Synthesis, characterization, and functional properties of a novel sodium carboxymethyl starch obtained from matured seeds of *Brachystegia eurycoma*

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Background and objective
Brachystegia eurycoma is an indigenous but underutilized legume crop in Nigeria. The aim of this study was to extract, purify, chemically modify, and characterize for its material and functional properties the starch from matured seeds of *B. eurycoma* and determine its suitability as an excipient in solid oral dosage formulations.
Materials and methods
Starch was extracted, purified, and subjected to carboxymethylation using modified methods. Micromeritic and rheological properties of the starch products were evaluated using differential scanning calorimetry, powder radiograph diffraction (XRD), Fourier-transform infrared spectroscopy, scanning electron microscopy, and thermogravimetric analysis techniques, which were used to characterize the preparations. Granules were prepared using the method of wetting and massing. The bulk properties, solubility, and the disintegrant properties were evaluated in isoniazid capsules and tablets.
Results and discussion
The results show good compressibility and excellent flow of the modified starch. The solubility of the modified starch was increased by 2–10 folds, whereas the swelling capacity of the native starch was increased up to 9.0–16 times. Both differential scanning calorimetry and XRD results have shown a reduction of crystallinity, whereas the Fourier-transform infrared spectroscopy indicated formation of carbonyl functional group and absence of interactions. Scanning electron microscopy revealed eruption and coalescence of starch granules after modification. XRD showed decreased thermal stability, and thermogravimetric analysis showed product decomposition at lower temperatures than the native starch. Disintegration was found to be faster with lower concentration of carboxymethylated starches.
Conclusion
The study concludes that derivatization of carboxymethylated starch from *B. eurycoma* seed yielded starch products with unique functional properties that could be explored for the formulation of controlled-release systems.

Keywords:
Brachystegia eurycoma, capsules, carboxymethylated starch, disintegrants, formulation variables, tablets

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Introduction

Modification of starches are processes used in tweaking the inherent characteristics of starch to obtain products with improved functionality. This can be achieved through physical, chemical, genetic, and enzymatic means [1].

Carboxymethylation of starch is a chemical process of modification that involves the substitution of the hydroxyl groups in starch molecules by carboxymethyl groups in the presence of monochloroacetic acid or its sodium salt (sodium monochloroacetic acid), sodium hydroxide (NaOH), and other chemicals [2]. The first step involves an equilibrium reaction between the hydroxyl groups of starch and NaOH, whereas the second step is where formation of carboxymethyl starch by substitution of the monochloroacetic acid or its sodium salt takes place [3]. Carboxymethylated starch is characterized by its degree of substitution (DS) which is the number of carboxymethyl groups formed in every anhydroglucose unit of the starch, and as there are 3 (three) hydroxyl groups in every anhydroglucose unit, values of DS lie between 0 and 3 [4]. Carboxymethyl starch are hydrophilic, soluble in cold water, and cause rapid disintegration when incorporated as disintegrants in tablet formulations.

The use of carboxymethylated starch for controlled release has been well expounded in the literature [5–8].

Unconventional plant sources are commonly being exploited for the development of starch for pharmaceutical use [9]; the plant *Brachystegia eurycoma* is a huge tree (115 ft) distributed mainly in the forest zones. In Nigeria, it is widely available in the Southern region, whereas in Cameroun, it is found in the Western region. The tree has a rough bark, spreading branches, pinnate leaves, petal-less flowers, and seeds that are flat in shape [10]. Its wood is often used as timber, its seeds are rich in numerous nutrients, and the seed flour is used as thickeners in soups [11]. The plant has also been explored for its ethnomedicinal use in the treatment of diseases such as tuberculosis, malaria, dysentery, leprosy, syphilis, and diabetes [12]. The seed gum has been explored as a binder for tablet formulations [13].

The aim of this study is to modify starch extracted from the seeds of *B. eurycoma* by carboxymethylation and evaluate its disintegrant property in isoniazid capsules and tablet formulations.

Materials and methods

Materials

Sodium metabisulphite, absolute ethanol and NaOH, monochloroacetic acid, glacial acetic acid, methanol, concentrated hydrochloric acid (HCl), and *B. eurycoma* seed starch were used in the study.

Collection and extraction of *Brachystegia eurycoma* seed starch

B. eurycoma seeds were purchased from the market in Uyo, Akwa Ibom State, Nigeria. They were sorted by picking out the bad seeds and dirt. The method of Joel *et al.* [7], was adopted for extraction of starch; the seeds were soaked in sodium metabisulfite solution (1% w/v) for 18 h. The seeds were then washed, and seed coats were removed and pulverized in a blender with distilled water. The pulverized mass was sieved using a muslin cloth, and the residue was discarded, whereas the filtrate was allowed to stand in a container for 24 h. After that, the supernatant liquid was decanted, and the sediment was air-dried and pulverized. Then, the extracted starch, *B. eurycoma* (BeS), was packaged in air-tight containers and kept in a desiccator until further use.

Preparation of carboxymethylated *Brachystegia eurycoma* starch

The process of Joel *et al.* [7] was adopted with slight modifications. A mixture of absolute ethanol (80 ml) and NaOH was made to obtain a solution of 2.5 M and placed in a water bath (50°C). *B. eurycoma* starch was prepared in distilled water (10%w/v) and incorporated into the hot ethanol-NaOH mix. Sodium monochloroacetic acid (5 g) was added to the mix and allowed to stand in the water bath for 1 h. After this, the pH of the mixture was determined and brought to neutral (pH 7) using glacial acetic acid. The mixture was filtered, and the sediment was washed liberally in methanol (99%v/v) and filtered again, and then the mass was washed again in acetone, filtered, and dried in the oven at 40°C for 48 h. The resulting product, carboxymethylated starch (BeS_1), was packaged in air-tight container and stored in a desiccator until further use. Other batches of carboxymethylated starches were produced according to the formula in Table 1.

