EFFECT OF ANNEALING, HEAT MOISTURE TREATMENT AND CITRIC ACID MODIFICATIONS ON THE CHEMICAL COMPOSITION, X-RAY DIFFRACTION PATTERN AND SCANNING MORPHOLOGYOF STARCHES FROM COCOYAM (*COLOCASIA ESCULENTA AND XANTHOSOMASAGITTIFOLIUM*)

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ABSTRACT

The effect of annealing, heat moisture treatment and citric acid on major physicochemical properties of cocoyam starch was investigated. This was done in order to establish the optimum processing condition with the aim of enhancing the utilization capacity of the starch. Starch extracted from red and white Cocoyam (Tania) *Xanthosomasagittifolium* and Taro *Colocasiaesculenta*cultivars respectively were subjected to modification using annealing, heat moisture treatment and citric acid methods. The modified and native starch samples were analyzed for chemical composition using standard methods, likewise the x-ray diffraction pattern and starch granules morphology of the samples were assessed. The result showed that amylose content ranged from 24.81 - 38.16%, protein 0.00 – 0.77%, ash 1.19 – 3.16 and carbohydrate 84.19 – 86.79%. The X-ray diffraction patterns of red and white cocoyam starches showed a strong peak at 15^{o}, 18^{o} and 23^{o} (2ø) indicating A typestarch. *Colocasiaesculenta*starch also displayedA typediffraction pattern with additional peak at 24^{o}. The modification methods used did not change the diffraction pattern of the starch samples but only influenced the intensity of the diffraction peak. The scanning electron micrographs showed that the starch granules were small to medium in size with most having irregular shape and smooth surface. Annealing and citric acid caused little noticeable fractures on some granules without compromising starch granules integrity. Heat moisture treatment however resulted in evident loss of starch granules integrity. The modification methods affected the physicochemical properties of cocoyam starch samples in a varying pattern which may qualify the starch samples for use in different food and non-food applications.

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***Keywords**:* Cocoyam starch, modification, morphology, chemical composition, x-ray diffraction pattern.

1.0 INTRODUCTION

Central Asia or Southeast is the origin of cocoyam, one of the world's six most significant root and tuber crops worldwide (FAO, 2012). Taro, ancient cocoyam, eddoe, arrowroot, macabo, and dasheen are all names for Cocoyams. Local farmers in Africa, Asia and Latin America produce bulk of the crop. Although it is a staple food crop in many places, cocoyam has been understudied and under-exploited despite its widespread use as a major calorie source (Mpotokwane*et al*., 2008). The edible roots of cocoyam are the primary reasons for its cultivation. The corm of the plant that gives rise to *Colocasia* is cultivated for human

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consumption. It is possible to manufacture flour out of dried corms or chips by slicing them and drying them. The expansion of crop regions and the introduction of new varieties in Nigeria have been spurred by the country's current economic difficulty and food insecurity scenario. When it comes to the root and tuber crops cultivated in Nigeria, cocoyam ranks third after cassava and yam (Okoye*et al*., 2008).

Grown mostly in the southeastern and southwestern regions of Nigeria, cocoyam (*Colocasiasp*and *Xanthosomasp*) are vital members of the roots and tubers family, (Onyenweaku*et al*., 2005). Poor farmers, most especially women, cultivate it in a variety of ways for food security, often in conjunction with other staples including sorghum, corn, plantain, bananas and vegetables. Due to the low levels of starch and high levels of digestible protein in cocoyam, it is one of the best therapeutic crop plants for controlling high blood sugar in people who have been diagnosed with the disease condition (Arene and Ene, 2007).

Starch has remained a major raw material in food and non-food industries where it is usually used as thickener or binder (Ashogbon and Akintayo, 2014).Mostly in the food industries, cassava is a major indigenous root crop and source of starch. However, the high demand for cassava products including its starch has created a wide gap between the demand and supply channel, hence the need to source for alternative root crop that can provide good quality starch for industrial use becomes imperative. Cocoyam is one of such crops that could be adopted for this purpose. However, cocoyam starch like many others from different root and tubers needs to be modified before it can be applicable in industries. Modification has been used as a way to reduce the deficiencies of native starch by introducing some specific functional properties lagging in them. Several methods are available for modification of starch; however, attention is gradually being focused on methods which do not involve the use of chemicals (Yousif*et al*., 2012). Such methods have been reported to be less expensive and with little or no hazards on human health. There are few reports on the effect of modification on the properties of cocoyam starch of different varieties. The focus of this study is to modify starch extracted from three cocoyam types using simple and safe modification methods. This study is expected to provide basic scientific information on potentials of modified starch in food applications.Thus the objective of the research was to investigate theeffects of annealing, acid hydrolysis and citric

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acid modifications onthe physical, functional, and pasting properties of starches of threecocoyam cultivars.

