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Selective Comparison of Native and Nanocrystals of White and Yellow Yam Starches

Abraham Olasupo OLADEBEYE[1,*], Aladesanmi Augustine OSHODI[2], Isiaka Adekunle AMOO[2] and Alias Abd KARIM[3]

[1]*Polymer Technology Department, Auchi Polytechnic, Auchi, Nigeria*
[2]*Department of Chemistry, Federal University of Technology, Akure, Nigeria*
[3]*Food Technology Division, Universiti Sains Malaysia, Penang, Malaysia*

([*]Corresponding author's e-mail: folabeye@yahoo.com)

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Abstract

Mild acid hydrolysis at 40 °C for 5 days was used to prepare nanocrystals of the native starches isolated from white yam (*Dioscorea rotundata*) and yellow yam (*Dioscorea cayenensis*). The shapes and granular sizes of the native starches were obtained by a scanning electron microscopy while their nanocrystals were examined through transmission electron microscopy. The nanocrystals were found to exist in the form of platelets. X-ray diffraction of the starch nanocrystals showed that they were of V-type crystalline structure whereas the native starches exhibited C_B-type crystalline structure. The nanocrystals were insoluble in all the solvents used, except in chloroform, where precipitates were formed. These starch nanocrystals could be potential precursors to nanocomposites and nanoparticle-based copolymers.

Keywords: Nanocrystals, native starches, solubility

Introduction

Starch, a biocompatible, biodegradable, non-toxic polymer [1], has, in recent times, gained increased attention in food and non-food applications due to a host of advantages, which include low density, cost effectiveness, abundant supply and environmental amity [2]. It is widely used in food, paper-making, fine chemicals, packing materials, pharmaceuticals, rubber and plastic industries [3]. The major botanical and commercial sources of starch are cereals, roots and tubers, and pulses [4]. Other sources, classified as minor, are legumes. In its native form, starch is unusable in some applications. The undesirable behaviours of native starch can be reduced or eliminated through modifications by reorganizing the structural arrangement of the starch granules, resulting in enhanced physicochemical properties.

Starch granules in the nanometer range have been shown to have unique and novel functional properties. Nanoparticles of native starch called nano starch or starch nanocrystals can be obtained by acid hydrolysis [5]. Worldwide commercial foods and food supplements containing added nanoparticles are becoming available. A major growth area appears to be the development of "nanoceuticals" and food supplements [6].

The application of nanocrystals of starch has gone beyond food. Interest in biomaterials and nanocomposites has now emerged [5-8]. The quest for nanocrystals of starch has increased the demand for starch for both food and non-food applications. Thus, high competition arises between the domestic and industrial uses of most commercial starches from normal and waxy corn, rice and potato. It is, therefore, imperative to explore alternative sources of starches to complement the ever-increasing demand in the global market. A good way of doing this is by exploring underutilized crops. Such crops, as found in the tropics, are hitherto, mostly for staple food.

In our previous work [9], we proposed lima bean and jack bean starches as alternative sources to the ever-increasing demands of starch for both food and non-food applications. The objectives of this research work were to (a) search for more alternatives to commercial starches from white yam (*Dioscorea rotundata*) and yellow yam (*Dioscorea cayenensis*), (b) prepare nanocrystals of the starches, and (c) investigate the morphology, crystalline nature and solubility of these nanocrystals. This is with the view to harnessing these starches as possible alternatives to non-renewable materials.

