



# **Effect of Annealing, Acid Hydrolysis and Branching Enzyme on *Dioscorea schimperiana* Starch Technological and Functional Properties**

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## **Authors' contributions**

*This work was carried out in collaboration between all authors. Author DAW designed the study, wrote the protocol, and wrote the first draft of the manuscript. Authors DDFF and LMS managed the analyses of the study. Author GI oriented the data analysis and validated the final writing of the paper. All authors read and approved the final manuscript.*

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## **ABSTRACT**

**Aims:** To assess its technological aptitude and functional properties, *Dioscorea schimperiana* starch was submitted to various treatment of technological importance and its properties was evaluated.

**Methodology:** For this aim, the starch extracted from *Dioscorea schimperiana* tubers and was submitted to annealing, acid hydrolysis and to a branching enzyme (1, 4- $\alpha$ -glucan branching enzyme). Afterward, Fourier-transform infrared spectroscopy (FTIR), gelatinization profile, physicochemical and functional properties of the samples was recorded.

**Results:** FTIR spectra show the introduction and withdrawal of bond in modified samples. The thermal properties (DSC) of starch were not affected by annealing (AS) and enzymatic treatment (EBS). No peak temperature and gelatinization profile were observed for acid hydrolyzed samples (AHS) on Rapid Visco Analyzer. Annealing and enzyme treatment lead to an increase of the starch peak viscosity of while reducing its breakdown. The functional properties of the starch such as

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swelling capacity, least swelling concentration and water binding capacity were increased by annealing. Acid hydrolysis significantly increases *in vitro* digestibility of *D. schimperiana* starch while no significant change was observed after annealing and enzymatic modification, thus presenting it as particularly resistant to digestion.

**Conclusion:** This study suggests that annealing can be considered for the production of *D. schimperiana* modified starch with high technological and functional properties.

**Keywords:** Starch; modification; technological properties; functional properties; *in vitro* digestibility.

## 1. INTRODUCTION

Starch is used as ingredient in the manufacturing process of various products. It affect texture, consistency, moisture, capacity of gel formation, film-forming behavior, homogeneity and adhesion [1]. In the food domain, starches are used as substitutes for the production of low-calorie foods, biodegradable packaging materials and thin films [1]. Native starches are considered as pure starch forms, obtained from various sources such as wheat, cassava and potato. Considering their chemical structure, these are long-chain carbohydrates insoluble in cold water but with a high swelling capacity when exposed to heat.

Due to the poor technological aptitude of native starch such as breaking down in acidic environments or when heated, the food manufacturers are more interested in starches with improved capacity, commonly known as modified starches. In 2018, the market of modified starch was estimated at USD 10.35 billion and it is projected to reach USD 12.67 Billion by 2023 [2]. The market is driven by the rise in demand for starch with high viscosity, stability to heat, acid, and shear, heat penetration and workability [3]. Several techniques such as physical (heat-moisture treatment, annealing, pre-gelatinization, high-pressure treatment, radiation, and sonication), chemical (cross-linking, substitution, acid hydrolysis, oxidation/bleaching) or enzymatic processes have been developed to produce this type of starch. Since starches that resist heating and acid treatments are highly appreciated, techniques such as annealing or acid hydrolysis were frequently employed [4]. However, in recent decades, enzyme modifications is highly appreciated for the production of functional starch for food domain since enzymes are consider safer and healthier for both the environment and consumers [5].

To meet the ever-increasing demand for starch from industries, research is nowadays focused

on unconventional sources. Yams are presented as an interesting alternative for this reason. Yam occupies an important position in the economic and social life of people living in Africa. It is estimated to comprise about 600 species, of which about 50 are cultivated for food [6]. As of 2017, global yam production was estimated to be about 72 million tons of which about 95% was grown in Africa, with Cameroon, being part of the ten highest producers [7]. Apart from being an economic cash crop and staple food crop in Africa, yam could serve as a sustainable source of starch for industrial uses due to their high starch content. This is particularly true for under-consumed and neglected species such as *Dioscorea schimperiana* in Cameroon.

*D. schimperiana* which is one of the eight yam species grown in Cameroon is not sufficiently exploited. A part of the tubers of this species is mostly eaten during times of scarcity. The rest of the tubers is left abandoned in the attics where they dry out or rot [8]. However, its starch content represents 75% of its dry mass [9]. Furthermore, *D. schimperiana* starch exhibit higher amylose content as compare to those of widely used yam species such as *D. dumetorum* [10]. To the best of our knowledge, no study has been done to produce high technological potential starch with *D. schimperiana* tubers. This study have therefore been designed to evaluate the effect of annealing, acid hydrolysis and enzymatic treatment on the functional properties of *D. schimperiana* starch.

## 2. MATERIALS AND METHODS

### 2.1 *D. schimperiana* Tubers and Chemical Procurement

*Dioscorea schimperiana* tubers were bought in the local market of Baham (West region of Cameroon) and brought to the laboratory.

The 1,4- $\alpha$ -Glucan branching enzyme (Enzyme Commission No. 2.4.1.18; Source organism: *Bacillus subtilis*, biological function: transfer a

segment of a (1, 4) -  $\alpha$ -D-glucan chain to a primary hydroxyl group in a similar glucan chain) used for the modification of starch was purchased from Prozomix, UK; while citric acid was purchased from Sigma Chemicals USA. All others chemicals used in this study were of analytical grade, unless and otherwise specified.

