# EFFECT OF ACETYLATION ON THE PHYSICOCHEMICAL PROPERTIES AND MICROBIAL LOAD OF *Manihot palmata* STARCH FROM MOKWA, NIGER STATE, NIGERIA

*[1]N. C. Nwakife, [2]H. I. Muhammad, [2]M. Musah, [3]E. J. Morah, [1]A. Andrew and [1]M. B. Mohammed,

[1]Department of Chemistry, Federal University of Technology, Minna, Nigeria
[2]Department of Chemistry, Ibrahim Badamasi Babangida University, Lapai, Nigeria
[3]Department of Pure and Industrial Chemistry, Nnamdi Azikiwe University, Awka, Nigeria

*Corresponding email: nkechi.nwakife@futminna.edu.ng
Phone Number: +2348065633472

## ABSTRACT

Starch expediency in industrial applications is well-defined by its physicochemical properties and functionality. Starch in its native form constitutes certain limitations. *Manihot palmata* starch extracted from cassava tubers by wet extraction method was modified by acetylation to improve its properties, using maize starch British Pharmacopeia (BP) as control. The effect of acetic anhydride $(CH_3CO)_2O$ concentrations on the physicochemical properties, as well as the microbial load of the native sweet cassava starch, were studied. The percentage yield of the native cassava starch was 41.56 %; however, the value increased with an increase in acetylation from 89.44 % to 89.92 % for acetylated cassava starch I (ACSI) and acetylated cassava starch II (ACSII) respectively. The acetylation of the cassava starch decreased the pH, moisture content, gelatinization temperature, browning and charring power, and increased amylose/amylopectin content, swelling power, solubility, iodine, acidity, and viscosity. The amylose/amylopectin content, pH, moisture content, viscosity, gelatinization temperature, browning and charring power, solubility in cold water, hot water and ethanol of the acetylated starches were significantly similar at $p < 0.05$ except for the percentage yield and swelling power. Also, the percentage yield, pH, moisture content, swelling power, viscosity, browning and charring power, solubility in cold water and hot water of the NCS and the acetylated starches (ACSI and ACSII) were significantly different at $p<0.05$. The amylose/amylopectin contents of the NCS and acetylated starches were significantly similar but differed from the maize starch. The gelatinization temperatures of the studied starches were significantly similar while the swelling powers differ significantly from each other at $p < 0.05$. The moisture content, and browning and charring power of the maize starch and acetylated starches were significantly similar at $p<0.05$ but differ from that of the NCS. The microbial loads of the native cassava starch decreased; with acetylation and an increase in the concentration of the acetic anhydride and serial dilution. However, the microbial loads increased with incubation time and were within the standard specifications but with much load in the unmodified than the modified starch. The results of the sensory parameters for the modified starches had better properties compared to that of the unmodified starch. Thus, acetylation improved its quality as a valuable raw material in the pharmaceutical and other food Industries.

**Keywords:** *Manihot palmata*, starch, microbial load, physicochemical properties and acetylation

## INTRODUCTION

Cassava (*Manihot esculenta crantz*) as a perennial crop is cultivated mostly in the tropical and sub-tropical regions of the world, over a varied range of environmental and soil conditions [1]. It is very tolerant of drought and heat stress with maximum yield on marginal soils [2]. It is an imperative dietary staple in many countries, where it provides food for more than 800 million people in Africa [3]. As a subsistent crop, cassava is the third most essential carbohydrate food source after rice and



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maize, providing more than 60 % of the daily calorific needs of the populations in tropical Africa and Central America [4]. According to Amelework *et al.* [5], cassava plays a vital role in alleviating food problems because it thrives and produces stable yield under condition in which other crops like yam and sweet potatoes fails and therefore plays a significant part in food security programs. Cassava as a versatile crop can be processed into diverse products such as starch, flour, beverages, and cassava chips for human and animal feed [6].

Starch also known as amylum, is the most abundant carbohydrate (polysaccharide) reserve in plants, second to cellulose, and is widely found in root-tubers [7] The main sources of food starch are yam, maize, amura, potato, cocoyam, wheat, barley, rye, cassava, millet, bambara groundnut, sorghum, banana, beans, acha (fonio), and rice [7-9]. Starch is a versatile compound with widespread applications across various industries. In sectors like food, textiles, cosmetics, plastics, casting, metallurgy, petroleum, chemical, adhesives, medicine, paper, and pharmaceuticals, starch plays a significant role. In the food industry specifically, starch serves as a thickener, clouding or gelling agent, and stabilizer for snacks, meat products, and fruit juices. Beyond food, it functions as an emulsifier, mixing agent, lubricant, and encapsulating agent, and it's used in coatings and sizing in paper, textile, and carports. Starch also finds applications as a colloidal agent, film former, absorbent, water binder, water retention agent, texturizer, fat-sparing agent, bone replacement implants, bone cement, drug delivery systems, and scaffolds in tissue engineering. [10].