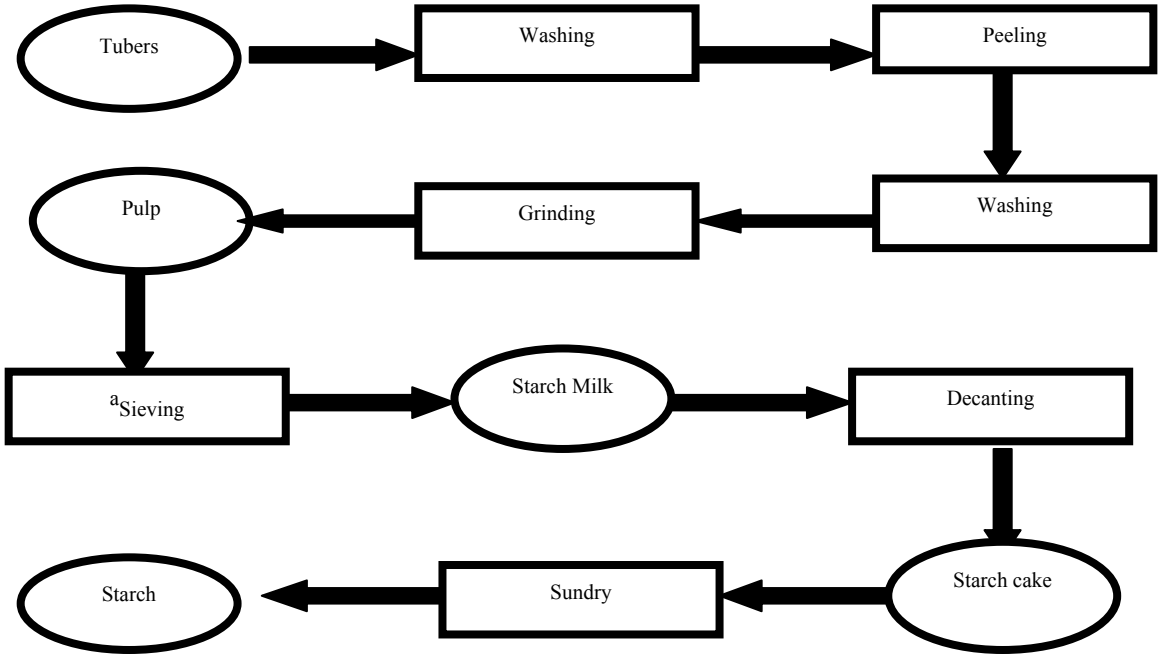
Materials and methods

Materials

The tubers of white yam (*Dioscorea rotundata*) and yellow yam (*Dioscorea cayenensis*) were purchased at Jattu Market, Etsako-West Local Government Area, Edo State, Nigeria. All the chemicals used were analytical grades and were used directly without further purification.

Isolation of native starch

The starches of white yam (*Dioscorea rotundata*) and yellow yam (*Dioscorea cayenensis*) tubers were extracted following the scheme shown in **Figure 1** [10].



[a]muslin bag was used for sieving.

Figure 1 Isolation of native starch from tubers.

Preparation of starch nanocrystals

An acid hydrolysis method was adopted. 37 g of native starch granules was mixed with 250 ml of 3.16 M H_2SO_4 [5]. The suspension was placed over a water-bath at a regulated temperature of 40 °C for 5 days. Continuous stirring was ensured by means of a homogenizer set at 100 rpm. After 5 days, the

suspension was washed by successive centrifugation in distilled water until neutral. The aggregate was freeze-dried at 4 °C with several drops of chloroform.

Methods

Scanning electron microscopy (SEM)

The starch samples were sprinkled onto aluminum specimen stubs with double-sided adhesive tape while the non-sticking portion was blown off. The samples were coated with a 30 nm layer of gold using a sputter coater [Polaron (Fisons) SC 515 VG Microtech, Sussex, UK]. The coated starch samples were observed using a Scanning Electron Microscope (FESEM Leo Supra 50VP, Carl-Zeiss SMT, Oberkochen, Germany). Images were captured at different magnifications for morphological studies.

Transmission electron microscopy (TEM)

The suspension of nanocrystals of starch was dispersed in ethanol and sonicated for homogeneity of the nanocrystals for 3 min, using Sonicor (Copiague, NT). A drop of dilute nanocrystal suspension was spread on a glow-discharged copper-coated TEM grid and was allowed to dry for 3 min. The preparation was negatively stained with 2 % w/v uranyl acetate, and was allowed to spread for 1 min. The grid holding the stained nanocrystals was placed in a petridish for 15 min to dry. The dry grid was observed using a Philips CM 12 microscope (FEI Company, Eindhoven, Netherlands) operating at 80 kV. Images were recorded on Kodak S0163 film.

