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Original Research Article

Acetylation and Physicochemical Properties of *Ampelocissus africana* (Wild Cassava Tuber) Starch for Enhanced Drug Delivery

Lawal G. Hassan[1]*, Nafiu Aminu[2], Y.M. Mustapha[3], Zainab I.S.G. Adiya[1], Ernest C. Agwaba[4], Mustapha Salihu[5]

[1]*Department of Pure and Environmental Chemistry, Faculty of Chemical and Life Sciences, Usmanu Danfodiyo University Sokoto, Nigeria*
[2]*Department of Pharmaceutics and Pharmaceutical Microbiology, Faculty of Pharmaceutical Sciences, Usmanu Danfodiyo University Sokoto, Nigeria*
[3]*Ibrahim Badamasi Babangida University Lapai, School of Preliminary and Continuous Education Ibeto Center, Magama Local Government Area, Niger State, Nigeria*
[4]*Department of Chemical Sciences, Faculty of Science, Clifford University Owerrinta, Abia State, Nigeria*
[5]*Department of Chemistry, School of Sciences, Shehu Shagari College of Education, Sokoto State, Nigeria*.

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ABSTRACT

Ampelocissus africana (wild cassava tuber) is a starch-rich tropical plant that has been used as a source of diet by humans for several years, particularly in some parts of Africa during times of famine. The stalk and foliage are used to treat many ailments. This research aims to extract and modify starch from wild cassava tuber for drug delivery applications. The starch was extracted and modified by acetylation reaction. The physicochemical properties of the resultant starch was evaluated following standard procedures. The isolated starch was odourless and tasteless, with a faint ash-off-white colour. The iodine test indicated a starch content of 10.12%. Scanning electron microscope (SEM) analysis revealed that the starch was globular and oval. Thermogravimetric analysis (TGA) result showed that the modified starch exhibited a decrease in thermal stability. The range of acetyl content and degree of substitution (DS) achieved in this study were 0.39% to 6.47% and 0.02 to 0.26, respectively. The swelling power of the modified starch tended to increase with an increase in DS. The Fourier Transform Infra-Red (FTIR) spectra indicated the presence of four significant absorption peaks: OH, C-H, C-O, and C=O, which signified the presence of the acetyl group in the starch. The findings revealed that the modified wild cassava tubers starch could be a potential excipient for enhanced drug delivery applications.

***Keywords*:** Wild cassava tuber, *Ampelocissus africana*, acetylation, modification, drug delivery, starch

Introduction

Polymeric materials play different roles as binders, matrix formers, drug release modifiers, film coating formers, thickeners or viscosity enhancers, stabilizers, suspending agents, gelling agents, and bioadhesives. The hydroxyl, amino acid, and carboxylic acid groups found in polymer molecules are among the reactive functional groups that can be employed to modify them chemically.[1] Polymers play a vital role in any dosage form as excipients. The positive qualities of natural polymers on the drug delivery system is that they should be compatible, non-toxic, stable, and economically significant. Excipients are utilized to create oral dosage forms and improve their physicochemical characteristics.[2] In the oral drug delivery system, natural polymers are used as rate-controlling, taste-masking, protecting, and stabilizing agents.[2]

Starch is sometimes consumed in its intact form and is usually used by industry in its native state. The three most common native starches, including wild cassava tuber starch (*Ampelocissus africana*), are limited in their uses because they are unstable in terms of changes in temperature, pH, and shear forces.[3]

*Corresponding author. E mail: hassan.lawal@udusok.edu.ng
Tel: +2348036076965

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Native starches are very susceptible to decomposition and retrogradation. Some starch granules are unreactive, insoluble in water at room temperature, and highly resistant to enzymatic hydrolysis, and lack functional properties.[3] The native starches are usually modified to improve their properties, such as solubility, texture, swelling ability, and ability to withstand high temperatures used in industrial processes.[4] Some techniques, such as acetylation and crosslinking reactions, have been developed to produce modified starches with unique characteristics.[4,5] These modifications change the starch polymer, rendering it highly flexible, alters its physicochemical properties and structural arrangement to increase its value for food and pharmaceutical uses.[6] Acetylation reaction is the most common chemical modification method, resulting in native starch esterification using reactive reagents such as anhydrous acetic acid, vinyl acetates, and octenyl succinates anhydrides (OSA) in the presence of an alkali which serves as a catalyst.[7] The modification by acetylation improves its physicochemical properties, such as solubility, swelling ability, and gelatinization compared to native starch.[8]

