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Characterization of Modified Tiger Nut (*Cyperus Esculentus*) Starches: Functional and Physicotechnical Properties

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ABSTRACT

Background and Objective: Starches and their modified products have a wide range of applications in pharmaceutical manufacturing and food processing industries. The purpose of this study was to characterize native and modified tiger nut (*Cyperus esculentus*) starch.

Methodology: The native starch (NCS) was extracted and subjected to acidic (ACS), pre-gelatinization (PCS) and enzymatic (ECS) modifications. The native starch was evaluated for phytochemical and elemental composition and characterized with the modified starches in terms of their physicochemical (organoleptic, chemical test, solubility, pH, amylose-amylopectin ratio, gel formation, moisture content, loss on drying, swelling, hydration and moisture sorption capacities, paste clarity, browning, charring and gelatinization temperatures) and physicotechnical (powder bulk and flow parameters) properties. The thermal and particle characteristics of the starches were also evaluated using differential scanning calorimetry and scanning electron microscopy (SEM), respectively.

Results: The starch extraction yield was 15.60%. Phytochemical and elemental composition were similar to other studies. Modified starches exhibited significantly ($p < 0.05$) better flow, lower moisture content, increased swelling and hydration capacity and lower gelatinization, browning and charring temperatures. A significant increase in swelling and hydration capacities were observed for ACS and PCS while a significant reduction in water sorption capacity was noticed for ECS. SEM showed differences in particle morphology (sizes, shapes and distribution) between the native and modified starches.

Conclusion: Modification of *C. esculentus* native starch resulted in products with improved functional and physicotechnical properties in terms of swelling, hydration and water sorption capacities as well as in flowability.

Keywords: *Acid modification, enzymatic, pre-gelatinization, tiger nut, starch.*

INTRODUCTION

Starch is a major energy source in many plants such as cereal grains (rice, maize, wheat and sorghum), tubers (potato and yam) and roots (arrowroot and cassava) [1]. Starches have extensive applications in food processing and pharmaceutical manufacturing industries [2]. The use of starch for industrial applications remains attractive because it is inexpensive, readily available and biodegradable [3]. The native form of starches have limited functionalities due to its physical and chemical properties. These inherent properties have been modified either by physical or chemical treatments to produce functionally tailored starches with improved functional attributes to meet specific applications [4].

Reports of several works on native starch modification abound in literature and some examples includes the modification of pea starch, where the modified product showed high industrial potentials in the confectionary, food and pharmaceutical industries [5]. Another study on modified *Colocasia esculenta* starch resulted in improved morphological characteristics, viscosity, swelling volume, solubility and gelatinization temperature [6].

Cyperus esculentus, also known as tiger nut or earth almond is both an annual and perennial plant, growing up to 3.0 feet in height and producing spherical tubers and rhizomes from its base [7]. It is found in the temperate zones within southern Europe as its probable origin but also grow naturally in many West African countries such as Nigeria, Ghana and Sierra Leone. In Nigeria, it is commonly cultivated in the northern states [8]. Tiger nuts have been established to contain starch, fats, proteins, vitamins (C and E) and minerals (potassium and phosphorous) [9]. It is consumed either for its milk, oil or starch and also used in the production of animal feed. It is reported to have a starch yield of about 10 to 39% [10,11], hence a source of significant quantity of starch that can be applied for pharmaceutical manufacture and drug production purposes.

The aim of this study was to subject the starch extracted from *Cyperus esculentus* tubers to various forms of modification and to characterize the native and modified starches using standard procedures and high resolution analytical methods.

METHODOLOGY

Ethanol, n-hexane and hydrochloric acid (JHD Chemicals Ltd. Guandang, China), sodium metabisulphite (Aditya Birla Chemicals, Thailand), iodine (ISE Chemicals, Japan), sodium hydroxide (Tianye Chemicals, China), A7595 α-amylase (Loba Chemie, India). Standard amylose fraction of starch was obtained from the Department of Chemistry, University of Port-Harcourt. All other reagents were of analytical grade.

Collection and identification of *Cyperus esculentus tubers*

Fresh tubers of *C. esculentus* were purchased from a local market in Rivers State, Nigeria. The sample was identified and authenticated by Dr. NL Edwin-Wosu, a taxonomist in the University of Port Harcourt. A voucher specimen (EH-C-012) was deposited at Ecoland Herbarium, Centre for Environmental Research, Conservation and Bioresources Development, Rivers State.

Defatting of *Cyperus esculentus* powdered tubers

About 14 kg of *C. esculentus* tubers was washed thoroughly to remove all adhering dirt and soil and sun-dried for several days to a constant weight. The dried tubers was milled using a ball mill into fine powders. The powdered sample was subjected to a continuous fat extraction in a Soxhlet extractor for 16 h using n-hexane as the defatting solvent. A rotary evaporator was used to remove the solvent from the sample at 67.0 ± 3.0 °C [12].