## 2.2 Starch Extraction

Starch was extracted from the yam using the modified method of Falade & Ayetigbo [11]. Tubers were peeled with a sharp knife, washed to clean off adhering soil, and weighed. The tubers were cut into small pieces and dropped into a 0.01% (w/v) sodium metabisulphite solution to prevent browning, and ground with a manual grinder. Water was added continuously to make slurry. The slurry was filtered through double muslin cloths, in distilled water and then dispersed in a 0.2% (w/v) NaOH solution. The slurry was allowed to settle and the supernatant was collected. The pellet was repeatedly washed with distilled water alongside gentle stirring, settling and decanting until a clear supernatant was obtained. The wet starch obtained after the decantation of all the supernatants was thinly spread onto trays and dried in a forced draught air oven drier (Jayedeep engineer, India) at 45°C for 24 hours. After weighing, the samples were milled to fine particle size by a conventional blender (Laboratory Mill 3100, Seedburo-Canada) and sieved through a 180 mm sieve, and kept aluminum bags.

## 2.3 Preparation of Modified Starches

### 2.3.1 Preparation of annealed starch (AS)

Annealing modification of yam starches was carried out using the method described by Falade & Ayetigbo [11]. A starch suspension in distilled water (1:2 w/v) was sealed in containers and heated in a water bath at 50 °C for 24 hours. After 24 hours incubation, the suspension was filtered through a Whatman N<sup>o</sup> 1 filter paper, air-dried at 30 °C for 72 hours, and stored in sealed polyethylene bag at 27 °C.

### 2.3.2 Preparation of acid hydrolyzed starch (AHS)

The chemical modification of the starch was carried out with acid following the method described by Falade & Ayetigbo [11] with minor modifications. A starch solution was prepared by suspending 30 g of native starch in 40 mL of

distilled water. One mL of NaOH (1 M) was added to increase the pH to 9. The alkalinized solution was maintained for 30 minutes with occasional manual shaking. Subsequently, 10 mL of a solution containing 15% citric acid (g/g dry starch) and 1% H<sub>2</sub>SO<sub>4</sub> (volume/mass of dry starch) were added. The mixture was kept for 5 hours at 27°C, then washed with 60 mL of distilled water, filtered, dried at 50°C for 24 hours, ground and packaged in sealed polyethylene bags at 27 °C until the use.

### 2.3.3 Preparation of enzyme branched starch (EBS)

This sample has been prepared as described by Kumar & Prabhasankar [12]. For this purpose, a suspension of starch (12%, W/V) was prepared and heated at 80°C for 10 minutes with constant stirring. Later sodium acetate buffer (0.5 M, pH 5.0) was added and stirred at room temperature (28°C) for 10 minutes. The suspension was diluted by adding distilled water and incubated with 1,4- $\alpha$ -glucan (0.64% starch basis) a thermostable branching enzyme from *Bacillus subtilis* for 5 hours in shaking water bath at 45°C. Later one volume of 95% ethanol was added and centrifuged at 3000 g for 15 minutes. The precipitate thus obtained was dispersed in distilled water and heated in a boiling water bath for 10 minutes. The sample was stirred continuously on a magnetic stirrer at room temperature (28°C) overnight. The mixture thus obtained was dried and the modified starch samples obtained were stored for further utilization.

## 2.4 Effect of Modification on Physicochemical Composition of Starch

Ash and protein content of the starches have been estimated by using AOAC [13] methods. Total phosphorous was estimated following the method described by Jayakody et al. [14].

The amylose content of starches was determined following the colorimetric method described by [15]. The starch sample (70 mg) was placed in a test tube and 10 mL of urea (6 M)-DMSO solution (1:9) was added with continuous stirring. The samples were heated for 10 minutes in boiling water and placed in an oven at 10°C for 1 hour and then cooled at room temperature. Then 0.5 mL of the solution was taken into a volumetric flask containing 25 mL distilled water and 1 mL of I<sub>2</sub>/KI (100 mg I<sub>2</sub> and 1000 mg KI in

50 mL distilled water) and final volume was made up to 50 mL using distilled water and mixed thoroughly. The absorbance of the samples was measured at 635 nm in a UV spectrophotometer, against a blank (prepared by allowing chemicals and distilled water to stabilize for 15 minutes).

$$\begin{aligned} \text{Blue value} &= (\text{Absorbance}/2 \text{ g solution} \times \text{mg starch}) \times 100 \\ \text{Amylose} &= \text{Blue value} \times 28.414 \end{aligned}$$

## 2.5 Effect of Modification on Starch Granule Morphology

The morphology of starch granules was examined using a scanning electron microscope (SEM, Hatfield, PA, USA). Starch was directly deposited on aluminum stub using double-sided adhesive carbon conductive tape and starch was coated with gold and examined in the scanning electron microscope under an acceleration voltage of 10 kV with a magnification of 2000 X. Granule size was determined by measuring the average diameter of starch granule by using the micrometer on the lens of the microscope [16].

## 2.6 Fourier Transformer Infra-Red Spectroscopy (FT-IR) Spectra of Samples

The FT-IR spectroscopy of starch samples was analyzed with the help of Perkin Elmer-Spectrum RX-FTIR (USA). The spectrum of samples was recorded in transmittance mode at the 4000-400  $\text{cm}^{-1}$  region at room temperature [17].

