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Modification of Starch Extracted from Cassava with Acidified Ethanol

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Author's contribution

The sole author designed, analyzed and interpreted and prepared the manuscript.

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ABSTRACT

Aim: The aim of this study was to chemically modify the starch extracted from *Manihot esculentus* with an objective of gaining insight into the structure and architecture of the native and modified starch.
Study Design: Experimental.
Methodology: Starch from tubers of cassava (*M. esculentus*) was extracted and chemically modified using a 95% ethanol solution containing 20% HCl at 50℃ for 1-4 hours. Structural changes in the native starch and its derivatives were evaluated using colorimeter on treatment with Iodine-KI solution, and the absorbance read at wavelength (λ=470 nm). The gelatinization temperature of the starch was also determined.
Results and Discussion: From the result, absorbance of the acidified ethanol modified starch decreased per hour after treatment with Iodine solution while the weight of recovered derivatives went accordingly. The gelatinization temperature of the modified starch increased with respect to the time taken.
Conclusion: In conclusion, there was an indication that acidified ethanol changed the starch structure and architecture with a corresponding effect on the gelatinization temperature, easy drying and reaction with Iodine solution. The process of modification of the starch gave rise ethyl-O-starch. In addition, starch modified with HCl/ethanol obeys the Lambert-beer law and the rate of

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hydrolysis of its molecular chains could be monitored and calculated. Therefore, cassava starch modified with acidified ethanol could find applications in food, textile and paper industries.

Keywords: Cassava starch; alcoholysis; gelatinization; Iodine-test; absorbance; time.

1. INTRODUCTION

Starch is a naturally occurring, non-soluble, biodegradable and abundantly available polysaccharide molecule. It can be extracted from tubers such as yam, potatoes, cassava, and cereals like maize, rice, millet and others. Starch, because of its abundance attracts industrial application and commercialization. It is made up of glucose monomers in a repeated unit in the range of 300-1000. Primarily, a starch molecule consists of two major polymers which are amylose and amylopectin. Amylose forms the straight chain linked together by α 1-4 glycosidic bond while the amylopectin constitute the branched point linked together by α-(1 → 4)-d-glucopyranose units with α-(1 → 6)-linkages at the branching points [1,2].

In most part of the world, starch serves as a major source of food. Besides, it has attracted serious industrial applications in textile, food, pharmaceutical, paper and wood industries. Starch in recent years are processed into biodegradable plastics which are more eco-friendly than those derived from petrochemicals [3,4]. The industrial selection of starch is made by considering its accessibility and also its physico-chemical characteristics, that vary depending on the source [5]. Therefore, for starch to meet maximum industrial applications, modification of its form and functions will be necessary [6].

The various starch modification techniques have been categorized into; physical, chemical, enzymatic and genetic modification so as to improve the physical-chemical properties of the starch molecules [7]. Although, the functionality of a starch depends on the molecular size, crystallinity degree, amylose content and, specially, on the viscosity properties [3,5] which could be achieved through modification of form and function of the native starch. The aim of this study was to chemically modify the starch extracted from *Manihot esculentus* with an objective of gaining insight into the structure and architecture of the native and modified starch by using iodine-based colorimetric methods as well as to enhance quick drying.

2. MATERIALS AND METHODS

2.1 Materials

The tubers of *M. esculentus* were obtained from the local market in Bayelsa State, and the starch was extracted in our laboratory. Ethanol, sodium metabisulphite, sodium hydroxide and hydrochloric acid used were of analytical grade as procured from their manufacturer.

2.2 Methods

2.2.1 Extraction of *M. esculentus* starch from the tubers

The skin was peeled off to expose the inner white layer which was then sliced into several smaller pieces and washed in 0.1% sodium metabisulphite before wet milling. The paste was saved using a muslin cloth and allowed to settle overnight. Thereafter, the supernatant was decanted leaving the past that has settled at the bottom of the container. The paste collected at the bottom was then washed thoroughly with 0.1% sodium metabisulphite which served as an antioxidant until the paste became white. The washed paste was then air-dried for 72 hours. Thereafter the dried starch was size-reduced using a mortar and a pestle and thereafter passed through a double-layered sieve size 250 µm and stored in an airtight container [8].