Determination of the degrees of substitution of carboxymethylated starches

The process used by Emeje *et al.* [9] was adopted with some modifications. Carboxymethylated starch (BeS_1) (5 g) was placed in a volumetric flask (250 ml), and then distilled water (50 ml) was added into the flask. The mixture was stirred using a magnetic stirrer and then phenolphthalein indicator (five drops) was added to it. NaOH solution (0.1 N) was used to titrate the starch mixture until a pink color was obtained. NaOH solution (0.45 N) was then added into the pink colored starch mixture, shaken for 30 min, and titrated with HCl (0.2 N) until the pink color disappeared. This procedure was repeated for the other carboxymethylated products (BeS_2–BeS_9) and the native starch (to obtain a blank titer). The DS

Table 1 Composition for the preparation of carboxymethylated starch

Batch	BeS_0 dispersion (% w/v)	Ethanol-NaOH mixture (M)	SMCA (g)	Time of reaction (h)
BeS_1	10	2.5	5	1
BeS_2	10	2.5	5	3
BeS_3	10	2.5	5	5
BeS_4	10	3.0	5	1
BeS_5	10	3.0	5	3
BeS_6	10	3.0	5	5
BeS_7	10	3.5	5	1
BeS_8	10	3.5	5	3
BeS_9	10	3.5	5	5

BeS_0, native *Brachystegia eurycoma* starch; BeS_1–BeS_3, carboxymethylated starch reacted with 2.5 M ethanol-NaOH mix for 1, 3, and 5 h, respectively; BeS_4–BeS_6, carboxymethylated starch reacted with 3.0 M ethanol-NaOH mix for 1, 3, and 5 h, respectively; BeS_7–BeS_9, carboxymethylated starch reacted with 3.5 M ethanol-NaOH mix for 1, 3, and 5 h, respectively; NaOH, sodium hydroxide; SMCA, sodium monochloroacetic acid.

was determined using the following formula:

$$\text{Percentacetyl} = \frac{(\text{BT} - \text{ST})\text{ml} \times \text{ACM} \times 0.043 \times 100}{\text{drysampleweight(g)}}. \quad (1)$$

Where BT=blank titer, ST=sample titer, and ACM=acid molarity.

Evaluation of micromeritic properties of the starch products

Bulk and tapped densities

The volume occupied by the starch products (5 g) in a measuring cylinder was noted, and bulk density (g/ml) was computed. The measuring cylinder was tapped 100 times. The volume occupied after tapping was noted, and tapped density (g/ml) was computed.

Hausner ratio and Carr's compressibility index

These were computed from the data obtained from bulk and tapped densities as follows:

$$\text{HR} = {}^{\text{tappeddesity}}/_{\text{bulkdensity}}. \quad (2)$$

$$\text{CI} = {}^{\text{tappeddensity}} - \text{bulkdensity}/_{\text{tappeddensity}} \times 100. \quad (3)$$

Angle of repose

The funnel method as described by Onyishi *et al.* [14] was used. The starch product was poured into a clamped funnel whose orifice had been closed. The height (h) and diameter (D) of the heap formed after the material was allowed to flow through the orifice was measured, and angle of repose (A) was calculated as follows:

$$\tan\theta - = {}^{2}h/_{D}. \quad (4)$$

Determination of true density

The specific gravity bottle method as described by Muazu *et al.* [15] was adopted. The weight of the specific gravity bottle filled with xylene was noted as X. The bottle was emptied, and then the starch product (5 g) was poured into the bottle filed with xylene. The bottle was allowed to stand for 10 min, and its weight was noted (Y). True density (ρ) was calculated using the following formula:

$$\rho = {}^{W}S/_{[(X+W)-Y]}. \quad (5)$$

Where S=specific gravity of xylene, 0.855, and W=weight of the starch product.

Determination of swelling capacity

The method of Iwuagwu and Okoli [16] was adopted; the volume occupied by the starch product (10 g) poured into a measuring cylinder (100 ml) was noted. Distilled water (85 ml) was poured into the measuring cylinder and mixed. The volume was made up with distilled water, and the suspension was left to stand for 24 h. The swollen volume was noted, and swelling capacity was calculated as the difference between the initial volume and the swollen volume.

Determination of hydration capacity

The starch product (1 g) was placed in a preweighed graduated test tube, and distilled water (10 ml) was poured into the test tube. The contents of the test tube were shaken and left to stand for 10 min. The starch suspension was centrifuged at 100 rpm, the supernatant discarded, and the residue was weighed [17]. Hydration capacity (HC) was calculated as follows:

$$\text{HC} = {}^{\text{weightofsediment}}/_{\text{innitialweightofmaterial}}. \quad (6)$$

Determination of pH

The pH of 1% w/v slurry of the starch product was determined at room temperature (28°C) using the Philips DZS-706 pH meter (Philips, Shanghai, China). Triplicate determinations were made, and the mean was computed.

Determination of solubility index

The method used by Ihegwuagu *et al.* [17] was employed. The starch product (1 g) was poured into a test tube containing distilled water (20 ml), and the tubes were placed in a water bath (60°C) for 30 min. The starch suspension was centrifuged at 1200 rpm for 20 min. The supernatant was decanted into a porcelain dish and dried in the oven. Solubility (S) was determined as follows:

$$S = {}^{\text{weightofdissolvedstarch}}/_{\text{weightofdrystarch}}. \quad (7)$$

Determination of viscosity

The method used by Natapulwat *et al.* [18] was adopted. Viscosity of 1% w/v starch suspension was determined using a viscometer (Brookfield, RVDV-II +PRO, 11 Commerce Boulevard, Middleboro, MA, USA) with a spindle number RV-02 and a speed of 60 rpm at 25°C.

Moisture sorption capacity

The method previously reported [19] from our laboratory was adopted with modification. In brief, 2 g of the sample was accurately weighed and evenly distributed over the surface of a 70-mm tarred Petri dish. The samples were then placed in a large desiccator containing distilled water [relative humidity (RH)= 100%] at room temperature, and the weight gained by

the exposed samples at the end of a 5-day period was recorded, and the amount of water sorbed was calculated from the weight difference.

Determination of gelatinization temperature

Thermograms were obtained from the differential scanning calorimeter (Model DSC 204 F1; Netzsch, Gerätebau GmbH Wittelsbacherstraße 42 95100 Selb, Germany). The method adapted by Emeje *et al.* [9] was used. Starch products were weighed and transferred into aluminum pans. Distilled water (9 µl) was poured into the aluminum pans, which were hermetically sealed. Distilled water (9 µl) was placed in the reference pans. The starch products were left to stand for 2 h then scanned between 26 and 180°C at a heating rate of 10°C/min under constant nitrogen flow.

Fourier-transform infrared spectroscopy

The infrared spectra of the starch products were obtained from 410 Nicolet using the Fourier-transform infrared spectroscopy (FTIR) spectrometer (Nicolet Magna 4 R 560, Minnesota, USA), within the frequency range of 4000–500 cm^{-1}.

Morphology

Micrographs of the starch products were obtained from the scanning electron microscope (JSM-5600 LV; JEOL, Tokyo, Japan) at an accelerating voltage of 1.0 kV.

Thermogravimetric analysis

Starch product (1.78 mg) was heated at 800°C at a rate of 10°C/min in the thermogravimetric apparatus (TGA-DTG-50; Shimadzu, Tokyo, Japan). Liquid nitrogen flowing at the rate of 20 ml/min was used as the purge gas.

