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Analysis of Starch from Non- Edible Root and Tubers as Sources of Raw Materials for the Synthesis of Biodegradable Starch Plastics.

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Abstract: The study investigated the physicochemical properties of starches from *Caladium bicolor* (wild cocoyam), *Icacina trichantha* (false yam), *dioscorea villosa* (wild sweet yam) and oyster mushroom. Amylose/amylopectin ratio, gel characteristics, hygroscopicity among other properties were examined. The study revealed that wild yam, sweet yam, false yam and oyster mushroom have total starch content of 86.6%, 82.8%, 58.4& and 49.3% respectively. False yam and oyster mushroom had high percentages of amylose (40.2 and 56.7%) while wild cocoyam and sweet yam had high amylopectin content (88.9% and 84.6%) respectively. The functional properties of the starch samples compared favourably with starches from edible sources. This study revealed that starches from non-edible sources like wild cocoyam, false yam, mushroom and sweet yam can be used in the synthesis of bioplastics with improved quality. This will reduce the amount of plastics littered in landfills and will also replace the use of edible starches in non-food purposes enabling edible starches to be available for human consumption and other food/medical applications.

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INTRODUCTION

Starch is the reserved carbohydrate stored as glucose in plants to meet energy needs (Carey, 2000). It is a natural polymer made up of hundreds of thousands of glucose units linked together by glycosidic bonds. The molecular formula of starch is $(C_6H_{10}O_5)_n$. It is the most common carbohydrate in human diets and is contained in large amounts in such as yam, cassava, wheat, corn, rice, potatoes, mushroom etc. (Israel, Sunday, Mansong and Ubong, 2016; Sunday, Israel and Odey, 2016).

Most starches used for industrial applications such as adhesives, non-food fillers, textile stiffening agents and bioplastics for packaging are extracted for edible plant sources regardless of their end use. This poses a problem as extraction of starch from edible sources reduces the availability of food. Generating starches from other sources for industrial purposes is the trending research as starch from non-edible sources have properties appropriate in replacement of starches from edible sources. Wild species of Cocoyam (*Caladium bicolor)*, Sweet yam (*Dioscorea Villlosa*), False yam (*Icacina trichantha)* and Oyster mushroom (*Pleurotus ostreatus*).

Plastic is an extremely versatile synthetic material made from the polymerization of organic compounds and can be moulded into shapes or fabricated in many different forms for use in commerce and industry. A plastic material can be hard, transparent, opaque, lightweight and even elastic. Examples of plastics include Low density Polyethylene (LDPE), High density polyethylene (HDPE), Polypropylene (PP), Polyvinylchloride (PVC), Polystyrene (PS), Polyetlylene terephthalate (PET), Polyvinylacetate (PVA), Polymethylmeth-acrylate (PMMA), etc. Plastics are used in our everyday life in form of bottles, garment bags, frozen food packages, toys, car fuel tanks, pipes, straws, computer cases, rain wears, shoes, shampoo containers, floor tiles, gloves, carpet, recording tapes, brushes, fishing lines, textiles, medical implants, optical lenses and electrical circuits; the list is endless.

Plastics are very useful materials and its applications increases as new products are developed to meet demands. Plastics play a pivotal role in the packaging industry. It is not surprising that the amount of plastic waste has increased over the decades. For instance, in Sabon-gari area of Kano City alone, high volume of plastic waste (17.6%) is generated in every fourteen days (Bichi and Amatobi, 2013). Waste generated in Lagos has increased to thirteen thousand metric tons (13,000) on a daily basis, twenty three percent (23%) being plastic waste (Akonni and Olasunkanmi, 2015). High volume of waste (55.20 tons) is generated daily in Oyo State, plastic waste accounting for twenty percent (20%) of the total solid waste (Abel, Afolabi and Okewole, 2007). With the price of oil rising and easily accessible reserves declining, alternative sources of fuel and of oil-based articles such as plastics are being explored across the world. The environmental concerns of the oil-based economy are also being widely voiced as companies and individuals attempt to reduce their carbon footprints.