2.0 MATERIALS AND METHODS

2.1 Sources of Materials

Matured freshly harvested white and red cocoyams (*Xanthosomasagittifolium)* and Coco-ghana (*Colocasiaesculenta)* cultivars were procured from the inIkole-Ekiti, Ekiti State, Nigeria. The equipment and reagents used were obtained from the laboratories in Food Technology Department, The Federal Polytechnic, Ado-Ekiti.

2.2 Methodology

2.2.1 Production of cocoyam starch

Fig. 1 depicted the processes required in making cocoyam starch.Lingering dirts were separated from the newly harvested cocoyam tubers and washed clean with potable water.After the cocoyam tubers were cleansed, they were peeled by hand using a stainless steel knife. The corms were then washed in clean water before being wet-milled. The cocoyam slurry was diluted with water (by a factor of 4:1), sieved through muslin fabric and left to rest for 5 hours while the starch solution was prepared. Two or three cycles of re-suspending in water, allowing the sediment to settle and then decanting to remove the white starch and left behind a transparent supernatant. After being sun-dried for three days, the cocoyam starch that had been extracted was milled using a Hammer mill machine and then stored in a plastic bag for further analysis.

2.3 Modification of starch

Each starch samples was subjected to modification using heat moisture treatment, annealing and citric acid methods.

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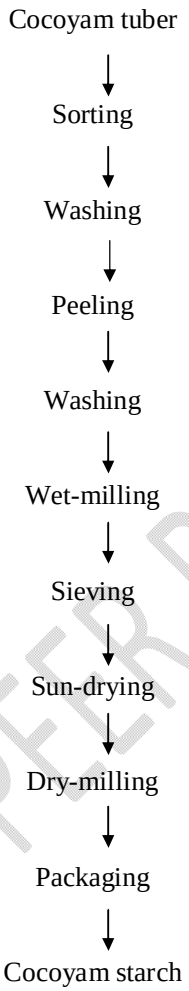

Figure 1: Flowchart for the production of cocoyam starch.

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2.3.1 Heat Moisture Treatment.

The method of Nadir *et al*., (2015) for heat-moist treatment was followed Starches extracted from red and white cocoyams *Xanthosomasagittifolium* and (*Colocasiaesculenta*) cultivars had their moisture levels brought up from 12.17 percent, 11.94 percent, and 11.73 percent, respectively, to 33 percent using distilled water. After 2 hours of mixing, the starch samples were considered to be of similar concentration. After that, the samples spent an hour in a hot air oven set to 120^0C. After being cooled, air dried, processed in a hammer mill, filtrated through a 0.5mm sieve, it was then packaged in a polyethylene bag, the samples were ready for analysis.

2.3.2 Annealing

Suspension of each starch's sample was made by combining the starch and distilled water (1:2 w/v) in a beaker, covering the beaker with aluminum foil and incubating it at 50^0C in a water bath for 16 hours. The incubated starch sample was dried at 40°C overnight, ground in a hammer mill, sieved through a 0.5mmmesh and stored in a polyethylene bag (Falade and Ayetigbo, 2015).

2.3.3 Citric Acid

The starch sample was suspended in distilled water (at a 3:4 w/v ratio) and the pH was adjusted with around 10 cc of 1M NaOH. After adding the alkali, the mixture was left alone for 30 minutes while being stirred by hand. Additions of citric acid (15%) and 1% hydrogen peroxide (by weight of dried starch) were made to complete 100ml with distilled water. After 5 hours at 27°C, it was washed with distilled water, filtered, dried at 50°C in a hot air oven for 24 hours, milled in a hammer mill, sieved through a 0.5mmmesh, and packaged in a plastic bag (Falade and Ayetigbo, 2015).

2.4 Analysis of Proximate Composition

2.4.1 Moisture Determination

A sample of 3g was obtained and dried to constant weight at 105°C for 14 hours in a porcelain crucible that had previously been weighed. The percentage of moisture in the sample was determined by how much it weighed before and after drying (AOAC, 2005).

$$\% \text{ moisture} = \frac{Weight\ loss\ (g)}{Sample\ Weight} \times 100$$

2.4.2 Total Ash Evaluations:

The ash content was assayed (determined) by the weight loss observed or that occurred when the extract (sample) was burned at a high temperature to heat all the organic matter without allowing

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appreciable decomposition of the ash constituents. Incineration is done in a muffle furnace set at a temperature of 550 °C for 6 hours (AOAC, 2005).

