Solid state characterization and rheological properties of native and modified *Bambara* groundnut (Vigna subterranean) starches.

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Received: March 20, 2017; *Accepted:* May 17, 2017

Technical Note

ABSTRACT

This study was designed to determine the suitability of starches from the native, pregelatinized and carboxymethylated *Vigna subterranean* (Bambara nut) for pharmaceutical applications by physicochemical, rheological, thermal, morphological and instrumental spectroscopic methods. The native starch was extracted from the Bambara nut, after which it was prepares in both pregelatinized and carboxymethylated forms. Microscopy revealed increased granular size after modification. Both pregelatinized and carboxymethylated Bambara starches showed improved flow properties and swellability compared to the native starch. Native Bambara starch had greater tendency to retrogradation, was more sensitive to heat and heat changes, which were reduced by both pregelatinization and carboxymethylation. DSC confirmed that carboxymethylated Bambara starch was the most thermally stable starch. The presence of functional groups and crystallinity were established by FTIR and XRD, respectively. This study shows that native and modified Bambara starches could be used as locally, readily available alternative excipients in pharmaceutical formulations.

KEY WORDS: *Vigna subterranean* starch, pregelatinization, carboxymethylation, solid state characterization, rheological properties, excipients

INTRODUCTION

Legumes contain about 60 % carbohydrates of which starch constitutes the major portion (1). Refined starches from several cereal, root and tuber crops according to Hoover and Sosulski (2) are used widely in industrial and food applications but legume starches, such as lentil starch, have few commercial uses.

Vigna subterranea (also known by its common names *Bambara* groundnut, *Bambara*-bean, Congo goober, earth pea, ground-bean, orhog-peanut) is a member of the family *Fabaceae*. The origin of the *Bambara* groundnut is West Africa

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and the main region of cultivation is the Sub-Saharan Africa's tropics (3). The *Bambara* groundnut, an underutilized crop predominantly grown in African countries, is very nutritious and contains 65% carbohydrates and 18% protein (4, 5).

Starch is one of the most widely used excipients in the manufacture of solid dosage forms. Researchers have tried to develop botanical starches for use as binders and disintegrants in tablet formulations (6, 7). It has been reported that the performance of starches as pharmaceutical excipients is dependent on their botanical source (8, 9). Efforts have been made to develop starches from locally available plant species with a view to discovering starches from botanical sources different from those already on the market (10, 11).

Native starches from several botanical sources have been characterized and their limited functionality as excipients in pharmaceutical and food industries have been described previously (12-13). However, the functional properties of starches for use as excipients may be improved through various modifications (14-17). Modified starches have promising industrial prospects because they are more biocompatible and environmentally friendly compared to synthetic polymers (18). Starch can be modified using chemical, enzymatic, or physical techniques (18). Starches can undergo many reactions characteristic to polyhydroxyl compounds. The introduction of small amounts of ionic or hydrophobic groups into the starch molecule alters the properties of starch including the solution viscosity, association behavior and shelf life stability (19, 20).

This study aims to investigate the suitability of the Bambara groundnut (an underutilized crop predominantly grown in African countries) for pharmaceutical applications (3, 5). Solid state characterization of pharmaceutical excipients is a prerequisite for successful drug formulation. The starch was characterized using microscopy, viscoamylography, FTIR, and X-ray powder diffraction, in order to investigate their potential uses as pharmaceutical excipients and determine the effect of modification on functional properties.

MATERIALS AND METHODS

Materials

Materials used included sodium chloride (BDH Chemicals Limited), acetic anhydride (BDH Chemicals Limited), hydrochloric acid (BDH Chemicals Limited), and Acetone (Merck Limited, Germany). Distilled water and starches of Bambara (*Vigna subterranean* Fabaceae) were prepared in the laboratory of the Department of Pharmaceutics and Industrial Pharmacy, University of Ibadan, Nigeria.

Methods

Collection and preparation of the plant material

The Bambara nuts (*Vigna subterranean* Fabaceae*)* were purchased from the Bodija market in Ibadan, Nigeria. The native starch forms were prepared in the laboratory through extraction with water using the method described by Young (21).

Preparation of the pregelatinized starches

Pregelatinized forms of the starches were prepared in the laboratory using the method described in the British Pharmaceutical Codex (22) and by Herman *et al.* (23). The product was stored in air tight, amber colored containers.