## 2.7 Characterization of Samples Thermograms

The DSC thermograms of the starch before and after modification were recorded on a calorimeter (DSC8000; Perkin Elmer, Inc., Shelton, CT USA). The starches were dispersed in distilled water (1: 3, m/v) in a hermetically sealed aluminum dish. The dish was placed in the apparatus and maintained at room temperature at 25°C for 1 hour to allow good homogenization. The capsule thus sealed was subsequently heated from 30-130 °C at a rate of 10°C/minutes. The apparatus was calibrated for temperature and enthalpy measurement with indium (H = 28.43 J/g) and an empty capsule was used as a reference. The operating software was used to calculate the heat capacity and to integrate the peaks. The starting and ending temperatures of

gelatinization were determined by the intersection of the tangents adapted to the main and secondary sides of the peak with the baseline. The melting enthalpies ( $\Delta H$ ) were calculated by integrating the total area of the melting peak. The transition characteristics were the initial gelatinization temperature ( $T_0$ ), the gelatinization peak temperature ( $T_p$ ), and the gelatinization end temperature ( $T_f$ ) [18].

## 2.8 Effect of Modification on the Functional Properties of Starch

### 2.8.1 Water binding capacity

The water binding capacity (WBC) defined as the amount of water retained by the sample under low speed centrifugation (300 g) was determined as described in the method of [19]. The samples (1 g) were mixed with distilled water (10 mL) and centrifuged at 2000 g for 10 minutes. The WBC was expressed in grams of water retained per gram of dry starch according to the following formula:

$$\text{WBC}(\%) = \frac{\text{weight of hydrated starch} - \text{weight of dry starch}}{\text{weight of dry starch}} \times 100$$

### 2.8.2 Swelling capacity and solubility index

The swelling capacity (SC) and the solubility index in water (SI) of the samples were determined according to the method of Rosell et al. [20] with slight modifications. Briefly, 50 mg of starch was dispersed in 1 mL of distilled water in an Eppendorf tube using a glass rod and then heated at 90°C for 10 minutes. The tube was cooled in an ice bath for 10 minutes and then centrifuged at 3000 g at 4°C for 10 minutes. The supernatants and residues were recovered and dried at 105°C to a constant weight. The dried residues and supernatants were weighed and SC and SI were calculated as follows:

$$\text{SI}(g/g) = \frac{\text{weight of supernatant}}{\text{weight of dry starch}}$$

$$\text{SC}(g/g) = \frac{\text{weight of the residue}}{\text{weight of dry starch}}$$

### 2.8.3 Paste clarity

The clarity of the samples was determined according to the method described by Reddy &

Seib [21]. About 50 mg of starch was mixed with 5 mL of distilled water in a glass tube and then heated at 95 °C for 30 minutes with stirring every 5 minutes and then cooled to room temperature. The samples were stored at 4°C for 3 days and then transmittance was measured at 650 nm against a water blank using UV spectrophotometer (Shimadzu model UV-160A Kyoto, Japan).

#### 2.8.4 Least gelling concentration

The Least gelling concentration was determined using the method of Zouari et al. [22] with slight modifications. Starch dispersions of 2%, 4%, 6%, 8%, 10%, 12%, 14%, 16%, 18%, 20%, 22% and 30% (w / v) were prepared with 5 mL of distilled water and heated at 90 °C for 1 hour in a water bath. The samples were cooled under tap water and held for 2 hours at 25 °C. The least gelling concentration is the concentration at which the sample in an inverted tube does not fall.

#### 2.8.5 Color analysis

The color of starch was measured using a colorimeter (Hunter, Labscan XE system, USA) with a 10° observation window and a D65 light source [17]. About 3 g of starch was arranged to cover the reading tank. The tank was subsequently deposited on the lens of the colorimeter. The coordinates L, a, b, defined by the International Commission on Illumination (CIE) were noted.

### 2.9 Effect of Modification on Pasting Properties

Pasting properties of starch samples were tested using a Rapid Visco Analyzer (RVA) (Newport Scientific Pty. Ltd, Australia). Samples were mixed with distilled water. The wet sample was inserted into the RVA which was switch on for 13 minutes in a heating and cooling cycle at a constant motor speed of 160 rpm. The mixture was maintained at 50°C for 1minute. Heated between 50 to 95°C within 4 minutes, and then at 95°C for 2 minutes. The slurry was finally cooled to 50°C within 4 minutes and held at 50 °C for 2 minutes. The pasting curves were recorded on RVA. The starch viscosity parameters measured were pasting temperature, peak time, peak viscosity, breakdown viscosity and setback viscosity. The results are expressed in centipoise (Cp) for all of the parameters except for pasting

temperature and peak time, which was expressed in °C and minute respectively.

### 2.10 In vitro Digestibility of the Samples

*In vitro* starch digestibility of starch was performed following Bharath & Prabhasankar [17] method. The starches (50 mg) were dispersed in 4 mL of sodium acetate buffer (pH 4.6, 0.4 M) containing amyloglucosidase and incubated in a water bath for 45 minutes at 60 °C. Later, the enzyme was inactivated by placing the tubes in a boiling water bath for 15 minutes. The tubes were cooled to room temperature and then centrifuged at 5000 g for 10 minutes. The glucose content of the supernatant was determined using the GOD-POD assay kit (Autospan, Span Diagnostics Limited, India). Absorbance was measured at 505 nm and the glucose concentration was converted to starch content using a conversion factor of 0.9.

