World Journal of Applied Chemistry
2017; 2(3): 109-115
http://www.sciencepublishinggroup.com/j/wjac
doi: 10.11648/j.wjac.20170203.16

Isolation and Characterization of Starch from Unripe Plantain (*Musa Paradisiaca*) Peels

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To cite this article:

Ezeh Vincentia Ogechukwu, Obidiegwu Martin Uzodinma, Uzoma Paul Chinonso, Obasi Henry Chinedu. Isolation and Characterization of Starch from Unripe Plantain (*Musa Paradisiaca*) Peels. *World Journal of Applied Chemistry*. Vol. 2, No. 3, 2017, pp. 109-115.
doi: 10.11648/j.wjac.20170203.16

Received: July 25, 2017; **Accepted**: August 2, 2017; **Published**: September 4, 2017

Abstract: Starch isolated from unripe plantain (*Musa Paradisiaca*) peels was characterised for proximate composition (moisture content, ash content and pH) and functional properties. The morphology of the starch granule and particle size were determined using scanning electron microscopy technique. The yield of starch from the unripe peels was 1.86% (1.86g/kg) on a dry weight basis, and the starch contained 12.60% moisture, 0.47% ash and had a pH value of 7.49 at 25°C. Studies on the functional properties gave values of bulk density, tapped density, particle density, water binding capacity, fat binding capacity and dispersibility as 0.46g/ml, 0.56g/ml, 1.18g/ml. 1.662 (166.2%), 1.3848 (138.48%) and 84% respectively. The morphology of the starch granule indicated a smooth, irregular, rod-like and highly elongated granule. The analysis of some of the flow properties of starch showed the good flowability property of plantain peel starch. Results obtained from proximate and physicochemical analysis portrayed the potentials of unripe plantain peel starch for industrial applications, especially in the pharmaceutical industries.

Keywords: *Musa Paradisiaca*, Proximate Composition, Functional Properties, Starch, Pharmaceutical Industries

1. Introduction

Starch is a polysaccharide of glucose stored as accumulated energy in many plants. The energy is built up during photosynthesis because of carbon fixation. And starch is stored in tiny granules in plant tissue and organs including leaves, roots, stems, shoots, fruits, and grains. It is also the second most abundant natural and biodegradable polymer after cellulose. Starch has attracted diverse applications ranging from food consumption to raw material for many industries. This is premised on its renewability, availability at low cost, high caloric value, excellent physicochemical properties and the ease of modification to other derivatives [1].

Different botanical sources have been exploited for the commercial production of starch. The majority of these which are food sources include corn, wheat, potato, cassava, rice, sorghum, barley, rye, yam, cocoyam, millet, etc. Corn starch represents the largest source of starch constituting 80% of global starch production [2]. Other minor sources include jackfruit, breadfruit, screw pine, sago palm, plantain, cowpea, arrowroot (Tacca species), etc. [3].

Naturally, starch was designed as energy reserves for plants. However, man has expanded the applications of starch beyond this original design [4]. Aside from man's food, starch plays a significant role in the food and beverage industries serving as thickener, emulsion stabiliser, film former, softener, texturizer, colourant and viscosity controller [4]. In the non-food industries like the paper industry, starch is employed as flocculants and retention aid, bonding agent, surface size, a binder for coatings and as an adhesive. In the textile industry, starches are used for warp sizing of fabrics before weaving, for sizing or finishing woven cloth and in printing certain types of fabrics [5]. Other applications are seen in the polymer industry, petroleum industry, water treatment industry, etc. As starch continues to garner more applications to itself, problems are created for food sufficiency. At present, about 54% of starch produced

globally is used for food use while 46% are used for non-food application [1]. Recently, research interests have focused on the possibilities of exploiting non-food sources of starch for industrial purposes to alleviate the competition with food consumption. For this reason, unripe plantain peel was used in this study.