Proximate analysis

Lipid content

The percentage lipid content (%) of the powdered *C. esculentus* tubers was calculated from the ratio of the weights of the extracted oil from the defatting process and the powdered sample and expressed as a percentage [12].

Protein content

The protein content of *C. esculentus* tubers was estimated using the Kjeldahl method to obtain the percentage nitrogen content using equation 1. Percentage crude protein was then calculated by multiplying the nitrogen value obtained with the nitrogen to protein standard conversion factor of 6.25 [12].

$$\%\ nitrogen = \frac{(Vol.of\ sample\ acid - Vol.of\ blank\ acid) \times Acid\ normality \times 1.4007}{Weight\ of\ sample(g)} \quad \ldots. \quad (1)$$

Extraction of *C. esculentus* starch

Using an earlier reported method, the defatted powdered *C. esculentus* tuber sample was steeped in 8 L of 0.08 % sodium metabisulphite ($Na_2S_2O_5$) solution at 30 °C for 48 h. The steep solution was decanted and the powdered pulp re-dispersed in 10 L of same solution. The dispersion was stirred for 5 min and left to stand for 10 h. Afterward, the dispersion was stirred and filtered with a muslin cloth. The filtrate was allowed to stand for 24 h and the starch sediment was collected after decanting the supernatant. The sediment was re-suspended in 2.4 L of distilled water, allowed to stand 8 h and the supernatant decanted. This procedure was carried out for another 16 h to ensure complete washing of the starch sediment. The resulting wet starch cake was air-dried overnight and subsequently oven-dried (Mammert Hot Oven, Schwabach Germany) at 45 °C for 8 h [13]. The extracted starch was weighed and the percentage yield calculated. The starch was labelled native *C. esculentus* starch (NCS) and then packed in air-tight plastic containers until use.

Phytochemical analysis

The following phytochemical tests was carried out on a 5.0 %w/v aqueous dispersion of the starch using standard procedures: alkaloids, carbohydrates, tannins, phlobatannins, flavonoids, saponins, phenolic compounds, proteins and amino acids and triterpenoids [14,15].

Elemental analysis

Ash value

A 25 g quantity of the native *C. esculentus* starch sample was ignited in the presence of oxygen in a crucible using a hot plate at 200 °C until charred. The charred starch was moved to a furnace and heated at 600 °C for 8 h. The sample was allowed to cool and the residue weighed. The ash value (As) was computed as the percentage of starch weight lost in the experiment.

Atomic absorption spectrophotometry (AAS)

One gram of the native *C. esculentus* starch was heated in a furnace to ash using the dry combustion method. The ash starch sample was transferred to a 100 ml volumetric flask, digested in 10 ml of 50% HCl and diluted to volume with distilled water. The digested sample was analysed in an atomic absorption spectrophotometer (AA 7000, Chimaz Japan) equipped with lamps for various metals.

Modification of starch

Acid hydrolysis

The acid hydrolyzed starch was prepared using a modified method of Atichokudomchai and Varavinit [16]. A 400 g sample of the native starch (NCS) was incubated in 800 ml (6.0 %v/v) HCl solution without stirring at 27 ± 1.0 °C for 192 h. The resulting suspension after hydrolysis was neutralized using 10 %w/v NaOH solution to terminate the reaction. The starch slurry was repeatedly suspended with distilled water, allowed to stand for 1.0 h and the supernatant decanted until the supernatant was neutral to litmus. The starch obtained was dried in a vacuum oven for 24 h at 40 °C, screened using a 125 µm mesh sieve and weighed. The percentage yield was calculated and modified starch labelled acid hydrolyzed *C. esculentus* starch (ACS).

Pre-gelatinization

The pre-gelatinized form of the starch was obtained using the method of Eraga et al. [17]. Aqueous starch slurry (20 %w/v) was prepared by dispersing 400 g of the native starch (NCS) in 2 L of distilled water and heated in a water bath thermostated at 80 °C with constant stirring for 15 min. The resulting slurry was then oven dried for 48 h at 40 °C. The dried flakes obtained were milled using a laboratory blender, screened through a 125 µm mesh sieve and weighed. The percentage yield was determined and the starch labelled pre-gelatinized *C. esculentus* starch (PCS).

Enzyme hydrolysis

The enzyme hydrolyzed *C. esculentus* starch was prepared using the method of Buwalda and Arends-Scholte [18]. A 400 g quantity of the tiger nut native starch (NCS) was dispersed in 1.0 L of distilled water to give a 40 %w/v starch slurry and placed on a water bath set to 55 °C. Alpha-amylase enzyme (0.8 g) was introduced at pH 5.7 to facilitate hydrolysis. The enzyme was inactivated after 5.0 h and the pH was neutralized using HCl and NaOH, respectively. The reaction mixture was rinsed several times with distilled water and centrifuged at 3000 rpm for 10 min. The resulting sediment was suspended in 95 %v/v ethanol, air-dried and then screened through a 125 µm mesh sieve. The percentage yield was calculated

and labelled enzyme hydrolyzed *C. esculentus* starch (ECS).