Radiograph powder diffraction

Structural characterization of the starch products was carried out as described by Emeje *et al.* [9], in the radiograph diffractometer (Siemens, Munich, Germany). Starch samples, packed in rectangular aluminum cells, were illuminated using CuKa radiation (l=1.54056 Å) at 45 kV and 40 mA. The samples were scanned between diffraction angles of 5 and 80° using scan step 0.1 and dwell time of 15 s. A nickel filter was used to reduce the size (kb) contribution to the radiograph signal. Three measurements were taken, and the degree of crystallinity was calculated according to the method of Manek *et al.* [19].

Preparation of isoniazid granules

Optimized batches of starch products (BeS_1, BeS_6, and BeS_8) based on their physicochemical properties were employed as disintegrants in isoniazid capsule and tablet formulations. The wet granulation method of massing and screening were employed in the preparation of granules (Table 2). Carboxymethylated starches were incorporated at concentrations of 2.5, 5.0, 7.5, and 10.0%w/w, whereas the native starch (BeS_0) and sodium starch glycolate (SSG) were used at a concentration of 10%w/w only. Appropriate quantities of isoniazid, the disintegrants, and diluent (lactose) were geometrically mixed in a mortar. The binder (starch mucilage) was used to wet mass the powdered mix and screened through a sieve with mesh size of 1.6 mm. The wet granules were dried in the oven at 40°C for 20 min and screened again through another sieve (mesh size; 1.0 mm) and dried again in the oven at 40°C for 20 min.

Table 2 Composition for the preparation of isoniazid granules

Ingredients (mg)	ISZD	LAC	MSM	BeS_0	BeS_1	BeS_6	BeS_8	SSG	MST	Total
FB0	100	195	50	50	–	–	–	–	5	400
FB1	100	232.5	50	–	12.5	–	–	–	5	400
FB2	100	220	50	–	25	–	–	–	5	400
FB3	100	207.5	50	–	37.5	–	–	–	5	400
FB4	100	195	50	–	50	–	–	–	5	400
FB5	100	232.5	50	–	–	12.5	–	–	5	400
FB6	100	220	50	–	–	25	–	–	5	400
FB7	100	207.5	50	–	–	37.5	–	–	5	400
FB8	100	195	50	–	–	50	–	–	5	400
FB9	100	232.5	50	–	–	–	12.5	–	5	400
FB10	100	220	50	–	–	–	25	–	5	400
FB11	100	207.5	50	–	–	–	37.5	–	5	400
FB12	100	195	50	–	–	–	50	–	5	400
FSG	100	195	50	–	–	–		50	5	400

FB0, tablet formulations containing 10%w/w BeS_0; FB1–FB4, tablet formulations containing 2.5, 5.0, 7.5, 10.0%w/w BeS_1, respectively; FB5–FB8, tablet formulations containing 2.5, 5.0, 7.5, 10.0%w/w BeS_6, respectively, FB9–FB12, tablet formulations containing 2.5, 5.0, 7.5, 10.0%w/w BeS_8, respectively; FSG, tablet formulations containing 10%w/w sodium starch glycolate; ISZD, isoniazid; LAC, lactose; MSM, maize starch mucilage; SSG, sodium starch glycolate.

Evaluation of isoniazid granules

The flow properties of the granules were determined using bulk and tapped densities, compressibility index, Hausner ratio, and angle of repose, as already described before.

Granule porosity (Pb)

This was computed from the data obtained from the true density (TrD) and tapped as follows:

$$\mathrm{Pb} = {}^{\mathrm{Td}}/_{\mathrm{TrD}}. \quad (8)$$

Encapsulation of isoniazid granules

Isoniazid granules (400 mg) were filled into empty hard gelatin shells and covered. The batches were labeled as CB1–CB4 for capsules containing granules prepared with 2.5, 5.0, 7.5, and 10.0%w/w BeS_1, respectively; CB5–CB8 for those prepared with 2.5, 5.0, 7.5, and 10.0%w/w BeS_6, respectively; CB9–CB12 for those prepared with granules prepared with 2.5, 5.0, 7.5, and 10.0%w/w BeS8, respectively, and CSG and CB0 for those prepared with SSG and native seed starch, respectively.

Preparation of isoniazid tablet formulations

The granules were lubricated with magnesium stearate (Table 2) and compressed into tablets using the single-punch tableting machine fitted with 10-mm punch and die set at a compression pressure of 15 kN. The ejected tablets were stored in a desiccator for 24 h to allow for elastic recovery before evaluation.

Evaluation of isoniazid capsules and tablets

Uniformity of weight

Ten tablets and 10 capsules were randomly selected, weighed, and the average weight was determined.

Tablet diameter and thickness

Diameter and thickness of 10 randomly selected tablets were determined using the Mitutuyo micrometer screw gauge, and the average was computed.

Tablet hardness

Hardness (kg/cm^3) of five randomly selected tablets was determined using the Monsanto hardness tester, and the mean was calculated.

Friability test

Five tablets were collectively weighed (W1), placed into the Erweka friabilator, and set to rotate at 25 rmp for 4 min. Afterward, the tablets were reweighed (W2), and friability (%) was calculated as follows:

$$F(\%) = {}^{W}1 - W2/_{W}1 \times 100. \quad (9)$$

Disintegration test

The disintegration tester (Erweka ZT4-4, ERWEKA GmbH, Pittlerstr, 45, 63225 Langen, Germany) was used. One tablet was placed in each of the six compartments of the disintegration tester, and the medium (0.1 N HCl) was maintained at 37±0.5°C during the test. The time taken for all the tablet particles to pass through the compartment's mesh was noted, and the average was determined. The same procedure was repeated for the capsules.

Statistical analysis

Statistical analysis was carried out using analysis of variance using GraphPad Prism (GraphPad Software Inc., San Diego, California, USA). Tukey–Kramer's multiple comparison test was used to compare the physicochemical parameters of the starches. At 95% confidence interval, P values less than or equal to 0.05 were considered significant.

Results

Flow properties of native and carboxymethylated starch products

The flow properties of *B. eurycoma* seed starch products (BeS_0–BeS_9) as determined by angle of repose and powder densities are presented in Table 3. All the starch products were found to have good to excellent

Table 3 Flow properties of starch products

Batch	Bulk density (g/ml)	Tapped density (g/ml)	Angle of repose (^{0})	Carr's index (%)	Hausner ratio
BeS_0	0.56±0.02	0.76±0.05	30.51±0.04	13.51±0.04	1.45±0.02
BeS_1	0.41±0.03	0.45±0.03	36.16±0.06	9.13±0.07	1.14±0.01
BeS_2	0.44±0.04	0.47±0.02	32.49±0.07	6.38±0.05	1.06±0.03
BeS_3	0.45±0.01	0.51±0.04	31.77±0.06	10.01±0.03	1.11±0.03
BeS_4	0.47±0.06	0.53±0.06	33.05±0.08	11.87±0.05	1.13±0.06
BeS_5	0.51±0.02	0.53±0.03	25.22±0.05	6.19±0.03	1.07±0.07
BeS_6	0.46±0.01	0.54±0.01	23.38±0.02	13.19±0.02	1.15±0.08
BeS_7	0.57±0.03	0.67±0.03	22.67±0.03	14.54±0.05	1.17±0.06
BeS_8	0.63±0.03	0.67±0.07	24.23±0.05	6.21±0.08	1.06±0.08
BeS_9	0.51±0.02	0.57±0.02	26.60±0.02	12.28±0.04	1.14±0.07

BeS, *Brachystegia eurycoma* starch.

flow (36.16 and 22.67°). Increasing the concentration of NaOH was observed to improve material flow indicated by the low values obtained. All the starch products had Carr's index between 6.19 and 14.54% (Table 3). The native starch was found to be nonfree flowing with Hausner ratio of 1.45, but all the carboxymethylated products were observed to be free flowing with Hausner ratio of 1.06 and 1.17.