Starch modifications have been found to change the surface properties, polarity and linearity of the molecular chains, the degree of substitution, the polymeric, granular, and crystalline structure, amylose to amylopectin ratio, solubility, viscosity, pasting, gelatinization, swelling, water absorption, emulsifying properties, water holding capacity, heat resistant behaviour, reinforcement of its binding, minimized syneresis of starch and improved thickening. Starch is readily converted physically, chemically, and biochemically into many useful products. Various chemical modification techniques include acid hydrolysis, oxidation, etherification, esterification, cationization, cross-linking, and graft polymerization. Chemical modification of starch is based on the chemical reactivity of the constituent glucose monomers which are polyhydroxyl and can undergo several reactions. Starch modification through esterification involves starch chain reactions with inorganic and organic acids, salts of inorganic acids, and anhydrides or chlorides of organic acids. In acetylation, hydrophilic hydroxyl groups are substituted with hydrophobic acetyl groups ($CH_3CO$-) to produce a specific ester using reactive reagents such as anhydrous acetic acid, and vinyl acetate in the presence of an alkaline catalyst ($NaOH$, $KOH$, $Ca(OH)_2$ and $Na_2CO_3$). Acetylation makes starch more hydrophobic and prevents the formation of hydrogen bonding between hydroxyl groups, and water molecules, helps starch swell at relatively low temperature, and weaken the granule



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structure, preventing molecular re-association and realignment required for retrogradation.

Native cassava starch despite its worth has limitations such as high moisture content, poor absorptivity, poor compressibility, low shear resistance, low thermal resistance, ease of thermal decomposition, and high tendency towards retrogradation (crystallization and aging of gels), which limit its use in some industrial and food applications. Also, starches are prone to microbial attack and this is occasioned by the physiological reactions and activities of microorganisms that enter the cassava roots through injuries during harvesting, the use of contaminated water and unhygienic practices during the traditional processing of cassava into starch which results in bacteria and mould contamination. Therefore, there is a need for modification and characterization of locally available cassava starch for possible industrial applications.

## MATERIALS AND METHODS

### *Sample Collection*

Matured tubers of sweet cassava (*Manihot palmata*) were obtained from a farm in Mokwa Local Government Area, Niger State, Nigeria.

### *Preparation, Pretreatment and Extraction of Cassava Starch*

Starch was extracted using the wet extraction method. The cassava tubers were peeled, washed, sliced, and milled in a grating machine to obtain smooth slurry. The slurry was further mixed with water to form free-flowing slurry which was then filtered through a muslin cloth. The retained solids were exhaustively rinsed on the muslin cloth with distilled water. The filtrate was allowed to stand overnight, the precipitate was collected, and the supernatant was discarded. The precipitate was suspended until white starch was obtained. The starch was dried in an oven. Finally, the starch was ground and sieved through a 100 µm-mesh sieve. The cassava starch was kept in a light resistance container and stored at 4 $^{o}$C for further analysis [11].

### *Preparation of Acetylated Starch (ACS)*

Using an analytical weighing balance, 100.00 g of the native cassava starch was dispersed in 500.00 $cm^3$ of distilled water and stirred magnetically for 20 minutes. The pH of the slurry was adjusted using 1.00 $moldm^{-3}$ HCl and 1.00 $moldm^{-3}$ NaOH until 8.0 was obtained. 10.20 g of acetic anhydride was added slowly and over a period of one hour to the mixture while maintaining pH range of 8.0-8.5. The reaction was allowed to proceed for 5 minutes after the addition of acetic anhydride. The pH of the slurry was finally adjusted to 4.5 using 0.50 $moldm^{-3}$ HCl. It was then filtered, washed four times with distilled water, and air-dried at 30±2.0 $^{o}$C for 48 hours [12]. The procedure was repeated for 15.20 g acetic anhydride. These were labelled accordingly as ACS I and ACS II.