Preparation of the carboxymethylated starch

Carboxymethylated forms of the starches were prepared using the method described by Kittipongpatana *et al.*, (15). The dried flakes of the starches recovered were ground using a mortar and pestle and screened through a number 120 mesh (125 μm) British Standard sieve. The dry product was weighed and stored in air tight containers.

Determination of the degree of substitution (DS) of carboxymethylated starch

The degree of substitution of the prepared carboxymethylated starch was determined as described by Jovanovic *et al.* (24).

Microscopic analysis

The starch powder was examined using a light microscope (BH-2 BHS, Olympus, Tokyo, Japan). The mean projected diameter, d, of 200 particles was determined.

Morphology

The surface morphology of the polymer was examined using Scanning electron microscopy (SEM). Samples were mounted on aluminium stubs using double sided carbon tape attached to a stub and coated with a gold film under vacuum in a sputter coater and observed under magnification with a JEOL JSM-6060LV Scanning Electron Microscope at 20 kV.

Determination of particle density

The particle density of the starches were determined using the pycnometer method as described previously by Odeniyi *et al.* (25). Xylene was used as the displacement fluid.

Determination of loose bulk and tapped bulk densities

The starch sample (30g) was weighed (M) and carefully transferred into a graduated measuring cylinder using a funnel. The loose bulk volume V_O was determine by measuring the height h_O, in cm formed by the bulk of powder without any disturbance and derived from Equation 1:

$$V_o = \pi r^2 h_o \qquad \text{Eq. 1}$$

Where, r is the radius of the graduated measuring cylinder. The tapped volume of each starch sample was determined by mechanically tapping the loose powder in the graduated cylinder. The volume was recorded after no significant change in the volume occurred. The volume at this point is the tapped volume $V_{t,}$ which may be determined from the final height h_t obtained after tapping as shown in Equation 2.

$$V_t = \pi r^2 h_t \qquad \text{Eq. 2}$$

The loose density ϱ_o and tapped density ϱ_t were determined by dividing the mass of powder (M) used for the analysis by obtained volumes (mass per volume formed by each sample in g/cm^3), that is V_o and V_t , respectively, using Equations 3 and 4:

$$\rho_{o} = \frac{M}{V_o} \qquad \text{Eq. 3}$$

$$\rho_{t} = \frac{M}{V_t} \qquad \text{Eq. 4}$$

The compressibility index, also known as Carr's index C, was determined for each powder starch sample using Equation 5:

$$C = \frac{100(V_o - V_t}{V_o} \qquad \text{Eq. 5}$$

The Hausner ratio, H of the starches was also determined from the ratio of loose bulk volume to tapped volume using Equation 6:

$$H = \frac{V_o}{V_t} \quad \text{Eq. 6}$$

Determination of solubility in water

Aqueous solubility was determined by the method described by Leach *et al.* (26).

Determination of swelling index

The swelling index at room temperature (25°C±2°C) was determined using the method described by Bowen and Valdino (27). Determinations were done in triplicate.

Determination of pH

Slurries of starch samples were prepared at 1% w/v concentration, by aqueous suspension of the starches in distilled water. The pH of the slurry was determined using a bench-top pH meter (pH-016, China).

Determination of water absorption capacity

The water absorption capacity (WAC), also known as water binding or water holding capacity, was determined using the method developed by Yamazaki (28), as modified by Medcalf and Gilles (29).

Determination of angle of repose

An opened ended cylinder was placed on a base of similar diameter. Starch powder (5 g) was allowed to flow freely through a funnel under gravity, to form a conical heap. The angle of repose was calculated using Equation 7:

$$\mathrm{Tan}\,\theta = \frac{h}{r} \quad \text{Eq. 7}$$

Where h is the height of the powder and r is the radius of the base of the cone. The angle of repose was determined from the mean of three calculations.

Moisture content

Starch powder (10 g) was weighed into a tarred dish with a removable lid and the moisture content determined according to AOAC guidelines (30).

Rheological assessment of the starches

Rheological properties of the starch forms were analyzed using the Rapid Visco Analyser (RVA) (Newport Scientific Pty. Ltd, Wariewood, Australia). Starch slurries (30% w/v) were heated in the instrument at a ramp rate of 1.5°C per minute from a starting temperature of 50°C to a temperature of 95°C at a shear rate of 960 RPM. The viscoamylograph plots were printed out for analysis.