Chemically modified starches are used extensively to overcome the variability of native starches and their lack of versatility over a wide range of processing conditions.[9] Wild cassava starch (*Ampelocissus africana)* is one of Africa's most abundant starchy tuberous roots. Its pharmaceutical application has been well explored. The present study was undertaken to modify starch extracted from wild cassava tuber (*Ampelocissusafricana*) for drug delivery applications.[10]

Materials and Methods

Materials

Acetic acid anhydride (99.5%) was purchased from Philip Harris Limited laboratory reagent. Sodium dihydrogentetraoxophosphate (v) dihydrates solution (98%), Trisodiumtetraphosphate (v) (99%). Methanol (99.8%). Chloroform (96.8%). sodium hydroxide (96%), Hydrochloric acid (36.5-38%) were purchased from the British Drug House (BDH) laboratory. FTIR Machine (Carry630 Agilent, USA), pH meter (Jenway 3510, Jenway, China), Electric balance (Shimazu aw320, Shimazu, Japan), SEM (phenom pro, phenom world Thermo Fisher, Schwisland).

Methods

Preparation of Acetylated Wild Cassava Tuber Starch

The methods reported by Mulhbacher *et al.* [11] and Das *et al.*[12] were adopted to prepare starch acetate (SA). The acetylation reaction was performed by mixing 20 g of dry wild cassava starch, 10 mL acetic anhydride (AA), and 10 mL sodium hydroxide in a 500 mL round bottom flask. Distilled water was used as a control.

Accurately weighed, 20 g dry wild cassava tuber starch was placed in a 500 mL flask, and then 30 mL of distilled water was added and shaken for 5 min to obtain a starch slurry. The starch slurry was placed on a hot plate at 40ºC, 45ºC, 50ºC, and 60ºC with constant stirring using a magnetic stirrer at 3000 rpm until a homogeneous slurry was obtained. Then 10 mL of sodium hydroxide (50% aqueous solution) and 10 mL acetic anhydride (AA) were slowly added dropwise over 20 min. The reaction vessel was sealed, and the reaction mixture was heated to 50ºC and held at this temperature for 2 h while stirring. The pH of the alkaline solution was re-adjusted to 4.5 with hydrochloric acid, after which the slurry was filtered using a Buchner funnel by vacuum filtration and washed several times with distilled water. The resultant filter cake was dried in an oven at 40ºC for 24 h. Then the material was powdered using mortar and pestle, passed through a 224-micrometer mesh sieve, and packed in an air-tight container.

Determination of Degree of Substitution

Exactly 1 g of starch acetate and 50 mL of 75% ethanol were mixed in a flask with a loose stopper. The mixture was stirred for 30 min in a water bath at 50ºC. After cooling to room temperature, 40 mL of 0.5M KOH solution was added to the mixture. The flask was tightened with a tight stopper and kept at room temperature with occasional shaking for 24 h to complete the saponification process. An excess of alkali in the solution was titrated with 0.5M HCl solution using phenolphthalein as an indicator. A blank test was performedby applying the same procedure. The percentage of acetyl group and degree of substitution (DS) was calculated according to equations (1) and (2);

$$Percentage\ of\ acetyl\ group = \frac{blank\ vol. sample\ vol. x\ molarity\ of\ HCl\ x\ 0.043}{sample\ weight} x100 \quad --eqn\ (1)$$

$$DS = \frac{162\ X\ \%\ of\ acetyl\ group}{4300 - (42\ X\ \%\ acetyl\ group)} x100 \quad ------eqn\ (2)$$

Where 162 is the molecular weight of the anhydrous glucose unit, 42 is the molecular weight of the replaceable acetyl group, and 4300 is the molecular weight of the acetyl group attached to 100 anhydrous glucose units.

Physical appearance

The colour and odour of native and modified wild cassava tuber starch were examined by a visual process with the naked eyes and smelled with the nose.

Solubility

The solubility of the starches was determined in water, methanol, and chloroform.[13] Accurately weighed, 2 g of starch was dispersed in 10 mL of each solvent and left to stand overnight. The supernatant solution was filtered, and 5 mL of the filtrate was taken and heated to dryness at 110ºC on a hot plate. The residue was weighed, and then the solubility was calculated using equation (3);

$$\text{Solubility} = \frac{weight}{volume} ------eqn\ (3)$$

Swelling Capacity of the Starch

Swelling experiments were conducted to determine the swelling power of acetylated wild cassava tuber starch using phosphate-citrate buffer solutions of desired pH (4.0–7.4) at 37ºC as a swelling medium.[14] The weighed mass of dry acetylated wild cassava tuber starch was dipped in the swelling medium and left to stand overnight. Acetylated wild cassava tuber starch was removed from the swelling medium by vacuum filtration through a polyamide filter (Sartorius, Spain). The resulting swollen acetylated wild cassava tuber starch (AS) was weighed. Equation 4 was used to calculate the swelling ratio in the form of a percentage (%).[15]

$$swelling\ ratio = \frac{Ws - Wd}{Wd} x100 ------eqn\ (4)$$

Where = Ws represents the weight in the swollen state of a sample at a given time, and Wd is the weight of the dry form of the sample.