### *Physical (Sensory) Properties of Starch*

Physical (sensory) Properties (taste, aroma, colour, and texture) were determined using the sense organs for the native cassava and acetylated starches.



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***Preparation of the Medium for Bacteria***

With the aid of an analytical balance, 1.00 g of the native cassava starch was dissolved in 10.00 $cm^3$ of pre-sterilized buffer sodium chloride peptone solution and the pH was adjusted to 7.0. Four test tubes were taken, and 9.00 $cm^3$ of the buffer sodium chloride peptone solution was transferred into all the test tubes and autoclaved at 121 °C for 15 minutes. The serial dilutions of $10^{-1}$, $10^{-2}$, $10^{-3}$ and $10^{-4}$ (dilution factors) were made by transferring 1 $cm^3$ of the previous dilution in the autoclaved test tubes with buffer solution. Then, 1.00 $cm^3$ each of the dilution factor was taken into pre-sterilized petri dishes, followed by the pour plate technique using Nutrient Agar as the media, and the plates were then incubated at 37 °C for 24 hours and 48 hours respectively. This was examined for microbial colony forming unit (CFU). The procedure was repeated for the maize and acetylated starches [13].

***Preparation of the Medium for Fungi***

With the aid of an analytical balance, 1.00 g of the native cassava starch was dissolved in 10.00 $cm^3$ of pre-sterilized buffer sodium chloride peptone solution and the pH was adjusted to 7.0 (Step 1). Four test tubes were taken, and 9.00 $cm^3$ of buffer sodium chloride peptone solution was transferred into all the test tubes and autoclaved at 121 °C for 15 minutes (Step 2). The serial dilution of the pretreated sample from step 1 was made for $10^{-1}$, $10^{-2}$, $10^{-3}$, and $10^{-4}$ (dilution factors) using the test tubes from step 2. 1.00 $cm^3$ of each dilution factor was taken into a pre-sterilized petri dish followed by pour plate technique using Sabouraud dextrose agar as the media and the plates were then incubated at 25 °C for 24 hours and 48 hours. These were examined for microbial colony forming unit (CFU) [12]. The procedure was repeated for the maize and acetylated starches.

***Physicochemical Properties of Native and Modified Starches***

The starch samples were analysed for pH, iodine, acidity, swelling capacity, solubility, moisture content, amylose/amylopectin content, viscosity, browning and charring temperature, gelatinization temperature, and percentage yield using standards methods.

***pH***

Using an analytical balance, 5.00 g of the native cassava starch was weighed into a beaker, 15.00 $cm^3$ of distilled water was added and this was properly mixed. The mixture was poured into boiling distilled water and made up to the 100 $cm^3$ mark. The slurry was allowed to cool. The pH of the slurry was measured using blue line digital pH meter (Schott GG8842/14pH). The pH meter probe was rinsed before and after the measurement. The determination was done in triplicates and the mean value was considered as the pH of the starch. The procedure was repeated for the maize and acetylated starches [14].



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***Acidity Test***

The native cassava starch (10.0 g) was weighed and added to 70 %v/v alcohol which was previously neutralized using phenolphthalein solution as an indicator. The mixture was shaken for an hour, filtered and 50.00 cm$^3$ of the filtrate was titrated with 0.10 moldm$^{-3}$ NaOH solution. The colour change was observed as the titration was done in triplicate. The mean value was considered. The procedure was repeated for the maize and acetylated starches [14].

***Moisture Content***

With the aid of a weighing balance, 5.00 g of the starch sample was weighed and dried to constant weight at a temperature of 105 ºC, the loss in weight was determined and expressed as percentages moisture content [15]. The procedure was repeated for the maize and acetylated starches.

Moisture content (%) = $\frac{w_1 - w_2}{w_1} \times 100$

***Amyloses/Amylopectin Content***

With the aid of a weighing balance, 20.00 mg of the native starch sample was taken and 10.00 cm$^3$ of 0.50 moldm$^{-3}$ KOH was added. The suspension was thoroughly mixed; the dispersed sample was transferred into a 100 cm$^3$ volumetric flasks and diluted to the mark with distilled water. 10.00 cm$^3$ of the solution was pipetted into a 50 cm$^3$ volumetric flasks, 5.00 cm$^3$ of 0.10 moldm$^{-3}$ HCl and 0.50 cm$^3$ of iodine reagent were added. The volume was made up to 50 cm$^3$ and the absorbance was measured at 625nm. The procedure was repeated for the maize and acetylated starches. The measurement of the amylose/amylopectin was determined from a standard curve [16].