FTIR spectroscopic analysis

Potassium bromide pellets of the samples were analyzed using a Perkin Elmer Spectrum RX-1 Fourier Transform Infrared Spectrometer (Perkin Elmer Ltd., Chalfront Road, Seer Green, Beaconsfield, Buckinghamshire, UK). Each spectrum was acquired by performing 32 scans.

X-ray diffraction analysis

The polymer samples were characterized by X-ray powder diffraction using a D2 Phaser diffractometer (Bruker AXS GmbH, Karlsruhe, Germany), with a sealed microfocus generator operated at 30 kV and 10 mA, producing Cu_{Ka} (λ_X = 0.1542 nm) radiation. The samples were scanned in Bragg-Brantano geometry, over a scattering (Bragg, 2θ) angle range from 5 to 60°, in 0.02° steps at 1.5° min^{-1}. Detection was accomplished with a Lynxeye 'silicon strip' multi-angle detector.

Differential Scanning calorimetry (DSC)

Samples of the polymer (5-10 mg) were placed in standard aluminium pans (40 µl) with a vented lid. The crimped aluminum pans were

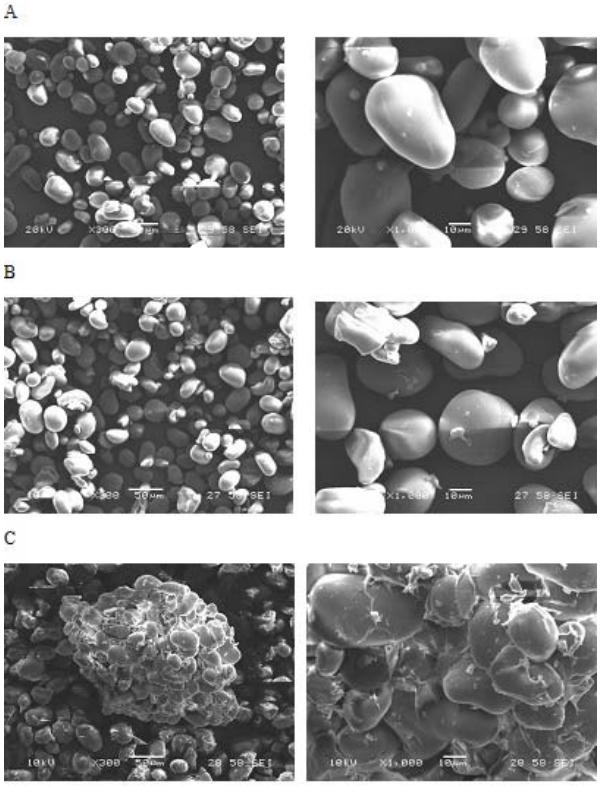

Figure 1 Scanning Electron Microscopy (1000×) of Native (A) and Carboxymethylated (B) and Pregelatinized (C) Bambara starches.

heated from 20 to 250°C at a scanning rate of 10°C/min using nitrogen as a purge gas in a DSC 1 (Mettler-Toledo, Switzerland). The enthalpy, onset temperatures and melting points of the samples were obtained using the software provided.

Statistical analysis

Statistical analysis was carried out using the Students' t-test and ANOVA, and the limit of significance was $p<0.05$ (Graphpad Prism version 6.00 for Windows, GraphPad Software, La Jolla California, USA, www.graphpad.com).

RESULTS AND DISCUSSION

The starch granules of the native starch form were circular or ovoid shapes. Pregelatinized and carboxymethylated forms were prone to aggregation but maintained individual granule structure (31). Although not significantly different, the mean projected particle diameter increased in the sequence, native< pregelatinized<carboxymethylated. The phenomenon is attributable to osmotic swelling (32, 33) and also explained the increased swelling and water absorption indices for the modified starches as compared to native starch ($p<0.001$) (Table1).

The effect of modification on granule morphology (Figure 1) was investigated using scanning electron microscopy. The size distribution of the polymers was similar with a general spherical or ovoid shape. The native and the carboxymethylated starch granules were discrete and had smooth surfaces while the pregelatinized starch formed aggregates with slightly wrinkled surfaces. 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 (34, 35).

The physical properties of the starch are given in Table 1. Modified starches had a lower Angle of repose, carboxymethylated starches exhibited lower Carr's index and Hausner ratio values indicative of improved flow properties suitable for pharmaceutical granulation (36,37,38). The pregelatinized and carboxylated forms exhibited significantly different flow properties.