As a result of their molecular stability, plastics do not easily breakdown into simpler components. This keeps plastics accumulated in the landfill and because they are non-degradable and have high resistance to chemical attack, they litter the environment resulting in serious environmental pollution (Wisa, Manasomboonphan and Suwannee Junyapoon, 2012). Plastic garbage litters the landfill, causes blocking of the city and affects soil fertility.

The search for alternatives to traditional petroleum-based plastics is progressing to the point that the source and the possible environmental impact is being addressed in the form of biodegradable plastics. Although photodegradable plastics continue to be explored (Zan, Fa and Wang, 2006), these alternatives must be constantly exposed to sunlight and so are not suitable for landfill disposal. Biodegradability in composters or municipal landfills is the goal.

In this context, production of biodegradable plastics in plants is an enviable goal. Plants naturally produce many polymers, such as starch or cellulose, and these have been exploited for plastics production.

Biodegradable plastics are plastics made from renewable biomass sources such as vegetable fats and oils, starches, cellulose or microbes, etc. which are capable of decomposing naturally when disposed. Unlike petroleum based plastics, biodegradable plastics have the following advantages:

- Reduces carbon footprint.
- Save energy during production.
- Uses only renewable sources. Non-renewable sources are not consumed. Starch plastics are made from biomass, which is a completely renewable resource. Biomass includes trees, plants, grass, and all organic materials that decompose. This may even include animal fats, meats, and other tissues.
- Reduces non-biodegradable waste, which pollutes the environment. Biodegradable packaging and biodegradable bags take less time to break down after being discarded.
- No health-damaging additives are contained, like phthalates or bisphenol-A. They do not alter the flavour and smell of food they store.

The nutritive value and physicochemical properties of starch determines its end use. The edible starchy plant are toxic free thus, their starches are relatively useful. Similarly, starches from non-edible sources are non-nutritive and may contain some toxins like Oxalic acids, hence, their starches have limited or no use. Nevertheless, these starches from non-edible sources can be used for non- nutritive purposes depending on their physicochemical properties. This study seeks to investigate the physicochemical properties of starches from non-edible sources to ascertain the applications in the synthesis of bioplastics.

MATERIALS AND METHOD

The reagent used in this study were of analytical grade. The sample items used are labelled as

A – Wild Cocoyam – *Caladium* bicolor – (Ikpong ekpo in Efik)

B – Wild sweet yam- *Dioscorea villosa* – (Anem ekpo in Efik)

C – False yam – *Icacina trichantha* – (Efik isong in Efik)

D – Oyster Mushroom - *Pleurotus ostreatus* – (Abubid udip in Efik)

(**A, B, C, D** will be used to represent these samples).

Sample Collection and Treatment

Wild species of sweet yam, mushroom, cocoyam and false yam were collected from a forest in Oboya Ikot Ita, Nsit Ibom Local Government Area in Akwa Ibom State. The forest has been uncultivated for many years and was overgrown with wild plants. The samples were thoroughly washed clean with water and were preserved in the laboratory.

The items were peeled with kitchen knife and washed. The clean tubers were cut into smaller pieces and pulverized.

Extraction of Starch from the Samples

Starch was extracted from the samples following the procedure described by Adikwu, 1998. 100g of the powdered sample was soaked in distilled water and strained through a piece of cotton fabric to extract the starch from the flour. The flour was washed three times to ensure effective extraction. The filtrates were allowed to settle for six hours. On complete sedimentation, the supernatant was decanted and the starch slurry was preserved for further analysis. This procedure was repeated for all four samples; **A** (wild cocoyam), **B** (wild sweet yam), **C** (false yam) and **D** (mushroom).

