Solid State Characterization of Two Tropical Starches Modified by Pregelatinization and Acetylation: Potential as Excipients in Pharmaceutical Formulations

**Adenike Okunlola[1,2], Sarafadeen Amusa Adebayo[2]
and Moji Christianah Adeyeye[2*]**

[1]*Department of Pharmaceutics and Industrial Pharmacy, University of Ibadan, Ibadan, Nigeria.*
[2]*Department of Biopharmaceutical Sciences, College of Pharmacy, Roosevelt University, Schaumburg, Illinois, USA.*

Authors' contributions

This work was carried out in collaboration between all authors. Author AO was the research investigator, took part in the design of the study and drafted the manuscript; author MCA designed the study and supervised the work; author SAA managed the literature search and correction of manuscript. All authors read and approved the final manuscript.

Original Research Article

ABSTRACT

Aims: To pre-gelatinize, acetylate and characterize *Dioscorea dumetorum* (bitter yam) and *Dioscorea oppositifolia* (Chinese yam) starches using solid-state parameters such as differential scanning calorimetry (DSC), X-ray powder diffraction (PXRD), Fourier transform infrared spectroscopy (FT-IR), nuclear magnetic resonance spectroscope (^{1}H-NMR) and scanning electron microscopy (SEM). The pH, swelling, density, flow and rheological properties of the starches were also determined. Commercial native corn and pre-gelatinized potato starches were used as standards.
Methodology: Pregelatinization and acetylation were conducted by thermal and chemical modification respectively. Acetylated starches of bitter and Chinese yams with degrees of substitution of 2.56 and 2.70 respectively were obtained. The presence of acetyl groups was

Corresponding author: Email: nabidogun@yahoo.com;

confirmed by FT-IR, ^{1}H NMR spectroscopy, SEM, DSC and PXRD.

Results: The DSC endotherms revealed that pre-gelatinized bitter and Chinese starches had higher gelatinization temperatures than the native forms (111.56 to 123.52°C and 101.14 to 108.88°C respectively), whereas the acetylated starches had lower gelatinization temperatures (84.69°C and 69.85°C respectively). The modified starches showed lower enthalpies (ΔH values 154.0 to 91.91 J/g and 263.57 to 120.70 J/g for the respective pre-gelatinized starches; 8.09 J/g and 7.02 J/g for the respective acetylated starches). Acetylation was confirmed by FT-IR and NMR spectroscopy. The PXRD spectra showed characteristic semi-crystalline structures of the native starches (2θ between 13°C and 23°C) were disrupted by acetylation and pregelatinization, forming amorphous structures. Viscosity of 5% slurry of the starches decreased with increasing shear rate; flow curves were typical of non-Newtonian shear thinning fluids. Bitter and Chinese yam starches had lower bulk density (0.33±0.02 and 0.38±0.01 gcm^{-3} respectively) and better compressibility (40±2.00 and 27.17±2.18% respectively) relative to commercial native corn, suggesting their potential effectiveness as binders in tablets. Pre-gelatinized yam starches had higher ($p<0.01$) swelling than their native forms and standards, suggesting their potential as disintegrants. Acetylation altered the nature of the starches from hydrophilic to hydrophobic and reduced swellability, suggesting their suitability for controlled release application.

Conclusion: Bitter and Chinese yam starches in their native, pre-gelatinized and acetylated forms could find applications as cheaper alternative excipients in various pharmaceutical formulations.

1. INTRODUCTION

Pharmaceutical excipients are pharmacologically inactive substances that are added to formulations to facilitate design and perform a wide range of functions in order to obtain desired properties for the finished drug product [1]. Many of the available excipients are sufficient to support typical formulation development. However, a significant number of drug entities currently being developed present formulation challenges that may require either the discovery of new excipients or new applications of existing excipients.

Starch is one of the most widely used excipients in the pharmaceutical industry because of its inertness, abundance and low cost. Commercial starches are obtained from cereals (corn, wheat and rice) as well as from tubers and roots (potato and cassava) [2]. It has been reported that the performance of starches as pharmaceutical excipients is dependent on their source [3,4]. Efforts have been made to develop starches from locally available plant species with a view to discovering starches from botanical sources different from the official ones [5,6]. New, underutilized tropical starches that could be explored for pharmaceutical applications are those obtained from the tubers of *Dioscorea dumetorum* Pax (bitter yam or trifoliate yam) and *Dioscorea oppositifolia* L (family Dioscoreaceae) (Chinese yam). Bitter and Chinese yams are food crops that are important sources of staple diet in many parts of Africa and Asia. The high starch contents of these crops readily present an opportunity for industrial application that could lead to increased earnings from the crops. Conversion into industrial starch is expected to add value to the crops and provide cheaper excipients for utilization in pharmaceutical industries.

Native starches can be modified to introduce new properties and or remove certain inherent undesirable characteristics. Thus, physical and chemical modifications have been applied to starch to improve their functional properties. Physical modification entails subjecting the starch to heat-moisture treatment and annealing, while chemical modification includes oxidation, acid thinning, hydroxypropylation and acetylation [7]. Pregelatinization of starches by thermal modification has been reported to improve viscosity and flow characteristics of starches and thus enhance their uses as excipients in tablet formulations [8]. Chemical modification of starch by acetylation can significantly affect the physicochemical and functional properties of starch [9] as the free hydroxyl groups on C2, C3 and C6 of the starch molecule are substituted with acetyl groups resulting in improved thermal stability, structural strength, reduced swellability and aqueous solubility.

Inherent limitations of native bitter and Chinese yam starches imply inability to meet all the requirements for excipients in applicable

pharmaceutical formulations. Hence, modifications by pregelatinization and acetylation could alter micromeritic properties and, hence, improve some of the inherent shortfalls of the native yam starches. Comparison of the modified starches with commercial pharmacopoeia starches of well-established physico-chemical properties [10] such as corn starch (in its native form) and potato starch (in pre-gelatinized form), would indicate the degree to which modifications have improved native yam starch properties for intended applications in dosage formulations. As of the time of this study, no known commercial starch acetate was available.