Characterization of the native and modified starches

The following characterizations were carried out on the native and modified starches.

Physicochemical properties

Organoleptic

The colour, odour, taste and texture of the starches were assessed by three assessors and the decision of a majority (at least 2 out of 3) of the assessors was taken.

Chemical (iodine) test

About 300 mg quantity of starch was added to 15 ml of water and boiled on a water bath set to 90 °C for 30 min. On cooling, two drops of 0.1 N iodine solution were introduced into the starch mucilage and the colour change was recorded [19].

Solubility profile and pH

The solubility of the starches was determined in distilled water, ethanol (95 %v/v) and 0.1 N HCl at room temperature using the gravimetric method. About 100 mg of the starch was dispersed in 100 ml of solvent in a beaker. The dispersion was filtered using a pre-weighed filter paper and the residue air-dried. The filter paper with the dried residue was weighed and the difference in weight was used as a measure of solubility of the starch powder.

The pH of a 2.0 %w/v dispersion of the starches in distilled water was read using a pH meter (PH 1100 Series, Eutech Instruments, Singapore) and recorded.

Amylose/amylopectin ratio

The amylose content of starch samples was determined by transferring 10 mg of sample into a 100 ml beaker and adding 10 ml of 0.5 N KOH solution before making up to volume with distilled water. A 5.0 ml aliquot of the mixture was measured into another 50 ml volumetric flask with the addition of 5.0 ml of 0.1 N hydrochloric acid and 0.5 ml of 0.1 N iodine and then made up to volume with distilled water. The absorbance of the resulting solution was taken at a wavelength of 625 nm and the amylose content extrapolated from the regression equation previously obtained from the calibration plot of a standard amylose sample.

The total starch content of the samples was determined with 0.5 g of the starch sample heated in 50 ml of distilled water at 67 ± 2 °C for 30 min. One millilitre of the resulting suspension was measured into a beaker and 9.0 ml of Anthrone reagent was added and the absorbance read at 620 nm. The starch content was extrapolated from the regression equation previously obtained from the calibration plot of a standard *C. esculentus* starch sample. Amylopectin content was calculated as the difference in values of total starch and amylose.

Gel formation

Two millilitres of distilled water was added to 1.0 g of the starch sample to obtain a smooth mixture of starch slurry. The mixture was stirred continuously with the addition of 10 ml of boiled water and then placed on a hot water bath to boil gently for 2 min and then cooled.

Moisture content

A 5.0 g quantity of the starch powder was weighed and spread evenly on a clean crucible of known weight. The sample was placed in an oven maintained at 60 °C. The sample was brought out intermittently, cooled in a desiccator and weighed until a constant weight was obtained between two successive weighings. The difference between the initial and final weights of the starch sample was recorded as the moisture content.

Loss on drying

Using the USP method [20], 1.0 g of starch sample was placed in a crucible of known weight and transferred to a hot air oven set at 105 °C for 5 h. The percentage loss in weight after cooling was calculated as the difference between the initial and final weights of the starch sample, divided by the initial weight, and the ratio expressed as a percentage.

Swelling capacity

The swelling capacity of the starch samples were determined using the method of Iwuagwu and Onyekweli [21]. The volume occupied by 5.0 g of powder after tapping was recorded. Thereafter, the powder was dispersed in 85 ml of distilled water and made up to 100 ml volume with water. On standing for 24 h, the volume of the sediment was noted. The ratio of the sediment volume to the powder tapped volume was computed as the swelling capacity.

Hydration capacity (water retention capacity)

A 0.5 g quantity of the starch powder sample was weighed into a 10 ml plastic centrifuge tube and 5.0

ml of water was added. The dispersion was shaken intermittently for 2 h and allowed to stand for 30 min. The dispersion was then centrifuged at 3000 rpm for 10 min and the supernatant decanted. The weight of the formed sediment was determined and the ratio of the sediment weight to the initial weight of the starch powder was computed as the hydration capacity.

Gelatinization temperature

Using the method of Attama et al. [22], 1.0 g of starch sample was suspended in 10 ml of distilled water and heated on an electric hot plate. The temperature of change from a suspension to mucilage was recorded as the gelatinization temperature.

Browning and charring temperatures

Some quantity of starch powder was packed into a capillary tube sealed at one end and tapped gently on the sealed end to form a powder column at the base of the capillary tube. The tube was inserted into the heating block of a melting point apparatus (Gallenkamp, London, UK) and the temperature raised at 1.0 °C/min until the starch sample turned brown. Raising of the temperature was continued until the brown starch sample was charred. The temperatures of browning and charring were recorded.