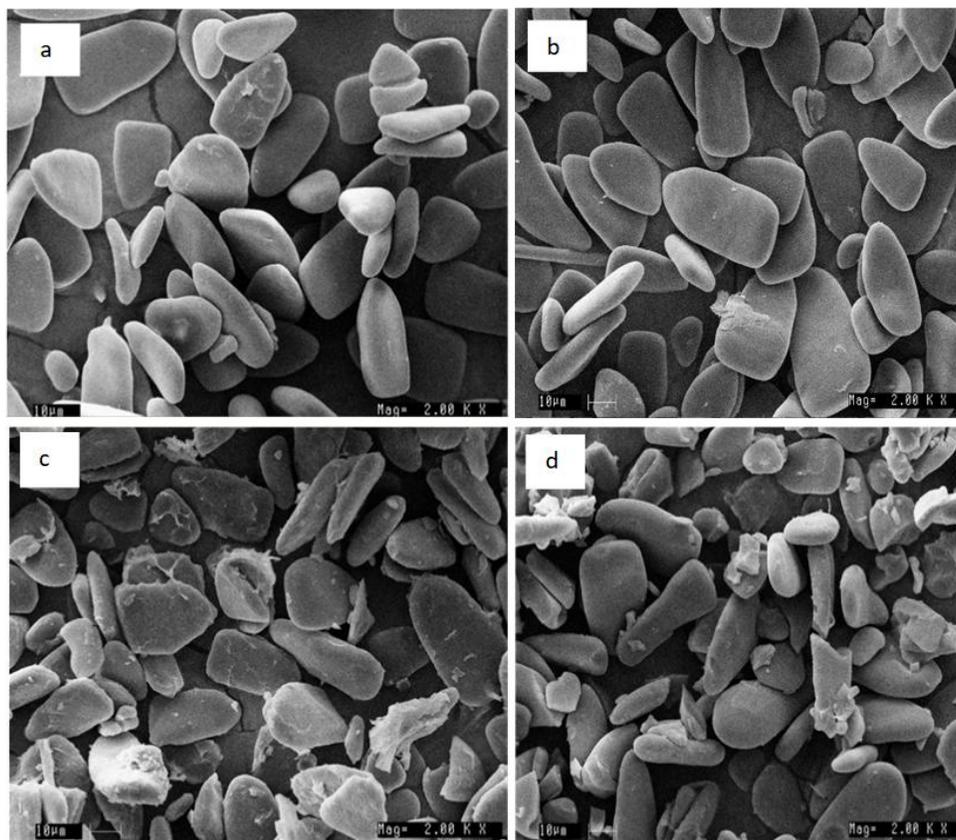
### 2.11 Statistical Analysis

All the measures were done in triplicate. For measurable analysis, to determine the effect of the modification on starch, the one way ANOVA at 95% of confidence interval was firstly done, then grouping was subsequently done using Duncan's multiple ranking. These analyses have been done by using the statistical software SPSS 17 while graphs were plotted by using the Excel software (2016).

## 3. RESULTS AND DISCUSSION

### 3.1 Effect of Modification on Starch Granule Morphology

The morphological characteristics of native and modified samples using scanning electron microscopy (SEM) are represented in Fig. 1. Microscopic analysis of *D. schimperiana* starch present granules with an ovoid shape similar to those of *D. rotoundata*, *D. alata* and *D. cayenensis* species as shown by Otegbayo, Oguniyan, & Akinwumi [16]. However, *D. schimperiana* starch granules size (17.2-28.7 µm) are smaller than that of *D. rotoundata* (28-47.25 µm), *D. alata* (28-40.25 µm), and *D. cayenensis* (41.25 µm) [16]. Since *D. schimperiana* starch has large granule according to Lindeboom et al. [23], they can be considered when high viscosity is needed.



**Fig. 1. *D. schimperiana* starch granule**

a. NS- Native starch; b. AS- Annealed Starch; c. AHS- Acid hydrolyzed Starch; d. EBS- Enzyme branched Starch

The treatments carried out cause various modifications to the starch granules of *D. schimperiana*. Annealing increases the size of the granules of the starch with no trace of gelatinization while acid hydrolysis and enzyme branching (AHS and EBS), damaged starch granule. Similar observations have been done by Adebowale et al. [24] on other yam species. The cracks or etches on starch granules may be due to acid and enzyme action on the side chains of the granule [25].

### 3.2 Effect of Modification on the Molecular Modification of Starch Granule

FTIR spectra of the starch samples after modification show the presence of a new band at wavenumbers 2895  $\text{cm}^{-1}$  and 1362  $\text{cm}^{-1}$  regardless of modification (Fig. 2). Various specific band at wavenumber 1328  $\text{cm}^{-1}$  have been observed for AS and EBS samples and at 485  $\text{cm}^{-1}$  for AHS and EBS. There was also a

missing band of wavenumber 1241  $\text{cm}^{-1}$  for all modified samples.

