 2 hours of incubation (Figure 9).

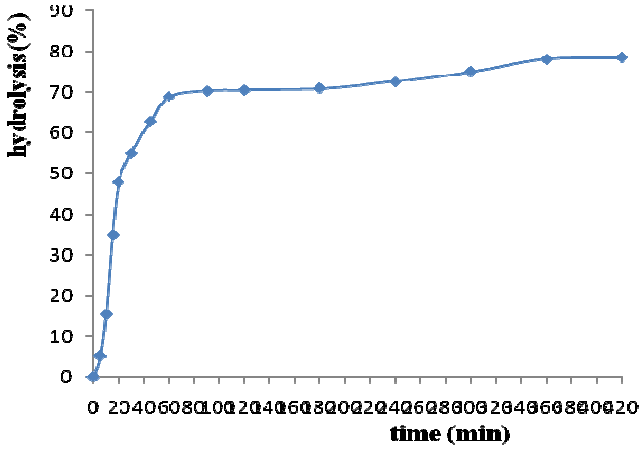

Figure 9: Hydrolysis of *Borassus aethiopum* starch by the digestive juice of the snail *Archachatina ventricosa*

Starch is one of the most abundant industrially produced polymers. But native starches are not widely utilized in the food industry due to their poor functional properties such as poor thermal, shear and acid stability, and high rates and extent of retrogradation (Jayakody and Hoover, 2008). Starches functionalities are been boosted by the ability to modify starch granules chemically, genetically and enzymatically. Therefore, most starches currently incorporated into foods are modified. Some literatures have reported that starch degrading enzymes have been used to modify the physicochemical properties of polysaccharides to achieve the desired functional properties (Olivia et al. 2010). The most important tool in providing a saccharide with a specific composition is the use of starch hydrolyzing enzymes. In the conventional starch processing industry, the starch is gelatinized and then it is hydrolyze by the amylases to produce glucose, maltose or a mixture of malto-oligosaccharides which have significant industrial uses (Goyal et al. 2005; Azad et al. (2009)).

This is possible because when starch is gelatinized the semi-crystalline nature of granules becomes totally amorphous and the starch becomes digestible by amylases (Hoover and Zhou, 2003; Konsula and Liakopoulou-Kyriakides, 2004; Tester et al., 2004a,b).

CONCLUSION

In this work, we have determined physico chemical components, the rheology of the starch young growths of *Borassus aethiopum* and the best conditions for its enzymatical hydrolysis. The result indicated that much more reducing sugar was released from gelatinized starch by action of the digestive juice of snail *Archachatina ventricosa* and there is also a link between gelatinization time and degradation. Thus, the *Borassus aethiopum* fresh paste would have potential applications in starch processing.

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