Moisture sorption capacity

A 0.5 g quantity of the starch was weighed separately and placed in four (4) pre-weighed Petri dishes. The samples were each placed in a desiccator (at room temperature) containing different solutions to simulate different relative humidity (RH); 100% RH (water), 84% RH (potassium chloride), 75% RH (sodium hydroxide) and 52% RH (magnesium nitrate). The difference in the initial and final weights of the starch sample after seven days was computed as the moisture sorption capacity of the starch at the respective relative humidity.

Paste clarity

A 4.0 %w/v dispersion was prepared by dispersing 1.0 g of starch powder in 25 ml of distilled water. Serial dilution was carried out with distilled water to obtain dispersions of varied concentrations (0.5, 1.0, 1.5, 2.0 and 2.5 %w/v). The amount of light transmitted through the dispersions was determined spectrophotometrically (Jenway, Staffordshire, UK) at a wavelength of 650 nm and their transmittance readings recorded.

Physicotechnical properties

Bulk and tapped densities

The bulk volume of the starch sample was determined by pouring a 20 g quantity into a calibrated 100 ml measuring cylinder and recording the volume occupied. The ratio of the weight of the sample to the volume was recorded as the bulk density. The measuring cylinder was tapped 100 times on a horizontal surface until a constant volume was obtained. The ratio of the weight of the sample to the consolidated or tapped volume was used as the tapped density.

Carr's index and Hausner's ratio

The difference between the tapped and bulk densities of the starch sample, divided by the tapped density and expressed as a percentage was recorded as the Carr's index while the ratio of the tapped to the bulk densities of the starch powder was recorded as the Hausner's ratio.

Flow rate and angle of repose

Using the method of Carstensen and Chan [23], a glass funnel was clamped to a retort stand at 5 cm distance from a horizontal surface with a clean white paper. Thirty grams of the starch sample was poured into the funnel with its efflux closed. The efflux was opened and the powders allowed to fall freely under the influence of gravity. The time taken for the entire sample to flow out was recorded. The flow rate was calculated as the ratio of the weight of starch sample to the time of flow. The height and base diameter of the heap of powder was measured and used in calculating the angle of repose employing Equation 2.

$$\theta = tan^{-1} (h/r) \quad \ldots . \quad (2)$$

Where h is the height of the heap of powder and r is the radius of the circular base

True/particle density

Using the solvent displacement method, a 25 ml glass pycnometer (specific gravity bottle) was filled with n-hexane and weighed (a). The bottle was emptied and rinsed with acetone before drying. About 0.5 g (b) of the starch sample was poured into the dried bottle, filled with n-hexane and weighed (c) after cleaning off the excess n-hexane from the bottle. The weights recorded were used to calculate the true density of the starch sample using Equation 3.

$$\rho = \frac{b}{[(a+b)-c]} \times S \quad \ldots . \quad (3)$$

Where ρ is the particle density and S is the specific gravity of n-hexane

Powder bulkiness, packing fraction and porosity

Using the following equations, the bulkiness, packing fraction and porosity of the starch samples were calculated.

$$Powder\ bulkiness = \frac{1}{Bulk\ density} \quad \ldots\ldots . \quad (4)$$

$$Packing\ fraction = \frac{Bulk\ density}{True\ density} \quad \ldots\ldots . \quad (5)$$

$$Powder\ porosity = 1 - \frac{Bulk\ density}{True\ density} \quad \ldots\ldots . \quad (6)$$

Thermal properties

The thermal characteristics of the samples were analyzed using a differential scanning calorimeter (Mettler, Philip Harris Ltd., England). A 3.5 mg quantity of starch sample was weighed into an aluminum pan and 2.45 µL of distilled water was added with a Hammitton micro-syringe to form a suspension. The pan was sealed hermetically, left to equilibrate for 1.0 h at room temperature and the seal pierced before heating at 60 - 300 °C at the rate of 10 °C/min under nitrogen gas at a flow rate of 70 ml/min.

Scanning electron microscopy (SEM)

The starch particle sizes and morphology were evaluated using a scanning electron microscope (JEOL-SEM, Instrument 7500F, Japan). About 2.0 mg of the starch powder sample was used and the sample was examined at ×4000 magnification.

Statistical analysis

Determinations were carried out in triplicate for all experiments and their results reported as mean ± standard deviation. The mean values obtained from the evaluation of the starch powder samples were subjected to student's t-test at 5 % level of significance using GraphPad InStat 3.10.

RESULTS

Results from the proximate analysis of *Cyperus esculentus* tubers showed that the tubers contains 5.97 and 7.15 %w/w of lipids and proteins respectively. The percentage yield of starch after the extraction of oil from the tubers was 15.60%.