According to the National Institute of Standards and Technology (NIST) database, the 2895  $\text{cm}^{-1}$  and 1362  $\text{cm}^{-1}$  bands represent respectively the C-H and C = O bonds of the carboxylate anions of the esters. Similar observations have been achieved by Hui et al. [26] on succinylated starches. This observation suggests that these bands are not specific to the treatments and may be the result of the molecular reorganization in the granule after treatments. Both annealing and enzyme branching modification introduce in *D. schimperiana* starch the -OH group of an alcohol function at wavenumber 1328  $\text{cm}^{-1}$ . While diethyl ether function at wavenumber 485  $\text{cm}^{-1}$  is present in acid hydrolyzed and enzyme branched samples. A similar observation has been done by Sridach et al. [27] after the treatment of starch with citric acid and polyvinyl alcohol. These authors have associated these functional groups to crosslinking in the starch molecule. This observation suggests that all the modifications

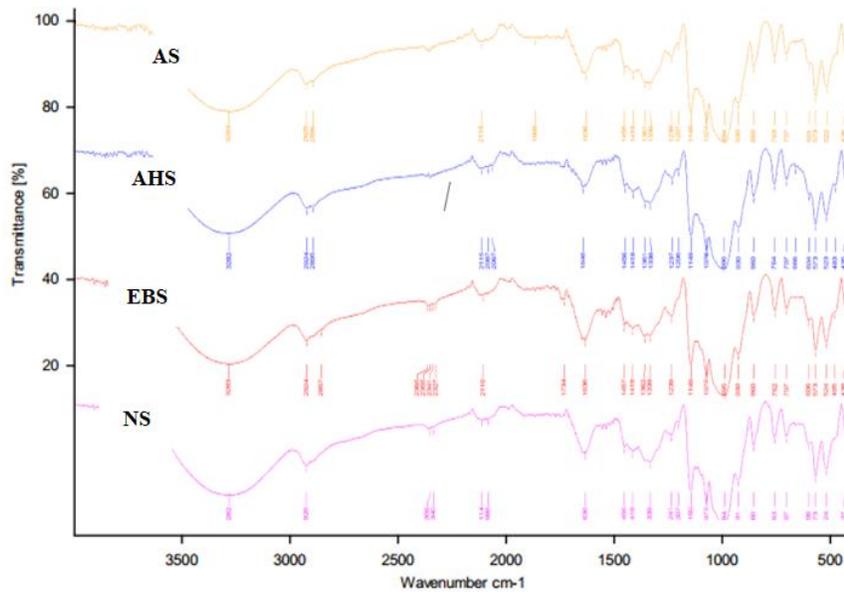
create crosslinking in *D. schimperiana* starch. The disappeared band at wavenumber 1241 cm<sup>-1</sup> could be attributed to a C = C double bond or an -O- ether stretching bond. This observation could be explained by the implication of this stretching bond in the crosslinking reaction.

### 3.3 Effect of Modifications on the Physicochemical Composition of Starch

The physicochemical composition of *D. schimperiana* starch is given in Table 1. The native starch has an amylose content of 16.3 mg/100 g db. Its ash, phosphorus and protein contents are respectively 1.3 g/100 g db; 48.2 mg/100 g db and 1.2 g / 100 g db. After modification, there was a significant decrease in ash, phosphorous and amylose content for AHS samples as compared to native starch.

Phosphorus and protein levels were unaffected by annealing and enzymatic modification.

*D. schimperiana* native starch composition is similar to other yams species (table 1). In fact, its amylose content (less than 20%) is similar to those of *D. dumetorum*, *D. esculenta* as shown by Zhu [1]. Functional properties of starch are generally attributed to its amylose content [16]. Thus, one might think that *D. schimperiana* offers the same technological guarantees as these other species. However, other constituents such as proteins, lipids, phosphorus compounds and ash can increase the peak viscosity of the starch [28]. The protein content of *D. schimperiana* starch are lower than that of white, water, yellow and bitter yam starches obtained by Falade & Ayetigbo [11] while its ash content is higher as compare to those starches. These differences could be due variety origin of the starch.



**Fig. 2. FT-IR spectra of *D. schimperiana* starch**

NS- Native starch; AS- Annealed Starch; AHS- Acid hydrolyzed Starch; EBS- Enzyme branched Starch

**Table 1. Physicochemical composition of *D. schimperiana* starch**

Samples	Ash (g/100 g db)	Phosphorous (mg/100g db)	Proteins (g/100g db)	Amylose (g/100g db)
NS	1.3±0.2 <sup>a</sup>	48.2±2.1 <sup>a</sup>	1.2±0.1 <sup>a</sup>	16.3±0.4 <sup>a</sup>
AS	0.9±0.1 <sup>c</sup>	44.2±1.9 <sup>a</sup>	1.2±0.1 <sup>a</sup>	15.2±0.1 <sup>a</sup>
AHS	0.4±0.1 <sup>c</sup>	29.6±3.2 <sup>b</sup>	1.0±0.0 <sup>a</sup>	0.7±0.1 <sup>b</sup>
EBS	0.9±0.1 <sup>b</sup>	41.7±1.2 <sup>a</sup>	1.1±0.0 <sup>a</sup>	13.6±0.1 <sup>c</sup>

NS- Native starch; AS- Annealed Starch; AHS- Acid hydrolyzed Starch; EBS- Enzyme branched Starch; db: dry basis

Number with different superscript in the same column are significantly different  $p < 0.05$

Acid hydrolysis causes a decrease of ash, phosphorus and amylose content of starch (Table 1). The same trend has been reported in other on oxidized *Dioscorea* starch by Awolu & Olofinlae [29]. Amylose content, as determined by iodine binding method after acid hydrolysis, has been shown to decrease significantly during the early stage of acid hydrolysis [30]. The amylose content even becomes undetectable after extensive hydrolysis [31] indicating that the length of residual linear chains is too short to form a blue iodine complex. This decrease is generally attributed to the preferential hydrolysis of amorphous regions within starch granules, due to the fact that amylose is largely located in the amorphous regions of starch granules. The reduction in ash could also be attributed to washing away of minerals during modifications. These modifications could be considered to improve digestibility of *D. schimperiana* starch since it is well establish that low amylose content leads to high digestibility suitable for the preparation of high energy food.