2.2.2 Preparation of HCl/ethanol hydrolyzed starch

The method of [9] was modified and used. Briefly, about 40 g of starch was suspended in 80 ml ethanol (95%) in a 250 ml conical flask. A hydrolysis reaction was initiated by adding 20 ml of concentrated HCl (20%) and allowed to proceed for an interval of 1-4 hours at 50^{o}C in a water bath. The reaction was stopped by neutralizing with 3.0 M NaOH until it was neutral to litmus paper. The sample was allowed to settle, the supernatant was decanted and the modified starch sample was collected, air dried and weighed.

2.2.3 Determination of effect of HCl/ethanol on starch

To determine the level of ethanolic modification of the native starch, 1.0 ml Iodine-KI solution was added to 0.5 g of modified sample and distilled water was added to 10 ml. The colour developed was measured at 470nm with a colorimeter, and the absorbance was recorded accordingly [10].

2.2.4 Gelatinization temperature of HCl/ ethanol hydrolyzed starch

1.5 g of the native and modified starch samples were dissolved in a beaker with 10 ml of distilled water, the mixture was stirred, a thermometer inserted and the beaker placed in a water bath. The dispersion was stirred continuously until its milky colour became transparent and thickened. This is the gel point and the temperature at this point was read off as the gelatinization temperature.

3. RESULTS AND DISCUSSION

The results of HCl/ethanol modified starch as shown by absorbance, the weight of the sample recovered, gelatinization temperatures at each time interval are presented in Figs. 1, 2, and Table 1 below. From the result, the observance of HCl/ethanol modified starch reduced with an increase in time. However, there was no observable effect of Iodine-test for starch hydrolyzed with only acid at the same time interval.

Table 1. HCl/ethanol modified starch

Time (hrs)	Weight of sample (g)
0	40.00
1	33.60
2	28.00
3	24.40
4	22.4

3.1 Discussion

From the results, starch sample was modified after it was subjected to these reaction conditions. The gradual change in colour of modified starch from the original blue-black at each time intervals was an indication of structural change. The blue-black colour reflected the ability of amylose and amylopectin chain segments to mobilize and interact with iodine to form inclusion helices which gave rise to the blue color [11]. The structural change was monitored colorimetrically on addition of Iodine solution, and could be linked with the interaction of native starch with acidify ethanol. This observation could be associated with the initial gradual disappearance of blue-black colour with time, until the colour was becoming purplish after four hours of reaction with acidified ethanol. The complete colour change from the original blue - black of the native starch seems to signal completion of the modification and introduction of functional groups.

$$C_2H_5OH + HCl \rightarrow H_2O + C_2H_5Cl$$

$$C_2H_5Cl + H\text{-}O\text{-}Starch \rightarrow C_2H_5\text{-}O\text{-}Starch_{(ethyl\ starch)} + HCl$$

From the reaction above, there was an introduction of an ethyl group into the glucose moiety of the starch resulting into substitution of the hydrophilic hydroxyl group of the starch by hydrophobic functional group-ethyl, yielding ethyl starch. However, the recovery of the acid indicated that the presence of HCl did not only help in hydrolysis but also catalyzed the reaction between the starch and ethanol.

The result also indicated that, no colour development was seen in the sample hydrolyzed with only HCl. From the result, the sample hydrolyzed with only acid looked much more soluble in water compared to the HCl/ethanol per hour. This means that the rate of starch hydrolysis is higher without ethanol. Therefore, addition of ethanol to the starch invariably means exchanging the water in the medium with ethanol thereby reducing surface tension and thus reducing the capillary forces and making the starch more hydrophobic by modifying its textural structure [9] as a result of the etherification process. This phenomenon explains why the ethanol modified starch got dried with mere air-drying for two days compared to the acid hydrolyzed starch, which took several days to dry under the same condition. As such, the presence of ethanol increased the hydrophobicity of the starch and enhanced easy drying.