Degree of substitution

DS was found to generally decrease with increase in molarity of NaOH, and the reaction time had little effect on the DS (Table 4). However, DS of all the carboxymethylated starches was found to be higher than reported for some starches [20–27]. There was a marked increase ($P<0.05$) in the viscosity of the carboxymethylated starch from 1 to 3 h (Table 4), after which, viscosity was found to decrease, although no correlation was found between viscosity of the starch products and their DS. Starch solubility, hydration capacity, and swelling index were found to increase upon carboxymethylation. The pH of all the carboxymethylated starches (7.48–9.43) was higher than that of the native starch (5.62). Results of the hydration and swelling studies show that there was a significant ($P<0.05$) increase in the hydration and swelling capacities after modification, implying that the modification resulted in weakening of associative forces in the starch molecules. This property introduced after carboxymethylation is very important, especially in the application of this starch either as a drug carrier or disintegrant in tablets and capsule formulations.

Radiograph diffraction

Radiograph diffraction patterns as presented in Fig. 1 show all the starch products have A-type diffraction patterns with major reflections at 20, 25, and 26° of diffraction angle 2θ, and small peaks at 6, 17, and 23°. These represent densely packed arrangement in the amylopectin region [19].

Differential scanning calorimetry

Results of DSC as shown in Fig. 2 confirm a material change in the native starch; gelatinization temperature of the modified starch was lower than the native. The values of the enthalpies for the native (BeS_o) and modified starches (BeS_3, BeS_6, BeS_8) were 49.5, 18.8, 22.0, and 21.6 J/(g×K), respectively. The modified starch shows evidence of less thermal stability as evidenced from the diffractograms.

Fourier-transform infrared spectroscopy

All the starch products were observed to have similar FTIR spectra (Fig. 3). The peak around 1500 cm^{-1} corresponds to scissoring of O-H water molecules, whereas the region below 1000 cm^{-1} represents skeletal vibrations of starch.

Thermogravimetric analysis

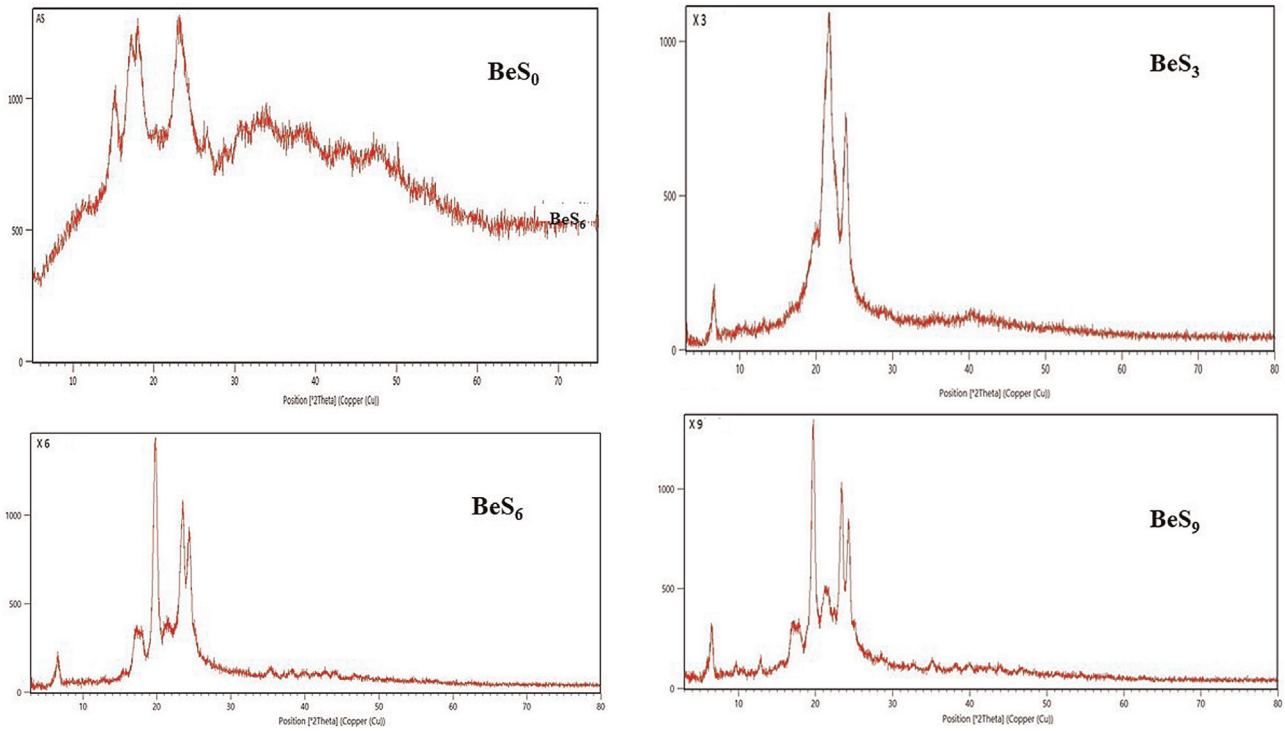
Figure 4 shows that the native starch was found to undergo maximum decomposition at temperatures between 300 and 490°C (DTGmax=390°C). Native starch was observed to start decomposing at 390°C, whereas the carboxymethylated products began to decompose at lower temperatures (250, 320, and 350°C) for BeS_3, BeS_6, and BeS_8, respectively.