The choice of excipients is extremely critical in the design of pharmaceutical formulations. These excipients must fulfill certain requirements such as compressibility, good binding functionality, powder crystallinity and acceptable flowability. The solid-state characterization of pharmaceutical excipients using a material science approach would provide vital pre-formulation data that could be used in quality-by-design and risk mitigation approach to dosage form design. The approach was emphasized by Brittain and Adeyeye [11] and demonstrated in an earlier investigation by Jain and Adeyeye [12]. Therefore; this study was designed to characterize particle morphology, thermal characteristics and crystallinity of native and modified bitter and Chinese yam starches. Other solid state characteristics such as densities and flow properties, as well as acidity (pH), viscosity and swelling of material dispersions in aqueous medium will be determined. FTIR analysis of native and modified starches would be used to confirm acetylation.

2. MATERIALS AND METHODS

2.1 MATERIALS

Tubers of Chinese and bitter yams were obtained from local farmers in Ibadan, Oyo State, Nigeria and authenticated (Forestry Research Institute Nigeria, FRIN) (FHI no: 109673 and 109674 respectively). Roquette 400L NF corn starch was a gift from Roquette America Inc, Chicago, IL, USA, while Prejel[TM] PA 5 PH pre-gelatinized potato starch was a gift from DFE Pharma, USA. All other reagents were of analytical grade.

2.2 METHODS

2.2.1 Extraction of starch

Fresh tubers of yam were peeled, washed with distilled water and then cut into small pieces. The yam pieces were soaked in distilled water containing 2%v/v sodium metabisulphite to prevent darkening and milled into a fine paste. The starch slurries were strained through muslin cloth and the filtrate was left to settle. The supernatant was decanted at 12 hours intervals and the starch slurry re-suspended in distilled water. The starch cake was collected after 72 hrs and dried in a hot air oven at 60°C for 48 hours. The dried mass was pulverized and then screened through a 250-µm mesh sieve [13].

2.2.2 Modification of starches

2.2.2.1 Pregelatinization (Thermal Method)

Aqueous slurry of starch was made with 10 g of starch powder in 100 mL of de-ionized water and then heated at 85°C with stirring for 20 min. The resulting paste was dried in a hot air oven at 60°C for 48 h. The dried mass was powdered in a laboratory mill and passed through a 250-µm sieve.

2.2.2.2 Acetylation (Chemical method)

Fifty grams of native starch was suspended in 550 mL of de-ionized water in a 1000mL conical flask [14]. The suspension was gelatinized by heating and stirring for 30 min at a temperature below 100°C over a hotplate. The gelatinized starch was precipitated with 1 litre of anhydrous ethanol, by stirring under a high shear homogenizer. The precipitated material was filtered and the residue washed with acetone, filtered again and dried. The dried powder was screened through a 125-µm sieve size.

Twenty five grams of the pre-gelatinized starch was dispersed in 200g of pyridine in a 1-litre round-bottom flask. One hundred grams of acetic anhydride was added to the dispersion. The flask was fitted to a rotary evaporator with attached reflux condenser. The round-bottom flask was immersed in an oil bath maintained at 100°C and rotated at low speed inside a fume hood. After 4 h, the reaction mixture was transferred to a beaker and cooled to room temperature. The product was precipitated with 1300ml of ethanol under high shear homogenization. The precipitate was filtered, washed well with ethanol to remove residual pyridine and then filtered again. It was dried in hot air oven (50°C) and passed through a 125-µm sieve.

2.2.3 Determination of degree of substitution

One gram of starch acetate and 50 mL of 75% ethanol were mixed in a flask with a loose

stopper. The mixture was stirred in a water bath at 50°C for 30 min. After cooling to room temperature, 40 mL of 0.5N KOH solution was added to the mixture. The flask was fitted with a tight stopper and kept at room temperature with occasional shaking for 72 h for complete saponification. An excess of alkali in solution was titrated with 0.5N HCl solution using phenolphthalein as the indicator. A blank test was performed applying the same procedure. The percent of acetyl group and degree of substitution (DS) were calculated as shown below [15]:

$$Acetyl\ group\% = \frac{(Blank\ (mL) - Sample\ (mL)) \times Molarity\ of\ HCl \times 0.043}{Sample\ weight\ (g)} \times 100 \tag{1}$$

$$DS = \frac{162 \times \%\ Acetyl\ group}{4300 - (42 \times \%\ Acetyl\ group)} \tag{2}$$

where 162 is the molecular weight of the anhydroglucose unit, 42 is the molecular weight of replaceable acetyl group and 4300 is the molecular weight of the acetyl group attached with 100 anhydroglucose units.

2.2.4 Morphology

The shape and size of the native and modified starch granules were observed using a scanning electron microscope (Hitachi SU8030 FE-SEM Tokyo, Japan) at an accelerating potential of 5.0 kV. All samples were sputter-coated with Au/Pd prior to examination.

2.2.5 ^{1}H NMR analysis

Starch (0.015g) was dissolved in 1mL of deuterated dimethyl sulphoxide (DMSO-d_6) for 6 hours with stirring. The solution was centrifuged at 10,000 X g for 5 hours and the supernatant was measured.^{1}H NMR spectra were recorded on a NMR spectrometer (400 MHz Agilent DD MR-400 system equipped with Agilent 7600 96-sample autosampler).

2.2.6 FT-IR analysis

The starches were analyzed by FT-IR (FTIR-Thermo Nicolet Nexus 870 Madison, WI, USA) in transmission mode. Transmission spectra were recorded using at least 64 scans with 8 cm^{-1} resolution in the spectral range 4000–400 cm^{-1}.

2.2.7 DSC analysis

Thermal characteristics of the starches were studied using a differential scanning colorimeter (TA Instruments DSC 2920 Newcastle, Delaware, USA) in sealed stainless steel pans. The sample and reference pans were heated from -20 to 250°C at a scanning rate of 5°C /min, held for 2 min at 250°C and cooled to -20°C at a rate of 20°C /min. The gelatinization enthalpy (ΔH) and glass transition temperatures (T_g) were then determined for each sample.

2.2.8 X-ray diffraction analysis

The X-ray diffraction pattern was recorded with a copper anode x-ray tube (Cu-Kά$_1$ radiation) using an X-ray diffractometer (Rigaku D-max Tokyo, Japan). The starch powders were packed tightly in sample holders and each sample was exposed to the X-ray beam at 40 kV and 20mA. The scanning region of the diffraction angle (2θ) was from 5° to 60°at step size count of 2 s.

2.2.9 Determination of pH

The pH of 1% aqueous starch slurry was measured at 21.5±0.5°C (Hanna H1 3220 pH/ORP meter Woonsocket, RI, USA).