Phytochemical, ash and elemental contents

Table **1** shows the phytochemical as well as the ash and elemental contents of the extracted *C. esculentus* starch. The phytochemical tests confirmed the presence of alkaloids, triterpenoid, fixed oils and starch. The elemental constituents analysis showed that the starch contain both monovalent and divalent cations with a predominance of calcium ions and no traces of heavy metals such as chromium, lead and nickel.

Physicochemical properties

Table **2** shows some physical and chemical properties of the native and modified *C. esculentus* starches. The starches were smooth to touch, tasteless, odourless and white to off-white in colour. The percentage yield of the modifications processes had the enzyme hydrolyzed starches giving the highest yield of 87.25%. The native starch tested positive to the iodine test while the modified forms were negative. They were all insoluble in the solvents tested and were slightly acidic in pH. Their pH conformed to pharmacopeial specification of 4.5 - 7.0.

All the starch samples were positive to gel formation and their amylose content ranged from 19.85 - 24.75%, in the order of PCS > ECS > ACS > NCS.

The starch samples moisture content ranged between 3.90 - 11.36% while their loss on drying values ranged between 3.12 - 5.15% with NCS having the highest value. Since starch rarely contains volatiles other than water, these values can be attributed to the presence of moisture in the starch samples. The results of their hydration capacities revealed an order of PCS > ACS > NCS > ECS while their swelling index was in the order PCS > ACS > ECS > NCS.

The gelatinization temperatures of the starches were within 60 - 68 °C with NCS exhibiting the highest temperature while PCS had the lowest. The browning and charring temperatures obtained ranged from 205 - 270 °C and the percentage moisture sorption capacity of the starch samples at different relative humidities was of the order of ECS < ACS < PCS < NCS, showing that NCS had the highest moisture sorption capacity at all relative humidities investigated.

Table 1. Phytochemical, ash and elemental constituents of *C. esculentus* starch.

Constituents	Component	Value
Phytochemicals	Alkaloids	+
	Carbohydrate	+
	Tannins	–
	Phlobatannins	–
	Triterpenoid/steroids	+
	Flavonoids	–
	Saponins	–
	Cardenolides	–
	Fixed oils	+
	Starch	+
	Anthraquinone (Free)	–
	Anthraquinone (Combined)	–
Ash content (%w/w)	Total ash	0.55
	Sulphated ash	0.50
	Acid insoluble ash	0.15
	Water insoluble ash	0.40
Elemental (ppm)	Lead	0.00
	Nickel	0.00
	Chromium	0.00
	Zinc	0.06
	Copper	0.06
	Aluminium	0.00
	Iron	2.69
	Sodium	4.25
	Magnesium	3.49
	Potassium	0.93
	Calcium	185.33

(+): Present, (–): Absent

Table 2. Physicochemical properties of the native and modified starches.

Property		NCS	ACS	PCS	ECS
Modification yield (%w/w)		-	62.00	71.75	87.25
Iodine test		Positive	Negative	Negative	Negative
Solubility	0.1 N HCl	Insoluble	Insoluble	Insoluble	Insoluble
	95 %v/v ethanol	Insoluble	Insoluble	Insoluble	Insoluble
	Distilled water	Insoluble	Insoluble	Insoluble	Insoluble
pH		5.50 ± 0.00	4.90 ± 0.1	5.50 ± 0.2	6.00 ± 0.1
Amylose content (%)		19.85 ± 0.12	21.61 ± 0.21	24.75 ± 0.14	22.19 ± 0.23
Amylopectin content (%)		80.15 ± 0.02	78.39 ± 0.30	75.25 ± 0.35	77.81 ± 0.15
Gel formation		Positive	Positive	Positive	Positive
Moisture content (%)		11.36 ± 0.21	5.16 ± 0.34	4.14 ± 0.27	4.87 ± 0.54
Loss on drying (%)		5.15 ± 0.18	4.52 ± 0.47	3.80 ± 0.19	3.12 ± 0.35
Swelling index		1.44 ± 0.13	2.50 ± 0.11	4.42 ± 0.21	1.59 ± 0.13
Hydration capacity		1.78 ± 0.02	3.18 ± 0.03	7.25 ± 0.02	1.43 ± 0.02
Gelatinization temperature (°C)		68.00 ± 0.04	62.00 ± 0.15	60.00 ± 0.11	62.00 ± 0.32
Browning temperature (°C)		240.0 ± 0.02	211.0 ± 0.35	223.0 ± 0.05	216.0 ± 0.03
Charring temperature (°C)		270.0 ± 0.75	247.0 ± 0.55	266 ± 0.77	249 ± 0.44
Moisture sorption (%)	52% RH	7.60 ± 0.26	5.10 ± 0.35	5.80 ± 0.41	2.30 ± 0.63
	75% RH	10.70 ± 0.44	5.90 ± 0.27	8.30 ± 0.32	2.90 ± 0.36
	84% RH	9.30 ± 0.22	5.40 ± 0.30	8.50 ± 0.33	3.40 ± 0.41
	100% RH	12.80 ± 0.19	7.40 ± 0.36	9.10 ± 0.41	3.90 ± 0.28
Transmittance (%)	0.5 %w/v	2.857 ± 0.002	2.567 ± 0.001	1.800 ± 0.003	2.295 ± 0.001
	1.0 %w/v	2.697 ± 0.002	2.495 ± 0.002	1.867 ± 0.001	2.393 ± 0.003
	1.5 %w/v	2.685 ± 0.003	2.648 ± 0.001	1.956 ± 0.002	2.378 ± 0.001
	2.0 %w/v	2.788 ± 0.001	2.494 ± 0.003	2.097 ± 0.001	2.254 ± 0.003
	2.5 %w/v	2.878 ± 0.001	2.419 ± 0.001	2.120 ± 0.003	2.287 ± 0.001