Table 4 Physicochemical properties of starch products

Batch	DS	Viscosity (mPa.s)	Hydration capacity	Swelling capacity (%)	pH	Solubility index (%)	Moisture sorption capacity (%)
BeS_0	NA	2.97±2.31	2.74±0.03	18.18±0.45	5.62 ±0.03	1.00±0.02	22.8±1.60
BeS_1	1.22 ±0.02	6.26±1.74	5.42±0.07	162.16±0.86	7.87 ±0.01	3.10±0.01	42.16±0.76
BeS_2	1.00 ±0.05	37.14±1.92	7.67±0.57	242.80±1.28	7.48 ±0.01	5.10±0.01	42.80±0.28
BeS_3	1.10 ±0.06	23.18±1.86	5.93±0.64	214.30±1.16	9.43 ±0.02	4.00±0.02	24.30±1.16
BeS_4	0.58 ±0.04	20.65±1.53	7.17±0.05	175.20±0.67	8.01 ±0.04	5.00±0.03	45.20±0.77
BeS_5	0.51 ±0.02	25.42±1.75	7.54±0.41	250.50±1.18	7.65 ±0.02	4.10±0.02	50.10±1.18
BeS_6	0.46 ±0.01	11.42±1.39	4.87±0.03	150.20±0.21	8.31 ±0.02	10.00±0.01	43.50±0.71
BeS_7	0.57 ±0.03	18.95±1.57	7.80±0.09	285.70±0.68	7.93 ±0.03	9.00±0.01	50.20±0.88
BeS_8	0.63 ±0.03	23.68±2.18	8.00±0.25	300.10±0.81	7.87 ±0.01	4.00±0.03	50.10±0.83
BeS_9	0.51 ±0.02	21.24±1.04	7.60±0.73	258.70±1.34	7.98 ±0.04	2.00±0.02	48.30±1.33

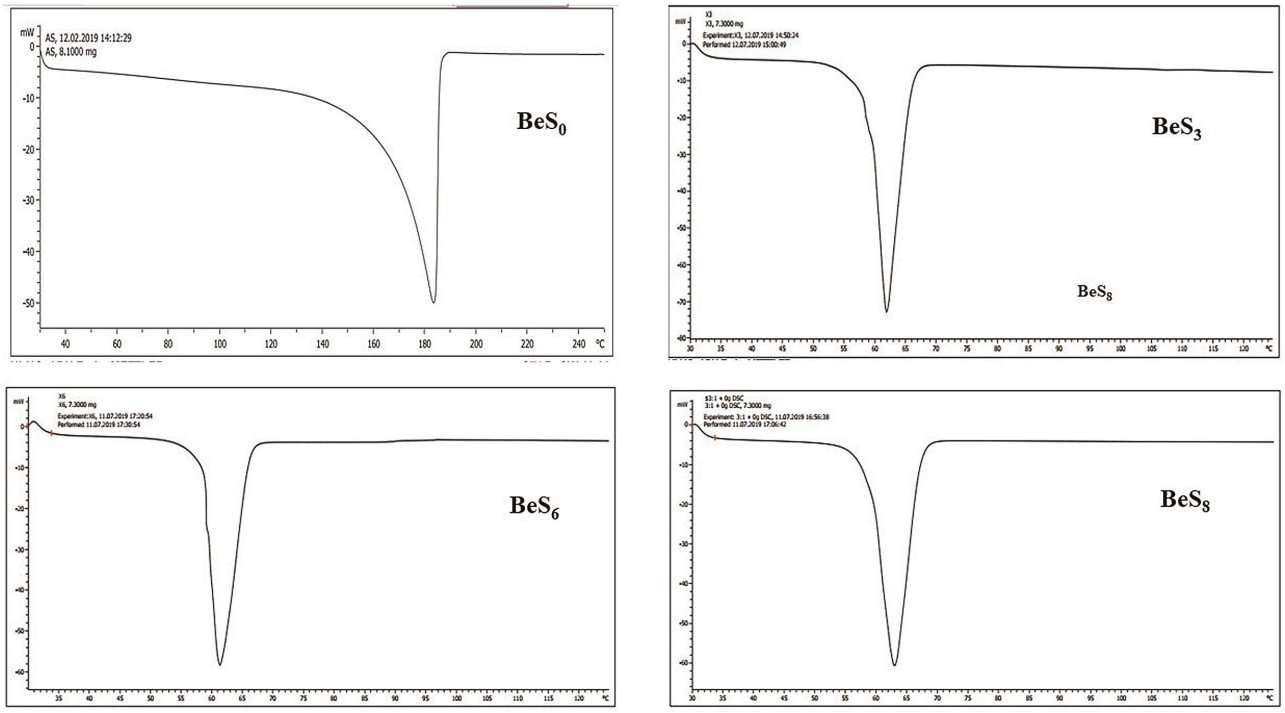
BeS, *Brachystegia eurycoma* starch; DS, degree of substitution.

Figure 1



XRD spectra of native *Brachystegia eurycoma* starch (BeS_0) and carboxymethylated starches (BeS_3, BeS_6, and BeS_9). XRD, radiograph diffraction.

Figure 2

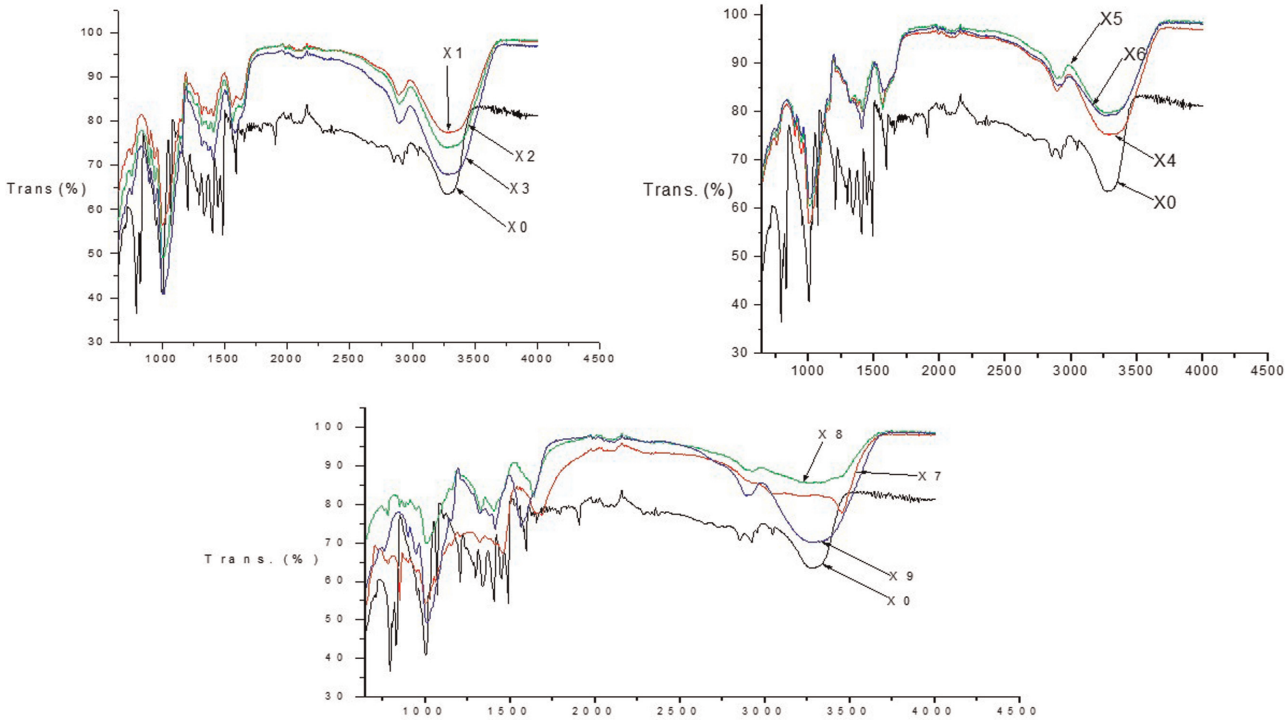


DSC spectra of native *Brachystegia eurycoma* starch (BeS_0) and carboxymethylated starches (BeS_3, BeS_6, and BeS_8). DSC, differential scanning calorimetry.

