



Studies on the isolation, structural and functional properties of Starch Succinate of Cocoyam (*Colocasia antiquorum*)

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ABSTRACT

Starch was isolated from Cocoyam (*Colocasia antiquorum*) and it was acylated using succinic anhydride to produce the starch succinate derivative. Starch succinate was prepared at various succinic anhydride concentrations. Ash content, protein and amylose contents were reduced following modification. No pronounced difference was observed between the X- ray pattern of native starch and modified starch samples, and the samples gave the characteristic B pattern of tuber starches. Modification of the starch by succinylation was confirmed by the presence of a characteristic band at 1734.06 cm^{-1} , this revealed that a carbonyl stretching vibration of an ester group is present in the succinylated cocoyam starch but absent in the native cocoyam starch. The granules were ellipsoid in shape with 15 to 140 μm in width and 25 to 190 μm in height. Studies on the functional properties also revealed that both swelling capacity and solubility increased with temperature increase. The pasting temperature was reduced after succinylation. Setback tendency of the native starch was increased after modification but reduced with increase in concentration of succinic anhydride.

Key words: Cocoyam starch; Succinylation; Blue value; Physicochemical properties.

INTRODUCTION

The high carbohydrate content of cocoyam and its degree of availability makes it a very good source of starch for both domestic and industrial uses in tropical Africa. The source of starch varies all over the world and it depends on the tradition and prevalent climatic conditions

When aiming at functional properties in starch, most commercial companies examine the characteristics of competitive starches in particular applications. For characteristics, which are unattainable with native starches, the only alternative is to look towards some form of value-addition or modification to achieve desired results [1]. Modifications are carried out to improve the functional and physicochemical parameters of the starches in various industries, particularly

where native starch itself cannot give optimal performance [2-3]. In recent times, many types of chemically modified starches have been prepared by acid hydrolysis, oxidation and etherification [4].

In order to increase the water resistance of starch, the hydrophobicity can be increased by succinylation of the hydroxyl groups. The degree of substitution (DS) is expressed as the average number of substituents per Anhydro Glucose Unit (AGU). The AGU is the monomer unit of the starch molecules.

Succinylation of starch is an esterification process achieved by using succinic anhydride as the esterifying reagent. It results in the introduction of succinyl groups onto the polymeric chain of starch.

The reaction produces starch esters that have found good domestic and industrial applications. The modification is also known to weaken the internal bonding that holds the granules together [5]. In addition starch succinate offers very desirable properties such as low temperature stability, high- thickening power, low gelatinization temperature and reduced tendency to retrograde. Succinylation is effected by treating aqueous starch slurry with succinic anhydride in the presence of an alkali, this results in the introduction of succinyl group onto the starch polymeric chain. This is known to weaken the intragranular bonds, thus making the resulting modified starch easily dispersible in water. Succinylation depends upon factors such as reactant concentration, reaction time, pH and presence of catalyst.

The aim of this work was to modify starch isolated from cocoyam by succinylation as means of improving its properties. The long term objective is that cocoyam a valuable source of starch which often perishes as a result of lack of storage technology, particularly in Africa, would be brought to full industrial utilization.

MATERIALS AND METHODS

Materials

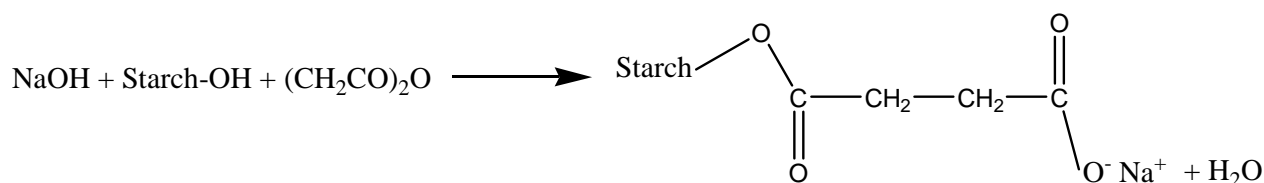
Cocoyam (*Colocasia antiquorum*) was purchased from a local market in Ogun State, Nigeria. All reagents used in this work were of analytical grade.

Starch extraction from cocoyam

The method employed for starch isolation is outlined in Fig. 1. Peeled cocoyam (15Kg) was washed thoroughly and used for starch extraction. The starch obtained was air dried for 48h at 30⁰C, after which it was ground to fine powder using a mortar and pestle, and then sieved.

Starch succinylation

The method of sathé and Salunkhe [6] was used. Starch (100g) was dispersed in 300ml of distilled water and stirred magnetically for 1hr. The pH of the slurry was adjusted to pH 9.0 using 1M NaOH. Different amount of succinic anhydride (3, 4, 7, 9 and 14g) were used to treat the starch suspension (to obtain starch succinate of five different degrees of substitution) over a period of 2 h while maintaining a pH range of 8.0 – 9.0. At the end of the reaction the pH of the slurry was adjusted to pH 6.0 using 0.5M HCl. The mixture was filtered, and the modified starch washed six times with distilled water and oven dried for 24hr at 50⁰C.



Scheme 1. Synthesis of Starch Succinate

Determination of degree of modification

The method of alkali saponification as described by Genung and Mallatt [7] was employed for the determination of succinyl content. A sample (1g) was weighed into a conical flask, 50mL of 75% EtOH was added, and the mixture was refluxed for 30min while maintaining a temperature of 50°C. After cooling to room temperature, 40mL of 0.5M NaOH was added. The flask was covered with aluminium foil and allowed to stand at room temperature for 72h with occasional shaking. Saponification occurred with the addition of NaOH, and the excess alkali was determined by titrating with 0.5M HCl using phenolphthalein indicator. Native starch was treated in the same manner to obtain a value for the blank. The percentage of succinyl group and the degree of substitution of the samples calculated using Eq 1 and 2 respectively.

$$\% \text{ Succinyl} = \frac{(\text{Blank titre} - \text{sample titre}) \times 0.1 \times \text{molarity of acid} \times 100}{\text{Weight of the sample}} \quad 1$$

$$\text{Degree of substitution (DS)} = \frac{162 \times \% \text{ succinyl}}{1000 - (99 \times \% \text{ succinyl})} \quad 2$$

Proximate analysis

Standard Association of Official Analytical Chemistry methods [8], were adopted for estimating moisture, ash, protein and amylose content.