Iodine test

The iodine test for starch identification was conducted according to the procedure of the British Pharmacopoeia.[16] An amount equivalent to 1 g of starch was boiled with 15 mL of water and allowed to cool. A few drops of 0.1 M iodine solution were added to 1 mL of the mucilage, and the colour change was recorded.

Granular mMorphology

A starch sample of 0.5 g was mounted on a slide in dilute glycerol and then examined under a light microscope.

Scanning Electron Microscopy (SEM)

SEM was employed to study the starch morphology. The analysis was performed by mounting the starch sample on metal stubs and coated with gold-palladium alloy (15 nm thickness) using the quorum Q150TES vacuum coating machine. The sample was observed using a scanning electron microscope at an acceleration potential of 15 kv; the equipment software captured images. Magnification of 500, 1000, and 2000 was used. The micro images obtained were used to detect any damage in starch granules and to define their forms.

Thermogravimetric Analysis (TGA)

To determine the thermal stability of both native and acetylated starch, thermogravimetric analysis was performed using computer controled thermogravimetric analysis (TGA). The temperature range was maintained at 30ºC to 600ºC and increased at 10ºC/min. The flow rate of nitrogen gas was 20 mL/min.

Fourier-Transformed Infrared Spectroscopic (FTIR) Analysis

The starches were analyzed by (FTIR) in transmission mode. Transmission spectra were recorded using at least 64 scans with 8 cm^{-1} resolutions in the spectral range of 4000- 400 cm^{-1}.

Results and Discussion

Preliminary Test

The extracted starch is a fine, light ash-colored powder with no flavor or smell (Table 1, Figure 1). A test on the native and acetylated starch with an iodine solution revealed that the solution turned blue-black. Additionally, the granular microstructure was examined using a scanning electron microscope, with the results displayed in photomicrographs.

Solubility and swelling power

The solubility and swelling power of native and acetylated wild cassava tuber starches are presented in Table 2. The swelling power of the acetylated *Ampelocissus africana* tuber starch tended to increase with increasing DS. This phenomenon resulted from the weakening of the intermolecular force of attraction due to the incorporation of acetyl groups, which reduces the content of hydroxyl groups.[17] These were similar to the result of Rahim *et al.*,[17] which revealed that swelling

power and solubility of acetylated arenga starches increased with increasing DS, indicating increased hydrophilicity and hydrophobicity of the starches. This also agrees with the work of Das *et al*., 2010, which showed that the swelling power of acetylated sweet potato starch increased with increasing DS from 0.018 to 0.058.[12]

SEM analysis of wild cassava tuber starch

SEM analysis of native and acetylated wild cassava tuber starch as shown in Figures 2 and 3 revealed that the granular starch morphology was rounded and oval but with different granular morphology as a result of chemical modification, this observation is consistent with the work of Aminu (2019) who reported that the particles of Triclosan and Flurbiprofen-loaded Nanogel were mainly spherical.[18]

Acetylation reactions

The extent of acetylation for various starches was primarily influenced by the number of acetyl groups introduced into the starch structure and this was expressed as degree of substitution (DS). According to Singh *et al*.,[19] the DS for acetylated starches varies considerably depending on the source. The characteristics of the starch and differences in the conditions for acetylation reactions may have led to this variation. The percentage (%) acetyl content and DS achieved by acetylation of the native wild cassava tuber starch (*Ampelocissus africana*) are summarized in Table 3.