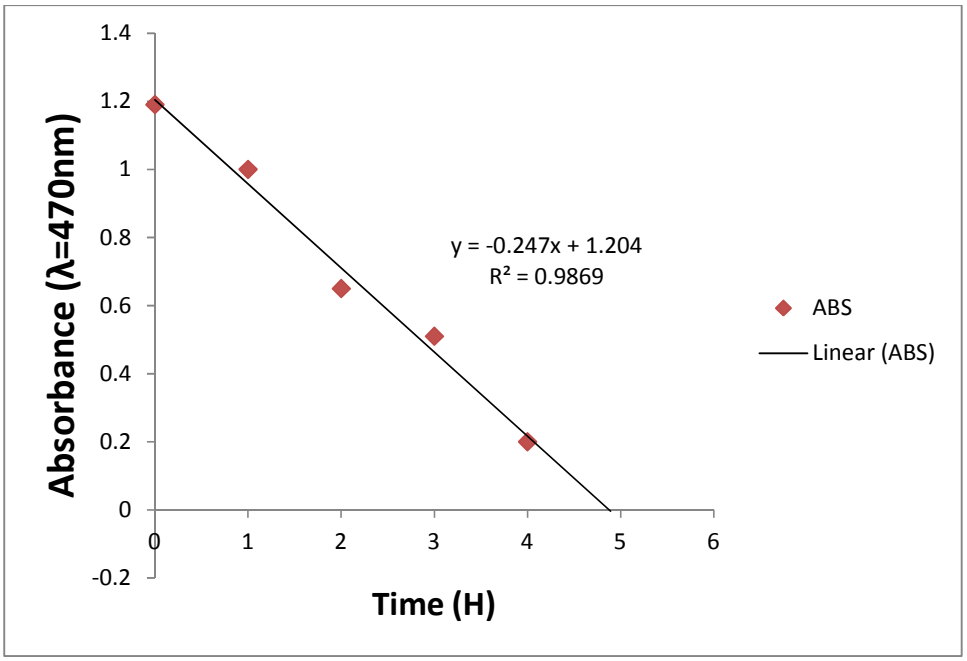

Fig. 1. Modified starch structural change as revealed by absorbance

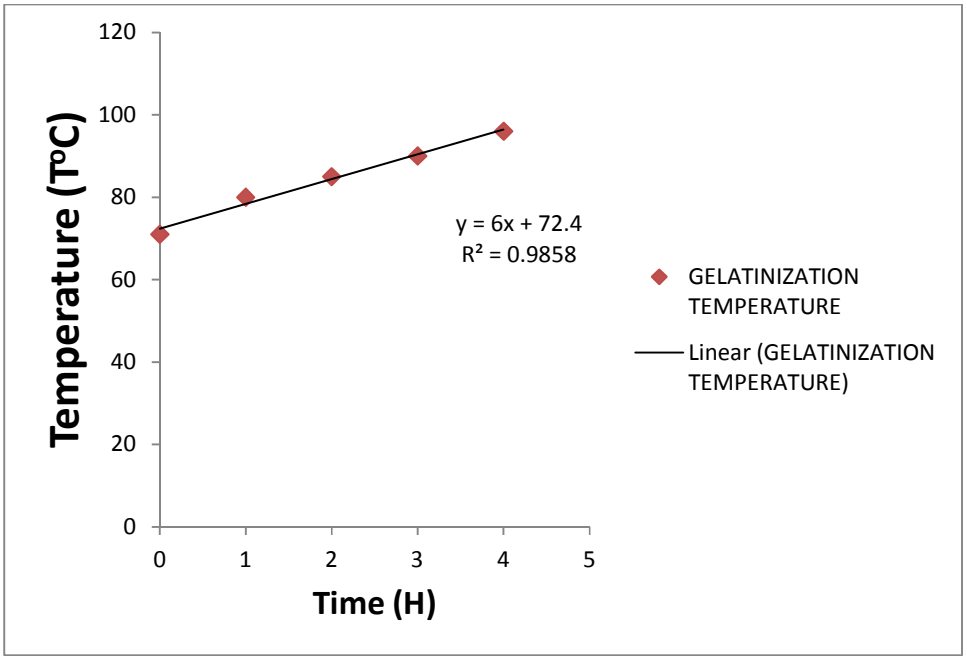

Fig. 2. Determination of gelatinization temperature of modified starch

According to [9], the HCl-ethanol modified derivative of *Psorelae esculenta* starch showed four different bands representing the skeletal mode vibration of the glucose pyranose ring when subjected to FTIR spectroscopy. The partial hydrolysis of the native starch produced a disaccharide maltose together with low molecular weight dextrans. Hydrolysis of the glycosidic linkage inside starch granules, in the presence of ethanol, are influenced by; the availability of water molecules, the acid participating in the hydrolysis and the availability and reactivity of

the glycosidic linkage. Therefore, the mechanism for the acid hydrolysis of starch granules suspended in alcohol involves the hydrolysis of the glycosidic bond with the water originally inside the granules [12].