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2.4.3 Determination of Fat

The procedure followed the guidelines established by the AOAC (2005). I weighed 5 g of the oven-dried sample and added it to the dry, sterile flour (W1), then rolled the mixture (W2). Petroleum ether (40-60°C) is poured into a round-bottomed jar until it reaches about 3/4 full. The sample is encased in a cartridge and heated in a Soxhlet apparatus with petroleum ether serving as an indicator for 6 hours; the condenser is set up as a Soxhlet and the reflux condenser is used to control the heat so that the solvent boils slowly. Once the extraction cylinder and condenser have been removed, the cartridge can be heated to 100 degrees Celsius for one hour, dried off in a desiccator, and reweighed (W3).

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$$\text{Fat (\%)} = \frac{w2-w1}{w2-w1} \times 100$$

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2.4.4 Crude Fibre

Reduced to about 2 grams in petroleum ether over the course of two hours. Several minutes are spent boiling it at reflux in a solution of 200 milliliters containing 1.25 grams of H_2SO_4 per 100 milliliters of solution. The acidity of the solution is neutralized by repeatedly filtering it through a cloth in a sieve and washing it with boiling water. The remaining residue is poured into a beaker and heated in a solution containing 1.25 g of NaOH per 100 ml for another 30 minutes. In order to remove any lingering odors, the last residue is washed and scrubbed under hot running water many times. After two washes in methanol, the residue was placed in a weighted furnace and heated to 105 degrees Celsius. The oven is heated up to 550 degrees Celsius and kept there for 2 hours. The angle, measured from a smooth, clean surface, is set at 1o (AOAC, 2005).

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The crude fibre is calculated as

$$\text{CF (\%)} = \frac{1a-1o}{weight\ of\ sample\ taken} \times 100$$

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Where; 1a = weight of empty crucible;

1o = weight of crucible and its content after incineration

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2.4.5 Crude Protein

A micro Kjeldahl digestion vial containing 1 gram of the sample and a selenium-enhancing pill were used to analyze the substance. A transparent solution is achieved by heating the mixture with electricity. After cooling the plate, transfer the solution to the distillation apparatus and add 5% boric acid to a 100 mL conical flask (temperature receiver) with 4% water and 1% methyl red indicator. An alkaline solution is reached by constantly adding a 50% NaOH solution to the sample solution until cloudiness appears. Boric acid is used for the distillation process, and the condenser is a transfer tube set at acid level. If the pinkish-blue solution persists throughout the distillation process, then ammonia is present. This process is repeated until there is roughly 50 ml left in the flask.

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2.4.6 Determination of Carbohydrate

Protein, lipid, ash, moisture, and fibre contents were subtracted from 100 using the AOAC, (2005) technique to determine the remaining value.

Calculation: Carbohydrate % = 100 – value of moisture, protein, lipid, ash and fibre.

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2.5 Determination of Amylose and Amylopectin Contents

Using the iodine binding method established by Okeet *al.*,(2013). The amylose contents of both natural and artificially altered cocoyam starchwere determined. In a 100 ml conical flask, we combined 100 mg of sample with 1 mL of 95% ethanol and 9 mL of IN NaOH. The flask was covered with foil paper and boiled in a water bath for 10 minutes. The mixture was heated, then cooled, and 100 mL of distilled water was added to get it to the desired volume. One milliliter (1 mL) of 1N acetic acid, two milliliters (2 mL) of 0.2% iodine solution (2% potassium iodide solution was used as solvent to prepare 0.2% iodine solution), and enough distilled water to bring the volume to 100 milliliters (mL) were added to the 5 mL aliquot in a separate 100 mL conical flask containing the original mixture. The absorbance at 620 nm was determined using a Shimadzu UV-120-01 spectrophotometer (Shimadzu Corporation, Japan). A blank made according to the instructions, but without the sample, was used to calibrate the spectrophotometer. The following formula was used to calculate the amylose content:

Amylose content (%) = (3.06) (A) (20);

Where A = Absorbance value

Amylopectin content was obtained by difference i.e. 100 – % amylose content

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2.6 X-ray Diffraction Measurement

Samples of both unaltered and altered cocoyam starch were X-ray diffracted using a copper Kα, 2λ λ = 1.540 µm and 1.544 Å; 40 kV; 35 mA) on a Siemens D5000 X-ray Powder Diffractometer (20_ Geometry, Madison, USA). The diffractograms were captured using a diffraction angle(2θ) of 1.5-600, a step size of 0.05, and a count time of 3 s. The crystalline nature of the starch samples was determined by analyzing the generated X-ray patterns and utilizing the instrument's software to identify peak positions.

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2.7 Scanning Electron Microscopy

A Quanta 200 environmental scanning electron microscope was used to capture images of the granular morphologies of both native and modified cocoyam starch samples (FEI Company, Hillsboro, OR, USA). Double-sided tape was used to evenly space the samples on the SEM specimen stubs. SEM micrographs acquired at 4000x magnification were used to examine the micrographs obtained at an accelerating potential of 15 kV in a low vacuum, which revealed interesting morphological traits.