(Mean ±SD)

NCS: Native starch, ACS: Acid-hydrolyzed starch, PCS: Pre-gelatinized starch and ECS: Enzyme-hydrolyzed starch.

Table 3. Physicotechnical properties of the native and modified starches.

Property	NCS	ACS	PCS	ECS
Bulk density (g/cm^3)	0.37 ± 0.01	0.46 ± 0.01	0.31 ± 0.01	0.29 ± 0.00
Tapped density (g/cm^3)	0.62 ± 0.01	0.58 ± 0.01	0.39 ± 0.01	0.34 ± 0.01
True density (g/cm^3)	1.09 ± 0.06	1.18 ± 0.05	1.48 ± 0.14	1.26 ± 0.13
Flow rate (g/s)	No flow	0.41 ± 0.03	0.63 ± 0.03	1.68 ± 0.03
Angle of repose (°)	No flow	37.10 ± 0.10	38.48 ± 0.67	32.97 ± 0.14
Hausner's quotient (%)	1.67 ± 0.02	1.25 ± 0.03	1.26 ± 0.02	1.18 ± 0.02
Carr's index (%)	40.11 ± 0.75	19.64 ± 1.86	20.34 ± 1.33	15.52 ± 1.40
Bulkiness	2.70 ± 0.03	2.17 ± 0.02	1.56 ± 0.01	3.45 ± 0.04
Packing fraction	0.34 ± 0.04	0.39 ± 0.01	0.21 ± 0.02	0.23 ± 0.03
Porosity (%)	65.53 ± 2.70	60.62 ± 2.66	78.83 ± 4.91	76.77 ± 2.33

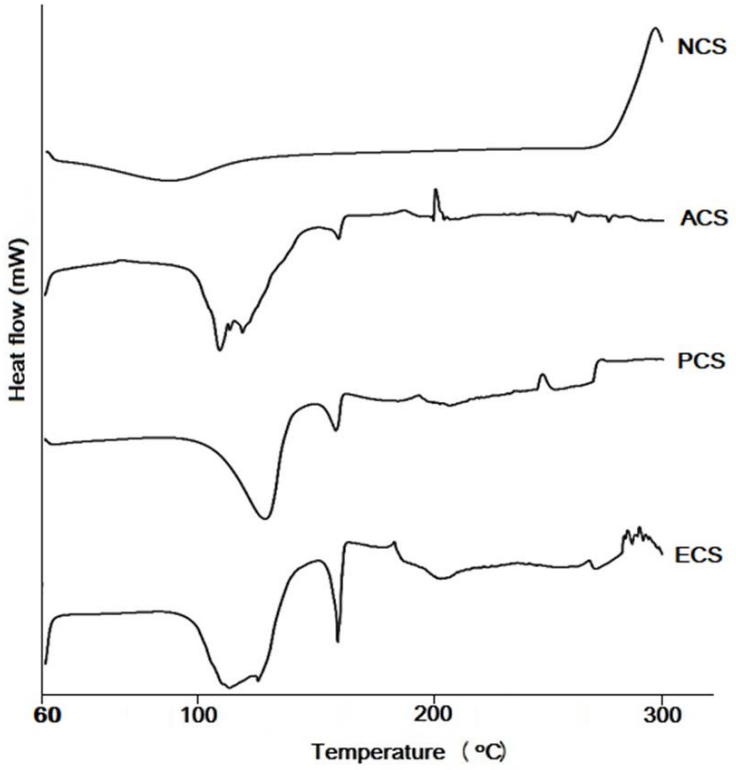

Figure 1. DSC thermograms of the native and modified starch powders.