X-ray diffraction patterns

The X-ray diffraction studies were carried out using a Siemens D5000 X-ray Powder Diffractometer (20° Geometry, Madison, USA). The starch nanocrystals were equilibrated with distilled water in a desiccator for 48 h before determination to improve resolution of the X-ray diffractogram pattern. The fine samples were filled into a sample holder and packed as densely as possible. The finished surface was smoothed and flushed. The samples were mounted into the X-ray diffractometer and copper Kα, 2λ (λ = 1.540 μm and 1.544 Å; 40 KV; 35 mA) was used to determine the X-ray pattern. The scan was made from a diffraction angle (2θ) of 1.5 to 70° at 0.05 step size with a count time of 3 s. From the resulting X-ray patterns, peak positions were identified using the instrument's software and these peak positions were used to determine the crystalline natures of the starch nanocrystals [11].

Solubility test

Native starch

100 mg of the starch sample was quantitatively and accurately weighed into a clean dried test tube and re-weighed. The starch sample was dispersed in 50 ml distilled water and mixed using a vortex mixer. The resulting slurry was heated at 90 °C for 30 min in a water-bath. The mixture was cooled to 28±2 °C and centrifuged at 2200 rpm for 15 min to separate the gel and supernatant. The supernatant was removed and poured in a dish for solubility determination. The supernatant was dried to a constant weight in an air-oven at 100 °C for 4 h [12].

$$\text{Solubility (\%)} = \frac{\text{Total Weight of Solubles}}{\text{Weight of Sample}} \times 100 \quad (1)$$

Starch nanocrystals

A previous method described for the determination of solubility of starch nanocrystals in organic solvents was adopted with some modifications [13]. Five organic solvents and water were used. The solvents used were toluene, xylene, acetic acid, trichloromethane (chloroform), ethanol and deionized water. A 5 mg/ml concentration of the nanocrystals was prepared with the solvents at room temperature. The resulting contents were slightly agitated and left undisturbed for about 30 min after which the contents were observed and photographs taken. The contents were left for 24 h to investigate any change in the solubility. No differences were observed in the solubility of the contents.

Statistical analysis

Duncan's least significant test was used to compare means at the 5 % significance level. Simple Pearson correlation and regression analysis was conducted using the Statistical Package for Social Sciences (SPSS) 17.0 software (SPSS Inc., Chicago, IL).

Results and discussion

Percentage yield

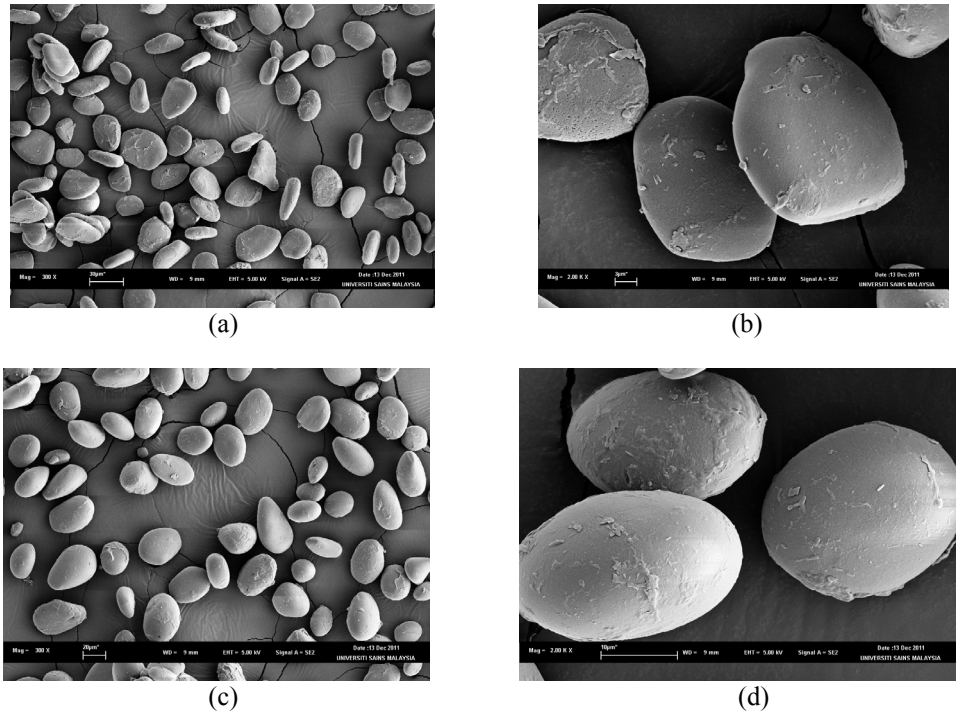
The percentage yield of native starch obtained from white yam was slightly higher than yellow yam whereas an opposite trend was observed for the starches in terms of their nanocrystals (**Table 1**). The differences in percentage yield of nanocrystals could be, apart from the botanical source, due to molecular interactions among the starch granules during acid hydrolysis. This observation was in agreement with the previous report [9].