FT – IR spectroscopy

The IR spectra of starches were run as KBr pellets on impact 330 Nicolet Avatar FT-IR spectrometer in the frequency range 4000 – 650cm⁻¹ at Central University Research Laboratory, Ladoké Akintola University of Technology, Ogbomosho, Nigeria.

Physicochemical properties**Wide-angle X-ray diffraction of starch granules**

X-ray diffraction measurement of the powder samples were performed on the MD-10 minidiffractometer at Center for Energy Research and Development (CERD), Obafemi Awolowo University Ile – Ife, Nigeria.

The powder sample was ground manually, sieved and loaded in glass capillaries (sample cuvette) of 0.3mm diameter. A photon wavelength of 1.54Å was used. The scanning region of the diffraction angle (2θ) was from 16° to 70°.

Starch granule morphology

To determine the granule morphology of the native and succinylated starches, each starch granule were taken and dusted onto a carbon sticker, then coated with gold using a sputter coater (Balzers Union, FL- 9496) for 30 min. Images were recorded using INCAPentaFET × 3 SEM fitted with Oxford ISIS EDS.

Oil and water absorption capacity

The method of Beuchat [9] was used to determine oil and water absorption capacity of starch. Distilled water (10mL) or oil (Kuok oils and PTE) Ltd, Lagos, Nigeria, density 0.9g/mL) was added to 1g of sample. The mixture was mixed thoroughly for 60s and allowed to stand for 30mins, after which time the volume of the supernatant was recorded.

Gelation studies

Starch dispersion (2-18% w/v) were prepared in test tubes with distilled water (5ml). The starch suspensions were thoroughly mixed for 5 min. The test tubes were heated for 30min at 80°C in a water bath, followed by rapid cooling under running cold tap water for 2hr. Least gelation concentration was determined as lowest concentration when the sample from the inverted test tube did not fall down or slip.

Effect of temperature on swelling power and solubility

Effect of temperature on swelling power and solubility was evaluated using the method of Leach, McCowen and Scoch [10]. 1.0g of starch sample was accurately weighed and quantitatively transferred into a clean dried test tube and weighed (W_1). The starch was then dispersed in 10ml of distilled water.

The resultant slurry was heated at 50°C, 60°C, 70°C, 80°C and 90°C respectively for 30min in water bath. The mixture was cooled and centrifuged at 500rpm for 15min. Approximately 5mL of the supernatant were dried to a constant weight at 110°C. The residue obtained after drying the supernatant represented the amount of starch solubilized in water. Swelling power was calculated as g per g of starch on dry weight basis.

The residue obtained from the above experiment (after centrifugal) with water it retained was quantitatively transferred to the clean dried test tube used earlier and weighed (W_2).

$$\text{Swelling of starch} = \frac{W_2 - W_1}{\text{Weight of starch}} \quad 3$$

Determination of the blue value and colour of succinylated starch – iodine complex

The samples were prepared according to the method of Gilbert and Spragg [11]. 0.1gram succinylated starch was weighed into a boiling tube, 10ml of distilled water added and heated in a boiling water bath for about 10minutes to solubilize the starch. This was brought down, cooled and quantitatively transferred into a 100ml standard volumetric flask and the volume made up to mark with distilled water.

5ml of the starch solution was taken in a 100ml standard flask, 1ml of 1M acetic acid added followed by 2ml of stock iodine (0.2g I_2 /2.0g KI/100ml) and the solution made up to mark with distilled water. The resulting colour was left for 20minutes to fully develop before the absorbance reading was monitored at 620nm with a Perkin-Elmer Lambda 3B double beam UV/visible spectrophotometer. Iodine solution of same concentration as above but without starch sample was used in the reference cell.

The colour of the succinylated starch – iodine complex was also monitored visually. The blue value was calculated according to the method of Gilbert and Spragg, using the formula:

$$\text{Blue value} = \frac{\text{Absorbance} \times 4}{\text{Concentration (mg/dl)}} \quad 4$$

Paste viscosity determination

Paste viscosity was determined using the Rapid Visio Analyzer (RVA). The RVA is a heating and cooling viscometer configured especially for testing starch based and other products requiring precise control of temperature and shear. It is ideal for measuring the viscosity of cooked pastes of starch based products since it can heat and cool samples rapidly in a controlled manner. Starch {3g} was dispersed in 25mL distilled water and quantitatively transferred into the canister.