Carboxymethylated forms of the botanical starch exhibited the highest values of swelling index (SI) and WAC, due to osmotic swelling (39). The pH values for the starches ranged between 5.18 and 6.61 (Table 1), conforming to pharmaceutical standards (38).

The moisture content of analyzed samples ranged between 10.11% and 10.53 % (Table 1), The ranges of moisture content of all the experimental starch samples fell within the typical values expected at 50% relative humidity (38).

Table 1 Physical parameters of the Native and Modified *Bambara* nut starches

FORM OF STARCH	NATIVE	PRE-GELATINIZED	CARBOXY-METHYLATED
Mean projected particle Diameter (µm)	8.69 ±4.69	9.35±5.71	13.23±7.37
Particle density (g/cm^3)	1.46±0.01	1.51±0.01	1.52±0.03
Bulk density (g/cm^3)	0.56±0.07	0.55±0.07	0.59±0.01
Tapped density (g/cm^3)	0.66±0.03	0.73±0.02	0.66±0.02
Hausner ratio	1.18±0.05	1.33±0.04	1.12±0.02
Carr`s index	14.00±11.23	24.82±3.94	10.98±1.93
Angle of Repose(˚)	64.13±0.52	48.12±1.36	42.51±1.39
Swelling index	0.42±0.01	0.62±0.04	1.78±0.23
Water absorption capacity %w/w	208.65 ±10.12	295.60 ±14.43	352.70 ±11.08
Moisture content (%w/w)	10.53±0.21	10.11±0.18	10.51±0.41
Solubility 80˚C (%w/w)	51.09±1.05	49.00±0.12	47.05±1.00
pH (1%w/v)	5.18±0.01	5.93±0.01	6.61±0.22
Degree of Substitution	-	-	0.25

The rheological properties of the studied starches are outlined in Table 2. The three most important parameters in characterizing viscosity profiles of starches are the peak viscosity, breakdown viscosity, and final viscosity (40).

Table 2 Rheological parameters of native and modified *bambara* nut starch

FORM OF STARCH	NATIVE	PREGELATINIZED	CARBOXY-METHYLATED
Peak viscosity (cP)	251.4	329.3	173.0
Trough viscosity (cP)	99.1	202.3	97.2
Breakdown viscosity (cP)	152.3	127.0	75.8
Final viscosity (cP)	293.2	374.5	196.8
Setback from Trough	2329	2066	1195
Setback from Peak	501	542	285
Peak Time (min)	4.13	4.40	4.73
Peak Temperature (˚c)	81.60	83.95	79.8

Both pregelatinized and carboxymethylated Bambara starch had lower values of breakdown viscosity and setback from trough compared to the native Bambara in the order Carboxymethylated< Pregelatinized< Native. Carboxymethylated starch had the least levels of peak, breakdown and setback viscosity, implying it has the highest resistance to heat change.

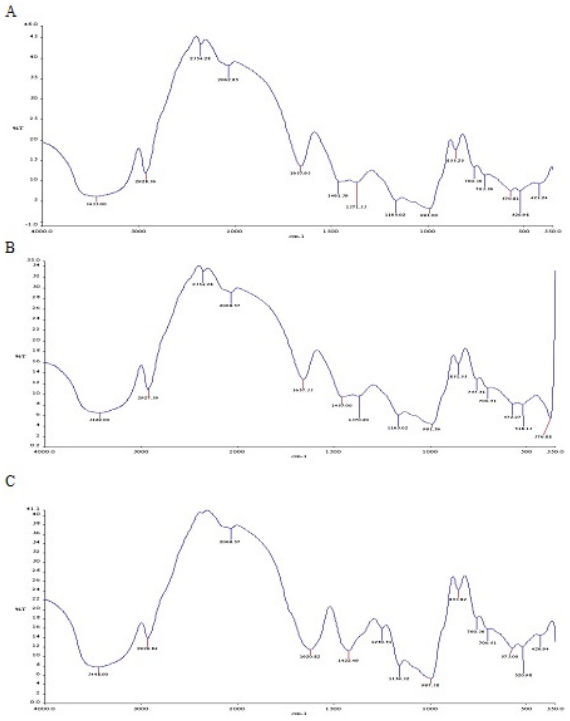

Figure 2 Individual FTIR Spectrum for (A) Native, (B) Pregelatinized and (C) Carboxymethylated *Bambara* starches.