Starch Content

The starch slurry was soaked in distilled water containing 0.1% Sodium trioxosulphate (VI) for 24 hours, followed by 0.1N H_2SO_4 for another 12 hours. The solution obtained was dried in an oven at 60^0C for 3 hours. The dried starch was weighed and stored in powder form in airtight bottles. This procedure was repeated for all four samples; **A, B, C** and **D**. percentage content of starch was determined using the formula below;

$$\%\,Starch = \frac{weight\ of\ dry\ starch}{weight\ of\ wet\ flour} X\ 100 \qquad (1)$$

Moisture Content in Starches (A. O. A. C., 1975)
Moisture content in the dried starch was determined by keeping weighed quantity of sample in a thermostat controlled oven at 105ºC for 24 hours. The dry weight of each sample was taken on a weighing balance. The percentage of the moisture content and dry mater was then calculated by the formula as presented below:

$$Moisture\ content\ (\%) = \frac{initial - final\ weight}{initial\ weight}\ X\ 100 \qquad (2)$$

Determination of Ash Content in Starch Samples
Total ash content was determined by igniting previously dried starch sample in a muffle furnace at 500°C for 8 hours. On cooling, the resulting ash was weighed and the percentage ash was calculated as:

$$\%Ash = \frac{weight\ of\ ash}{weight\ of\ dry\ starch}\ X\ 100 \qquad (3)$$

pH Determination of Starch Samples (Adebayo and Itiola, 1998)
An electronic pH meter was initially standardized using buffer solution 4 and 9 to serve as calibration response. The electrode of the pH meter was rinsed with distilled water and inserted in the starch sample slurry. The pH value of the sample was recorded.

Hydration Capacity of Starch Samples (Radley, 1990)
Dry starch sample (0.5g) was placed in a centrifuge tube. Distilled water was added from an automatic burette, the tube was covered with paraffin and the content was shaken for 2 minutes. The mixture in each tube was left for an additional 3 minutes and centrifuged at 5000rmp for 10 minutes. The supernatant was decanted and the sediment was weighed. The weight of water absorbed and retained was determined as the gain in weight of the dry sample.

Gelatinization Characteristics (Alves, Silva and Grossmaan, 1999)
Dry starch (3g) was measured into a beaker and melted with 60ml of distilled water. The resulting solution was heated in a water bath and stirred continuously until the solution began to form gel. The temperature of the gel was recorded as the gelatinization temperature.
The viscosity of the starch gel was determined by pouring gel into 50ml burette and the reference time (t_1) for gel to flow out of the burette was recorded.

Solubility of Starch Samples (Zobel and Stephen, 1995)
The solubility of the starches was tested in water, ethanol, chloroform and diethylether at 30^0C. The starch (0.5g) was added to 10ml of the each solvent and stirred for 10 minutes. The solubility was observed as the solute gradually mixed with the solvent.

Hygroscopity of Starch Samples
A modified method of Adebayo and Itiola (1998) was adopted in this determination. The starch powders (1g) was kept in a petridish and placed in a dessicator containing only distilled water. The amount of moisture absorbed was measured from the weight gain at specific time intervals of 24 hours up to 192.The moisture sensitivity of the starches were studied as a function of time for over 192 hours.

Determination of Amylose and Amylopectin in Starch Samples (Majzoobi, Connock, Hill and Harding, 2003)
Starch sample (1g) was dispersed in 10ml of distilled water containing 20ml of 0.16M NaOH. The mixture was swirled gently until the suspension clears. The solution was allowed to stand for 5minutes after which 4ml of 5% NaOH was added and the pH of the solution adjusted by adding 5 drops of 1M HCl. The resulting solution was allowed to stand for 15 hours.
The mixture was centrifuged ad 10,000 rpm for 10 minutes. The supernatant was decanted and quantified as V_t. The supernatant (8ml) was saturated with 4ml of 1-butanol and stirred and allowed to stand for 2hours before centrifuge at 5000rpm for 15 minutes. The precipitated amylose butanol complex was allowed to stand for 3 hours and the supernatant siphoned by suction pump while the amylose precipitate was collected by filtration and dried at 80^0C in the oven. The amylose fraction was allowed to cool and weighed (W). The quantification of the amylose was calculated using the formula below:

$$\%\ Amylose = \frac{V_t\ X\ W}{V_a}\ X100 \qquad (4)$$

Where V_t = total volume of supernatant
W = weight of amylose obtained
V_a = volume of aliquote amylose (8ml)