3.0 RESULTS AND DISCUSSION

3.1 Chemical Composition of Modified Cocoyam Starch

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Table 1 shows the results of the chemical composition of the treated cocoyam starches. Starch samples differ significantly ($P<0.05$) in terms of moisture content, an indicator of maintaining quality. Starch had a moisture percentage that varied from 10.74% to 12.17%. Low-moisture flours (those having a moisture content of 14% or less) are more resistant to spoilage during storage (Hayma, 2013). As a result, the moisture levels in the flour samples are well within the parameters for safe, long-term storage and subsequent processing. In terms of ash content, values ranged from 1.19 to 3.16%, with RCTR (red cocoyam treated with citric acid) having a substantially ($P<0.05$) greater value at 3.16% than the rest of the samples, and GNAT (Ghana native) having the lowest value at 1.19%. It has been found that oxidation and reduction reactions in minerals cause damage to food products with high ash concentrations (Sri-Wahyuni*et al.*, 2017). It is possible that the treatment's lack of effect on the cocoyam starch's ash level is due to the fact that different cocoyam kinds were employed.

The protein contents of modified starch showed high protein content than the native starch. The protein contents ranged between 0.00 - 0.77% and showed significant different among the samples.

red native starch showed no protein content while GANN (Ghana annealing treatment) had the highest protein content of 0.77%, followed by GNAT (Ghana native) of 0.64%. The treatment of starch with annealing treatment increased the protein content of cocoyam. The result also revealed that Ghana varieties contain more protein than other varieties of cocoyam. Since amino acids account for roughly 60% of total nitrogen, Tewe and Lutaladio (2014) speculate that protein content may rise following analysis as a result of these compounds in the starch samples. The crude fibre measures the cellulose, hemicelluloses and lignin content of the starch. Annealing treatment increases the crude fibre contents of the samples significantly ($P<0.05$). The value ranged from 0.09 - 0.98%. GANN showed the highest value of crude fibre of 0.98%, followed by WANN of 0.96% than other samples. There was no trace of fat contents among the samples except GCTR (Ghana citric treatment) which had 0.032%, this indicates that cocoyam is not a fatty food product.

The range of 84.19–86.79 percent reported for the carbohydrate content of *P. esculentus* starch by Temple *et al.*, 2011 was also found in Cocoyam starch. Carbohydrate content in starch can be altered by annealing, while carbohydrate content can be altered by heat and moisture treatment. The values of the samples were significantly different from one another ($P < 0.05$). The samples had amylopectin concentrations that went from 24.81 to 38.16. The amylose content of the starch was decreased by heat moisture treatment and citric acid, although the Annealed starch had the highest value of all the treated starches. The pH of the samples ranged from 3.87 to 8.42. It showed that all samples were acidic in nature except WANN (White annealing treatment) and RANN (Red annealing treatment) that were alkaline and this could be as a result of annealing treatment given to the sample.

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Table 1: Chemical Composition of Modified Starches (%)

	Moisture	Ash	Fat	Fibre	Protein	Carbohydrate	Amylose	pH
RNAT	11.94±0.01^{c}	1.91±0.00^{h}	ND	0.10±0.00^{h}	0.00±0.00^{j}	86.04±0.08^{c}	24.81±1.4^{i}	4.17
RANN	11.64±0.01^{e}	2.14±0.01^{g}	ND	0.50±0.01^{c}	0.53±0.01^{c}	85.48±0.02^{g}	26.91±0.08^{h}	8.10
RHMT	10.74±0.01^{k}	2.26±0.00^{f}	ND	0.09±0.00^{i}	0.12±0.00^{i}	86.79±0.31^{a}	38.16±0.18^{a}	6.93
RCTR	11.32±0.01^{g}	3.16±0.01^{a}	ND	0.09±0.00^{i}	0.17±0.02^{h}	85.26±0.01^{f}	35.04±0.02^{c}	4.07
WANT	12.17±0.01^{a}	1.80±0.01^{i}	ND	0.21±0.00^{d}	0.15±0.01^{h}	85.69±0.01^{d}	33.40±0.12^{e}	4.05
WANN	11.29±0.01^{h}	1.75±0.00^{j}	ND	0.96±0.01^{b}	0.57±0.01^{c}	85.43±0.01^{e}	35.95±0.63^{c}	8.42
WHMT	11.13±0.01^{i}	2.31±0.01^{e}	ND	0.12±0.01^{g}	0.34±0.02^{f}	86.08±0.01^{c}	28.77±0.14^{g}	4.79
WCTR	11.40±0.01^{f}	2.77±0.00^{c}	ND	0.11±0.01^{h}	0.25±0.01^{g}	85.46±0.01^{e}	29.82±0.06^{f}	3.97
GNAT	11.73±0.01^{d}	1.19±0.01^{k}	ND	0.13±0.01^{f}	0.64±0.02^{b}	86.30±0.02^{b}	34.37±0.58^{d}	4.38
GANN	12.15±0.01^{a}	1.91±0.07^{h}	ND	0.98±0.01^{a}	0.77±0.01^{a}	84.19±0.07^{i}	37.82±0.12^{b}	4.50
GHMT	12.06±0.01^{b}	2.54±0.01^{d}	ND	0.20±0.00^{e}	0.13±0.01^{i}	85.06±0.00^{h}	35.64±0.00^{c}	4.91
GCTR	10.98±0.01^{j}	2.98±0.03^{b}	0.032±0.01^{a}	0.13±0.00^{f}	0.43±0.00^{d}	85.45±0.04^{e}	28.67 ±0.58^{g}	3.87