2.2.10 Solubility

Solubility of the starches was determined in water, methanol and chloroform. Two grams of starch was dispersed in 10 ml solvent and left to stand overnight. The supernatant solution was filtered and 5 ml sample of the filtrate was taken and heated to dryness at 110°C on a hot plate. The residue was weighed and the solubility was calculated as % w/v [16].

2.2.11 Determination of swellability

The swellability of the starches at room temperature (25±2°C) was determined using a modification of the method described by Bowen and Vadino (1984) [17]. Starch powder (5g) was placed into a 100ml measuring cylinder and the volume occupied was noted (V_1). Deionized water (90ml) was added; the dispersion was shaken for 2 minutes and then made up to volume. The slurry was allowed to stand for 24 hours before the sedimentation volume was read (V_2). The swelling index was calculated as V_2/V_1. Determinations were done in triplicates.

2.2.12 Powder density measurements

The particle densities of the starches were determined using Multivolume pycnometer 1305 (Micromeritics Inst. Corp. Norcross Georgia,

USA). The bulk density of each starch powder at zero pressure (loose density) was determined by pouring 10g of the powder at an angle of 45° through a funnel into a glass measuring cylinder with a volume of 50ml. The bulk density was measured as the ratio of mass to volume occupied by the starch. The tapped density was measured by applying 100 taps to 10g of starch sample in a graduated cylinder at a standardized rate of 38 taps per minute from a height of 2.54 cm [18].

2.2.13 Flow properties of starches

The flowability of the starches was assessed using Hausner's ratio and Carr's index. The Hausner`s ratio was determined as the ratio of the tapped density to the bulk density. The Carr's index (% compressibility) was calculated as follows:

$$Carr's\,index = \frac{(Tapped\,density - Bulk\,density)}{Tapped\,density} \times 100 \quad (3)$$

Flow property was also assessed by determining the angle of repose. Starch powder (5g) was allowed to flow freely through a funnel under gravity, to form a conical heap. The angle of repose was calculated from:

$$Tan\,\theta = h/r \qquad (4)$$

where h is the height of powder and r is the radius of the base of the cone. The angle of repose values represents means of three determinations.

2.2.14 Rheological properties

The Brookfield viscometer (DV-III ULTRA model, spindle no 04) was used to measure the viscosity. Starch slurry (5% w/v) was placed in the sample tube (100 ml capacity). The velocity of the spindle was varied from 50 to 250 rpm and the viscosity of the starches was measured at room temperature (25±1°C) as a function of shear rate to shear Stress. An apparent viscosity, commonly used to describe the flow behavior of non-Newtonian fluids was applied using the relationship:

$$\frac{\tau}{\gamma} = \frac{Shear\,stress}{rate\,of\,shear} \qquad (5)$$

The flow behavior was analyzed with a standard mathematical model, the Power law (also called Ostwald-de Waele equation) using equation 6:

$$\mu_a = K\left(1/n\right)^n \times \left(4\pi N\right)^{n-1} \qquad (6)$$

which can be expressed in logarithmic form as:

$$\ln(\mu_a) = (n-1)\ln 4\pi N + \ln(K) - n\ln(n) \qquad (7)$$

where μ_a is the apparent viscosity (Poise), N the spindle speed (rps), K is the consistency index (ps^n) and n is the flow behavior index.

The values of ln (μ_a) and ln ($4\pi N$) were fitted to obtain a linear relationship; and from the slope and intercept of the best fit line, the flow behavior index "n" and consistency coefficient "K" respectively were determined.

2.2.15 Statistical analysis

Statistical analysis was done to compare the properties of the starches using the analysis of variance (ANOVA) on a computer software GraphPad Prism© 4 (Graphpad Software Inc. San Diego, CA, USA). Tukey-Kramer multiple comparison tests were used to compare the differences between the starches. At 95% confidence interval, probability (p-values) less than or equal to 0.05 were considered significant.

3. RESULTS AND DISCUSSION

3.1 Morphology

Scanning electron images of all starch forms are shown in Fig. 1. The values of the mean particle size of the different starch types are presented in Table 1. The SEM images of bitter and Chinese yam in their native forms showed polygonal granules with mean particle sizes of 3.30±0.71μm and 7.55±1.10 μm respectively. Corn starch on the other hand had particles that were angular or polyhedral in shape with a mean particle size of 14.30±1.10. Comparison of the micrographs obtained for native and modified yam starches revealed that pre-gelatinization and acetylation of starch disrupted the granular structure of the native starches.

The pre-gelatinized starches had significantly (p<0.01) larger structures that were gel-like, aggregated and irregular while those of the acetylated starches were significantly (p<0.01) larger, more fibrous, irregular aggregates. These observed shapes and morphology are consistent with those reported in literature [19-22]. The

ranking of the mean granule size of all native starches was corn > Chinese > bitter while that for the pre-gelatinized starches was Chinese > potato starch > bitter. Particle size has been reported to have significant effect on the densification of the starches during die filling, particle rearrangement, fragmentation propensity and elastic/plastic deformation [23,24].