Physicotechnical properties

The physicotechnical parameters of the native and modified starches are presented in Table **3**. The bulk and tapped densities of the starches are in the order of ECS < ACS < NCS < PCS while their true density follows the order of NCS < ACS < ECS < PCS.

There was a direct relationship between particle packing and their density values, thus the modified starches showed better inter-particulate spacing than the native starch.

The results of the flow rates and angles of repose of the starches indicate that ECS had the best flow (1.68 g/s, 32.97°) of the modified starches while the flow rate and angle of repose of NCS could not be determined due to its high cohesiveness. Also, the Hausner's ratio and Carr's index of ECS were the lowest, corresponding to good flow and the values for NCS were the highest, indicating a poor flow.

Thermal properties

The DSC thermograms of the native and modified starch powders are shown in Figure **1**. The thermogram of the native starch showed a wide endothermic trough at about 90 °C while the modified starches exhibited varying sharp troughs and peaks at different temperatures. The modified starches showed a first and second trough at about 120 and 155 °C with corresponding transition temperatures at 90 and 150 °C, respectively.

These varied troughs and peaks can be attributed to different levels of degradation of the amylopectin glycosidic linkages in the native starch molecule resulting in a molecule with varied crystallinity, hence the lower gelatinization temperatures exhibited by the modified starches.

Scanning electron microscopy (SEM)

The scanning electron micrographs of the native and modified starch powders at ×4000 magnification are shown in Figure **2** while information on their particle morphology are presented in Table **4**. The micrographs showed differences in particle morphology between the native and modified starches. The pre-gelatinized starch had the largest particle size based on its area and volume measurements and this could be the result of agglomeration caused by the pre-gelatinization process.

Table 4. SEM Particle Information of The Native and Modified Starch Powders.

Particle property	NCS	ACS	PCS	ECS
Circle equivalent diameter (µm)	7.24	6.31	6.53	9.84
Major axis (µm)	8.8	8.26	8.82	12.5
Minor axis (µm)	6.01	4.9	4.91	8.03
Circumference (µm)	29.1	28.7	31	47.8
Convex hull (µm)	25.7	23.7	25.6	37.9
Circumscribed circle diameter (µm)	9.97	9.57	10.4	14.5
Area (µm²)	45.3	36.2	36.7	79.5
Volume by area (µm²)	258	194	187	560
Pixel count	10557	8395	8531	18460
Aspect ratio	0.68	0.612	0.567	0.653
Circularity	0.644	0.518	0.493	0.445
Convexity	0.899	0.852	0.854	0.809
Elongation	0.32	0.388	0.433	0.347
Grayscale	134	153	137	137

NCS: Native starch, ACS: Acid-hydrolyzed starch, PCS: Pre-gelatinized starch and ECS: Enzyme-hydrolyzed starch.

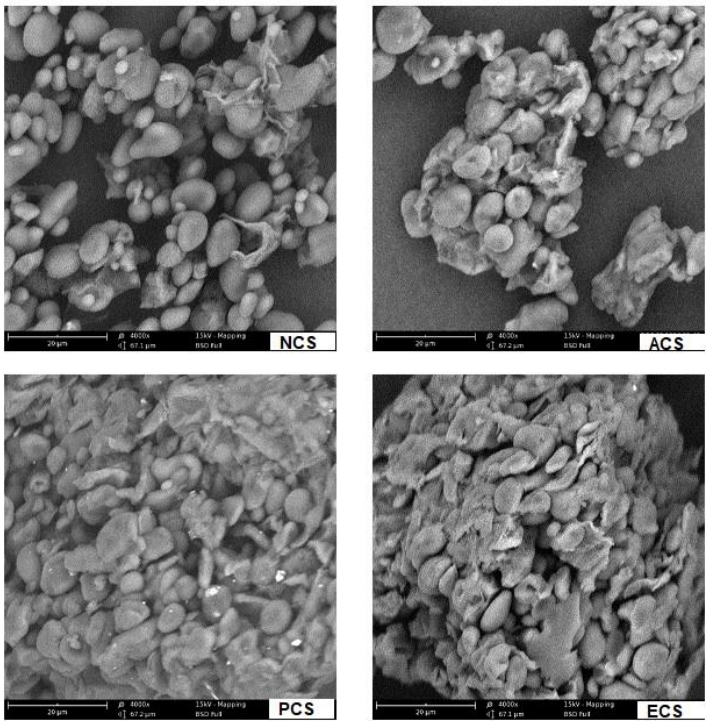

Figure 2. Scanning electron micrographs of the native and modified starch powders.

DISCUSSION

The native and modified starch products of *C. esculentus* tubers were characterized in the study. Preliminary proximate analysis of the tubers used in the study revealed lipids and proteins contents comparable to other studies where their results showed a range of 7.46 - 17.00 and 6.06 - 8.51 %w/w for crude lipids and proteins, respectively [24,25]. These results also show that *C. esculentus* tuber is a good source of proteins and lipids (cold-pressed oil). Protein functions in body development and tissue repair while the oil is ideal for different purposes including cooking, cosmetics, biodiesel, textiles etc [26].