Table 1 Percentage yield of native starch and starch nanocrystals.

Starch	% Yield	
	Native	Nanocrystals
White yam	42.66	6.22
Yellow yam	41.72	6.78

Morphologies of native and nanocrystals

The starch granules of the native starches observed by SEM varied in shape (**Figure 2**). The mean granular sizes of the native starches, measured with the aid of a Scanning Electron Microscope (FESEM Leo Supra 50VP, Carl-Zeiss SMT, Oberkochen, Germany) were 18.20 and 21.77 μm for white yam and yellow yam starch, respectively. Irregular polygonal shapes were observed for white yam starch while yellow yam starch granules were oval, ellipsoidal granules mixed with spherical granules. The roughness of granule surface was not due to damage, but the presence of surface proteins, which could be removed by intensive purification of the starch samples. However, this suggests that the processes of extraction and drying had no damaging effect on the starches. Observations on the unaltered shapes and surface characteristics of starch granules have been reported [14,15].

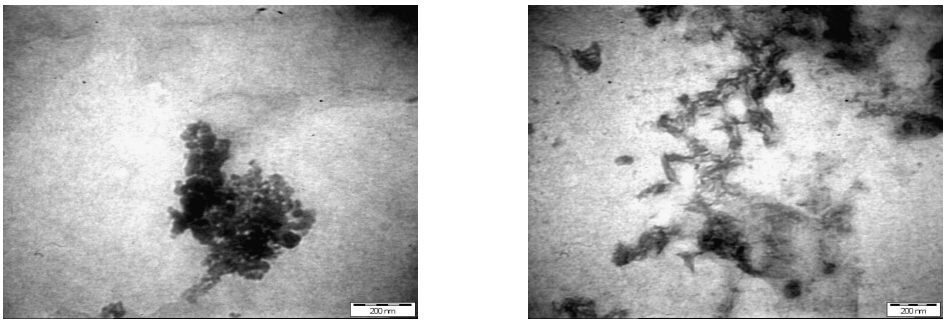


(a) (b)

(c) (d)

Figure 2 Scanning electron micrographs of (a) white yam starch at 0.300K×, 30 μm; (b) white yam starch at 2.00K×, 3 μm; (c) yellow yam starch at 0.300K×, 20 μm and (d) yellow yam starch at 2.00K×, 10 μm.

Figure 3 shows the transmission electron micrographs of the nanocrystals of white yam and yellow yam starches. Platelets were formed as a result of crystallization of the amylopectin molecules of the starches after mild acid hydrolysis for 5 days. The platelets were not discrete molecules, but existed in aggregates. These observations were in line with the report for waxy corn starch nanocrystals [16]. The nanocrystals of white yam starch nanocrystals appeared as aggregates of lamellar fragments stacked together while those of yellow yam were aggregates of fibre-like of parallelepiped blocks (**Figure 3**).