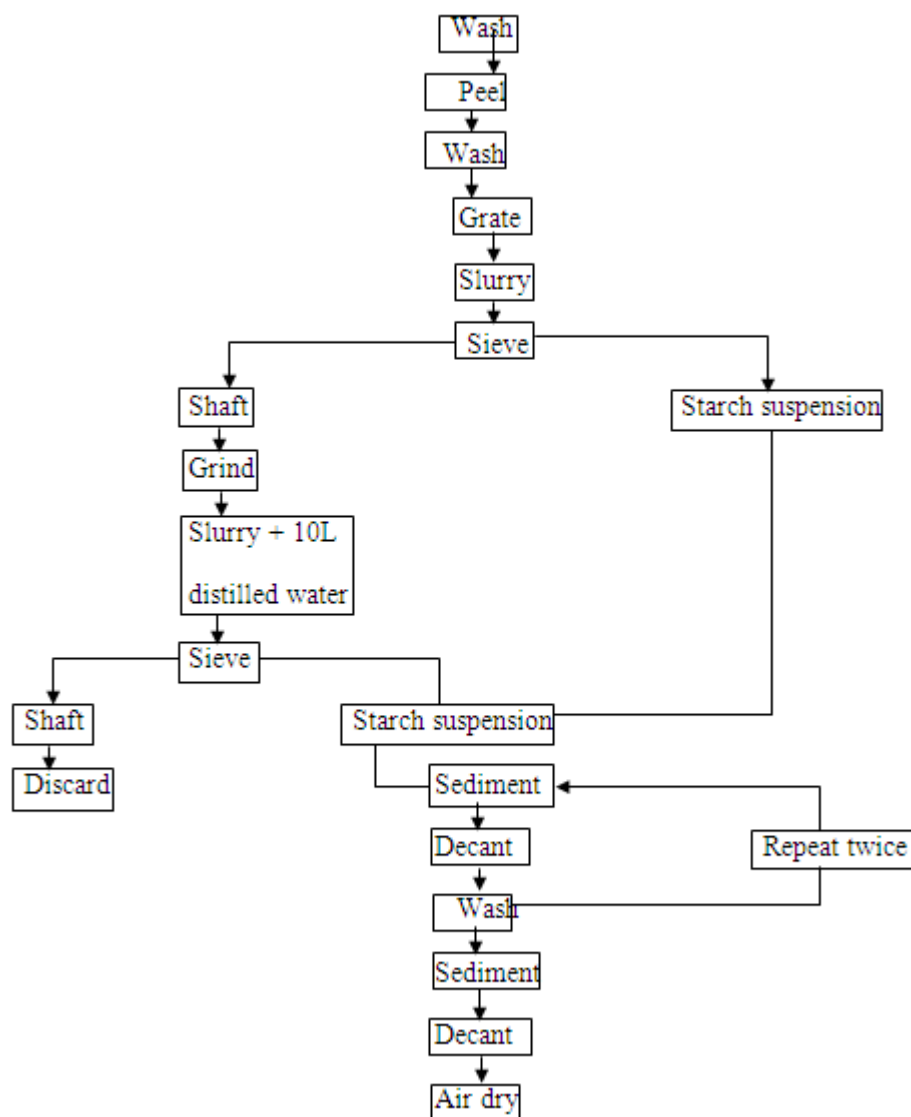


Fig 1: Schematic diagram for extraction of cocoyam (*colocasia antiquorum*).

RESULTS AND DISCUSSION

Degree of modification

The results of the degree of modification of the derivatised starches are presented in Table 1. The results indicate that % succinylation increased with increase in concentration of succinic anhydride.

This suggests that increase in concentration of succinic anhydride favours the production of starch succinate. This observation might be as a result of increases in reaction contact between the starch and the acylating agent, which facilitated formation of starch derivatives with higher degree of substitution [12 -13].

Proximate analysis

The results of the proximate analysis of derivatised and native white cocoyam starch presented in Table 2. The result indicates increase in moisture content with increased in degree of succinylation [14]. Protein, ash and amylase contents reduced with increase in degree of succinylation following succinylation.

Introduction of succinyl groups enhanced hydrophobic capacities of derivatised starches, and this probably facilitated increases observed in the % moisture level. The increase in moisture content after succinylation has been reported [15]. Reduction in ash, protein and amylose contents are due to structural disintegration that took place during chemical modification processes. Functional properties of starches depend on the amylose content to a large extent. Amylose (25.45%) content of native starch is in agreement with ranges of values reported for other tuber starches, 25.49% for potato, and 22.8%, 21.6% for *D. alata* and *D. cayensis*, respectively [16].

Oil and water absorption capacity

Oil and water-absorption capacity of native and derivatised starches are presented in Figure 2. The results indicate that all the starches had more water absorption capacities than oil absorption capacities. This result also indicate that starch succinylation facilitated increased in water absorption capacity.

The introduction of bulky functional groups and their electrostatic repulsion facilitated percolation and absorption of water within the starch matrices. Increase in water absorption capacities following esterification reaction have been reported in the literature [13].

Gelation studies

In Table 3, gelation properties of native and succinylated starches are presented. The results indicate a reduction in gelation properties of the native starch after succinylation. The least gelation concentration (LGC) increased from 6 (native starch) to 14 (SNT₅). The succinyl group substitution on starch molecules affected the intragranular force interactions. During succinylation some of the hydroxyl hydrogen of the starch molecule is replaced by the succinyl groups, thus interferes with the strength of hydrogen bonding resulting in the formation of weak gels in succinylated starch [17] It is reasonable also that intermolecular electrostatic repulsion after introduction of succinyl group reduced gel cohesion.

Swelling power and solubility

Result of the effect of temperature on swelling power and solubility presented in Figure 3 and 4, respectively. The results indicate that both swelling power and solubility were temperature dependent, and values increased with increase in temperature [18]. The succinylated starches were more water soluble than the native starch. It is also reasonable that as the temperature of the medium increases, starch molecules become more thermodynamically activated, and the resulting increase in granular mobility enhances penetration of water which facilitates improved swelling capacities. Similar observations have been reported earlier for starches of rice [16].

Blue value and colour of starch-iodine complex

The blue value and colour of starch-iodine complex is presented in Table 4. The blue value decreases with increase in DS. When starch is derivatised there is a change in the molecular organisation within the granules, and since starch retrogradation is a phenomenon primarily associated with amylose molecule, the effective changes must have been those associated with amylose units.

Starch reacts with iodine to form a blue color. Studies on starch fractions have shown that the blue colour observed with iodine is due to the staining of amylose. Amylopectin itself is stained violet-red with iodine.

Wide-angle X-ray diffraction of starch granules

The wide angle X-ray diffraction of a native cocoyam starch and representative's succinylated starch (SNT₁ and SNT₅) is presented in Figure 5, 6 and 7 respectively.

The diffractogram of the native starch is the B-type, typical of tuber starches. Generally, tuber starches have been shown to exhibit a 'B' type X-ray pattern having diffraction peaks at 5.5-5.6⁰, 14.1⁰, 16.0⁰, 17.0⁰, 19.7⁰, and 24⁰ 2θangles. 'A' type starches mainly cereals exhibit reflections at 15.3⁰, 17.0⁰, 18.0⁰, 20.0⁰ and 23.4⁰ 2θangles.[19-20].

All the samples gave the characteristic 'B' pattern of cocoyam starch with strong peaks at 16.13⁰, 16.28⁰, 16.35⁰, 17.22⁰, 17.43⁰, 17.44⁰ and 19.880, 2θ.

No pronounced difference was observed between the X-ray pattern of the unmodified and modified starches apart from increased peak intensities in the succinylated starches.