Data was presented as mean ± standard deviation (S.D). Mean with different superscript along the same column are significantly different (P<0.05)

RNAT: Red native
RANN: Red annealing treatment
RHMT: Red heat moisture treatment
WNAT: White native
WANN: white annealing treatment
WHMT: White heat moisture treatment
GNAT: Ghana native
GANN: Ghana native
GHMT: Ghana heat moisture treatment

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RCTR: Red citric treatment WCTR: White citric treatment GCTR: Ghana citric treatment

ND:-Not Detected

3.2 X-Ray Diffraction Pattern of Modified Cocoyam Starches

The X-ray diffractograms are shown in appendix I to XII. The analysis of the starch samples was carried out to investigate the difference in the X-ray diffraction pattern and crystallinity of starches extracted from the three cocoyam varieties and also to examine how the different modification methods affected the X-ray pattern. The X-ray diffraction pattern has a link with the functional properties of starch. The native starches of the three varieties of cocoyam used in this study exhibited relatively the same X-ray diffraction pattern; however, there was difference in the intensity of the diffraction peaks. Native white and red cocoyam starches present strong peaks at 15^{o}, 18^{o} and 23^{o} (2θ) while the native starch of Ghana cocoyam, apart from peak at 15^{o}, 18^{o} and 23^{o} (2θ), also showed an additional small peak at 24^{o} (2θ). Oladebeye*et al.*, (2013) reported similar peaks at 14.95^{o}, 16.95^{o} and 22.10^{o} and 15.25^{o}, 17.30^{o} and 17.40^{o} (2θ) for native white and red cocoyam starches respectively. A-type starches has been reported to exhibit strong diffraction peaks at 15^{o}, 17^{o}, 18^{o} and 23^{o} (2θ) while B type starches present diffraction peaks at 15^{o}, 17^{o}, 20^{o}, 22^{o} and 24^{o} (2θ) with a characteristic peak at 5.6 ^{o} (2θ) which usually differentiate it from A type starches. C-type starch is a mixture of both A- and B-type crystalline structures (Pinto *et al.*, 2015; Cai*et al.*, 2014). With the absence of the typical peak of B-type diffraction pattern at 5.6 ^{o} (2θ), the X-ray diffraction pattern of the three native starch samples could therefore be classified as A- type diffraction pattern. Himeda*et al.*, (2012) reported similar A-type X-ray diffraction pattern for taro. A type diffraction pattern of the samples suggest that the amylopectin fraction of the starch samples are packed in a more compact structure and have shorter chain. The different modification methods used did not caused any major change in the diffraction pattern. All modified sample have similar X-ray diffraction pattern with their respective native samples, however, the modification affected the intensity of the peak. For white cocoyam and Ghana cocoyam starches, HMT samples showed higher intensities than native samples; when annealing and citric acid treatment were applied the intensities reduced. All the modified red cocoyam starch samples showed higher peak intensities.

The difference in peak intensities may be linked to the extent to which each modification affected the crystalline region of starch granules. The reduction in peak intensities may indicate a loss of the crystalline array probably due to breaking of hydrogen bonds leading to rearrangement which may not be in perfect parallel array (Klein *et al.*, 2013). On the other hand the increase in peak intensities could be attributed to promotion of displacement of double

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helices between starch crystals by moisture and thermal energy leading to structural rearrangement and a more packaged and ordered crystalline array (Hoover, 2010; Klein *et al.*, 2013; Pinto *et al.*, 2015a).