Functionally, ethanol as a precipitating agent influences the solubility of proteins by reducing the dielectric constant of the medium and the salvation effect of water molecules surrounding the protein is altered; the interaction of protein molecules is increased; and agglomeration and precipitation of protein occur. In essence, the activity of the ethanol in the medium was to agglomerate the protein molecule, but concomitantly affected the acid (HCl) hydrolysis of the starch by removing necessary water molecules. As a result, the inter- and intra-molecular hydrogen bonding of the starch molecule is significantly altered when treated with HCl/ethanol [9]. Besides, the method of acid hydrolysis of starch is difficult for practical applications because its hydrolytic reaction cannot be monitored and corrosive nature. Nano-sized starch particles can be obtained by precipitating the starch solutions with some organic solvents such as ethanol. Ma and co-workers used ethanol to precipitate a pre-cooked native starch, and the starch nanoparticles at a size of 50-100 mm were derived [13]. With the combination of a complex formation of n-butanol and starch, and an enzymatic hydrolysis, Kim and Lim also generated starch nanoparticles of 10-20 mm in size [14].

Moreover, the HCl-ethanol modification of starch obeys Lambert-Beer law; absorbance is directly proportional to the concentration of the absorbing specie at constant light path length. With this law we understand the level of starch modification by acidified ethanol. There was a gradual disappearance of the blue-black colour on addition of iodine-KI solution to the modified starch. This occurrence in relation to Lambert-Beer law established the effect of the acidified ethanol on starch amylose and amylopectin and also gave a picture of the starch structure and architecture.

That is;

$$A \alpha c \times l \quad (I)$$

$$A = \varepsilon \times c \times l \quad (II)$$

Where A is absorbent, c is the concentration of the sample, *l* is the light path length and ε is the absorption coefficient.

The above expression can be related to the starch modification with either chemical or enzyme as catalyst. In such situation, the level of modification of a known mass of starch at a given temperature is directly proportional to the time taken in the presence of a catalyst.

Mathematically;

$$\frac{A \times m \times 1000}{T^o} \alpha Q \times l \times t \quad (III)$$

i.e;

$$\frac{A \times m \times 1000}{T^o} = \varepsilon \times Q \times l \times t \quad (iv)$$

To determine the rate of modification;

$$A = \frac{\varepsilon \times Q \times l \times \Delta t \times \Delta T^o}{Me \times 1000} \quad (v)$$

A is the level of modification, Q is catalyst, m is concentration of sample in gram per liter, T^o is temperature and ε is the absorption coefficient in milimolar.

Since absorbance (A) is an indication of the level of appearance or disappearance of the coloured sample, therefore, the rate of modification (A) of starch corresponds to the appearance or disappearance of blue-black colour of the native/modified starch. This occurrence in relation to Lambert-Beer law established the effect of the acidified ethanol on starch amylose and amylopectin and also gave a picture of the starch structure and architecture. However, for starch samples, the temperature should be below gelatinization temperature.

The gelatinization behaviour of the starch was also observed to have changed from what it used to be. The result above showed that, modification increased the gelatinization temperature. Normally, when starches are heated above 60℃ in water the starch granules swells as the water penetrates the granules and the hydrogen bonds weaken and burst thereby releasing the amylose strain out of the granules into the water which thicken to form the gel. The increase in temperature observed could be attributed to the fact that, the ethanol rendered the modified starch a little hydrophobic [7] by hindering the penetration of water molecule thereby increasing gelatinization temperature than the original starch.

Additionally, the reduction in the sample' weight per hour could be associated with the fact that, starch polymer with higher molecular weight, amylopectin, was reduced further to smaller oligosaccharide molecules such as dextrin.

4. CONCLUSION

From the foregoing, critical examination of the modification of starch extracted from cassava with acidified ethanol was conducted. There was an indication that, the presence of acidified ethanol significantly changed the starch structure and architecture by etherification process with a corresponding effect on the gelatinization temperature, hydrophobicity-easy drying as well as reaction with Iodine solution. The process of modification of the starch gave rise ethyl-O-starch. In addition, starch modified with HCl/ ethanol obeys the Lambert-beer law and the rate of hydrolysis of its molecular chains could be monitored and calculated. Based on these observed features and further research, cassava starch modified with acidified ethanol could find applications in biomedical engineering, pharmaceutical, food, textile and paper industries.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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