Infrared spectra

Infrared spectra of native and succinylated starches are presented in Figure 8. The broad band between 3600cm⁻¹ and 3000cm⁻¹ is assigned to O-H stretching and it is due to hydrogen bonding involving the hydroxyl groups on the starch molecules. The band at 2963.28 cm⁻¹ is assigned to CH₂ symmetrical stretching vibrations. The succinylated starch derivative shows new band at 1734.06cm⁻¹. This new band indicates carbonyl stretching vibration of the ester group i.e evidence that succinylation has taken place.

Granule morphology

Scanning electron microscopy was used to investigate the granule morphology of the native starch as well as the developments in succinylation (Figure 9). Studies revealed that the native cocoyam starch has shell and ellipsoid shapes with sizes ranging from 15 to 140 μm in width and 25 to 190 μm in height. As observed, the conditions of modification significantly altered the granular structure of the succinylated starch. The alkaline environment during the succinylation process accounts for the structural changes. This result also suggests that starch crystallinity was altered and this allowed the esterifying agents to have more access to the starch molecules for succinylation processes.

Pasting characteristics

The pasting properties of native and derivatised starch samples are presented in Table 5. After succinylation, pasting temperature of native starch is reduced. Reduction in pasting temperature following succinylation reactions have been reported in literature [15].Reduction in pasting temperature following derivatization is a consequence of structural weakening and disintegration during the modification process.

When a starch granule is heated in excess water, it leads to further granule swelling and this results in formation of a viscous starch paste. However, increases were observed in the peak viscosity value of native after succinylation. This observation lends credence to similar results that have been reported.[21].

The results also indicate an increase in the breakdown value which is a measure of fragility of the starch. Following modifications, the modified starches become partially degraded and as a result could not maintain the integrity of the starch granule. This therefore accounts for the higher breakdown value observed in modified starches.

The setback value, an index of retrogradation tendency in the starch paste was increased after modification but reduced with increase in concentration of succinic anhydride. Reduction in setback value with increase in concentration of succinic anhydride could be attributed to limitations caused by the succinyl group introduced in the starch molecules. Although in the literature reduction in the setback values after succinylation have been reported [15], increase in setback values after modification might be as a result of the starch source.

Table 1: Degree of modification of starch succinate showing % succinyl groups

Sample	% succinyl	DS
SNT ₁	0.25	0.04
SNT ₂	0.35	0.06
SNT ₃	0.65	0.11
SNT ₄	0.85	0.15
SNT ₅	1.25	0.23

Table 2: Proximate composition of native and succinylated cocoyam starch

Sample	% M. C	% Ash	% Protein	% Amylose content
NT	7.77	1.09	0.458	25.45
SNT ₁	7.79	1.09	0.222	25.22
SNT ₂	8.84	1.08	0.197	25.08
SNT ₃	8.88	1.06	0.178	24.75
SNT ₄	9.35	1.04	0.125	24.55
SNT ₅	9.50	1.02	0.101	23.65

*Means within columns with different letter are significantly different { $p < 0.05$ }
All values are means of triplicate determinations + or – standard deviation.*

Table 3: Gelation properties of native and succinylated cocoyam starch derivatives

Concentration (% w/v)	NT	SNT ₁	SNT ₂	SNT ₃	SNT ₄	SNT ₅
2	- Liquid	- Liquid	- Liquid	- Liquid	- Liquid	- Liquid
4	- Viscous	- Liquid	- Liquid	- Liquid	- Liquid	- Liquid
6	+ Gel	- Liquid	- Viscous	- Liquid	- Liquid	- Liquid
8	+ Gel	+ Gel	- Viscous	- Liquid	- Liquid	- Liquid
10	+ Firm gel	+ Gel	+ Gel	- Liquid	- Viscous	- Liquid
12	+ Firm gel	+ Gel	+ Gel	+ Gel	- Viscous	- Viscous
14	+ Very firm gel	+ Firm gel	+ Gel	+ Gel	+ Gel	+ Gel
16	+ Very firm gel	+ Firm gel	+ Firm gel	+ Gel	+ Firm gel	+ Firm gel
18	+ Very firm gel	+ Very firm gel	+ Firm gel	+ Firm gel	+ Very firm gel	+ Very firm gel
LGC	6	8	10	12	14	14

LGC: Least Gelation Concentration

Table 4: Blue value and colour of native and succinylated starch-iodine complex

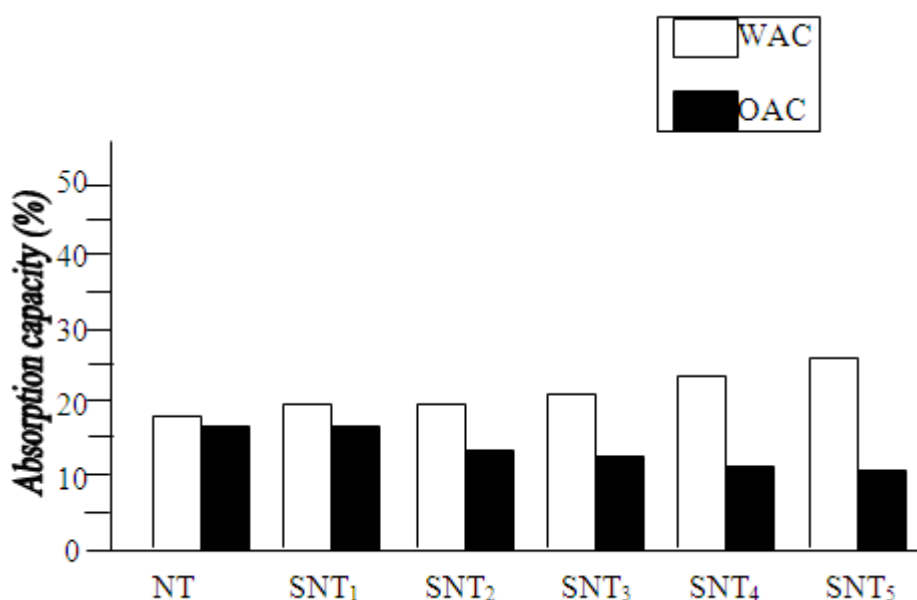
Sample	DS	Absorbance	Blue Value	Colour of starch iodine complex
NT		0.465	0.372	Blue black
SNT ₁	0.04	0.439	0.351	Blue black
SNT ₂	0.06	0.420	0.336	Blue black
SNT ₃	0.11	0.395	0.316	Blue black
SNT ₄	0.15	0.350	0.280	Blue black
SNT ₅	0.23	0.345	0.276	Blue black

Means within columns with different letter are significantly different ($p < 0.05$)

All values are means of triplicate determinations + or – standard deviation.