***Swelling Capacity***

The method described by Agbo and Odo [17] was used to determine the swelling capacity. One (1.00) g of the native starch sample was weighed into a test tube, 10.00 cm$^3$ of distilled water was added, and the mixture was heated in a water bath at a temperature of 50 ºC for 30 minutes with continued shaking. At the end, the test tube was centrifuged at 1500 rpm for 20 minutes in order to facilitate the removal of the supernatant which was carefully decanted, and the weight of the starch paste taken. The procedure was repeated for the maize and acetylated starches.

The swelling capacity was calculated as follows:

Swelling power = $\frac{weight\ of\ starch\ paste}{weight\ of\ dry\ starch} \times 100$

***Gelatinization Temperature***

This was evaluated using the method of Agbo and Odo [17]. One (1.00) g of the native starch sample was weighed into a 20 cm$^3$ beaker, 10.0 cm$^3$ of distilled water was added and the dispersion was heated on a hot plate. The gelatinization temperature was taken using a digital thermometer suspended in the starch slurry. The procedure was repeated for the maize and acetylated starches.

***Browning and Charring Temperature***

The method of Omojola *et al.* [18] was used. The native starch sample was put into a capillary tube, the browning and charring temperature was determined using a melting point apparatus.



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***Solubility***

The method of Ikegwu *et al* [19] with slight modifications was used. Two (2.00) g of the native starch sample each was dispersed in 10.0 $cm^3$ cold water, hot water and ethanol. These were left to stay overnight. Five (5.00) $cm^3$ each of the clear supernatant solutions was taken and heated to dryness over a water bath. The weight of each of the dried residues with reference to the volume of the solution was determined accordingly as the percentage solubility of the starch in the solvents. The procedure was repeated for the maize and acetylated starches.

$$\text{Solubility (\%)} = \frac{dry\ supernatant\ weight}{initial\ sample\ weight} \times 100$$

***Iodine Test***

This was carried out using the method described by Muazu *et al*. [14] with slight modification: 1.00 gram of the native cassava starch was suspended in 50.0 $cm^3$ of distilled water. This was boiled for 1 minute and allowed to cool. To 1.00 $cm^3$ of the mucilage's, 0.05 $cm^3$ of iodine solution was added and the absorbance was taken at 625 nm. The procedure was repeated for the maize and acetylated starches.

***Viscosity***

The native cassava starch (10.0 g) was prepared in 10.0 $cm^3$ of cold distilled water to form slurry. 250 $cm^3$ boiled water was added to the slurry and the resultant mixture was heated to about 70 ºC on a thermostatic water bath till translucent mucilage was formed. The viscosity of the starch mucilage was determined using a rotational viscometer (KINEMATICA, AG Type Viscometer + L, Switzerland) with spindle number 4 at shear rate of 20 rpm at room temperature. Slurries of acetylated and maize starches were prepared by reconstituting the appropriate amount of starches in cold water [20].

***Percentage Yield***

The percentage yield of the starch was determined by the method of Hasmadi *et al.* [21] Cassava was combined with water at a proportion of 1:10 and vigorously blended until smooth slurry was obtained. Following the blending, the slurry was filtered through a double layer of cheesecloth. The filtered mixture was then left undisturbed for two hours to facilitate the sedimentation process, causing the starch to settle while the liquid on top was poured out and discarded. The resulting sediment was then dried at 45°C for 24 hours and placed in an airtight container for later analysis. The percentage of extraction yield was calculated using the formula:

$$\text{Percentage Yield} = \frac{\text{Starch weight (g)}}{\text{Cassava weight (g)}} \times 100$$

***Data Analysis***

The analyses were carried out in triplicate. Data were subjected to a one-way analysis of variance (ANOVA) and Duncan Posthoc test was used to determine the significant difference at 95 % confidence level using the statistical software, Statistical Package for Social Sciences, SPSS.