3.3 Scanning Electron Micrographs of Modified Cocoyam Starches

The scanning electron micrographs of the starch samples are presented in fig 1. The micrographs of native white and red cocoyam starches showed small to medium size granules, most of which have irregular shape with smooth surface and few were spherical in shape. The starch granules of Ghana cocoyam were majorly smaller is size with irregular shape. With citric acid treatment of red cocoyam starch there was only little fractures on the surface of few granules; granules of annealed starch samples showed noticeable fractures on some granules however the integrity of the granules was not compromised. Heat moisture treated granules exhibited noticeable fractures on most granules with evident loss of integrity and aggregation of starch granules due to deformation, fracturing and collapse of some granules. Similar aggregation of pinhao starch granules after heat moisture treatment was reported by Pinto *et al.*, (2015b). Scanning electron micrographs of modified white cocoyam starch samples were similar to that of modified red cocoyam starch however with reduced severity. In heat moisture treated white cocoyam starch aggregation of starch granules was barely noticed, this may suggest that starch granules of white cocoyam starches have higher structural integrity than those of red cocoyam starch. This observation is in agreement with the report of Arinola, (2019) that white cocoyam starch granules were able to maintain their structural integrity during heating when compared with starch granules of red cocoyam starch. For Ghana cocoyam starch granules the extent of fracture on the granules surface increased from citric acid treated sample to heat moisture treated sample and then to annealed sample. In annealed sample aggregation of some granules was noticed. The effect of these modification methods on the structure of cocoyam starch granules is manifested in the different physicochemical properties displayed by the various modified starch samples.

Comment [User77]: Fig. 1 is a flowchart, not the micrographs

Suggestion:
Micrographs of similar samples can be grouped as a figure e.g. RNAT, RANN, RHMT and RCTR (Fig. 2), etc

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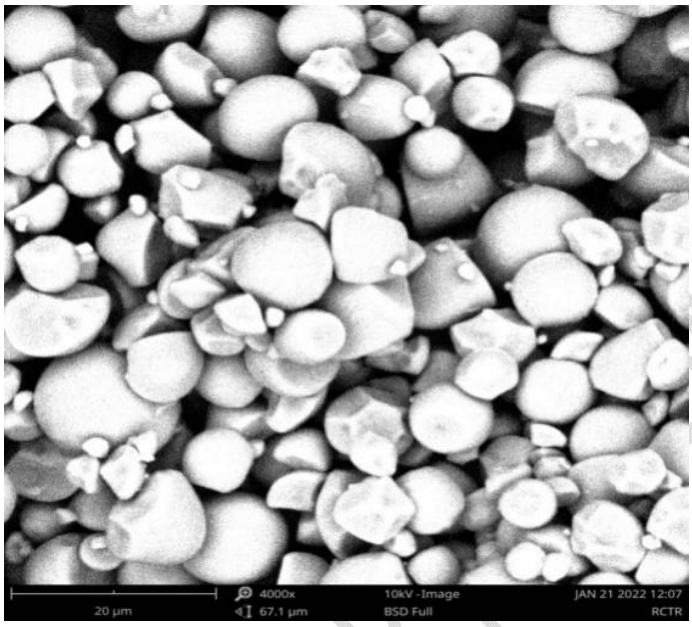
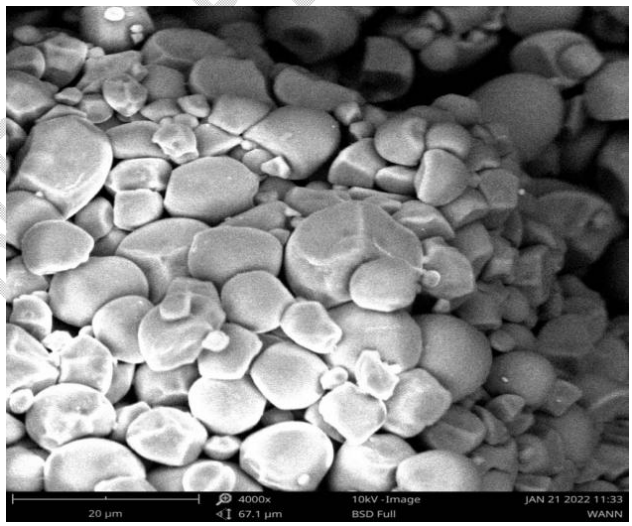
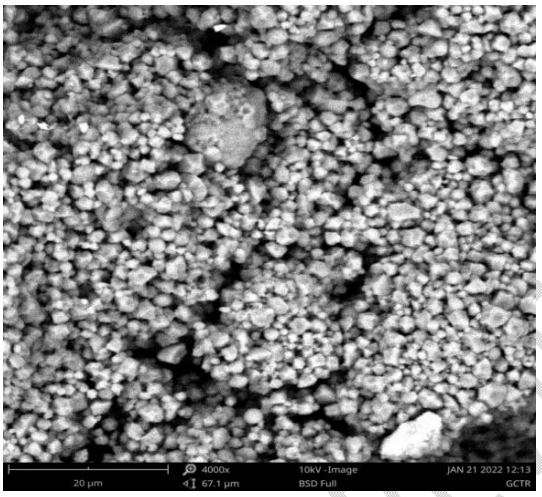