Table 5: Pasting properties of native and representatives of succinylated starch derivatives

Sample	Peak (RVA)	Trough (RVA)	Breakdown (RVA)	Final Visc (RVA)	Setback	Peak Time (min)	Pasting Temp (°C)
NT	231.92	164.17	67.75	216.67	52.50	4.27	85.60
SNT 1	246.25	174.25	72.00	273.25	99.00	4.80	83.80
SNT 2	262.58	163.25	99.33	260.17	96.92	4.47	83.15
SNT 3	313.33	181.92	131.42	273.08	91.17	4.20	83.15

**Fig 2: Water and Oil absorption capacity of native and succinylated starch cocoyam.**

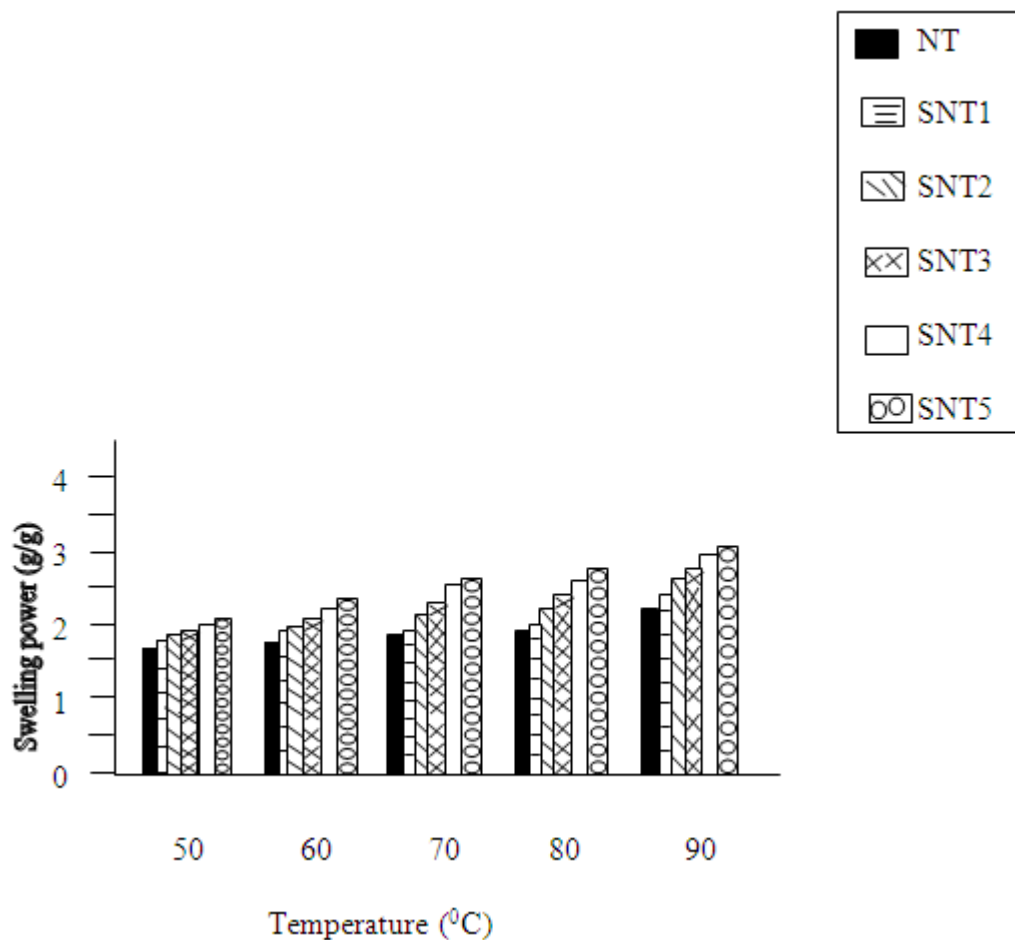


Fig 3: Effect of temperature on swelling power of native and succinylated starch cocoyam