Particle shape and size can influence compaction characteristics as they affect the packing behavior of the starches. This is because there is a tendency for particle rearrangement to occur in the initial stages of the compaction process [23]. Starches with regular shape and finer particles tend to have a higher number of particles per unit weight that is indicative of a higher potential of achieving homogeneity when mixing the substance with active pharmaceutical ingredients [3].
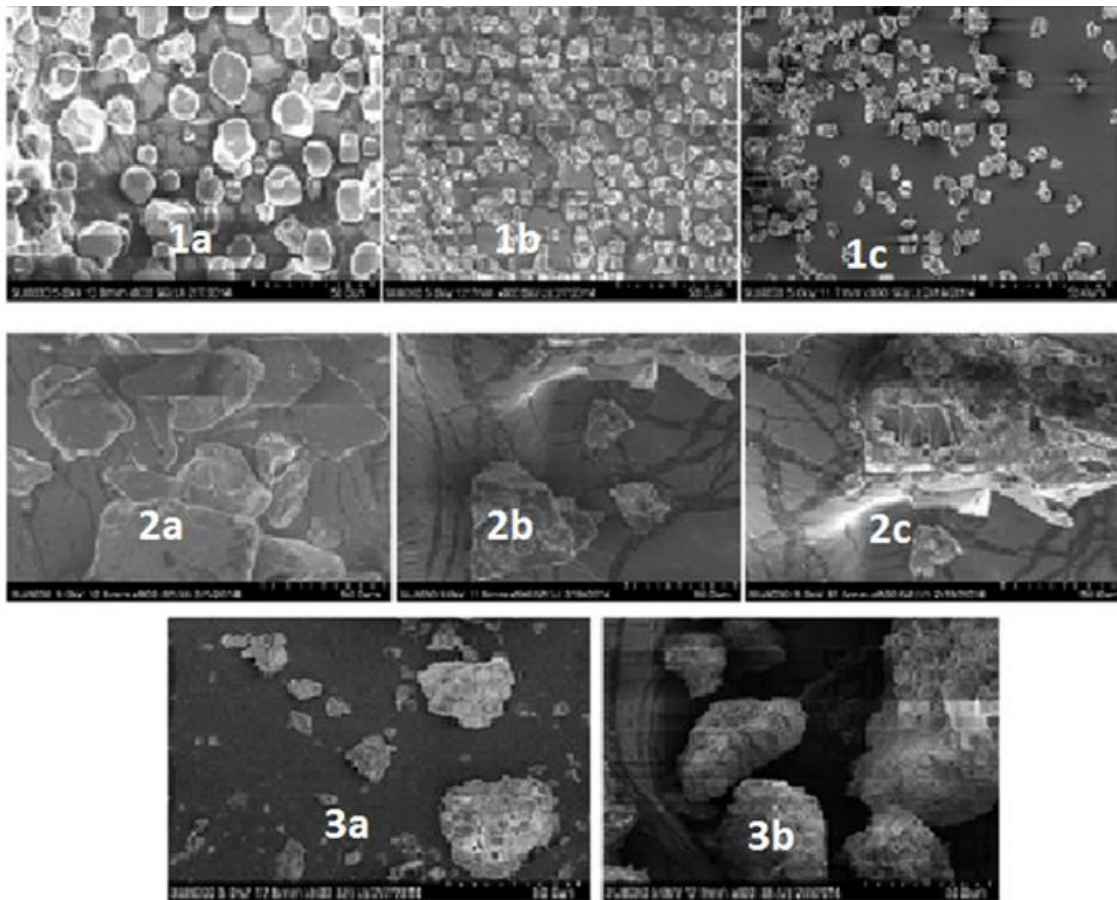

Fig. 1. SEM of: 1 (a) commercial native starch; (b) native bitter yam starch; (c) native Chinese yam starch; 2 (a) commercial pre-gelatinized starch; (b) pre-gelatinized bitter yam starch; (c) pre-gelatinized Chinese yam starch and 3(a) acetylated bitter yam starch and (b) acetylated Chinese yam starch. Mg x 800

Table 1. Material properties of starches (mean ± sd, n = 3)

Starch	Particle shape	Particle size µm	pH	Swelling	Bulk density gcm^{-3}	Tapped density gcm^{-3}	Hausner's ratio	Carr's index	True density gcm^{-3}	Angle of repose °
Bitter yam										
Native	Polygonal	3.30±0.71	5.14±0.03	1.04±0.04	0.33±0.02	0.56±0.01	1.67±0.03	40.00±2.00	1.50±0.01	55.03±4.50
Pregel	Irregular aggregates	17.90±2.00	6.08±0.06	3.33±0.12	0.39±0.01	0.48±0.01	1.23±0.03	18.75±1.75	1.52±0.02	52.22±3.80
Acetylated	Irregular fibrous aggregates	15.90±1.25	5.20±0.05	0.94±0.03	0.28±0.01	0.33±0.02	1.17±0.00	14.77±2.22	1.54±0.00	46.40±2.12
Chinese yam										
Native	Polygonal	7.55±1.10	5.00±0.10	1.17±0.03	0.38±0.01	0.52±0.02	1.38±0.01	27.27±2.18	1.51±0.01	53.06±2.82
Pregel	Irregular aggregates	83.95±3.60	5.90±0.10	4.71±0.10	0.46±0.01	0.59±0.01	1.31±0.01	23.64±1.70	1.53±0.00	40.70±3.23
Acetylated	Irregular fibrous aggregates	62.82±0.10	5.14±0.05	1.05±0.05	0.33±0.01	0.39±0.01	1.15±0.00	13.33±1.25	1.48±0.00	37.23±1.06
Corn	Polygonal	14.30±1.10	5.74±0.05	0.95±0.03	0.46±0.01	0.62±0.01	1.38±0.01	27.27±2.12	1.48±0.01	57.51±2.25
Pregel potato	Irregular aggregates	37.15±2.20	5.88±0.02	2.75±0.05	0.56±0.01	0.63±0.01	1.13±0.01	11.11±1.00	1.50±0.00	43.53±1.30

3.2 ^{1}H-NMR Analysis

Acetylated bitter and Chinese yam starches with degrees of substitution of 2.56 and 2.70 respectively were used. The proton resonances of the anhydroglucose units showed changes in the acetylated yam starches when compared to their native forms. The starch backbone signals were at 3.6-5.5ppm while those due to the methyl protons of acetate were at 1.9-2.1 ppm (Fig. 2). The peaks observed at 2.5 and 3.1 ppm are due to DMSO-d_6 and water respectively [25]. The peaks at 1.05 ppm, indicated that the hydrogen atoms of the hydroxyl groups in the native starches were substituted with acetyl groups. The intensity of the peak was greater for Chinese yam starch acetate due to its higher degree of substitution (DS). During acetylation, free hydroxyl groups on C_2, C_3 and C_6 of the starch molecules were substituted with acetyl groups and this modification has been reported to improve thermal stability and structural strength while reducing starch swellability and solubility [26,27]. High acetyl substituted starch with a degree of substitution (DS) of > 2 is of research interest because of their thermoplasticity, however, the FDA requires acetylated starches of DS 0.01 -0.2 for food applications [28].