The results of the FTIR analysis are presented in Figure 2. The –OH functional group can be detected on an FTIR spectrum by a broad stretching between 3300 and 3600 cm^{-1}. This stretch can be identified on the FTIR spectrum of the starches under study. The presence of the C=O group in the starches is suggested by a stretching vibration in the range 1850-1540 cm^{-1}. Other functional groups such as C=H peak near 3000 cm^{-1} and aromatic stretching around 1600-1500 cm^{-1} are also noticeable in the spectra for the starches. Carboxymethylation of the starch was confirmed by a sharp peak at around 1600 cm^{-1} which is typical for C=O stretching. The characteristic carbonyl singlet peak of carboxymethylated starch at 1422.49 cm^{-1} for carboxymethylated *Bambara* was observed. The peak at around 1419cm^{-1} was also typical of a

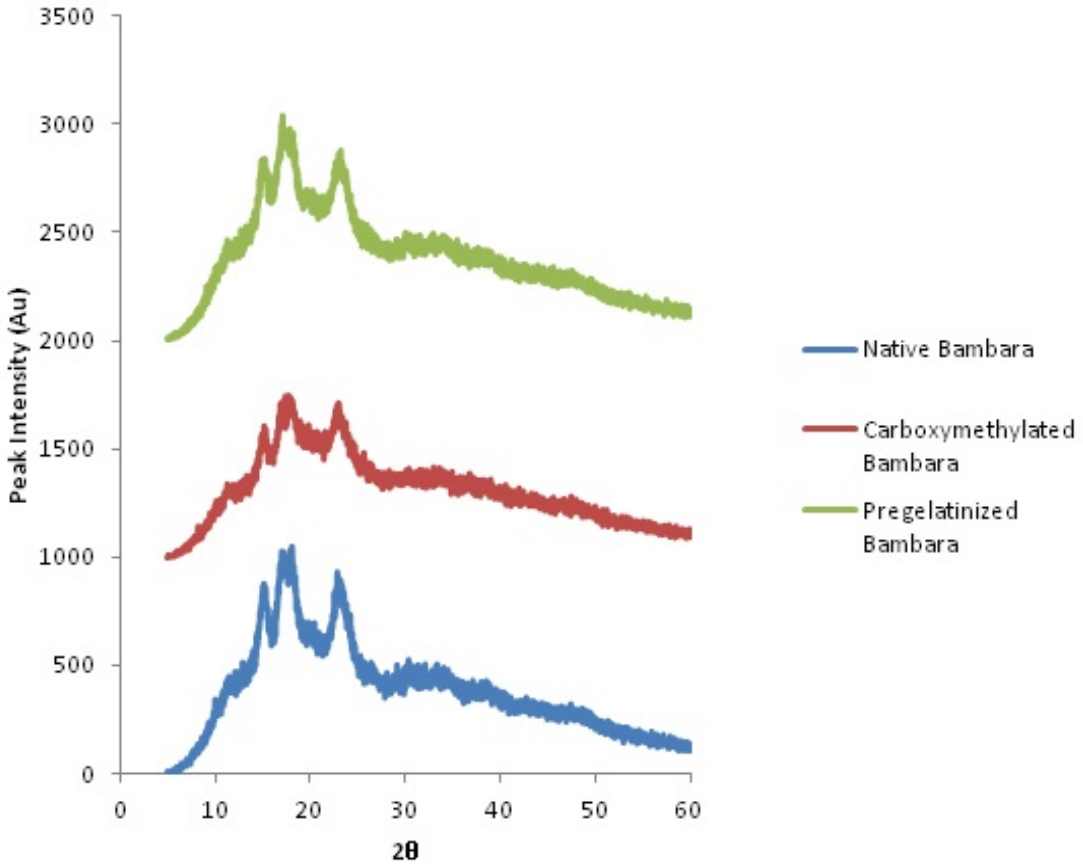

Figure 3 Combined XRD diffractograms for Native and Modified Bambara starches of Bambara starches.

carbonyl group (41). These bands were similar to the observations reported reported for carboxymethylated potato, corn and maize starches. The broad band between 3600 and 3000 cm^{-1} is assigned to O-H stretching and it is due to hydrogen bonding involving the hydroxyl groups in the starch molecules. Peaks at 2900 cm^{-1} and 2360 cm^{-1} may be due to CH_2 asymmetric stretching (42).