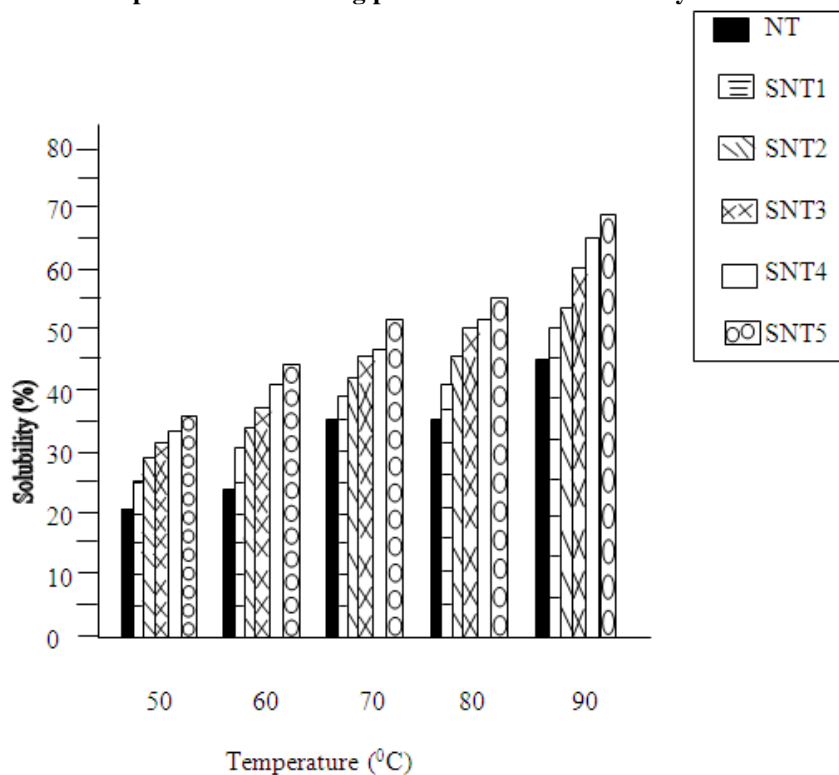
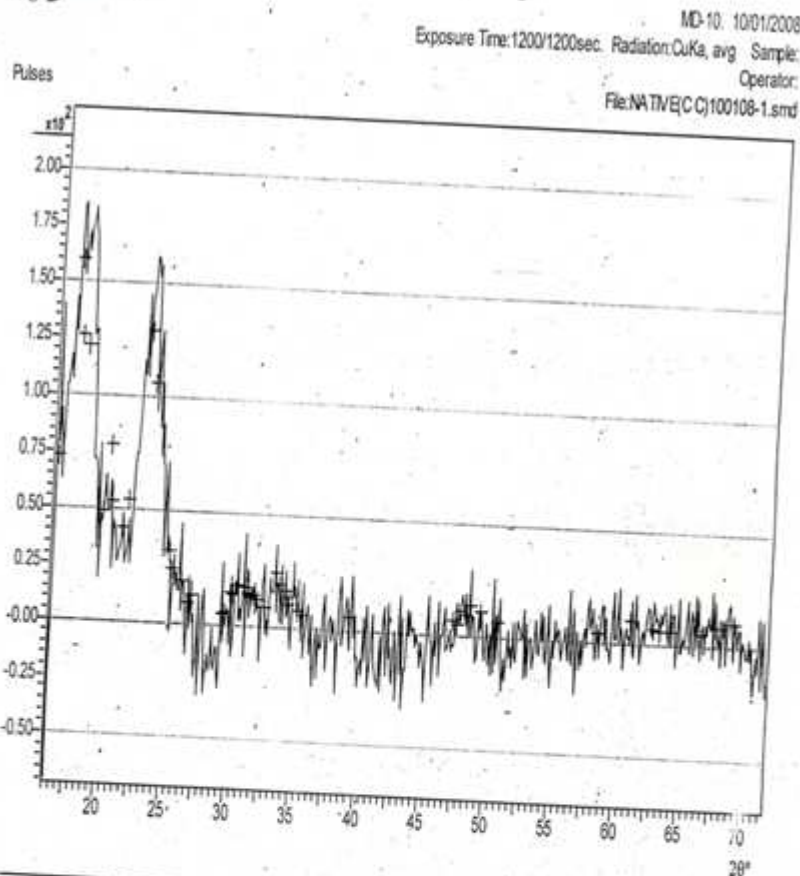


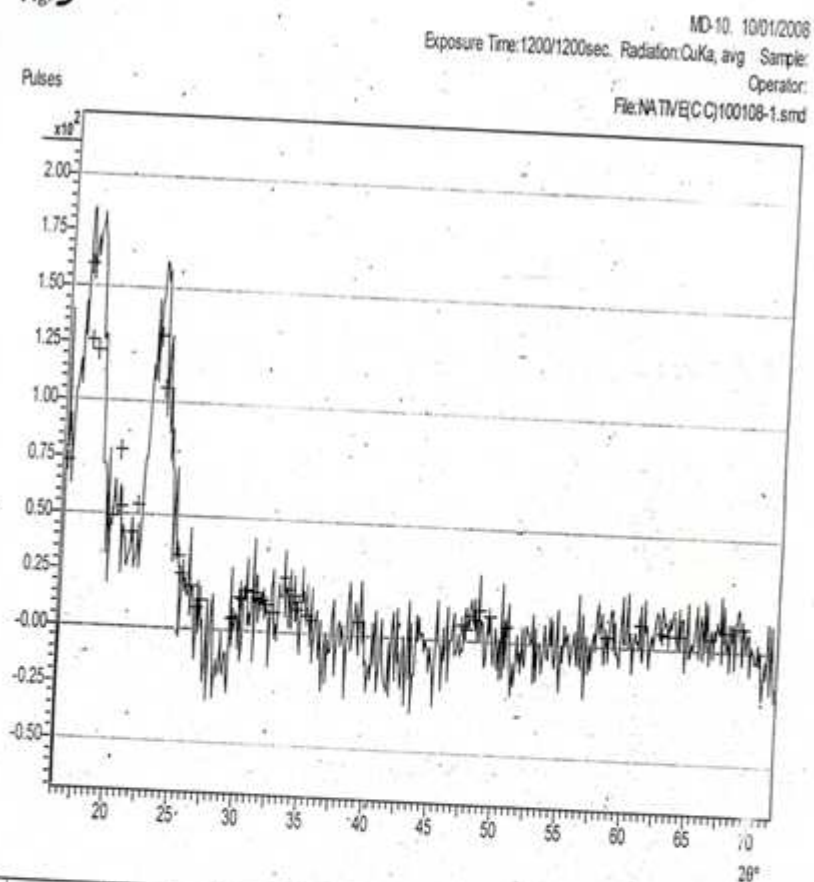
Fig 4: Effect of temperature on solubility of native and succinylated starch cocoyam