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## RESULTS AND DISCUSSION

**Table 1: Physical Properties of the Standard Maize Starch, Native and Modified Cassava Starches**

| Properties | MS | NCS | ACSI | ACSII |
|---|---|---|---|---|
| Odour | Odourless | Odourless | Odourless | Odourless |
| Colour | White | Off white | White | White |
| Taste | Tasteless | Tasteless | Tasteless | Tasteless |
| Texture | Smoother | Smooth | Smoother | Smoother |

**KEY:** MS- Maize starch, NCS- Native cassava starch, ACSI- Acetylated cassava starch one ACSII- Acetylated cassava starch two

The sensory parameters of the standard maize starch, native and modified cassava starch are presented in Table 1. The results showed that the colour and texture of the NCS were improved as they became whiter and smoother respectively after chemical modification. This implies that the physical properties of the NCS were tampered with during the acetylation. The whiter appearance is induced by the removal of impurities and other non-starch components while the smoother texture is as a result of the enhanced solubility and increased swelling capacity as the NCS particles absorb more water and swell uniformly. The result obtained for the modified cassava starches agreed with the result of maize starch describe by British Pharmacopeia [22]. This was similar to the observation of Muazu *et al.* [14] for Kaffir potatoes starch.

**Table 2: Microbial Properties (Bacteria) of the Standard Maize Starch, Unmodified and Modified Cassava Starch (CFU/g)**

| Starch | Dilution media | Dilution Factor | | | | |
|---|---|---|---|---|---|---|
| | | $10^{-1}$ | $10^{-2}$ | $10^{-3}$ | $10^{-4}$ | Control |
| MS | Nutrient agar at 24hr incubation | 06 | 03 | 01 | 0 | 0 |
| | Nutrient agar at 48hr incubation | 11 | 06 | 04 | 01 | 0 |
| NCS | Nutrient agar at 24hr incubation | 36 | 29 | 13 | 08 | 0 |
| | Nutrient agar at 48hr incubation | 58 | 32 | 20 | 15 | 0 |
| ACSI | Nutrient agar at 24hr incubation | 09 | 07 | 06 | 01 | 0 |



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| | | | | | | |
|---|---|---|---|---|---|---|
| | Nutrient agar at 48hr incubation | 16 | 14 | 10 | 03 | 0 |
| ACSII | Nutrient agar at 24hr incubation | 08 | 07 | 05 | 01 | 0 |
| | Nutrient agar at 48hr incubation | 16 | 13 | 10 | 02 | 0 |

**KEY:** MS- Maize starch, NCS- Native cassava starch, ACSI- Acetylated cassava starch one ACSII- Acetylated cassava starch two

The results of the microbial load of the extracted native cassava, standard maize and modified cassava starches are as shown in Table 2. The native cassava starch has the highest microbial load which might be due to bacteria and moulds contamination from injured roots during harvesting and other activities such as handling and processing. The microbial load of the cassava starches decreased with increase in acetylation and serial dilution. This is attributed to the purifying efficiency of the acetylation and reduction in quantity of the microorganism in the media. However, the CFU/g of the cassava starches increase from 24 hrs to 48 hours incubation and were below 100 CFU/g. The British Pharmacopeia [22] specified that the total aerobic count for starch to be used as an excipient in pharmaceutical dosage formulation should not exceed 100 colonies forming unit (CFU) per gram for synthetic and semi-synthetic substances. Also, the total aerobic count should not exceed 1000 colonies forming unit per gram for excipient from natural origin such as cassava starch. This implies that the results of the microbial load of the starches in this study are in compliance with the specification by BP [22].

**Table 3: Microbial Properties (Fungi) of the Standard Maize Starch, Unmodified and Modified Cassava Starch at different Time of Incubations and Serial Dilutions**

| **Starch** | **Dilution media** | **Dilution factor at** | | | | |
|---|---|---|---|---|---|---|
| | | **$10^{-1}$** | **$10^{-2}$** | **$10^{-3}$** | **$10^{-4}$** | **Control** |
| MS | Sabouraud dextrose agar at 24hr incubation | 04 | 03 | 01 | 0 | 0 |
| | Sabouraud dextrose agar at 48hr incubation | 09 | 07 | 04 | 0 | 0 |
| NCS | Sabouraud dextrose agar at 24hr incubation | 43 | 36 | 18 | 15 | 0 |



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| | | | | | | |
|---|---|---|---|---|---|---|
| | Sabouraud dextrose agar at 48hr incubation | 67 | 51 | 23 | 21 | 0 |
| ACS1 | Sabouraud dextrose agar at 24hr incubation | 15 | 14 | 09 | 05 | 0 |
| | Sabouraud dextrose agar at 48hr incubation | 18 | 16 | 10 | 09 | 0 |
| ACSII | Sabouraud dextrose agar at 24hr incubation | 14 | 12 | 09 | 05 | 0 |
| | Sabouraud dextrose agar at 48hr incubation | 17 | 14 | 11 | 08 | 0 |