The X-ray diffractograms for the native, carboxymethylated, and pregelatinized starch forms of the experimental starch is given in Figure 3. The samples exhibited an A-type pattern, with the major crystalline peaks at around 15°, 17° and 23° (2θ). This is despite the fact that *Bambara* is a legume crop. Previous work on the crop revealed that *Bambara* may possess wide angle X-ray diffractograms showed typical 'A' pattern characteristic of cereal starches, but significant differences were observed between the X-ray pattern of native and modified starches (43). Carboxy-methylation appeared to cause a reduction in peak intensities, leading to broader peaks in mainly the amorphous regions to give less intense peaks at the crystalline regions of 15°, 17°, 23° (2θ) than for the native form of the starch. Carboxymethylation (CMS) of *Bambara* starch did not produce any significant alteration in its crystalline character which may be due to the low DS of the carboxymethylated form. The loss of crystallinity is important in some applications, like preparation of hydrogels, as increase in the amorphous region would mean enhanced ability of the starch to absorb water (44). Pregelatinized Bambara starch did not show any thermal transitions

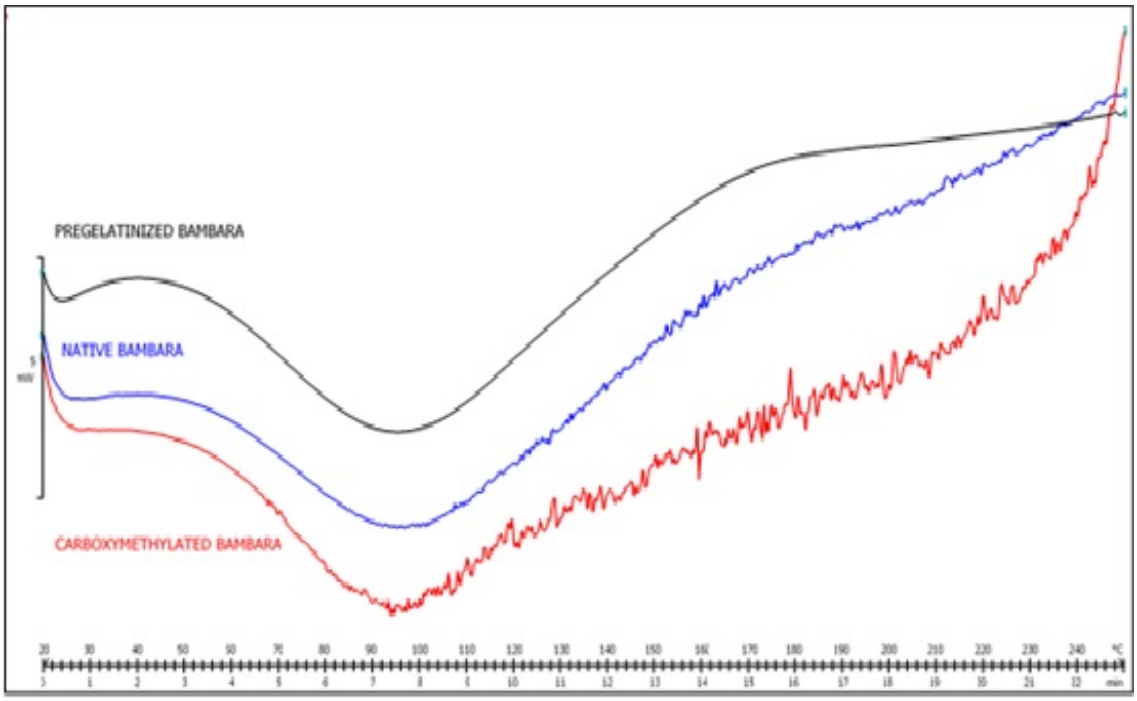

Figure 4 DSC thermogram of native and modified *Bambara* starches**.**

across the temperature range analysed, while the native and carboxymethylated starches did (Figure 4).

CONCLUSIONS

Pregelatinization and carboxymethylation caused significant changes in the physical, rheological, and structural properties of native Bambara starch. Rheological properties of the modified Bambara starch showed that both pregelatinized and carboxymethylated forms of the starch had a high resistance to heat change and a lesser tendency to retrograde. While the native and pregelatinized forms can be used as binders in tablet formulations, the carboxymethylated form may be suitable as a disintegrant in tablet formulations and as a matrix former at greater concentrations for sustained release formulations. Locally sourced and readily available Bambara nut starch, has the potential to be utilized as an excipient in pharmaceutical formulations.

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