3.3 FT-IR Analysis

The FT-IR spectra of the native and modified starches showed characteristic absorption bands at 992, 929, 861, 765 and 575 cm^{-1} (Fig. 3). The bands are due to the entire anhydroglucose ring stretching vibrations. Broad bands at 3000-3600 cm^{-1} correspond to O-H stretching while the peaks at 2950 and 1647 cm^{-1} correspond to C-H stretching and δ (O-H) bending respectively. The FT-IR band at 1042 cm^{-1} was observed to be present for all native starches. The spectra of the modified starches indicated the formation of amorphous structure resulting in decrease in the ordered structure of native starches. New bands at 1700 cm^{-1} (Stretching C=O), 1375 cm^{-1} (Stretching C-CH$_3$) were observed for the acetylated yam starches as had been previously reported [29]. FT-IR bands at 3400 cm^{-1} (Stretching O–H) and 1083 cm^{-1} (C-O-C bond stretching) were weak, confirming the replacement of the hydroxyl groups in the starch molecules with acetyl groups.

3.4 DSC Analysis

The enthalpy of gelatinization reflects the loss of molecular order [30]. The endothermic peaks in the DSC curve of the pre-gelatinized bitter and Chinese yam starches shifted slightly to the right with increase in the peak temperature (from 111.56 to 123.52°C and 101.14 to 108.88°C respectively (Fig. 4). There was decrease in enthalpy for the bitter and Chinese yam starches (ΔH values 154.0 to 91.91 J/g and 263.57 to 120.70 J/g respectively). Native starch is partly crystalline in nature and thus the magnitude
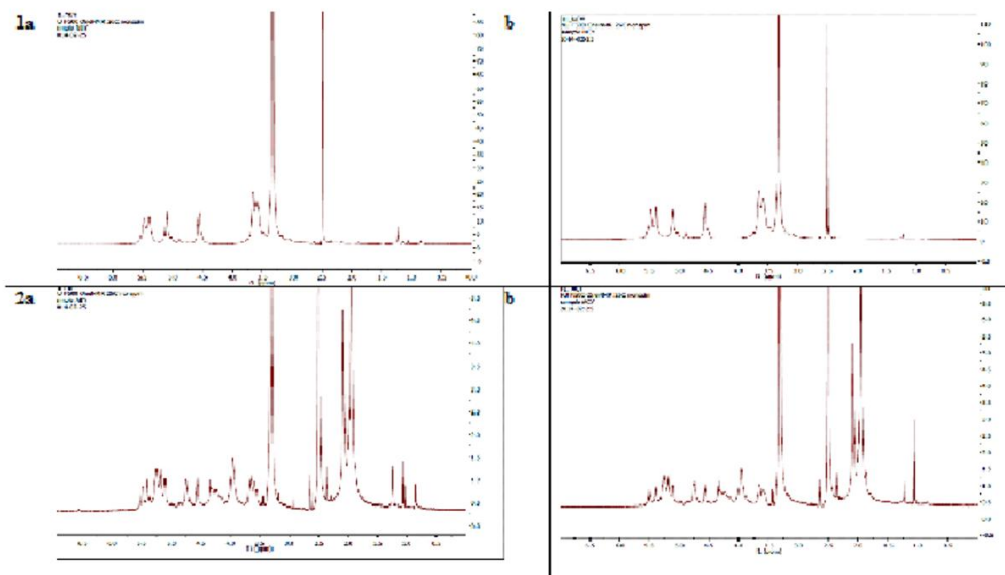

Fig. 2. NMR spectra of: 1 (a) native bitter yam starch; (b) native Chinese yam starch; 2 (a) acetylated bitter yam starch; (b) acetylated Chinese yam starch

of the amorphous region is lower than that of the pre-gelatinized starch, which could account for the differences observed in the gelatinization temperatures and enthalpies [31,32].

On the other hand, for the acetylated bitter and Chinese yam starches, DSC curves shifted towards the left with reduction in T_p (111.56 to 84.69°C and 101.14 to 69.85°C respectively) and marked reduction in ΔH values (154.0 to 8.09 J/g

and 263.57 to 7.02 J/g respectively). This change can be explained by the inter-molecular hydrogen bonds which decreased with the replacement of hydroxyl groups by acetyl groups. The increase in the free volume within the molecules due to the introduction of bulk groups that allows more molecular mobility also contributes to the reduction in ΔH of starch with acetylation [33]
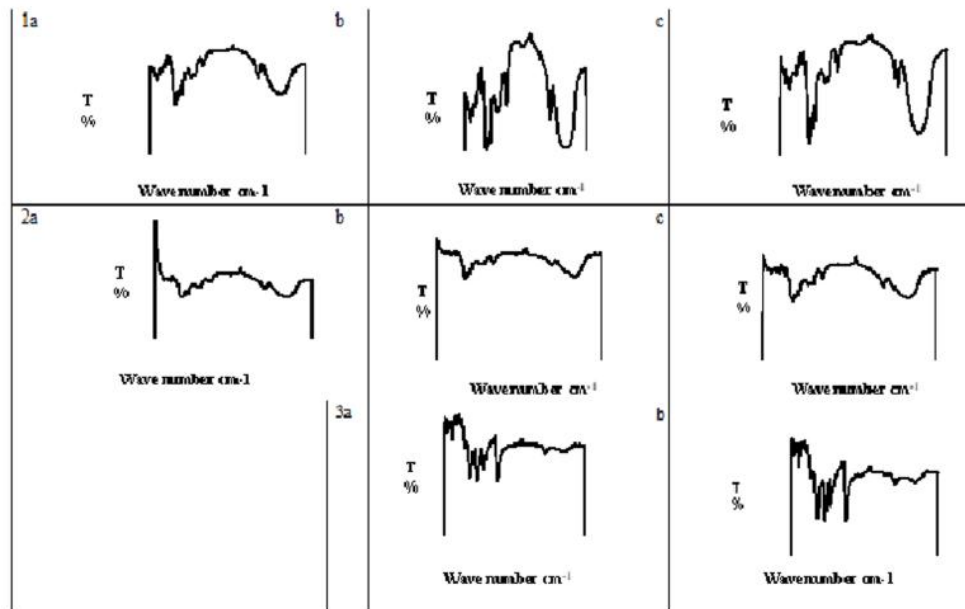