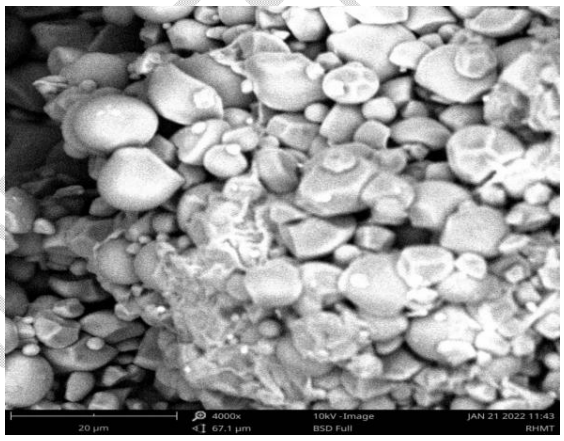
RCTR



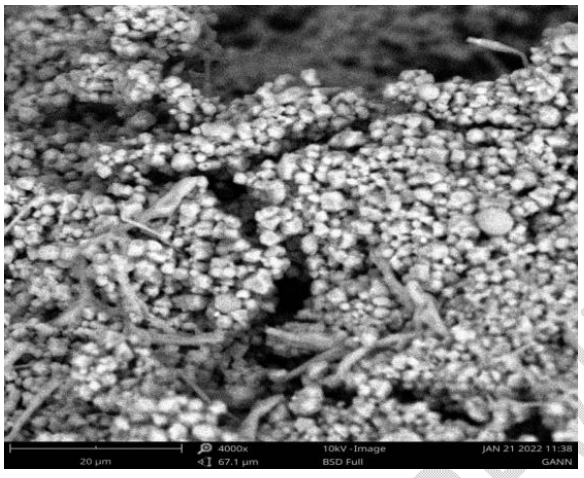
RNAT



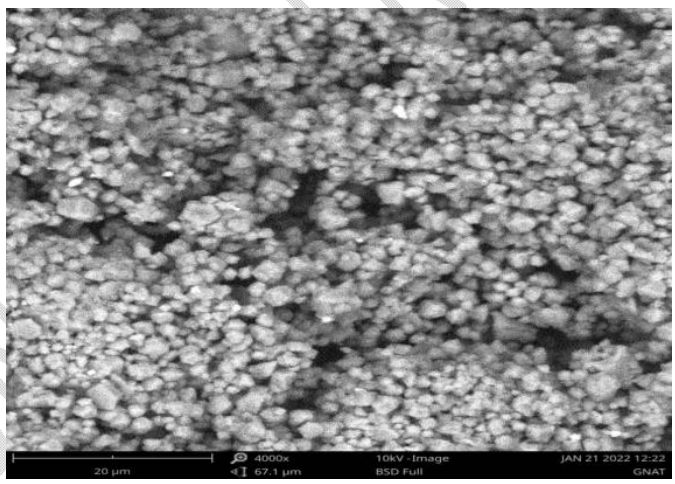
GCTR



RHMT



GANN



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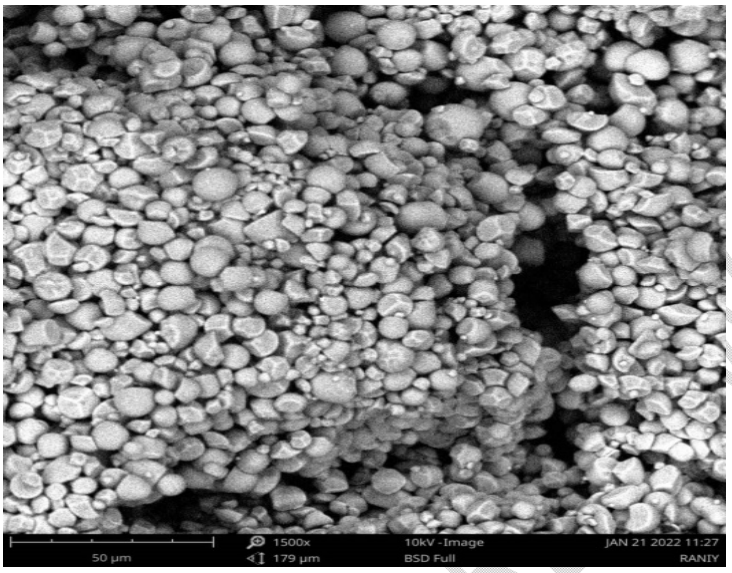