Fig. 5

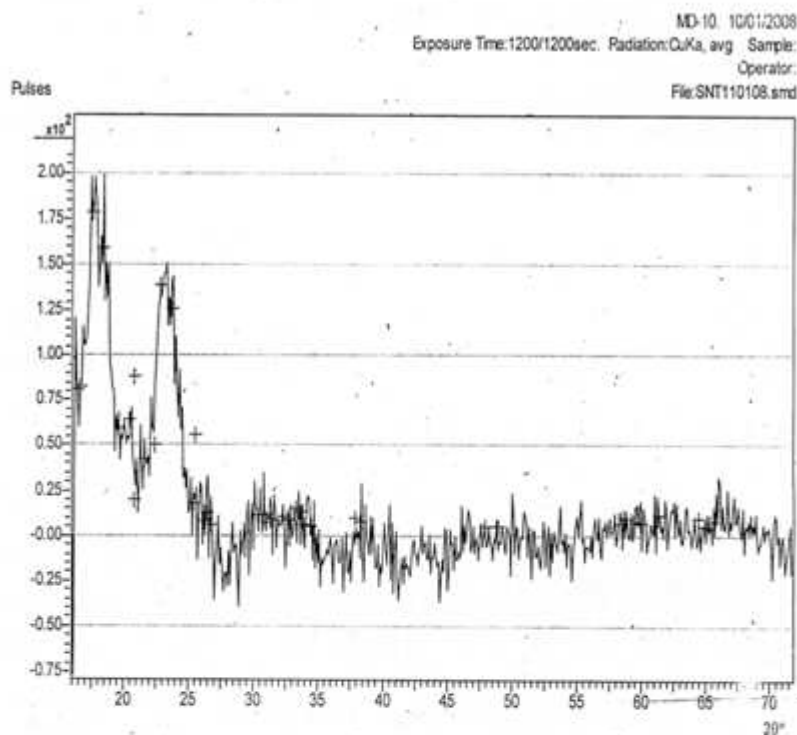


$2\theta^\circ$	d	I _{int}	I _{max}	I _{rel}	I _{corr}	FWHM
16.28	5.44286	-151	-10.2	-3.3	-3.5	0.1973
17.43	5.08688	4606	78.4	100.0	100.0	0.78497
18.29	4.85122	490	39.9	10.6	10.2	0.1645
17.81	4.98140	3197	45.1	69.4	68.2	0.94723
19.88	4.46551	-124	-34.7	-2.7	-2.4	0.047804
20.34	4.36688	-273	-5.8	-5.9	-5.3	0.62496
20.60	4.31075	-873	-30.1	-19.0	-16.8	0.38826
21.45	4.14180	-614	-42.2	-13.3	-11.5	0.19421

Fig. 5

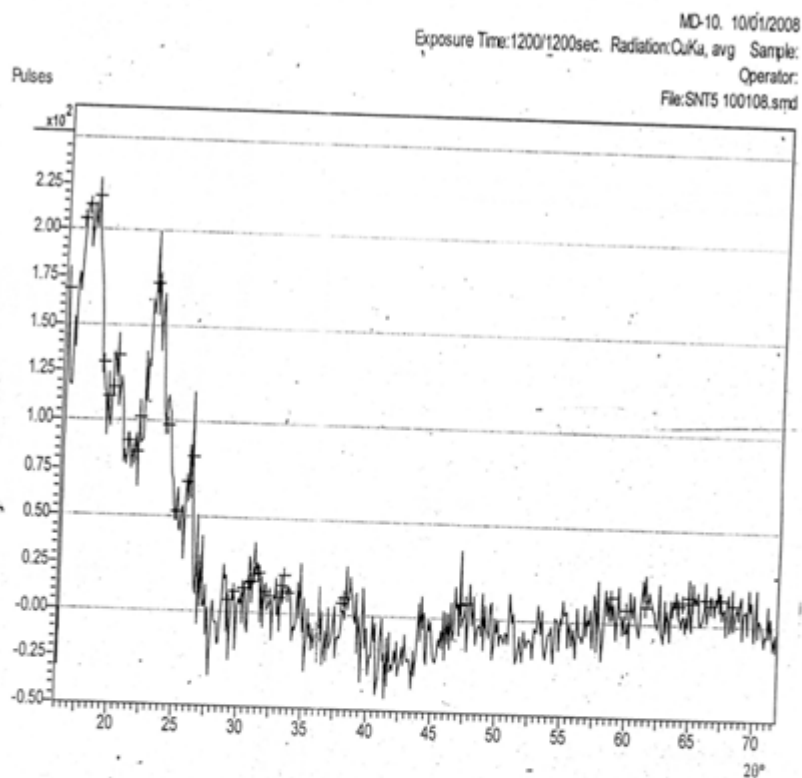


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2θ°	d	I _{int}	I _{max}	I _{rel}	I _{corr}	FWHM
16.35	5.42150	17	7.8	0.2	0.3	0.028587
16.54	5.36040	480	10.3	6.4	7.4	0.62506
17.44	5.08448	6767	105.4	90.0	100.0	0.86055
18.31	4.84437	5692	86.7	75.5	80.6	0.87715
19.56	4.53908	-514	-14.0	-6.8	-6.9	0.4899
20.40	4.35311	-136	-9.7	-1.8	-1.8	0.18657
20.80	4.27009	5729	14.1	76.0	74.3	5.4501
21.52	4.12843	-19	-32.2	-0.3	-0.2	0.0080153
20.73	4.28511	-789	-53.7	-10.2	-10.0	0.19137
22.39	3.97053	-425	-23.8	-5.6	-5.3	0.23801
22.72	3.91370	3177	90.4	42.1	39.2	0.46945
25.42	3.50372	6368	7.2	64.4	74.4	12.026
23.65	3.76127	5873	77.8	77.9	71.0	1.0093
25.36	3.51149	687	18.8	9.1	8.0	0.48925

Fig. 6



$2\theta^\circ$	d	I _{int}	I _{max}	I _{rel}	I _{corr}	FWHM
16.13	5.49307	6191	169.4	77.2	85.0	0.48865
17.22	5.14815	7560	206.8	94.3	97.8	0.48865
17.53	5.05863	7848	214.7	97.9	100.0	0.48865
18.23	4.86646	8016	219.3	100.0	99.0	0.48865
19.11	4.64440	4786	130.9	59.7	57.1	0.48865
19.47	4.55863	4141	113.3	51.7	46.7	0.48865
19.85	4.47290	4308	117.9	53.7	50.0	0.48865
20.16	4.40543	4915	134.5	61.3	56.5	0.48865
21.05	4.22040	3304	90.4	41.2	37.0	0.48865
21.75	4.08648	3120	85.4	38.9	34.3	0.48865
21.93	4.05306	3767	103.1	47.0	41.2	0.48865
23.07	3.85449	6332	173.2	79.0	67.3	0.48865