**KEY:** MS- Maize starch, NCS- Native cassava starch, ACSI- Acetylated cassava starch one
ACSII- Acetylated cassava starch two

The fungi properties of the standard maize starch, native and modified cassava starches are as shown in Table 3. The NCS has the highest fungi CFU/g than the acetylated and maize starch. This could be as a result of high moisture content in the native cassava starch (Table 4) and must have provided more conducive environment for the growth of fungi. Fungal proliferation can lead to discolouration of the cassava starch, production of off smells and give rise to rotten taste. The result also shows that the total fungi count for the maize starch, native and acetylated starches decrease with increase acetylation and serial dilution and were below 50 colonies forming unit (CFU) per gram as specified by BP [22] except for the native cassava starch that was 67 and 51 colonies forming unit (CFU) per gram at dilution factor of $10^{-1}$ and $10^{-2}$ respectively after incubation at 48hrs. This is an indication of its potential to spoil and might lead to food poisoning if consumed. British Pharmacopeia [22] states that for starch to be used as an excipient in pharmaceutical dosage formulations, the total fungi count for synthetic and semi-synthetic substance should not exceed 50 colonies forming unit (CFU) per gram and for excipient from natural origin. This implies that the result of the fungi load for the extracted cassava starch after modification is in compliance with specification [22]. Thus, sweet cassava starch collected from Mokwa L.G.A., Niger State after modification can be used as an excipient in pharmaceutical formulation.

**Table 4: Physicochemical Properties of the Standard Maize Starch, Unmodified and Modified Cassava Starch**

| Properties | MS | NCS | ACSI | ACSII |
|---|---|---|---|---|
| Percentage yield (%) | -- | 41.56±0.12$^{c}$ | 89.44±0.12$^{b}$ | 89.92±0.12$^{a}$ |
| Amylose/amylopectin content (% w/w) | 19.00±0.58$^{a}$ | 17.00±0.58$^{b}$ | 17.40±0.12$^{b}$ | 17.50±0.12$^{b}$ |



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| | | | | |
|---|---|---|---|---|
| pH | 5.78±0.12 c | 8.76±0.12 a | 6.09±0.12 bc | 6.18±0.12 b |
| Moisture content (% w/w) | 5.60±0.12 b | 8.38±0.12 a | 5.59±0.12 b | 5.58±0.12 b |
| Swelling power (%) | 8.77±0.12 a | 7.01±0.06 d | 8.06±0.06 c | 8.51±0.06 b |
| Solubility in cold water (% w/v) | 2.20±0.12 b | 2.04±0.12 b | 2.86±0.12 a | 2.89±0.12 a |
| Solubility in hot water (% w/v) | 19.67±0.12 b | 15.00±1.15 c | 23.40±0.12 a | 23.42±0.12 a |
| Solubility in Ethanol (% w/v) | 1.90±0.12 a | 1.88±0.12 a | 1.91±0.12 a | 1.97±0.12 a |
| Iodine test (at 625nm) | 0.72±0.12 a | 0.67±0.12 a | 0.60±0.12 a | 0.50±0.12 a |
| Titratable Acidity ($cm^3$) | 1.40±0.12 a | 0.80±0.12 b | 1.10±0.12 ab | 1.30±0.12 a |
| Viscosity (mpas) | 3036.13±5.77 a | 2016.60±5.77 c | 2481.90±11.55 b | 2488.65±11.55 b |
| Gelatinization temperature ($^0C$) | 59.00±5.77 a | 65.00±5.77 a | 58.00±5.77 a | 57.00±5.77 a |
| Browning and charring power ($^0C$) | 195.00±11.55 b | 265.00±11.55 a | 215.00±11.55 b | 205.00±11.55 b |

Means on the same row with different superscripts are significantly different at $p<0.05$

**KEY:** MS- Maize starch, NCS- Native cassava starch, ACSI- Acetylated cassava starch one

ACSII- Acetylated cassava starch two

The result of the physicochemical properties of the standard maize starch, native and modified cassava starches is as shown in Table 4.