### 3.4 Effect of Modifications on the Thermal Properties of Starch

The thermal properties of the samples are recorded in table 2. The starting ( $T_o$ ), peak ( $T_p$ ) and end ( $T_f$ ) gelatinization temperatures of the native starch are respectively 73.6; 77.3 and 81.1 °C. The gelatinization enthalpy ( $\Delta H$ ) is 15.1 J/g. Annealing (AS) and enzymatic modification (EBS) do not influence the parameters  $T_o$ ,  $T_p$ , and  $T_f$ . While a significant decrease in the enthalpy of gelatinization ( $\Delta H$ ) was observed after annealing and enzymatic modification. No value for those parameters was recorded after acid hydrolysis.

*D. schimperiana* starch has high transition temperatures ( $T_o$ ,  $T_p$ ,  $T_f$ ) which are similar to other *Dioscorea* species as shown by Adamovicz et al. [32]. This result is not surprising since *D. schimperiana* starch amylose content is similar to

those species and it has been demonstrated that starch with high amylose content requires a higher temperature and energy for the gelatinization process [33]. This high energy demand for gelatinization comes from the fact that in solution, the linear structure of amylose is rapidly aligned to form more extensive hydrogen bonding resulting in high resistance to gel formation.

Except for the acid hydrolyzed sample, all the modified samples show typical endothermic transitions of a gelatinization process. This observation has already been done on other yams species and suggests that acid hydrolysis would have completely altered starch amylose, thus removing its ability to form a gel hence the absence of a typical endothermic profile. Furthermore, the significant drop in enthalpies of gelatinization for the AS and EBS samples can be explained by the fact that these treatments would have led to the creation of bonds in the starch, thus creating a sufficient disruption of some double-helices during treatment and weaken enthalpy without provoking starch gelatinization. Similar behavior has already been observed by Hornung et al. [34] on starch from a wild species of *Dioscorea* sp. after oxidation. This observation suggest that native, annealed and enzyme treated *D. schimperiana* starch could be suitable in any other food process that request heating.

### 3.5 Effect of Modification on Functional Properties and Color of *D. schimperiana* Starch

The functional properties of the samples and their color are shown in Table 3. *D. schimperiana* native starch exhibit a water binding capacity of 2.7 g/g. Its solubility index and swelling capacity are estimated to 0.9 g/g and 3.0 g/g respectively. Its lowest gelling concentration is 12.0% and its paste clarity is 12.7%. The colour characteristics was: L = 81.9; a = 0.5 and b = 4.8. As expected,

**Table 2. Effect of modification on thermal characteristics of *D. schimperiana* starch**

Samples	$T_o$ (°C)	$T_p$ (°C)	$T_f$ (°C)	$\Delta H$ (J/g)
NS	73.6±0.5 <sup>a</sup>	77.3±1.7 <sup>a</sup>	81.1±2.0 <sup>a</sup>	15.1±1.0 <sup>a</sup>
AS	74.1±1.2 <sup>a</sup>	75.7±1.3 <sup>a</sup>	78.9±1.4 <sup>a</sup>	12.5±0.1 <sup>b</sup>
AHS	ND	ND	ND	ND
EBS	71.9±0.2 <sup>a</sup>	76.9±1.0 <sup>a</sup>	80.6±2.9 <sup>a</sup>	12.7±0.8 <sup>b</sup>

NS- Native starch; AS- Annealed Starch; AHS- Acid hydrolyzed Starch; EBS- Enzyme branched Starch;  $T_o$ - Initial temperature;

$T_p$ - Peak temperature;  $T_f$ - Final temperature;  $\Delta H$ - Enthalpy; ND- Not Define

Number with different superscript in the same column are significantly different  $p < 0.05$

annealing leads to a significant increase in the water binding capacity (4.1 g/g), a decrease of the solubility index (0.4 g/g) and the least gelling concentration (10.0%). Acid hydrolysis causes a decrease in swelling capacity (1.3 g/g), followed by an increase of the least gelling concentration (22.0%). The paste clarity, characterizing the ability of starch retrogradation, shows that native starch retrogrades faster than annealed (AS- 14.9%) enzyme branched (EBS- 17.0%) and the more important effect observed after acid hydrolysis (AHS- 37.6%). Acid and hydrolysis enzyme treatment lead to an increase of luminescence respectively (L = 85.1 and L = 84.2).

Based on the analysis of the functional properties of *D. schimperiana* starch, water binding capacity, solubility index, and paste clarity of native *D. schimperiana* starch are higher than those of *D. rotoundata* and *D. alata* species obtained by Otegbayo, Oguniyan & Akinwumi [16]. Variability in species (size of starch granules, amylose content, etc...) could explain these differences. These properties predispose this starch to be used as a gelling agent or thickener in frozen desserts made from sugar and in pastries.