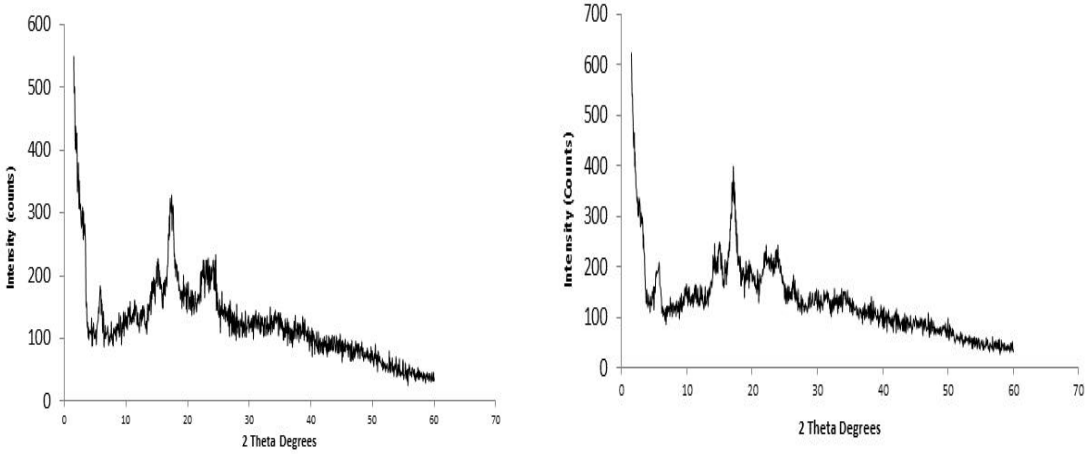
(a) (b)

Figure 3 Transmission electron micrographs of negatively stained preparations from (a) white yam, (b) yellow yam starch granules treated with H_2SO_4 at 40 °C under continuous stirring (200 nm).

Irrespective of the shape of the nanocrystals, it has been reported that all nanocrystals can be considered as potential fillers in nanocomposites [17]. This, therefore, suggests that all the starch nanocrystals prepared in this study are potential fillers in nanocomposites. Starch nanocrystals obtained by acid hydrolysis of potato and waxy maize starch granules have been used as fillers in a synthetic polymeric matrix and appeared to be an interesting reinforcing agent in natural rubber [8], polylactic acid, and polycaprolactone for getting nanocomposites with unique properties [18] and pharmaceuticals. Starch nanocrystals can, henceforth, serve as replacement for the use of petrochemicals in food and other applications.

