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Effect of Modifications on the Chemical Composition, X-Ray Diffraction Pattern and Scanning Morphology of Starches from Cocoyam (*Colocasia esculenta* and *Xanthosoma sagittifolium)*

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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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) *Xanthosoma sagittifolium* and Taro *Colocasia esculenta* 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

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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-type starch. *Colocasia esculenta* starch also displayed A-type diffraction 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.

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

1. INTRODUCTION

Central Asia or Southeast is the origin of cocoyam, one of the world's six most significant root and tuber crops worldwide [1]. 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 [2]. 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 consumption. 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 [3].

Grown mostly in the southeastern and southwestern regions of Nigeria, cocoyam (*Colocasia sp* and *Xanthosoma sp*) are vital members of the roots and tubers family [4]. 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 [5].

Starch has remained a major raw material in food and non-food industries where it is usually used as thickener or binder [6]. 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 desirable functional properties absent in the native starch. Several methods are available for modification of starch; however, attention is gradually being focused on methods which do not involve the use of chemicals [7]. Such methods have been reported to be less expensive and with little or no hazards on human health. Annealing and heat moisture treatment are two hydrothermal physical methods; annealing involves heat treatment of native starch with moisture content above 35% while heat moisture treatment involves same treatment at moisture content below 35%. Citric acid modification on the other hand involves the use of citric acid, an organic less hazardous acid, in the modification of native starch. 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 the effects of annealing, acid hydrolysis and citric acid modifications on the physical, functional, and pasting properties of starches of three cocoyam cultivars.

2. MATERIALS AND METHODS

2.1 Sources of Materials

Matured freshly harvested white and red cocoyams (*Xanthosoma sagittifolium)* and Coco-ghana (*Colocasia esculenta)* cultivars were procured from the in Ikole-Ekiti, Ekiti State, Nigeria. The reagents used were of analytical grade obtained from the laboratories in Food Technology Department, The Federal Polytechnic, Ado-Ekiti.

2.2 Methodology

2.2.1 Extraction of cocoyam starch

Freshly harvested cocoyam corms were washed, peeled, washed and wet milled. The resulting slurry was diluted with water (1:4 respectively), sieved through muslin cloth and the filtrate was allowed to stand for 5 hours. The starch sediment was washed thrice with water, sundried, milled using hammer mill and then packaged in high density polyethylene 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.

2.3.1 Heat moisture treatment

The method of Nadir et al. [8] for heat-moist treatment was followed. The moisture contents of starches extracted from red and white cocoyams (*Xanthosoma sagittifolium)* and *Colocasia esculenta* cultivars were adjusted to 33 % by adding distilled water. After this the samples were heated in hot air oven at 120^{o}C for one hour, cooled, air dried, milled in a hammer mill, sieved through a 0.5 mm aperture and packaged in high density polyethylene bag.

2.3.2 Annealing

Suspension of each starch 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^{o}C 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 0.5 mm mesh and stored in a polyethylene bag [9].

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 mL 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 100 mL 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 0.5 mm mesh, and packaged in a plastic bag [9].

2.4 Analysis of Proximate Composition

Proximate composition of the starch samples was determined according to AOAC methods [10], carbohydrate was determined by difference.

2.5 Determination of Amylose and Amylopectin Contents

Amylose content of the samples was determined using the iodine binding method established by Oke et al. [11]. The amylose contents of both native and modified cocoyam starch were determined. 100 mg of sample was mixed 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.

2.6 X-ray Diffraction Measurement

The X-ray diffraction studies were carried out using a Siemens D5000 X-ray Powder Diffractometer (20_ Geometry, Madison, USA). The cocoyam starch samples were equilibrated with distilled water in a dessicator for 48 h before attempts to improve the resolution of the X-ray

diffractogram pattern. The fine samples were filled into a sample holder and packed as densely as possible. The finished surface was smoothed and flushed. The samples were mounted into a X-ray diffractometer and copper Ka, 2k (k = 1.540 lm and 1.544 Å; 40 kV; 35 mA) was generated to determine the X-ray pattern. The scan was acquired at a diffraction angle (2h) of 1.5–600 at 0.05 step size with a count time of 3 s. From the resulting X-ray patterns, peak positions were identified using the instrument's software, and these peak positions were used to determine the crystalline nature of the starch samples.