Morphology

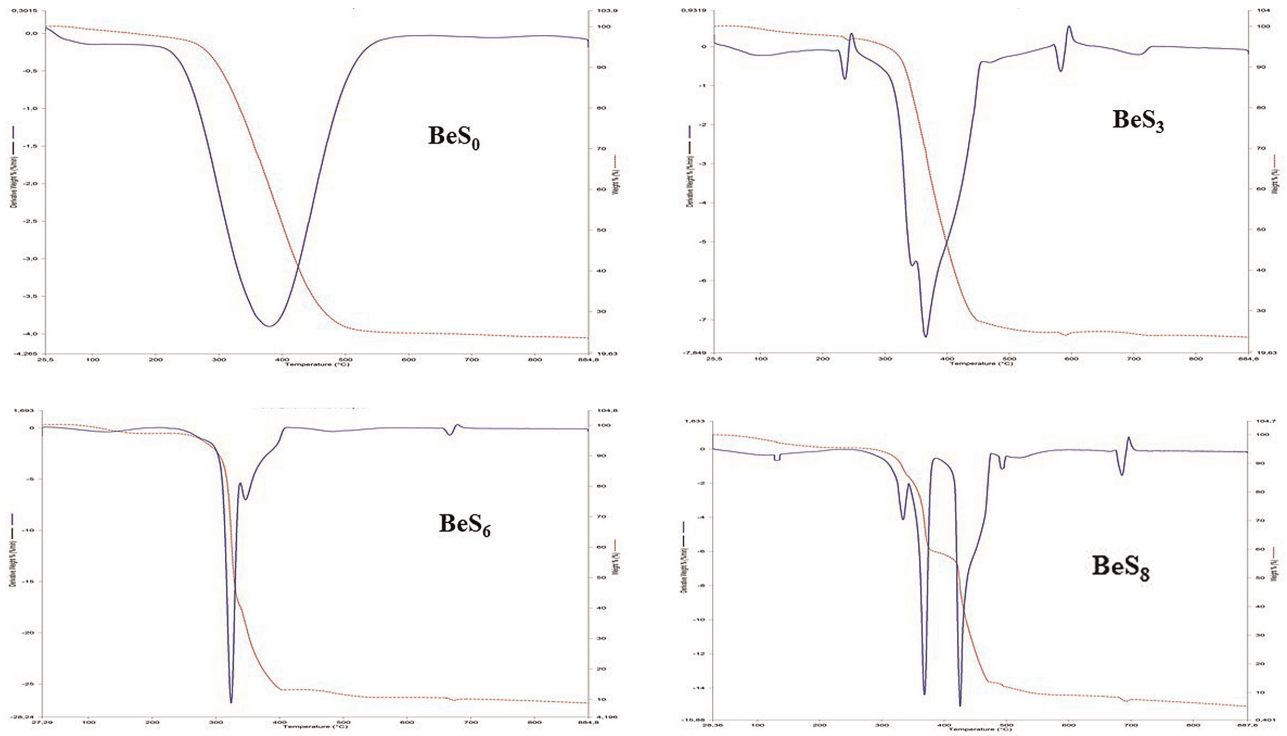
The morphology of the starch products shows partial eruption and coalescence of the carboxymethylated starch granules compared with the discrete polygonal granule shapes observed with the native starch (Fig. 5). Similar studies also documented this finding [2].

Figure 3



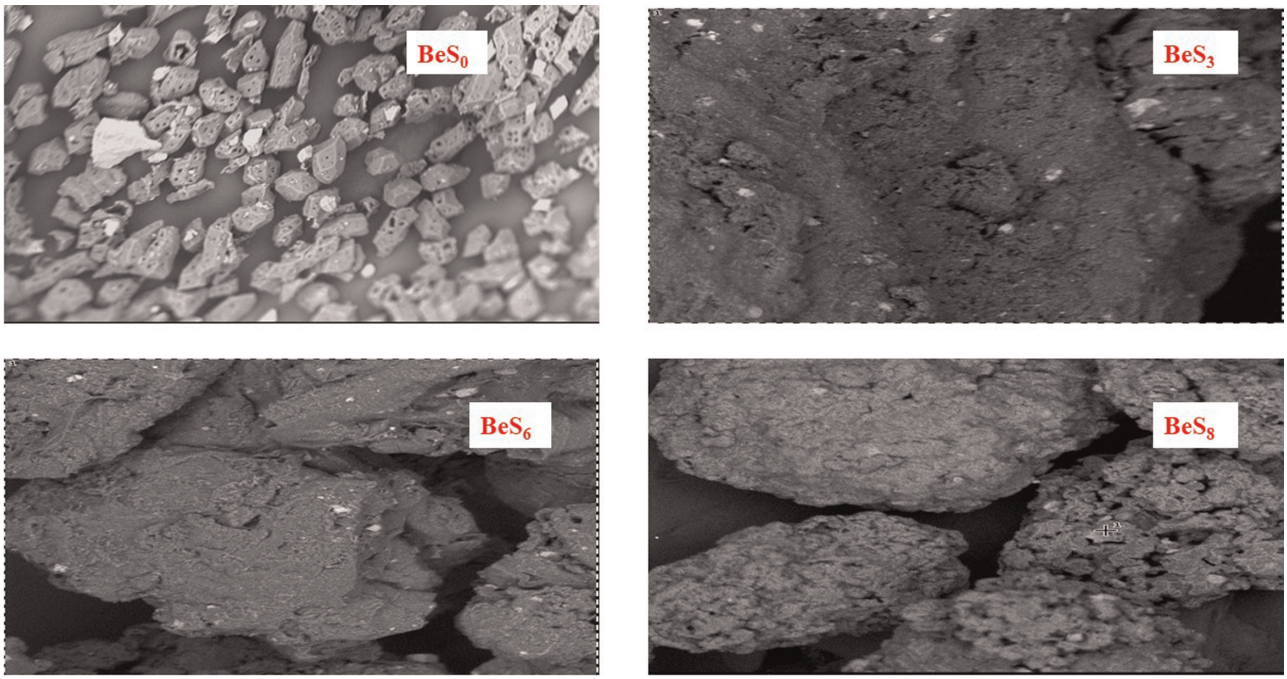
FTIR spectra of native *Brachystegia eurycoma* starch (X0) and carboxymethylated starches; X1–X9 (BeS_1–BeS_9). FTIR, Fourier-transform infrared spectroscopy.