% Amylopectin = 100 - %Amylose (5)

RESULTS AND DISCUSSION

Table 1: Variation of Moisture and Starch Content in the Samples

Samples	Moisture content (%)	Starch content (%)
A	62.7	86.6
B	76.9	82.8
C	62.5	58.4
D	46.8	49.3

A= wild cocoyam B= wild sweet yam C= wild false yam D= mushroom

Physicochemical Properties of the Starch Samples

The physicochemical properties of the starch samples are presented in Table 2. Differences in water content and the ratio of amylose to amylopectin of various affects the physical and barrier properties of starch bioplastics (Tanetrungroj and Prachayawarak, 2015). The research revealed that starch A had the highest starch content of 86.6%. The results of water absorption properties can also be described in terms of the biodegradable property. Starch plastic polymers could absorb water from the soil TPS component, leading to the biodegradation of TPS polymer by the microorganisms present in the soil. The most rapid degradation would be the sample D starch polymer since the mushroom starch consists of high amylose content (Table 2) that can be easily decomposed (Sunday *et al,* 2016).

Moisture Contents

Moisture content of starch is the total quantity of vapour present in it. The higher the moisture content, the lower the amount of dry solids in the flour. The maximum limit for moisture in starch flour is 14% (Oladayo, Umunna, Joseph and Oluwaseguun, 2016) and 12% according to African Organization for Standardization of cassava starch. Higher values of moisture in starch causes caking of the starch, affects its texture and encourages the growth of microorganisms which cause odours and off flavour.

Moisture contents of the starch as presented in table 2, increased in the order of A-B-C-D (6.5, 9.8, 11.5 and 12.2% respectively). The moisture content for mushroom (46.8%) is smaller than 87.6% reported by Anno, Konan, Kouadio, Dué, Kouamé (2016). Although, the obtained results are comparably than 0.27% in cassava starch reported by Oladayo *et al* (2016), the moisture contents in the starch samples were within acceptable limit. This indicates that the starches is less prone to fungal and microorganism infections and thus, can be stored at room temperature. This makes them amenable for utilization of bioplastics in food packaging.

Amylose and Amylopectin

From the result presented in Table 2, the amylose content of the starches increased in the order A, B, C and D. Sample C and D had the highest amylose content (40.2% and 56.7%), this is higher than 34.21% for cassava starch reported by Oladayo *et al* (2016).

High amylose content of the starch samples indicates decrease in crystallinity and gel tackiness; increased amorphous regions, gel hardness, higher pasting temperature and amylose aggregation/spoilage tendencies. High amylose starches have increased tendency for water absorption.

Amylopectin content increased with an increase in amylose, this shows that one is a function of the other and both properties are important in food preparation and development.

Amount of amylose and amylopectin present in starch influences the mechanical property of the starch polymer. High amylose starch presents higher stiffness and hardness than high amylopectin starch due to the comparatively less branching structures (Marie and Imada, 2004 as cited in Tanetrungroj e*t al* (2015). Similar observations were reported for high amylose rice film that was stiffer, less elastic than those of low amylose rice film, prepared from casting method (Cano et al., 2014).

Gelatinization Temperature

The gelatinization temperature of starch is known to affect the efficiency of starch hydrolysis. The gelatinization of A (wild cocoyam) was 81^{o}C, B (wild sweet yam) was 80^{o}C, C (false yam) was 71^{o}C and D (mushroom) was 77^{o}C (Table 2). The result compared favourably with 75.5^{o}C for cassava starch reported by Oladayo *et al* (2016).