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. RESULTS AND DISCUSSION

3.1 Chemical Composition

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. The moisture contents of starch samples 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 [12]. 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 [13] speculated 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 % reported for the carbohydrate content of *P. esculentus* starch by Temple et al., 2011 was also found in Cocoyam starch. The values of carbohydrate content of the samples were significantly different from one another ($P < 0.05$). The range of amylose was 24.81 to 38.16%. The amylose content of the starch was reduced 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.

3.2 X-Ray Diffraction Pattern of Modified Cocoyam Starches

The X-ray diffractograms are shown in Figs. 1 to 12. 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*.,* [14] 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 [15,16]. 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. [17] 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 [18]. On the other hand the increase in peak intensities could be attributed to promotion of displacement of double helices between starch crystals by moisture and thermal energy leading to structural rearrangement and a more packaged and ordered crystalline array [19, 18,15].

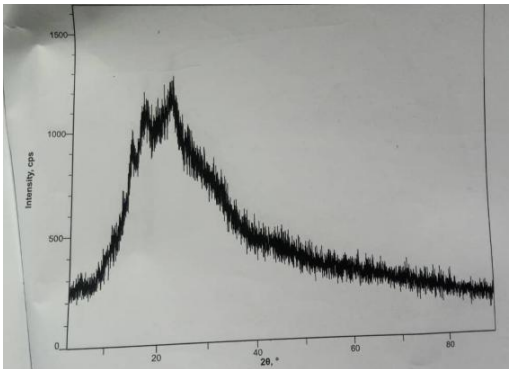

Fig. 1. RNAT

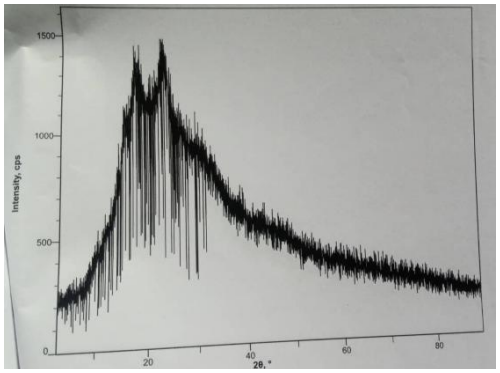

Fig. 2. RCTR

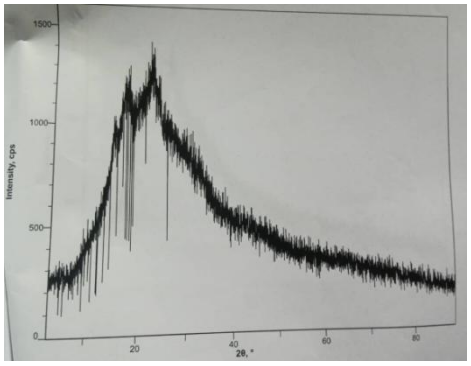

Fig. 3. RANN

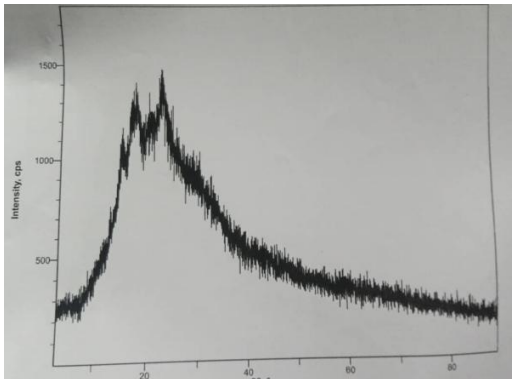

Fig. 4. RHMT

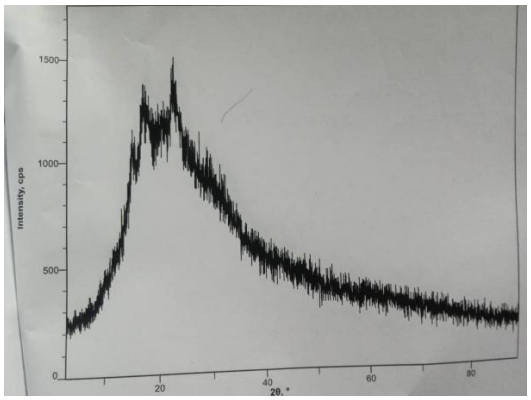

Fig. 5. WNAT

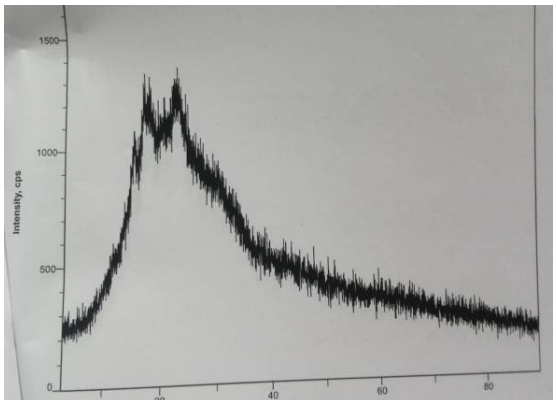

Fig. 6. WCTR

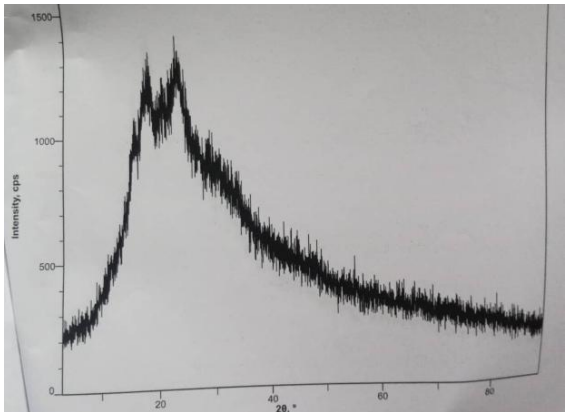

Fig. 7. WANN

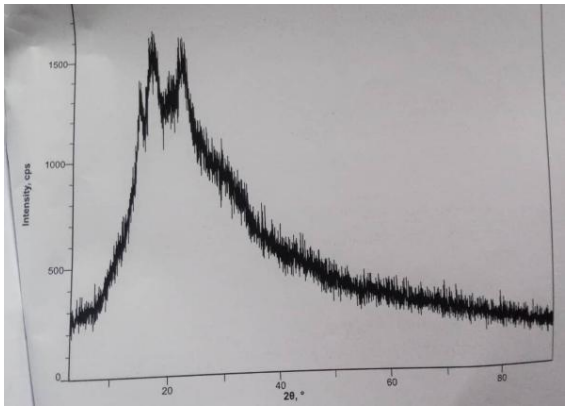

Fig. 8. WHMT

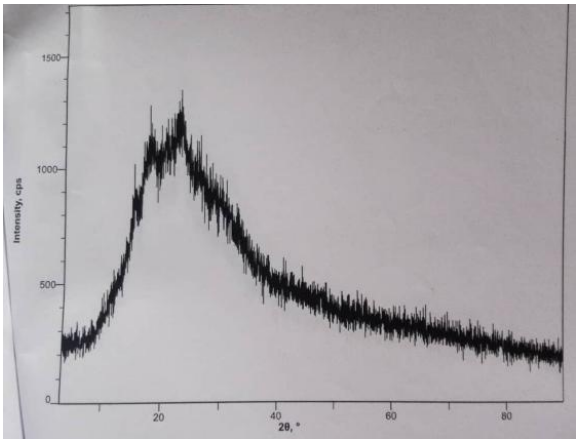

Fig. 9. GNAT