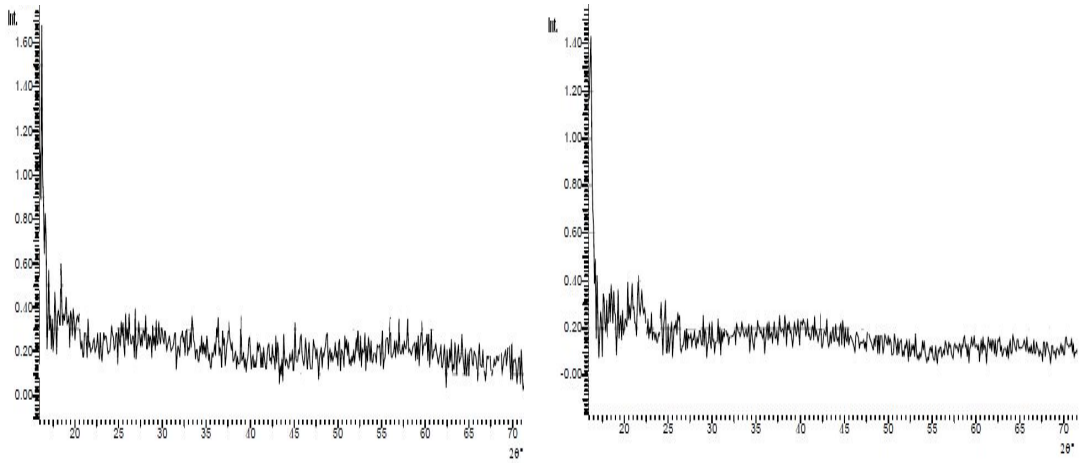
X-ray patterns

X-ray diffraction peaks for the native starches appeared at 5.80°, 15.05° and 17.40° 2θ for white yam starch, corresponding to interplanar d-spacing of 5.80 Å, 5.82 Å and 5.04 Å; 5.75°, 14.25° and 17.10° 2θ for yellow yam starch, corresponding to interplanar d-spacing of 5.75 Å, 5.91 Å and 5.20 Å (**Figure 4**). The strongest and broadest diffraction peaks appeared at 2θ = 17.40° and 17.10° for native starches of white yam starch and yellow yam starch respectively. These reflections indicated that these native starches were of B-type crystalline nature. Starches of roots and tubers have been reported to exhibit maximum X-ray diffraction peaks at 2θ = 17° [19].



(a) (b)

Figure 4 X-ray patterns of native (a) white yam starch (b) yellow yam starch.



(a) (b)

Figure 5 X-ray patterns of nanocrystals of (a) white yam starch; (b) yellow yam starch.

The X-ray patterns of the starch nanocrystals prepared from the native starches are presented in **Figure 5**. In all, there were no significant peaks. However, a weak peak appeared at $2\theta \approx 17°$ for white yam starch nanocrystals. The possibility for these observations could be that mild acid hydrolysis, by which starch nanocrystals were produced, resulted in the formation of amylase-lipids complex in the amorphous lamella. This could make the amorphous regions larger than the crystalline region. The V-type is relatively amorphous with a few weak lines that show crystallinity [20].

It is suggested that the relative amorphosity of the starch nanocrystals is not as a result of its high water content. This assertion is true based on the fact that X-ray powder diffraction is usually done on hydrated starch samples [4]. Hydration is accomplished by equilibrating the sample in a desiccator maintained at a certain relative humidity and temperature.

Solubility test

Table 2 shows the solubility values of the native starches. The solubility value of white yam starch (2.77±0.04 %) was higher than the value obtained for yellow yam starch (2.29±0.04 %). Solubility represents the amount of solubilized starch molecules present at a certain temperature.

Table 2 Solubility of native starches[a].

Starch	Solubility (%)
White yam	2.77±0.04[b]
Yellow yam	2.29±0.04[a]

[a]Results are expressed as means±standard deviations (n = 3). Values in the same column with the same superscript letters are not significantly different ($p < 0.05$).

The results of the solubility test of starch nanocrystals with 6 different solvents (both organic and inorganic), namely toluene, xylene, trichloromethane (chloroform), acetic acid, ethanol and de-ionized water are presented in **Table 3**. The solubility test of the starch nanocrystals is vital for the incorporation of nanocrystals of starches in aqueous form into nanocomposite matrices. Of all the solvents used in

testing the solubility tendency of starch nanocrystals, trichloromethane (chloroform) showed a tendency to form light precipitates (black blocs) with all the nanocrystals. These observations are in league with insoluble dispersions reported for nanocrystals of waxy starch in common organic solvents such as Starch nanocrystals of waxy corn *N*,*N*-dimethylformamide, acetone, carbon tetrachloride and toluene [13].

Table 3 Solubility test of nanocrystals of starches.

Starch	Solvent					
	Toluene	**Xylene**	**Chloroform**	**Acetic acid**	**Ethanol**	**De-ionized water**
White yam	Insoluble	Insoluble	Precipitate	Insoluble	Insoluble	Insoluble
Yellow yam	Insoluble	Insoluble	Precipitate	Insoluble	Insoluble	Insoluble

Dispersion of starch nanocrystals as aqueous suspensions prior to their incorporation into nanocomposite matrix has been a challenge [13]. This is due to its poor solubility in organic solvents, and this singular obstacle, has limited the application of starch nanocrystals as a reinforcing phase in a wide variety of polymers.

Conclusions

This research work has unveiled alternative sources of starch for ever-increasing demand of starch for industrial applications. The crystals of native starches of white and yellow yam cultivars transformed from B-type to V-type diffraction patterns by means of mild acid hydrolysis. It is suggested that the evidence of these nanocrystals, among other things, is a step towards meeting the quest for nanotechnology in all fields.

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