Fig. 3. FT-IR spectra of: 1 (a) native commercial starch; (b) native bitter yam starch; (c) native Chinese yam starch; 2 (a) pre-gelatinized commercial starch; (b) pre-gelatinized bitter yam starch; (c) pre-gelatinized Chinese yam starch; and 3 (a) acetylated bitter yam starch and (b) acetylated Chinese yam starch
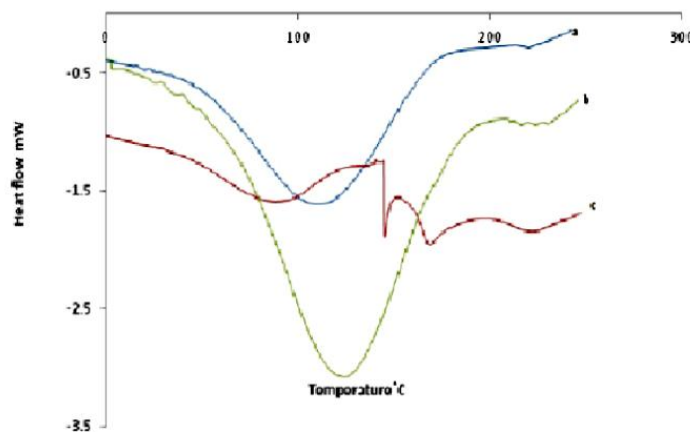

Fig. 4. Typical DSC thermograms of: (a) native; (b) pre-gelatinized and (c) acetylated bitter yam starch

3.5 XRD Analysis

Native granular starches have X-ray diffraction patterns that have been classified as A (for cereals such as corn) and B (for roots such as potato) polymorphs [34]. In the A-type starches, A and B_1 short chains in the crystalline structure are less stable and more susceptible to rearrangements, whereas B-type starches present higher proportions of long B chains, which extend for two or more clusters and stabilize the internal structure of granules becoming more resistant to enzymatic action [35]. Starches with A-type polymorphism are more susceptible to amylases action than starches with B-type polymorphism.

The native *Dioscorea* starches typically showed strong reflections at 2θ of between 13° and 23° (Fig. 5). These diffraction pattern values are interpreted as intermediate diffraction pattern between A and B and they have been referred to as C-type diffraction pattern which are not true crystalline polymorphs but rather a mixture of A and B polymorphs [36]. The native bitter yam starch had comparable diffraction pattern as the commercial corn starch (left panel). The pre-gelatinized yam starch also had similar pattern as the commercial pre-gelatinized. In contrast, the acetylated starches showed decrease in crystallinity when compared to native starches, with more peaks being disrupted, and a shift of a peak to a lower 2θ of about 9°. This correlated with NMR and FTIR observations.

3.6 Other Material Properties of Starches

The material and physico-chemical properties of the starches are presented in Table 1.

One of the physico-chemical properties of starch important to their application is pH value [37]. Pregelatinization of yam starches resulted in significantly higher (p<0.05) pH than those of native and acetylated starches.

Pregelatinization of starches resulted in increased aqueous solubility while acetylation resulted in decreased aqueous solubility. Acetylated starches were readily soluble in chloroform.

Pregelatinization also resulted in significantly higher swelling power (P<0.01) but acetylation resulted in reduction in swelling of starches. The ranking of the swelling power for the pre-gelatinized starches was Chinese>bitter> commercial pre-gelatinized potato, indicating that yam starches had significantly higher (p<0.05) swelling than the commercial pre-gelatinized potato starch. Differences observed in the swelling power values of the starches were probably due to the starch species, degree of macromolecular disorganization and variations in the degradation of starch during thermal treatments. Generally, the intrinsic swelling power has been recognized as a qualitative assessment of potential disintegrant effects of starches though it may not be considered to be an absolute index of disintegrant efficiency.
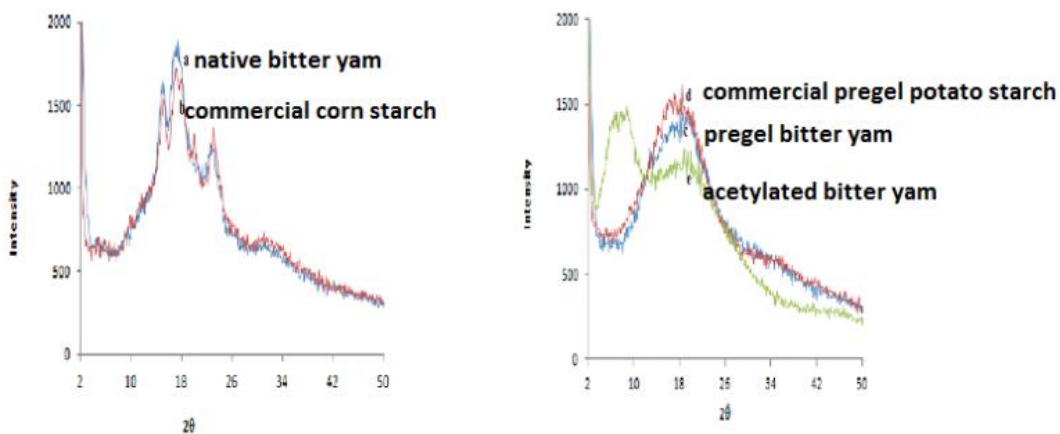

Fig. 5. XRD profiles of: (a) native bitter yam starch; (b) commercial native corn starch; (c) pre-gelatinized bitter yam starch; (d) commercial pre-gelatinized starch and (e) acetylated bitter yam starch

3.7 Density and Flow Properties

The bulk density of a powder describes its packing behavior while the tapped density indicates the rate and extent of packing that would be experienced by a material during the various unit operations of tableting [23]. Generally, pre-gelatinization resulted in higher values of bulk density while acetylation produced lower values (Table 1). Higher bulk density may be advantageous in tableting because of a reduction in the fill volume of the die. The differences observed in these values could be due to the difference in particle shape and particle size distribution, both of which affect the packing arrangement of particles.

The Hausner's ratio (tapped to bulk density) provides an indication of the degree of densification which could result from vibration of the feed hopper, for example, during tableting. Higher values of Hausner's ratio predict significant densification of powders. The ranking of Hausner's ratio was generally native starch>pre-gelatinized starch>acetylated starch, suggesting that the acetylated starches exhibited highest degree of densification with tapping.

Flowability is of important consideration in tableting since a uniform flow ensures minimal weight variations in tablets produced. Carr's index is a measure of flowability and compressibility of a powder. The lower the Carr's index, the better the flowability but the poorer the compressibility [38]. The ranking of Carr's index was generally native starch>pre-gelatinized> acetylated starch. The result indicates that modification of the starches resulted in improved flow. The angle of repose is another qualitative measure of the cohesiveness or the tendency of powdered materials to flow. Angle of 30° or below usually indicates that the powder is free flowing. Angles of repose of 40° or above indicate poor flow.