Figure 4



TGA spectra of native *Brachystegia eurycoma* starch (BeS_0) and carboxymethylated starches (BeS_3, BeS_6, and BeS_8). TGA, thermogravimetric analysis.

Figure 5



SEM of native *Brachystegia eurycoma* starch (BeS_0) and carboxymethylated starches (BeS_3, BeS_6, and BeS_8). SEM, scanning electron microscopy.

Table 5 Flow properties of Isoniazid granules

Batch	Bulk density (g/ml)	Tapped density (g/mL)	Angle of repose (^{0})	Carr's index (%)	Hausner ratio	Porosity
FB0	0.40±0.02	0.45±0.03	36.74±0.03	11.11±0.01	1.14±0.04	0.68±0.17
FB1	0.42±0.04	0.47±0.06	25.30±0.09	10.64±0.06	1.12±0.05	0.68±0.18
FB2	0.42±0.07	0.47±0.04	27.55±0.12	10.64±0.02	1.12±0.02	0.64±0.20
FB3	0.42±0.03	0.50±0.02	26.57±0.05	16.00±0.05	1.19±0.03	0.57±0.17
FB4	0.44±0.05	0.53±0.05	23.20±0.2	16.68±0.01	1.20±0.01	0.62±0.13
FB5	0.50±0.06	0.56±0.10	26.57±0.05	10.71±0.09	1.12±0.09	0.57±0.16
FB6	0.56±0.03	0.57±0.18	28.07±0.08	10.71±0.04	1.12±0.05	0.57±0.18
FB7	0.53±0.02	0.60±0.13	26.57±0.07	16.01±0.11	1.17±0.03	0.60±0.13
FB8	0.56±0.01	0.57±0.09	26.38±0.03	10.70±0.03	1.12±0.07	0.57±0.09
FB9	0.56±0.04	0.63±0.14	28.07±0.01	8.93±0.04	1.09±0.06	0.63±0.14
FB10	0.56±0.06	0.58±0.12	26.57±0.06	19.64±0.07	1.24±0.02	0.58±0.12
FB11	0.50±0.05	0.59±0.01	27.55±0.04	15.25±0.03	1.18±0.01	0.56±0.15
FB12	0.43±0.07	0.48±0.05	25.64±0.01	10.41±0.01	1.12±0.08	0.67±0.17
FSG	0.42±0.03	0.56±0.04	27.30±0.04	25.01±0.03	1.33±0.06	0.62±0.27

Flow properties of isoniazid granules

All the granules exhibited good flow properties as revealed by the Hausner ratio, Carr's index, and angle of repose (Table 5). The capsules had uniform weights between 500 and 540 mg.

Evaluation of isoniazid capsule formulations

Capsule disintegration time was found to be between 1.55 and 2.45 min, with no appreciable difference between the disintegration times of BeS_3 and BeS_6 formulations (Table 6). Capsules prepared with 2.5% w/w BeS_8 were found to have similar disintegration times with that of CSG-containing SSG, but those prepared with the native starch (BeS_0) had the shortest disintegration time (5 min). The capsules were found to have shorter disintegration times compared with the tablet formulations.

Evaluation of isoniazid tablet formulations

The hardness or crushing strength of all the tablets formulated passed the minimum requirement of 4 kgF. However, tablets formulated with carboxymethylated starch samples were notably harder than the tablets formulated with native starch. Tablets formulated with native starch were observed to disintegrate more rapidly (5 min) than those prepared with the

Table 6 Properties of Isoniazid capsules

Batch	Uniformity of weight (mg)	Disintegration time (min)
CB0	540±0.01	1.59±0.01
CB1	500±0.02	2.20±0.03
CB2	520±0.00	2.24±0.01
CB3	510±0.00	2.25±0.01
CB4	500±0.01	2.15±0.01
CB5	530±0.02	2.45±0.06
CB6	520±0.02	2.25±0.02
CB7	500±0.01	2.30±0.34
CB8	510±0.01	2.45±0.02
CB9	520±0.02	1.55±0.13
CB10	500±0.02	2.20±0.09
CB11	530±0.01	2.25±0.34
CB12	510±0.00	2.45±0.17
CSG	510±0.01	1.89±0.01

Table 7 Properties of isoniazid tablet formulations

Batch	Uniformity of weight (mg)	Hardness (kg)	Friability (%)	Disintegration time (min)
FB0	410±0.02	5.6±0.66	0.57±0.13	5.0±1.45
FB1	420±0.07	7.0±1.00	0.51±0.08	9.2±0.53
FB2	410±0.06	9.0±1.00	0.68±0.52	9.2±0.53
FB3	440±0.03	9.5±2.50	0.52±0.07	12.09±0.84
FB4	440±0.01	9.3±1.25	0.41±0.19	12.13±1.50
FB5	420±0.08	6.0±1.00	0.76±0.52	14.0±0.93
FB6	410±0.02	8.0±0.80	0.62±0.73	19.51±1.11
FB7	400±0.03	7.1±0.10	0.55±0.02	25.47±1.43
FB8	420±0.01	6.8±0.35	0.39±0.04	26.35±1.83
FB9	420±0.00	6.6±0.4	0.85±0.12	9.45±2.05
FB10	410±0.02	7.0±0.2	0.83 ±0.02	10.03±2.43
FB11	0.39±0.03	6.5±0.3	0.91 ±0.055	22.30±1.63
FB12	0.40±0.04	7.2±0.6	0.89±0.03	28.02±3.54
FSG	430±0.02	4.5±0.50	0.97±0.31	10.0±0.73

carboxymethylated starches (9.20 and 28.02 min) or those prepared with SSG (10 min) (Table 7).