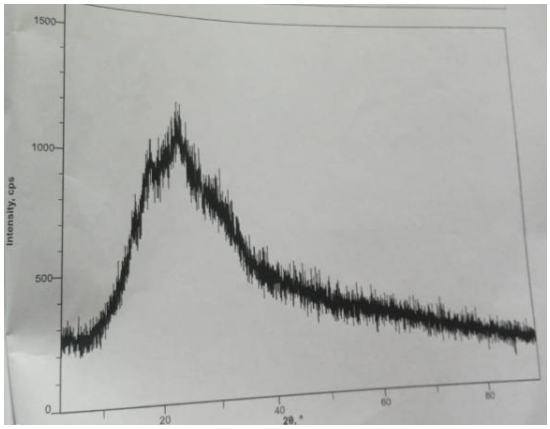

Fig. 10. GCTR

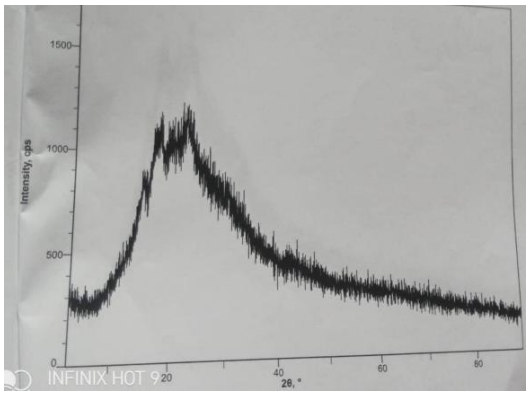

Fig. 11. GANN

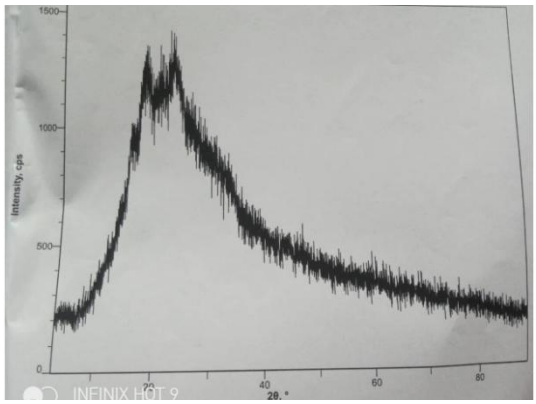

Fig. 12. GHMT

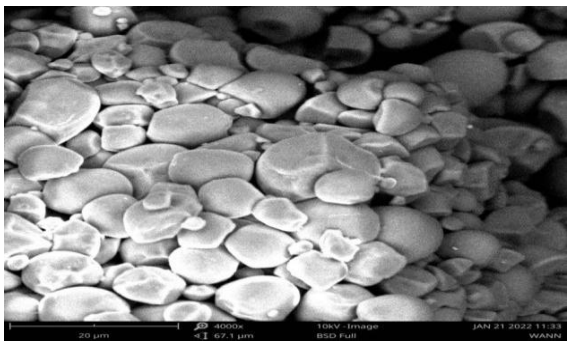

Fig. 13. RNAT

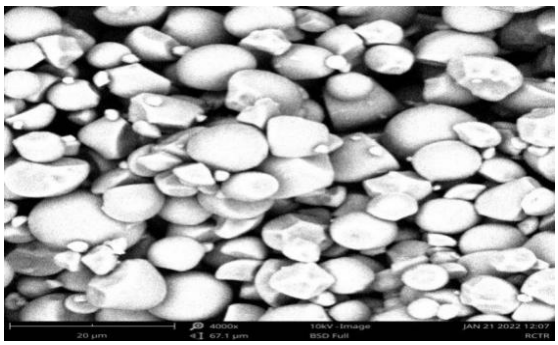

Fig. 14. RCTR

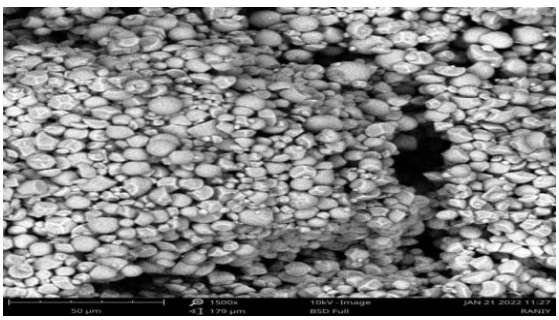

Fig. 15. RANN

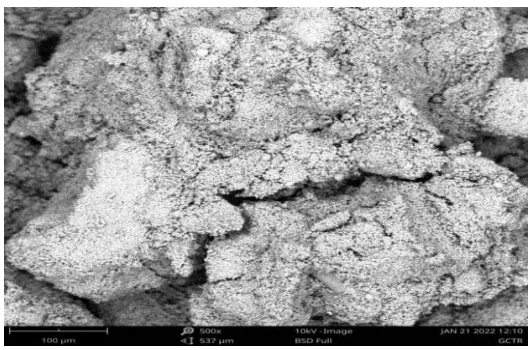

Fig. 16. RHMT

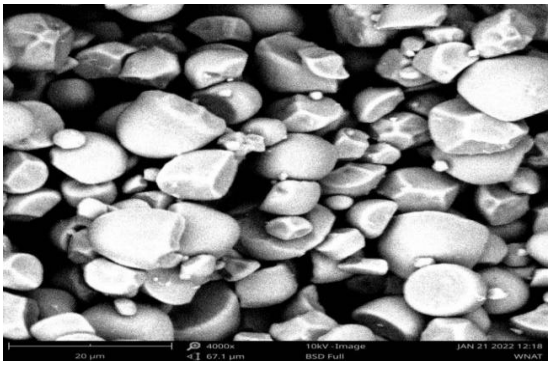

Fig. 17. WNAT

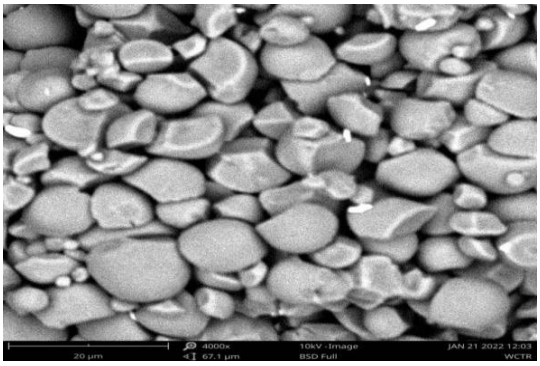

Fig. 18. WCTR

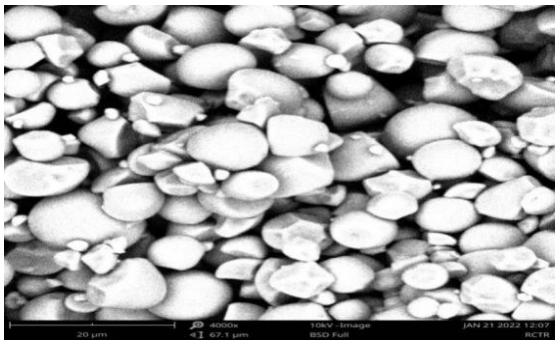

Fig. 19. WANN

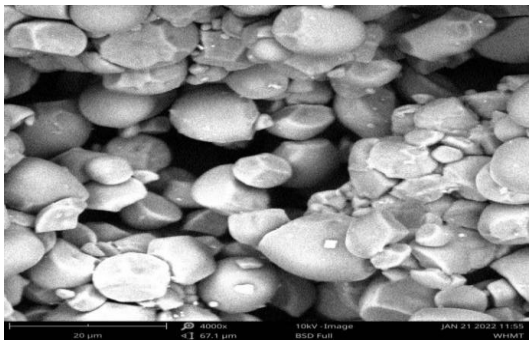

Fig. 20. WHMT

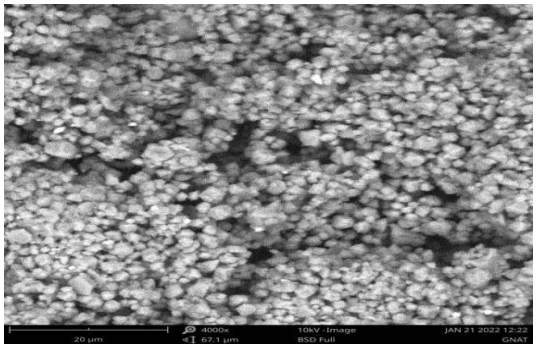

Fig. 21. GNAT

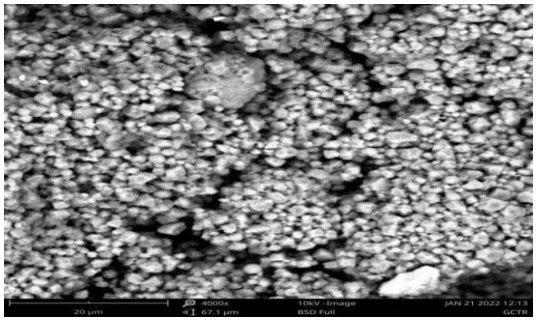

Fig. 22. GCTR

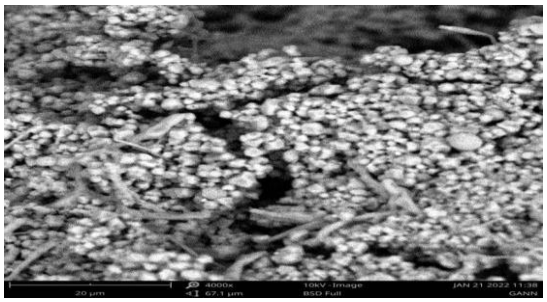

Fig. 23. GANN

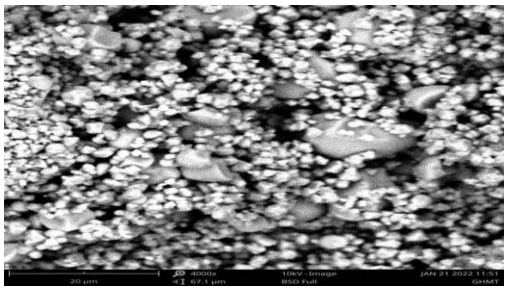

Fig. 24. GHMT

3.3 Scanning Electron Micrographs of Modified Cocoyam Starches

The micrographs of the starch samples are presented in Figs. 13 to 24. 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., [15]. 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

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±0.01^{g}
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±0.01^{b}
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±0.02^{c}
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±0.01^{g}
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±0.01^{g}
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±0.00^{a}
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±0.02^{d}
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±0.02^{h}
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±0.01^{f}
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±0.01^{e}
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±0.02^{d}
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±0.01^{h}

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 *WNAT: White native* *GNAT: Ghana native*

RANN: Red annealing treatment *WANN: white annealing treatment* *GANN: Ghana native*

RHMT: Red heat moisture treatment *WHMT: White heat moisture treatment* *GHMT: Ghana heat moisture treatment*

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

ND:-Not Detected

Arinola, [20] 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.

4. CONCLUSION

The results of the present research have shown that modified starches of cocoyam could find usefulness in food and non-food products based on the nutritional composition and pH of cocoyam starch. Starch granule integrity was most drastically altered by heat and moisture treatment, as seen by changes in x-ray diffraction pattern. The modified cocoyam starch samples disclosed here represent a potential new supply of starch for the food and non-food industries.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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