The ranking of angle of repose was generally native>pre-gelatinized>acetylated and this result shows that pre-gelatinization and acetylation improved the flow properties of the starches. The packing and cohesive properties of starches influence the various aspects of powder processing such as milling, blending, flow from hoppers, compression and packing into capsule shells or containers. The native forms of bitter and Chinese yam starches had smaller granule sizes, lower bulk density and higher values of Hausner's ratio and Carr's index in comparison to those of the commercial corn starch, indicating better compressibility. This suggests their usefulness as binders in tablet formulations.

3.8 Rheological Properties

Viscosity is an important material property that contributes to a fluid's performance, and often is the main source of problems in handling, processing, and application. Determination of rheological properties can be used to simulate the material's performance during processing and for quality control. Fig. 6 shows typical curves of apparent viscosity versus shear rate for 5% w/v slurry (a concentration at which most starches are utilized as binders and disintegrants in tablet formulations) of all starch materials at 25°C. The viscosity of the starches decreased with increase in shear rate and the flow curves obtained are typical of a non-Newtonian shear thinning (pseudo-plastic) fluid. Over the entire range of shear rates, reasonably linear behaviors were obtained with no evidence of a yield stress at the earlier periods of shear stress application. The data fitted the power law equation and the values of n (flow behavior index) and K (consistency index) are shown in Table 2. R^2 were generally > 0.9 for all starches. The values of n were less than 1 indicating shear-thinning behavior.

Table 2. Rheological characteristics of starches

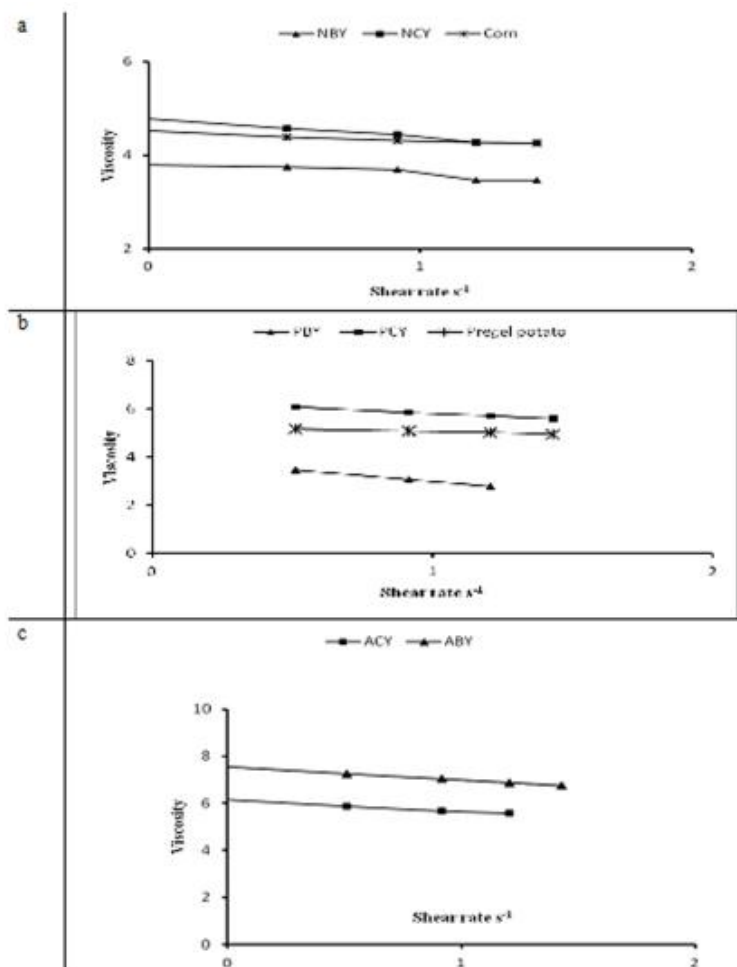| Starch	Flow behavior index N	Fluid consistency index K	Correlation coefficient
Bitter yam			
Native	0.93	44.24	0.997
Pregelatinized	0.50	270.93	0.950
Acetylated	0.44	189.56	0.990
Chinese yam			
Native	0.59	118.68	0.993
Pregelatinized	0.51	461.23	0.993
Acetylated	0.46	576.05	0.999
Corn	0.81	90.76	0.970
Pregel potato	0.97	91.50	0.990

Fig. 6. Viscosity profile of (a) native; (b) pre-gelatinized and (c) acetylated starches (5%w/v at 25°C)

4. CONCLUSION

Solid state characterization of pre-gelatinized and acetylated bitter and Chinese yam starches revealed that there were significant changes in the material nature, flow, swelling and rheological properties of the native forms of the starches upon modification. The native forms of bitter and Chinese yam starches had smaller granule sizes, lower bulk density and higher values of Hausner's ratio and Carr's index than commercial corn starch, indicating better compressibility. This suggests their usefulness as binders in tablet formulations. Modification of the yam starches by pregelatinization resulted in improved flowability and significantly higher swelling when compared to the native forms and the commercial pre-gelatinized potato starch,

suggesting their potential usefulness as disintegrants. Bitter and Chinese yam starches modified by acetylation showed potential for use as controlled delivery polymers because of the reduced swelling and hydrophobic properties. Thus, bitter and Chinese yam starches in their native and modified forms can find application as excipients in various pharmaceutical formulations.

CONSENT

Not applicable.

ETHICAL APPROVAL

Not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Ansel HC, Allen LV, and Jr Popovich NG. Pharmaceutical dosage forms & drug delivery systems, 9th ed., Lippincott Williams & Wilkins; 2011.

2. Freitas RA, Paula RC, Feitosa JPA, Rocha S, Sierakowski MR. Amylose contents, rheological properties and gelatinization kinetics of yam (*Dioscorea alata*) and cassava (*Manihot utilissima*) starches. Carbohydr Polym. 2004;55:3–8.

3. Riley CK, Adebayo AS, Wheatley AO and Asemota, HN. The interplay between yam (*Dioscorea sp.*) starch botanic source, micromeritics and functionality in paracetamol granules for reconstitution. Eur J Pharm & Biopharm. 2008;70:326–334.