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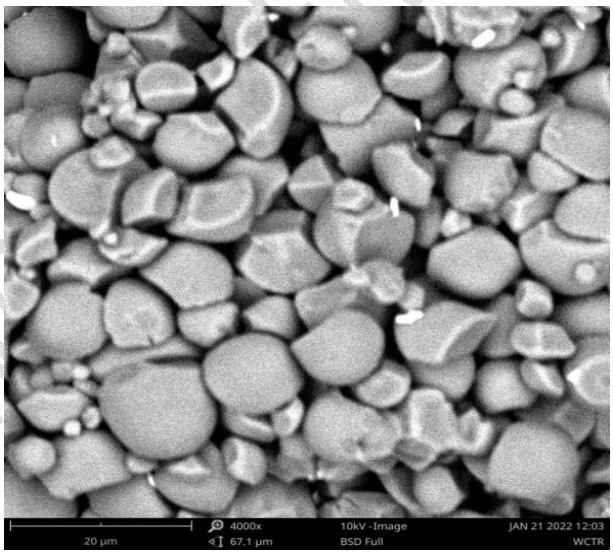
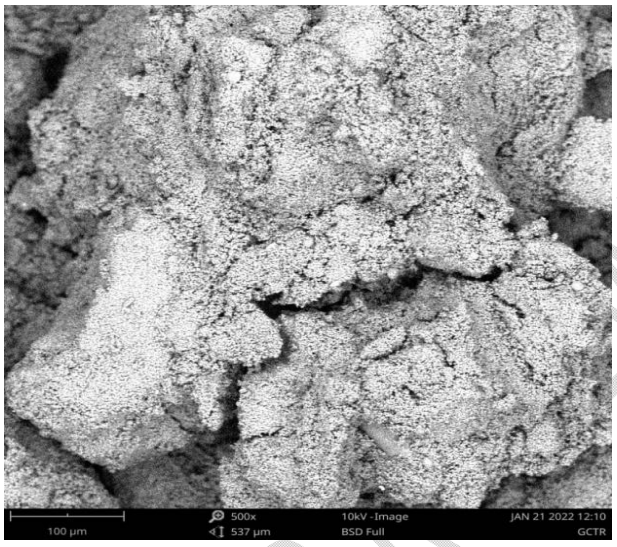


WCTR



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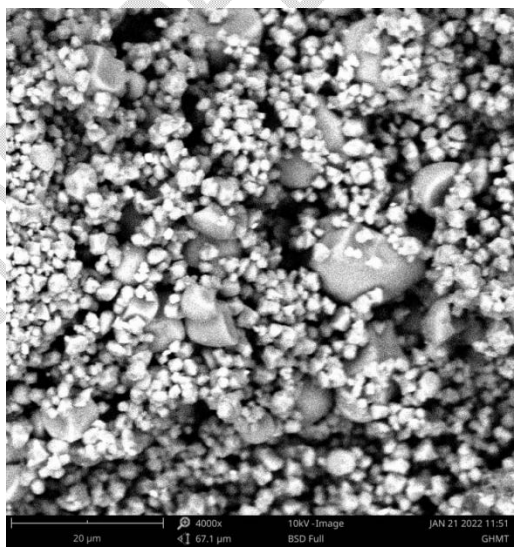
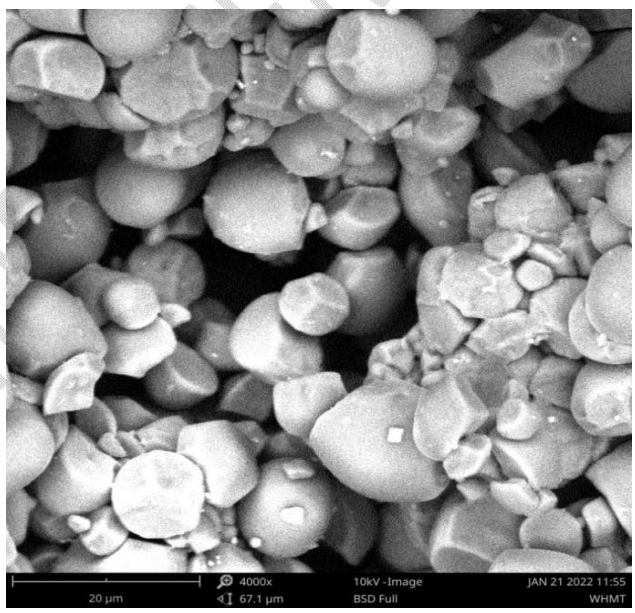


GHMT

WNAT



WHMT

4.0 Conclusion

The results of the present research have shown that natural and artificially modified starches can be used in a wide variety of food and non-food products based on the nutritional composition of cocoyam starch, which may be useful in the preparation of a variety of dishes. Starch granule integrity was most drastically altered by heat and moisture treatment, as seen by changes in x-ray diffraction pattern and a few physicochemical parameters. The modified cocoyam starch samples disclosed here represent a potential new supply of starch for the food and non-food industries.

Comment [User78]: Kindly reconstruct

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Kindly correct it accordingly

Page 5: [2] Comment [User37] **User** **5/13/2023 9:58:00 PM**

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Kindly indicate the unit of sample weight

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