Annealing has increased its gelling properties (water binding capacity, swelling capacity and least gelling concentration), while reduce the solubility index. According to Mweta, Kalenga-Saka and Labuschagne [35], a high swelling power of starches is generally coupled with high water binding capacity and low solubility index. This observation confers to annealed samples a potential be used as a gelling and texturizing agent in pastries because of their high capacity to form a gel.

The increase of solubility index of starch after acid hydrolysis have already been reported by Falade & Ayetigbo [11]. This result suggest that acid hydrolysis is also a good mean to improve the solubility of this starch. Because of its high

solubility, the acid hydrolyzed sample can be used as a thickening agent in the formulation of beverages.

Although the effect of branching enzyme has not yet been documented on yam starch, it is believed that this treatment causes modifications similar to annealing (creation of new bonds in the granule, cross-linking

Paste clarity of the starches varied significantly (12.70–37.60%) between native and modified starch.

The paste clarity was significantly increased by acid hydrolysis of the native starches. Increased clarity of acid-hydrolyzed starch could be due to the reduction of starch molecular weight and concentration caused by the introduction of citrate groups [11]. The increase of paste clarity after modification also shows that the treatment causes a decrease of its retrogradation capacity which is generally considered as a technological limit.

Color is an important parameter to appreciate the quality of starch, especially for its industrial use. In this study, we found that *D. schimperiana* native starch has better color characteristics as compared to potato starch (L = 69.45, a = 2.28, b = 14.73) as shown by Abdelmaguid [36]. Similar observations have been done by Abiodun et al. [37] on trifoliate yam starch. This observation suggests that the yellowish color of the *D. schimperiana* yam tuber does not affect starch. The genetic diversity between yam and potato starches could explain these differences. Moreover, annealing has improve starch overall color. This observation is different from those obtained by [37] after pre-gelatinization and oxidation of trifoliate yam starch. It appears that *D. schimperiana* starch color is not affected by heat treatment. This could be a useful characteristic of much industrial application.

**Table 3. Effect of modifications on the functional properties and color of starch**

Sam ples	Water binding capacit y (g/g)	Solubility index (g/g)	Swelling capacity (g/g)	Least swelling concentrati on(%) (m/v)	Paste clarity (%T <sub>650nm</sub> )	Colour		
						L	a	b
NS	2.7±0.0 <sup>b</sup>	0.9±0.1 <sup>a</sup>	3.0±0.0 <sup>a</sup>	12.0	12.7±0.0 <sup>a</sup>	81.9±1.3 <sup>a</sup>	0.5±0.1 <sup>a</sup>	4.8±1.0 <sup>a</sup>
AS	4.1±0.0 <sup>a</sup>	0.4±0.0 <sup>b</sup>	3.3±0.0 <sup>a</sup>	10.0	14.9±0.0 <sup>b</sup>	81.1±0.9 <sup>a</sup>	0.6±0.1 <sup>a</sup>	3.7±0.8 <sup>b</sup>
AHS	1.1±0.1 <sup>c</sup>	1.0±0.1 <sup>a</sup>	1.3±0.0 <sup>b</sup>	22.0	37.6±0.1 <sup>d</sup>	85.1±2.2 <sup>b</sup>	1.1±0.1 <sup>b</sup>	6.3±0.5 <sup>c</sup>
EBS	2.6±0.1 <sup>b</sup>	1.0±0.0 <sup>a</sup>	2.9±0.0 <sup>a</sup>	12.0	17.0±0.0 <sup>c</sup>	84.2±0.7 <sup>b</sup>	0.6±0.1 <sup>a</sup>	4.9±0.9 <sup>a</sup>

NS- Native starch; AS- Annealed Starch; AHS- Acid hydrolyzed Starch; EBS- Enzyme hydrolyzed Starch;  
Number with different superscript in the same column are significantly different p<0.05

### 3.6 Effect of Modification on the Pasting Properties of Starch

Modified starch samples were subjected to rheological characterization using Rapid Visco-Analyzer (table 4). *D. schimperiana* native starch gelatinizes after 6.5 minutes at a temperature of 77.2°C. Its peak viscosity is 4140.0 mPa·s, the breakdown is 802.0 mPa·s and the setback viscosity is 2720.0 mPa·s. The viscosity parameters of *D. schimperiana* starch are lower than those obtained by Falade & Ayetigbo [11] on four species of yam. These differences could be due to the amylose content of these species which is high as compared to *D. schimperiana*.

Annealing and enzymatic modification lead to an increase of peak and setback viscosity while decreasing the breakdown viscosity correlated to an increase of pasting temperature. Acid hydrolysis decreases these parameters, and starch remains in liquid form through the RVA pasting profile temperature cycle of heating and cooling. Similar observations have been done by Takata et al. [38], Falade & Ayetigbo [11]. These observations could be related to more cross-linkages formed by the molecular rearrangement among starch chains during annealing and enzyme branching as shown by FTIR. Hence the amylose leaching was largely reduced and thus more heat was required to disintegrate the structure, thus reduce the breakdown viscosity. Even though the phenomenon has been observed on other yams starch, it should be noted that annealing and enzyme branching is very useful to reduce the retrogradation capacity of *D. schimperiana* starch. Concerning acid hydrolysis, the observation can be explained by the drastic reduction of amylose in starch as shown by its physicochemical composition. This position was supported by previous report that acid modification caused partial disbranching of amylopectin molecules thereby weakening the internal cohesion within starch matrix [39]. EBS and AS samples could be suitable to develop

thickening agents for products that needs long storage.