The percentage yield of the native cassava starch is 41.56% on dry basis starch, while after modification, the percentage yield increased to 89.44 % and 89.92 % for ACSI and ACSII respectively. The percentage yield of both the NCS and the acetylated starches (ACSI and ACSII) were significantly different at $p < 0.05$. This observation might be due to the substitution of the hydroxyl groups on the starch molecules and also the effect of swelling of starch polymer as a result of increased acetylation. Previous works on chemically modified African star apple kernel (*Chrysophyllum albidnum*) starch and starch from *Musa paradisiaca* L. [23] also showed similar pattern. In addition, the percentage yield of the native cassava starch in this study was higher than 10.50 % and 20.00 % for yam and cassava respectively reported by Oni *et al.* [7].

The amylose/amylopectin contents of the starches in this study range are 17.0-19.0 %. The modified cassava starches (ACSII and ACSI) had higher values (17.50 and 17.40 % respectively) of amylose/ amylopectin content compared to the native starch (17.0 %) and were lower than the 19.0



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% recorded for the maize starch. The amylose/amylopectin contents of the NCS and acetylated starches were significantly similar but differed from the maize starch at $p<0.05$. This can be attributed to the presences of the acetyl groups that interfered with the amylose/ amylopectin fractions of the starch [24] and possibly affected the absorption of iodine for the amylose/amylopectin estimation, thereby leading to an increase in the value of amylose/amylopectin content. Also, the mean difference between the NCS and acetylated starches could result in the modified starches showing high volume of expansion and a high degree of flakiness. Agbo and Odo [17], also reported an increase in amylose/amylopectin content with an increase in acetylation.

pH is an indicator of the degree of acidity and alkalinity of substance. It is necessary that the pH of starch be near neutrality to be used effectively in industries where changes in pH of product are required. The pH of the MS, ACSI and ACSII were slightly acidic (5.18, 6.09 and 6.18 respectively) and were within the acceptable limit of 4.5-8. The pH of the maize starch was more acidic than the acetylated starches. The pH of NCS (8.76) was slightly alkaline and was a little above the acceptable limit. More so, the results showed that the pH decrease in acidity with increase in acetylation and this could be as a result of the reaction with the acetic anhydride. The nearness of the modified starches to pH 7 could be advantageous because neutral pH might decrease tendency of interaction of excipient with active pharmaceutical ingredient [14]. The pHs of the acetylated starches were significantly similar but differ from the NCS. ACSI was significantly similar to the maize starch at $p<0.05$.

Moisture content is a very important physicochemical property as it affects other properties of material such as flow property and stability of a product [25]. High moisture content in formulations might interfere with active ingredient, and therefore, its regulation is very important. The moisture contents for the MS, NCS, ACSI and ACSII did not exceed the limit of 15.0 % as specified in the British Pharmacopeia [22]. However, NCS had the highest moisture content (8.38 %) while the ACSII had the least value (5.58 %) which might be as a result of the hydrophilic hydroxyl groups being substituted with hydrophobic acetyl groups. The moisture content of the maize starch (5.60 % ) and acetylated starches; ACSI and ACSII (5.59 % and 5.58 % respectively) did not differ significantly at $p<0.05$ but differ from that of the NCS. This implies that the acetylated starches compared favourably with the standard maize starch. The moisture content of the cassava starch decreased with an increase in acetylation thus increased purity of the modified starches. A similar trend was observed for both cassava and yam starches [7], modified starches from acha (*Digitaria stapf*) grains [26] and chemically modified potato, corn and taro starch [27]. The low moisture contents of the modified ACSI and ACSII also indicate a possible prolonged shelf life and keeping quality of the starches because they will be less prone to



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microbial attack. Similar observations of low moisture content have also been reported by Dolas *et al.* [8], although with slight difference which could be as a result of different method used in heating at different temperature and time. However, the moisture content of the native cassava starch (8.38 %) and the acetylated starches: ACSI (5.59 %) and ACSII (5.58 %) in this study were lower than 13.75% and 12.45 % reported by Oni *et al*. [7] for cassava and yam starches respectively.

The standard maize starch has swelling power of 8.77%. This is higher than the swelling power of ACS II (8.51%) which is also higher than ACSI (8.06%) and NCS (7.01%) showing that the swelling capacity was enhanced with increase acetylation. This could be as a result of the acetyl groups introduced into starch molecules by acetylation leading to structural reorganization due to steric hindrance; a repulsion between starch molecules, hence enabling an increase in water percolation occasioned by their ability to form hydrogen bonds within the amorphous regions of granules. The swelling powers of all the studied starches differ significantly from each other at $p < 0.05$. The trend in this study is deviant from the finding of Agbo and Odo [17] and in agreement with [9]. An increase in swelling power suggests that a starch is suitable for use as a disintegrant in pharmaceuticals. Modified cassava starches could potentially replace maize starch in tablet formulation, given their swelling properties within the temperature range typically used for tablet production. Also, high swelling power results into high digestibility and ability to use starch in solution suggesting improved dietary properties and the use of starch in a range of dietary applications.