Water Sorption (Hygroscopicity Property of the Starch Samples)

Moisture sorption capacity is used to determine the degree of moisture sensitivity of the cassava starch and it reveals the relative physical stability of the bioplastic formulated with the starch when stored under humid conditions (Oladayo *et al,* 2016).

Moisture sorption behaviour of the starches is presented in Table 3. The values show that the hygroscopicity of the starches increased in the order A, B, C and D on the first day (24 hours). The total moisture absorbed by the starch samples compare favourably with the value (4.65g) reported for maize starch by Adebayo and Itiola (1998) at 33% RH for 7 days. It was noticed that water absorption ability of the samples decreased rapidly after the first day, this is an indication that the starches can be used as packaging materials.

The major main role of food packaging is often to avoid or least to decrease moisture transfer between the food and the surrounding atmosphere, or between two components of a heterogeneous food product. In order to achieve this, the water vapour permeability or absorption should be minimal (Piyada, Waranyou and Thawien, 2013). Report by Piyada *et al* (2013) revealed that water absorption property of starch from tuber crops is better that rice starch nanocrystals.

The research results revealed that starch D has the lowest water permeability (Figure 1) and can therefore be used extensively in food packaging.

Table 2: Physicochemical Properties of the Starch Samples

Starch sample	Ash (%)	Hydration capacity	pH	Moisture Content	Gel viscosity	gelatinization temperature (ºC)	Amylose content (%)	Amylopectin Content (%)
A	0.6	93.6	7.8	6.5	1.2	81	11.1	88.9
B	0.3	113.2	7.6	9.8	1.4	80	15.4	84.6
C	0.1	69.6	8.2	11.5	4.1	71	40.2	59.8
D	1.2	58.4	8.5	12.2	7.3	77	56.7	43.3

Table 3: Amount of moisture absorbed by various starch samples

Time (hours)	**A**	**B**	**C**	**D**
24	2.32	2.38	2.41	2.30
48	0.13	0.10	0.13	0.01
72	0.03	0.08	0.04	0.01
96	0.02	0.02	0.02	0.01
120	0.01	0.01	0.01	0.01
144	0.01	0.01	0.01	0.01
168	0.01	0.01	0.01	0.01
192	0.01	0.01	0.01	0.01

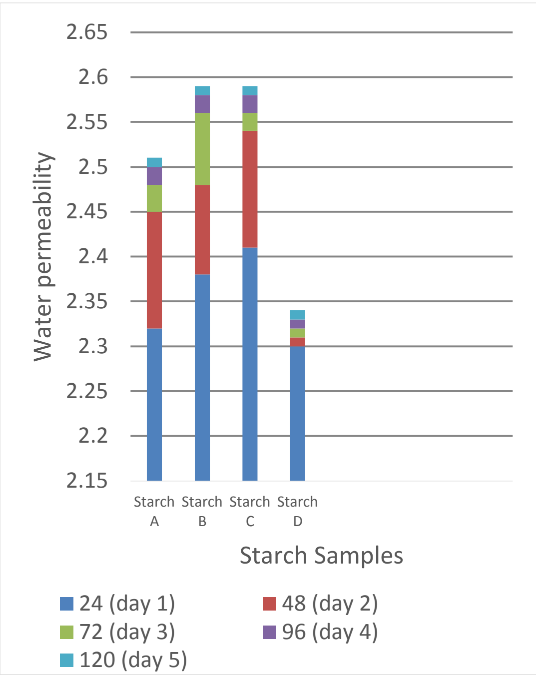

FIGURE 1: Water permeability of the starch samples for five days.

Conclusion

The low cost and abundant availability of starch encourages its incorporation into variety of products. Chemical modification of starch polymers for non-edible sources can lead to enormous useful derivatives. Starches from wild coco yam, sweet yam, false yam and mushroom can be used as a biodegradable additive or replacement material in commodity plastics, especially, garbage bags and food packaging.

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