Fig. 7

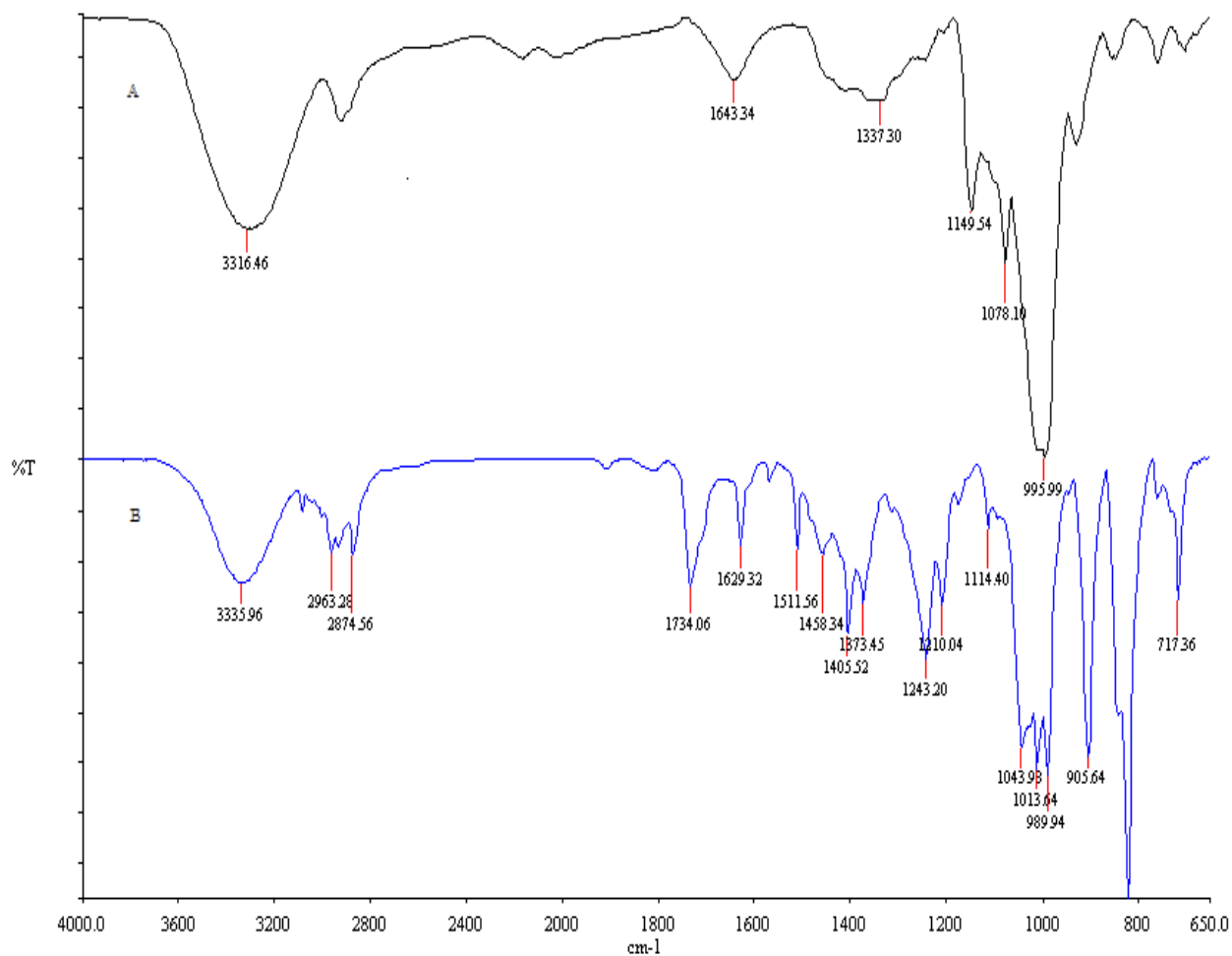


Fig. 8 Infrared spectra of native (A) and a representative succinylated cocoyam starch (B)

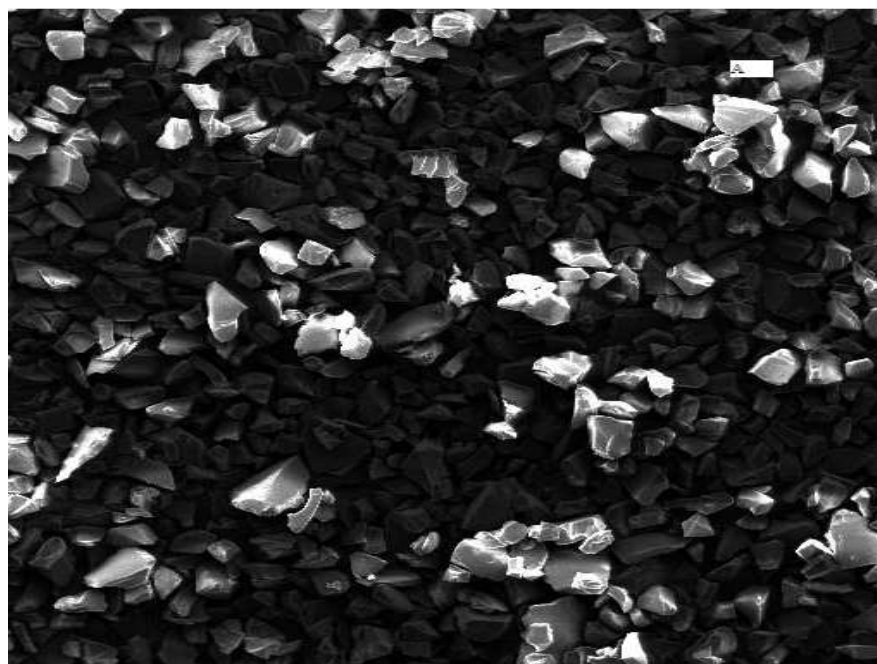




Fig. 9 (A) The scanning electron micrographs of native cocoyam starch. (B) A representative succinylated cocoyam starch

CONCLUSION

The interest concerns investigations on relatively cheap but under-explored starch resources as well as modifying them for valued-added technical applications.

The study revealed that % succinylation increased with increase in concentration of succinic anhydride. In the present investigation, to be précised, from the results obtained in Table 3, it can be established that there is a reduction in gelation properties of the native starch after succinylation. Modification of starch is indeed very useful; this is obvious from the results of the analysis of the modified and native starches.

Succinylation improved the functional properties of a native cocoyam starch; these properties are very invaluable in many industrial applications.

Acknowledgment

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