Discussion

The flow properties of *B. eurycoma* seed starch products (BeS_0–BeS_9) as determined by angle of repose and powder densities (Table 3) show the ability of the materials to flow freely; angle of repose values less than 30° indicate that the materials have excellent flow, those between 31 and 35 indicate good flow, whereas values between 36 and 40 and those more than 40° indicate fair and poor flow, respectively [19]. All the starch products were found to have good to excellent flow (36.16 and 22.67°). Increasing the concentration of NaOH was observed to improve material flow indicated by the low values obtained. Carr's compressibility index is used to assess the ability of a material to deform when pressure is applied and values less than or equal to 15% are suggestive of excellent flow, whereas Hausner ratio indicates the cohesive nature of the material, and values less than or equal to 1.25 signify that the material is free flowing [19]. All the starch products possess excellent flow (Table 3). The native starch was found to be nonfree flowing, whereas the carboxymethylated products were observed to be free flowing, showing that modification by carboxymethylation conferred better flow abilities attributable to minimal interparticulate friction. The DS was found to generally decrease with increase in molarity of NaOH, and the reaction time had little effect on the DS (Table 4). This result is contrary to other reports, where increase in reaction time was found to increase the DS [7,9] but it is in tandem with the reports of Tijsen *et al.* [20], Sangseethog *et al.* [21], and Khalil *et al.* [22]. This difference may be attributed to the difference in the biological sources of starch and the method of extraction. The DS of carboxymethylated starches influences how it can be used; DS less than 0.1 can be employed in the food industries, whereas those with DS more than 0.1 can be used as in the pharmaceutical industries. However, DS of the carboxymethylated starches was found to be higher than reported for some starches [23–25].

There was a significant ($P<0.05$) increase in the viscosity of the carboxymethylated starch from 1 to 3 h (Table 4) after which viscosity was found to decrease, although no correlation was found between viscosity of the starch products and their DS. Starch solubility, hydration capacity, and swelling index were found to significantly ($P<0.05$) increase upon carboxymethylation; this could be attributed to the presence of hydrogen bonds in the newly formed hydrophilic groups [28]. The pH of all the carboxymethylated starches (7.48–9.43) was higher than that of the native starch (5.62), implying that the introduction of the carboxymethyl group gave rise to less acidic starches. Radiograph diffraction patterns as presented in Fig. 1 show all the starch products have A-type diffraction patterns with major reflections at 20, 25, and 26° of diffraction angle 2θ, and small peaks at 6, 17, and 23°. These represent densely packed arrangement in the amylopectin region [19]. Numerous treatments have been proposed for the analysis of moisture sorption by starch. The most prominent among them being the BET equation, developed by Brunauer, Emmett, and Teller. However, the BET model suffers from the limitation that it is only valid at low RH, and does not accurately predict the moisture sorption at the upper end of the RH scale. As a result, various modifications of this model have been proposed, which improve the fit at high humidity [19]. To

overcome this limitation, scientists have proposed models such as the use of high RH (100%); it involves realistic parameters and provides a good fit to moisture sorption data at the highest RH.

The gelatinization, glass transition, and thermal stability of the native and modified starches monitored with DSC confirms and corroborates the results of thermogravimetric analysis and FTIR that there was a material change, that is, a new material was formed. Lower gelatinization and enthalpy give indication of the strength of thermal stability of a material and possible application in the food and pharmaceutical industry. Although a highly thermostable or heat-resistant starch may be desirable as excipient in the production of drugs by dry compaction, less stable products may be useful as emulsifying agents in the food industry.

All the starch products were observed to have similar FTIR spectra (Fig. 3). It shows a consistent peak at 3600 and 3000 cm^{-1} for all the products, which corresponds to O-H stretching due to hydrogen bonding between the starch molecules. The peak around 1500 cm^{-1} corresponds to scissoring of O-H water molecules, whereas the region below 1000 cm^{-1} represents skeletal vibrations of starch. Although there was no appearance or disappearance of peaks in the carboxymethylated products, shifting and broadening of the peaks were observed, suggesting the presence of possible modification.

As can be seen from Fig. 4, the native starch was found to undergo maximum decomposition at temperatures between 300 and 490°C (DTGmax=390°C). It was observed to start decomposing at 390°C, whereas the carboxymethylated products began to decompose at lower temperatures (250, 320, and 350°C) for BeS_3, BeS_6, and BeS_8, respectively. This implies that the carboxymethylated starches are less thermostable at relatively high temperatures (>350°C). The morphology of the starch products shows partial eruption and coalescence of the carboxymethylated starch granules compared with the discrete polygonal granule shapes observed with the native starch (Fig. 5). Similar studies also documented this finding [2]. As can be seen from Table 5, all the granules exhibited good flow properties as revealed by the Hausner ratio, Carr's index, and angle of repose, and the capsules had uniform weights between 500 and 540 mg, which can be attributed to good granule flow. Capsule disintegration time was found to be between 1.55 and 2.45 min, with no appreciable difference between the disintegration times of BeS_3 and BeS_6 formulations. The capsules prepared with 2.5%w/w BeS_8 were found to have similar disintegration times with that of CSG-containing SSG, whereas those prepared with the native starch (BeS_0) had the shortest disintegration time (5 min). Comparing the capsule and tablet formulations, it was discovered that the former had significantly ($P<0.05$) shorter disintegration times. This could be owing to the influence of compression pressure in the tablets, which leads to increased particle bond strength; it could also be attributed to the rapid dissolution of the hard gelatin capsules in the disintegration media (0.1 N HCl).The hardness or crushing strength of the tablets passed the minimum requirement for uncoated tablets. However, tablets formulated with carboxymethylated starch samples were notably harder than the tablets formulated with native starch. This could be because of stronger hydrogen bonds that bind the particles of CMS in the formulated tablets. It is well expounded in pharmaceutical technology that the dominating bonding types that adhere particles together in a tablet made of dry powders by direct compression such as reported herein are considered to be distance attraction forces, solid bridges, and mechanical interlocking. Distance attraction forces here include van der Waals forces, hydrogen bonds, and electrostatic forces. Usually, these bonds act between surfaces that are separated by some distance, and hydrogen bonds particularly occur primarily through electrostatic interaction and may occur both intramolecularly and intermolecularly. Biopolymers such as microcrystalline cellulose and starch and their derivatives are examples of materials where hydrogen bonds are considered important for the tablet strength; this in our opinion may be a contributory factor here. All the tablets were within official limit of specification of less than 1%. Tablets formulated with native starch were observed to disintegrate more rapidly than those prepared with the carboxymethylated starches or those prepared with SSG. This could be attributed to formation of viscous gel layer of the carboxymethylated starch upon contact with water, which impeded penetration of fluid into the tablets, leading to prolongation of the time taken for the tablet to break-up. It could also be owing to the fact that the disintegrant activity of SSG, a carboxymethylated starch, is optimum only at low concentrations (0.2–5%). The binding effect of the carboxymethylated starches was found to be more pronounced in acidic conditions [24,26–28].

Conclusion

Derivatization of carboxymethylated starch from *B. eurycoma* seed yielded starch products with varying degrees of substitution. The time of reaction and concentration of NaOH played a key role in

determining the DS. The derivatized starches possessed higher swelling index and viscosity than the native starch and could be employed as a disintegrant in solid dosage forms at lower concentrations. Conversely, the unique property of the derivatized starches at higher concentrations could be explored for the formulation of controlled-release systems.

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Conflicts of interest
There are no conflicts of interest.

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