As shown in Table 3, the range for the percentage acetyl content and DS obtained in this study were around 0.39 – 6.47% and 0.02 – 0.26, respectively. Acetyl content and DS increased with the concentration of acetic acid anhydride and time. The study by Ayucitra (2012) suggested that repeated treatment with acetic acid anhydride may be used if a higher degree of acetylation is desired.[20]

FTIR analysis of both native and acetylated starch

FTIR of acetylated and native wild cassava (*Ampelocissus africana*) tuber starch is shown in Figures 4 and 5. The FTIR spectra indicated the presence of four major absorption peaks: O-H stretch, C-H stretch, C-O stretch, and C=O stretch vibrations, which absorbed around 3300 cm^{-1}, 2900 cm^{-1}, 730 cm^{-1}, and 1700 cm^{-1}, respectively. The results were similar to the findings of Aminu *et al*., 2021.[21]

Thermogravimetric analysis (TGA)

The thermograms for native cassava starch (NCS) and acetylated cassava starch (ACS) are shown in Figures 6 and 7, respectively. The results suggest some increase in crystallinity of the starches. The additional endothermic peaks in ACS samples indicated granule swelling and crystallite melting over the gelatinization range. The ACS showed percentage decompositions of 39.11% and 89.41% successively. The results also indicated that the maximum decomposition occurred within 59.06ºC - 824.09ºC range. Whereas, successive decompositions for NCS were 7.18% and 35.20%, and the temperature range of maximum decomposition was within 103.29ºC - 928.68ºC. The TGA result showed a decrease in the thermal stability of the modified starch compared to the native starch.

Figure 1: The powder of native starch of wild cassava (*Ampelocissus Africana*) tuber

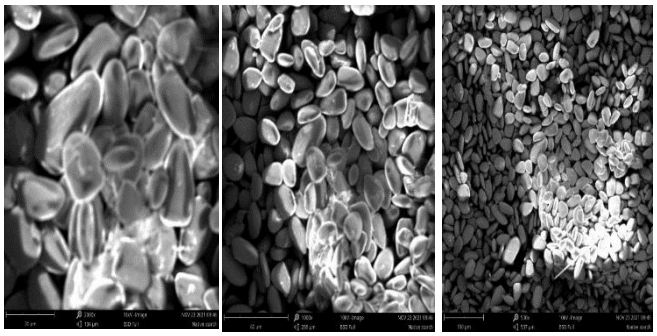

Figure 2: Photomicrographs of Scanning Electron Microscope (SEM) of Native Starch displayed at 2000, B 1000, and C 500 magnifications

Table 1: Results of the Preliminary Test on the Native and Acetylated Cassava Starch

S/No	Analysis	Native starch	Acetylated starch
1	Yield	10.12	
2	Texture	Fine	Fine
3	Odour	Odourless	Odourless
4	Taste	Tasteless	Tasteless
5	Colour	Light ash colour	Milk colour
6	Iodine test	++	++
7	Granular Mophorlogy	Spherical and oval shapes	Spherical, oval shapes and rough
8	pH	7.51	4.51

Table 2: Physicochemical properties of the acetylated starch

Brands	Concentration (M)	Solvent	Temperature (ºC)	Swelling power (%)	Solubility (M)
NS	Nil	Distilled water	Nil	71.5	0.002
C	0.250	Distilled water	60	83.5	0.060
E	0.625	Distilled water	60	95.0	0.120
I	1.000	Distilled water	50	176.0	0.150

Table 3: Conditions and Results of the Acetylation Reaction

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Serial number	Experiment	Concentration (M)	Time (hour)	Temperature (°C)	percentage of acetyl group	Degree of substitution
1	A	0.250	1	40	0.39	0.02
2	B	0.250	2	50	0.56	0.02
3	C	0.250	3	60	0.86	0.03
4	D	0.625	1	50	2.46	0.09
5	E	0.625	2	60	2.80	0.11
6	F	0.625	3	40	1.72	0.07
7	G	1.000	1	60	2.72	0.11
8	H	1.000	2	40	2.16	0.08
9	I	1.000	3	50	6.47	0.26
10	J	1.000	2	50	5.60	0.22

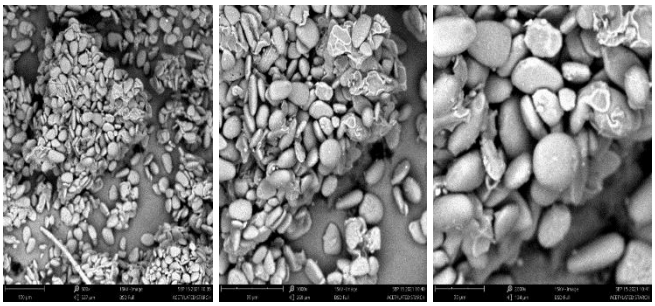

Figure 3: Photomicrographs of Scanning Electron Microscope (SEM) of Acetylated Starch displayed at 500, 1000, and 2000 magnification