4. Ogaji IJ, Nep EI, Audu-Peter JD. Advances in natural polymers as pharmaceutical excipients. Pharm Anal Acta. 2012;3:146. DOI:10.4172/2153- 2435.1000146.

5. Adebayo AS, Itiola OA. Evaluation of breadfruit and cocoyam starches as exo-disintegrant in a paracetamol tablet formulation Pharm. Pharmacol. Commun. (London). 1998;4:385-389.

6. Okunlola A, Odeku OA. Evaluation of starches obtained from four *Dioscorea* species as binding agent in chloroquine phosphate tablet formulations. Saudi Pharm J. 2011;19:95–105.

7. Neelam K, Vijay S, Lalit S. Various techniques for the modification of starch and the application of its derivatives. International Research Journal of Pharmacy. 2012;3(5):25-31.

8. Odeku OA, Picker-Freyer, KM. Analysis of the material and tablet formation properties of four *Dioscorea* starches Starch/Stärke. 2007;59:430-444.

9. Moorthy SN. Acetylation of cassava starch using perchloric acid catalysis Starch/ Starke. 1985;37:307-308.

10. British Pharmacopoeia. Her Majesty's Stationery Office, London. 2012;1 and 2.

11. Preformulation in solid dosage form development, Moji Christianah Adeyeye and Harry G. Brittain (editors). Informa Healthcare Publisher; 2008. ISBN: 9780824758097

12. Jain A, Adeyeye CM. Characterization of various substituted sulfobutyl ether-β-cyclodextrin inclusion complexes. Int. J. Pharm. 2001;212:177-186.

13. Young AH. Fractionation of starch. In: Whistler RL, Bemiller JN, Pashall EF (editors.). Starch Chem. Technol., 2nd ed. London: Academic Press. 1984;249-283.

14. Singh AV, Nath LK. Evaluation of acetylated moth bean starch as a carrier for controlled drug delivery IntJ BiolMacromol. 2012;50(2):362–368. DOI: 10.1016/j.ijbiomac.2011.12.011.

15. Ogawa K, Hirai L, Shimasaki C, Yoshimura T, Ono S, Rengakuji S, Nakamura Y, Yamazaki I. Simple determination method of degree of substitution for starch acetate. Bull Chem Soc Jpn. 1999;72:2785-2790.

16. Iwuagwu MA, Onyekweli AO. Preliminary investigation into the use of *Pleurotus tuberregium* powder as a tablet disintegrant. Trop. J. of Pharm. Res. 20021(1):29-37.

17. Bowen FE, Vadino WA. A simple method for differentiating sources. Drug Dev. Ind. Pharm. 1984;10:505-511.

18. British Standard 1460: British Standard Institution, London; 1967.

19. Amani NG, Bulẽon A, Kamenan Aand Colonna P. Variability in starch physicochemical and functional properties of yam (*Dioscorea*sp) cultivated in Ivory Coast. J. Sci. Food Agric. 2004;84(15): 2085-2096.

20. Singh J, Kaur L, Singh N. Effect of acetylation on some properties of corn and potato starches. Starch/ Starke. 2004;56(12):586-601.

21. Narkon KN, Tongdang T, Sirivongpaisal P. Crystallinity and rheological properties of pre-gelatinized rice starches differing in amylase content Starch/Starke. 2009;61:101-108.

22. ZhiGang L and Yong-Chen S. Preparation of acetylated normal and amylase content maize starch with intermediate degrees of substitution in aqueous solution and their properties J. Agric & Food Chem. 2012;60:9468-9475.

23. Wray PE. The physics of tablet compression revisited. Drug Dev & Ind Pharm. 1992;18:627-658.

24. Ruiz A, Paronen P. Time-dependent densification behavior of cyclodextrins. J Pharma Pharmacol. 1996;48:790-797.

25. Zhigang L, Zidan. Homogeneous synthesis and characterization of starch acetates in

ionic liquid without catalysts Starch/Starke. 2012;64:37-49.

26. Roper H. Applications of starch and its derivatives. Carbohydr. Eur. 1996;15:14-21.

27. Singh AV, Nath LK. Evaluation of acetylated moth bean starch as a carrier for controlled drug delivery. Int J Bio Macromol. 2012;50(2):362-368.

28. de Graaf R A, Broekroeflofs A, Janssen LBB. Starch/ Starke 1998;50:198–205.

29. Harvey AC, Adebayo AS, Wheatley AO, Asemota HN, Riley CK. Effects of acetylation on the micromeritics of yam (*Dioscorea* sp.) starch powder for pharmaceutical. Application West Afr J Pharm. 2012;23(2):40–50.

30. Tester RF, Morrison WR. Swelling and gelatinization of cereal starches II. Waxy rice starches. Cereal Chem. 1990;67:558.

31. Bean MM, Yamazaki WT, Donelson DH. Wheat starch gelatinization in sugar solutions, fructose, glucose and sucrose: Cake performance. Cereal Chem. 1978;55:945-952.

32. Chung HJ, Lee EJ, Lim ST. Comparison in glass transition and enthalpy relaxation between native and gelatinized rice starch. Carbohydr Polymer. 2002;48:287-298.

33. Singh J, Kaur L and McCarthy OJ. Factors influencing the physico-chemical, morphological, thermal and rheological properties of some chemically modified starches for food application – A review. Food Hydrocolloids. 2007;21(1):1-22.

34. Anastasiades A, Thanou S, Loulis D, Stapatoris A, Karapantsios TD. Rheological and physical characterization of pre-gelatinized maize starches. Journal of Food Engineering. 2002;52:57–66.

35. Jane J. Current understanding on starch granule structure. J App Glycosci. 2006;53(3):205-213.

36. Brunnschweiler J, Luethi D, Handschin S, Farah Z, Escher F, Conde-Petit B. Isolation, physicochemical characterization and application of yam (*Dioscorea* spp.) starch as thickening and gelling agent. Starch/ Stärke. 2005;57:107-117.

37. Shimelis E, Meaza M, Rakshit S. Physico-chemical properties, pasting behavior and functional characteristics of flours and starches from improved bean (*Phaseolus vulgaris* L.) varieties grown in East Africa. Agricultural Engineering International. 2006;15(8):1-19.

38. Carr RL. Evaluating flow properties of solids. Chem. Engr. J. 1965;72:163-168.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history.php?iid=860&id=14&aid=7086