### 3.7 Effect of Modification on *in vitro* Digestibility of *D. schimperiana* Starch

Digestibility is an important factor in choosing the use of a food matrix for the formulation of functional foods. Fig. 3 shows the *in vitro* digestibility of *D. schimperiana* starch before and after modification. The results reveal that the acid hydrolyzed sample (AHS) is 10 times more digestible than the native starch and the other samples (EBS and AS).

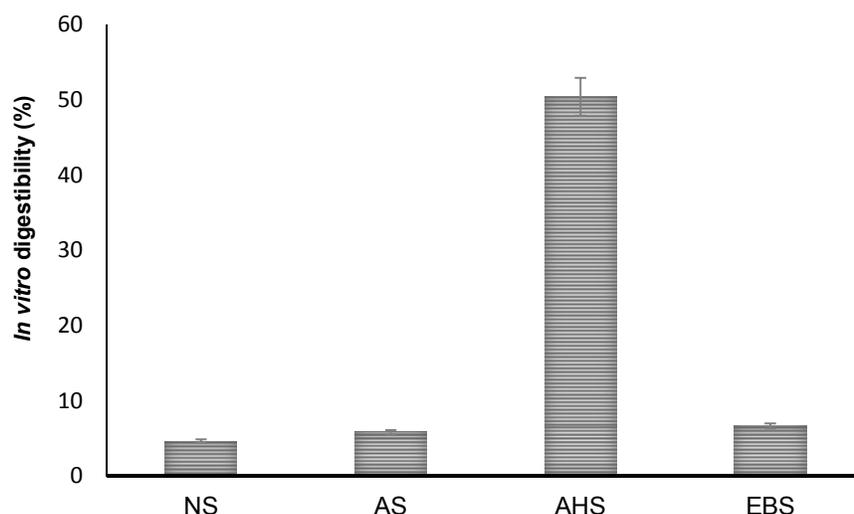
The digestibility of *D. schimperiana* native starch is lower than that of *D. cayenensis*, *D. alata*, *D. rotundata* and *D. esculenta* species [40]. In general, digestibility is influenced by the size of the starch granules and the amylose content [40]. Acid hydrolyzed samples exhibit the lowest amylose content and the highest digestibility percentage. According to [40], starches with a low amylose content are more digestible, whereas starches with a high amylose content are less digestible. Since the amylose content of *D. schimperiana* is similar to that of those species, the strength of the binding bond between starch and the other compounds may explain this difference.

Even after modification (EBS and AS) *D. schimperiana* starch is less digestible than that of *D. cayenensis*, *D. alata*, *D. rotundata* and *D. esculenta* as shown by Riley et al. [40]. A low digestibility has been observed for cassava starch after similar treatments by Hung et al. [41]. These authors have observed the increase of resistant starch content. This result suggests that *D. schimperiana* starch exhibit similar behavior with cassava starch which is one of the must use starch in industry against annealing. Moreover, the low digestibility of *D. schimperiana* starch is an advantage for the development of low glycemic index food.

**Table 4. Effect of modifications on pasting properties of *D. schimperiana* starch**

Samples	PV (mPa·s)	BD (mPa·s)	SB (mPa·s)	P time (min)	P temp (°C)
NS	4140.0	802.0	2720.0	6.5	77.2
AS	5500.0	654.0	3820.0	4.2	61.9
AHS	75.0	2.1	13.9.0	5.3	68.2
EBS	4950.0	628.0	2840.0	5.5	73.5

PV- Peak viscosity; BD- Breakdown; SB – Set Back; P time - Peak time, P temp- Peak temperature; NS- Native starch; AS- Annealed Starch; AHS- Acid hydrolyzed Starch; EBS- Enzyme hydrolyzed Starch



**Fig. 3. In vitro digestibility of *D. schimperiana* before and after modification**

NS- Native starch; AS- Annealed Starch; AHS- Acid hydrolyzed Starch; EBS- Enzyme branched Starch

#### 4. CONCLUSION

Different pretreatments including annealing, acid hydrolysis, and enzyme branching was applied to investigate the technological and functional properties of *D. schimperiana* starch. Characterization of this native starch reveals that its particle size and its physicochemical composition are similar to other *Dioscoreae*. In addition, this starch has a high gelatinization temperature and it is low digestible. After modification, it appears that acid hydrolysis has the most important effect on the molecular structure of this starch, manifested by a decrease in amylose content, water retention capacity, swelling capacity, and retrogradation capacity, while increasing its in vitro digestibility. The pasting properties (peak viscosity, setback viscosity, and breakdown) of the starch have been significantly improved by annealing and enzyme treatment. In general, *D. schimperiana* native starch is weakly digestible and could be considered for the formulation of low glycemic foods. Acid hydrolysis could be useful to produce syrups from this starch for industrial applications. Furthermore, annealing and enzymatic treatment could lead to the production of thickeners because of the increase in swelling capacity that they cause.

#### DISCLAIMER

The products used for this research are commonly and predominantly used products in our

area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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