Solubility of starch increases with increase in swelling power. This is because as starch granules swell, the intragranular bonds are weakened and the loosely held starch molecules mostly amylose leach out into the continuous medium. The starches were more soluble in hot water, followed by cold water and least soluble in ethanol. The solubility values of the acetylated starches were higher than the unmodified native starch in ethanol, cold and hot water. The solubility values of the modified cassava starches compared favourably well to the maize starch although with slight difference. Statistically, the solubility's of the acetylated starches (ACSI and ACSII) in cold water were significantly similar but differed from those of the NCS and maize starch; which were significantly similar. Also, the solubility's of the acetylated starches in hot water were significantly similar but differed from those of the NCS and maize starch; that significantly differ from each other. More so, the solubility's in ethanol of the studied starches were significantly similar at $p < 0.05$. The solubility of the native cassava starch also increases with increase acetylation. Similar trends were observed by Dolas *et al.* [8] and Olayinka *et al.* [9]. This might be due to the presence of hydrophilic substituting groups that retain water [8]. The 15.00 % solubility value in hot water for the native cassava starch in this study was lower than the 25.18 % for native cassava starch



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reported by Dolas *et al.* [8]. This might be due to the heating temperature and time.

The MS had the highest viscosity (3036.13 mpas) followed by ACSII (2488.65 mpas), ASCI (2481.90 mpas), and NCS (2016.60 mpas). The higher viscosity of the MS could be due to the degree of modification and high level of amylose/amylopectin content. The viscosities of the acetylated starches were significantly similar but differ from the NCS and maize starch; that differ significantly. The viscosity of the native cassava starch increased with increase in acetylation and could be due to the increase in amylose/amylopectin content of the modified starches. This is an indication that the modified starch will be good for pharmaceutical dosage formulation and also for food industry especially where the viscosity of a starch is a prerequisite. These results agreed with the findings on modified Starch from Banana done by Bello-Pérez *et al.* [23].

Gelatinization is the process whereby starches in the presences of water undergo an irreversible change under heat. The starch granules absorb water and swell up thereby forming a paste rather than a dispersion which it forms in cold water. The ACSI and ACSII were observed to have gelatinization temperatures of 58°C and 57°C respectively while the NCS has a gelatinization temperature of 65°C. This implies that the modified starches will gelatinize faster than the unmodified starches. So at lower temperatures, the modified starches will form a gel/paste. MS and NCS have gelatinization temperatures of 59°C and 65°C respectively which were within the range of gelatinization temperature commonly observed for starches. The gelatinization temperature of the studied starches has no significant difference at $p < 0.05$. Gelatinization temperature decrease with increase acetylation and has similar trend with the findings of [17].

The browning and charring power of the acetylated starches and maize starch were significantly similar but differ from the NCS. The browning and charring temperature decreased with increase acetylation. The acetylated starches were observed to have lower browning and charring temperature than that of the unmodified. However, these were higher than that of the maize starch. This showed that the acetylated starches can be heated to a higher temperature without changing colour or charring when compared to the maize starch. This quality will make the acetylated starches preferable in industries that use starch at higher temperatures. Omojola *et al* [18] observed a similar pattern of decrease in browning and charring temperature after modification.

## CONCLUSION

The effect of acetylation on the physicochemical properties and microbial load of the cassava starch was studied. On the basis of the result of this study, it is evident that the modification processes were effective in altering the characteristics of the native cassava starch (NCS). Increasing acetylation and serial dilution decreased microbial loads in starch, meeting standard specifications, but unmodified starch had higher loads than modified starch.



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Acetylation of native cassava starch decreased pH, moisture content, gelatinization temperature, browning, and charring power, while increasing yield, amylose/amylopectin content, swelling power, solubility, viscosity, iodine test results, and acidity. The starch locally collected from Mokwa Local Government Area could compete favourably with maize starch B.P as pharmaceutical excipients and therefore may serve as an alternative in the pharmaceutical and other food industries.

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