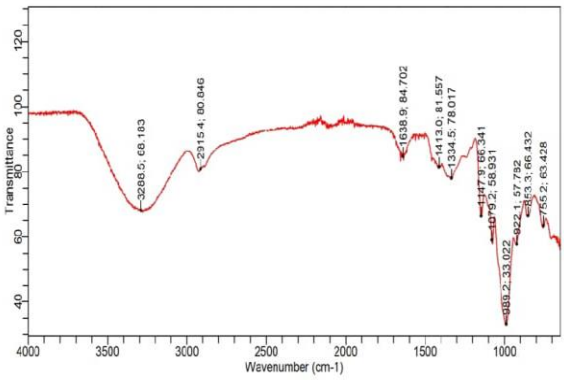

Figure 5: FTIR Spectrum of Acetylated Starch

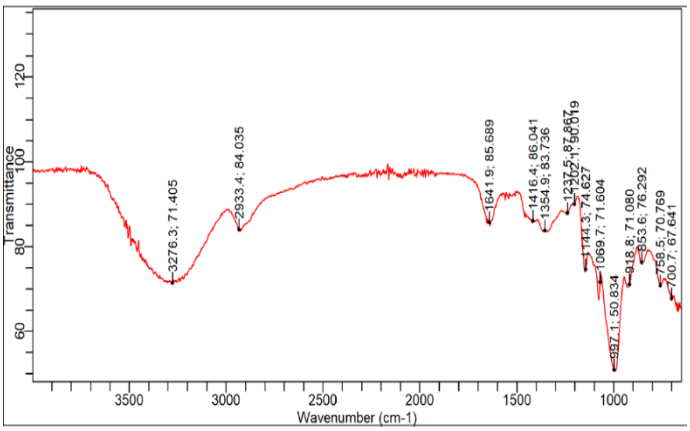

Figure 4: FTIR Spectrum of Native Starch

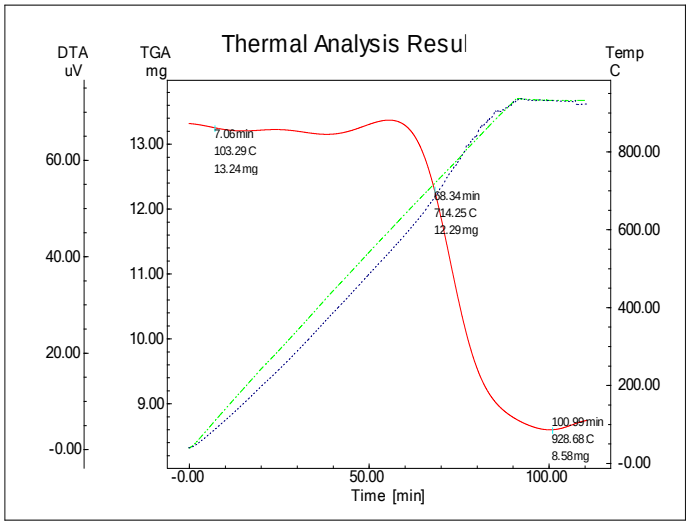

Figure 6: Thermogravimetric Analysis of Acetylated Starch

Conclusion

In this study, the native starch extracted from wild cassava (*Ampelocissus africana*) tuber was modified and evaluated for physicochemical properties. The isolated starch was odourless and tasteless, with a faint ash-off-white colour. From the results obtained from this study, the acetylated wild cassava starch had better physicochemical parameters than the native starch. The findings revealed that the modified wild cassava tubers starch could be a potential excipient for enhanced drug delivery applications.

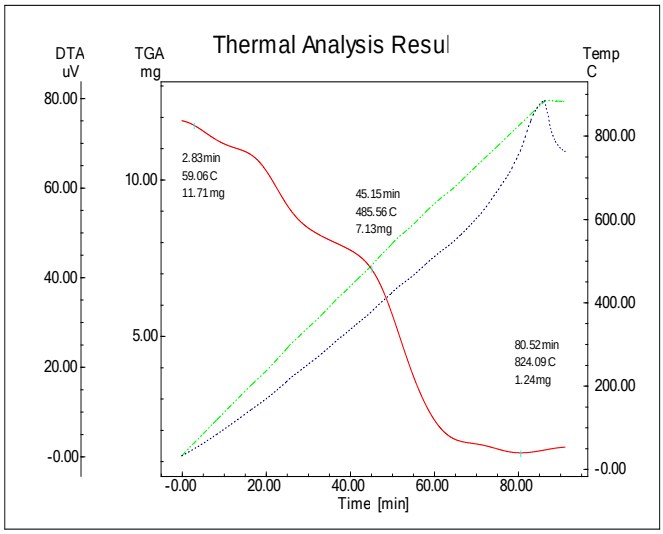

Figure 7: Thermogravimetric Analysis of Native Starch

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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