This yield is low when compared to the 21 - 37% obtained from previous researches [27-29]. The low yield may be attributed to initial oil extraction from the tubers, reducing the amounts of extraneous materials accompanying the starch extract.

The phytochemical contents of the extracted starch have also been confirmed by previous studies [28-30]. The low ash content of the starch is an indication of a high level of purity. Ash content represents the non-volatile inorganic components remaining after exposure to a high decomposition temperature. The extracted starch met the British Pharmacopeia specification of ash content value below 0.6% for starch samples [31].

Additionally, the elemental constituents of the starch showed that it is also devoid of heavy metal contamination. There are limits to the amount of permissible contaminants that may be introduced from pharmaceutical raw materials into finished products during processing. The elemental constituents of starch have been reported to influences several of the starch characteristics such as swelling potential or capacity and paste viscosity [32].

Although the exact tolerable limits of possible impurities are not specifically stated in the Pharmacopoeia, it is presumed that unusual contaminants are not to be tolerated. Heavy metals are not desirable in formulated products because they are capable of forming stable covalent complexes with body proteins as a result of their variable valence state. These complexes function as catalysts in inducing auto-oxidative reactions. Hence, the pharmacopoeia places stringent limits on the levels of heavy metals permitted in pharmaceutical materials and products.

Furthermore, the modification processes of the starch yielded reasonably high amounts. The lowest yield obtained from acid hydrolysis could be the result of loss of material during the several washings and straining to achieve neutral pH. Attainment of neutral or close to neutral pH is important in the modified products in order to prevent reactions with pH-sensitive compounds.

The modified starches exhibited an increase in their amylose content though the increase was not significantly ($p > 0.05$) different from that of the native starch. Their solubility and easy access to the amylopectin side chains of the starch molecule makes it most likely to undergo modification. Also, the moisture content values of the starches were less than 15.0%, hence these values were within British Pharmacopeia specification [19]. The moisture content of the native starch was however higher than the modified forms implying that the modified starches can be stored for longer periods as low moisture content signifies high stability on storage, protection from microbial growth and a high yield of dry weight.

This implies that PCS with a hydration capacity of 7.25 can absorb water 7.25 times its own weight, making it a good candidate for use as a disintegrant [33]. There was a direct correlation between the swelling indices and hydration capacities of the starches. This correlation further reinforces the possible disintegrant ability of the modified starches as both parameters favours tablet disintegration.

The percentage moisture sorption capacity of the starches that showed NCS having the highest moisture sorption capacity at all relative humidities implied a high sensitivity of NCS to atmospheric moisture. Modification of the native starch may have influenced its moisture sensitivity because the modified starches displayed significantly reduced sensitivity to moisture. Also, the low transmittance values from the starches' paste clarity may affect the aesthetic appearance of products when the starches are used. The higher paste clarity of the native starch may be attributed to its higher amylopectin content. Previous research has reported that starches with high amylopectin content are easily dispersed and exhibit higher transmittance and clarity [34].

However, the powder properties of the starches indicated an increase in flow among the modified products. This increase in flowability of the modified

starches was significant ($p < 0.05$) with the following order; NCS < PCS < ACS < ECS. The bulkiness, packing fraction and porosity results of the starches showed that the modification process increased the bulkiness and porosity of ECS while only increasing the packing fraction of ACS and porosity of PCS. These parameters have been reported to be influenced by particle sizes and their distribution in the powder bed [35,36], hence it would be expected that the particle sizes and distributions of the modified starches would be affected by the modification process.

Particle information from the SEM studies showed the aspect ratio of the starches to be in the order of NCS > PCS > ACS > ECS while their circularity was of the order of NCS > ACS > ECS > PCS and their convexity took the order of NCS > ECS > ACS > PCS. The enzyme hydrolyzed starch (ECS) was the most elongated of the starches while the native starch (NCS) was the least. As powder flow is greatly influenced by particle size, shape and size distribution, the larger particle sizes of ECS and PCS may have been responsible for their improved flow characteristics. These results confirm that modification of native *C. esculentus* starch significantly affected the morphology of the particles. The variations in the starch particle morphology may be attributed to varied amylose and amylopectin content, which directly exerts control over starch particle shape and size [37,38].

CONCLUSIONS

The modification of the native starch of *C. esculentus* resulted in products with improved functional and physicotechnical properties in terms of swelling, hydration and water sorption capacities as well as flowability. Scanning electron microscopy analysis showed differences in particle morphology between the native and modified starches while thermal analysis revealed a more crystalline modified starch.

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