Unripe plantain peels (skins) are waste materials left after the pulp is consumed. Food processing and other applications that convert unripe plantain pulp into starch, noodles, chips, flour, bread and other food ingredients, etc. generate a significant amount of peels that are discarded as waste. In rural areas, the peels serve as feed for livestock. In the cities where these ruminants are scarce, the peels constitute an environmental nuisance. The peel contains 35-40% (i.e. 35-40g/100g) of the weight of the pulp (wet basis) [6] and possesses high nutritional value. On a dry basis, the peel holds 6-10% protein, 6-12% ash, 2-6% lipids, 11- 39% starch, and 33-43% total dietary fibre (TDF). The TDF comprises 5-13% soluble dietary fibre (SDF) and 7-36% insoluble dietary fibre (IDF) [7]. Pectin ranging from 13.0-21.7 g/100g and gums (xanthan, arabic, guar, etc.) are present in the SDF. But in the IDF, the cellulose, hemicelluloses, and lignin are included with contents varying from 7-12 g/100 g, 6.4-9.6 g/100 g and 6.4-8.4 g/100 g, respectively [7]. Studies have also shown that the peels are good sources of polyphenols, carotenoids, and other bioactive compounds which are beneficial to human health [8]. Ighodaro [9] observed that the peels contain mineral elements like sodium, calcium, magnesium, potassium and iron, all in high amounts while nitrogen and phosphorous were present in little amounts. The high level of calcium (147.3g/100g) as suggested may be of great physiological significance especially in part of the world were muscle weakness, increased nervous system irritability and spontaneous action potential generation in neurones are relatively rampant. The peels also contain a substantial amount of iron and are important in areas of blood formation and overall improvement of the haemopoietic system. Plantain peels are also rich in essential amino acids (leucine, valine, phenylalanine and threonine) and polyunsaturated fatty acids particularly linoleic acid and α- linolenic acid [10].

Much interest has not been given in the study of the properties of starch isolated from unripe plantain peels. Hence, this study aims at examining the physicochemical and morphological properties of starch isolated from unripe plantain peels.

2. Materials and Method

2.1. Materials

Unripe plantain peels were sourced from wastes generated at Ochanja Market, Onitsha Anambra State, Nigeria. Analytical grade chemicals were used and as received. The chemicals include Sodium Metabisulphite (Loba Chemie; M. W 190.10), distilled water, xylene (Wharefedale Laboratories, England; M. W. 106.17, 0.862-0.864g/ml) and vegetable oil.

2.2. Method

Isolation of starch from unripe plantain peels

The peels were chopped into pieces (about 0.5cm x 0.5cm) with a stainless kitchen knife and washed severally with tap water to remove sand and other adhering foreign particles. The peels were further rinsed twice with distilled water to remove any other glued impurity. The cut peels were steeped for 1h in 1.5% (w/v) sodium metabisulphite solution (peels to solution ratio = 100g: 200ml) at a pH of 5.2 and temperature of 40±1°C. The steeping is done to reduce pulp and release starch granules in the peels. The plantain peel-bisulphite solution was blended using an industrial blender and allowed to stand for 30min at room temperature. The mixture was filtered twice with a three-layered muslin cloth in the first case and four layered muslin cloths in the second case. The filtrate could stand overnight to enable the settling of the starch. The supernatant was decanted, and the starch sediment was centrifuged using a Bosch Centrifuge (800D) at 3000 rpm for 15min. The mucilage was scrapped off and the starch obtained was rinsed with distilled water severally. The rinsed starch was collected on Petri-dishes and then, sun dried for three days. It was further oven-dried at 50°C for 1h and pulverised to powder with an electric grinder. The process flow chart for the extraction of the plantain peel starch is given in Figure 1.

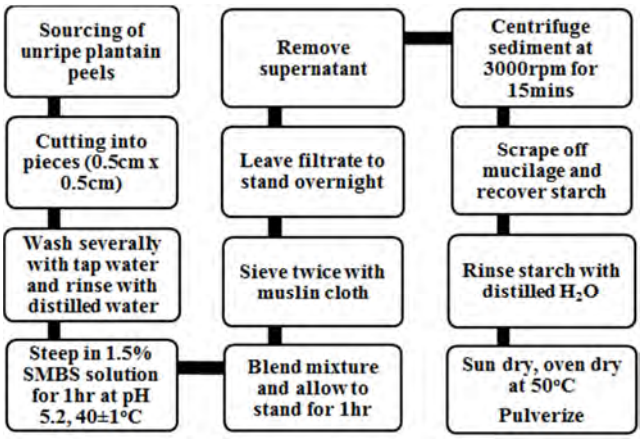

Figure 1. *Process flow chart for plantain peel starch extraction.*

2.3. Characterization of Unripe Plantain Peel Starch (UPPS)

2.3.1. Determination of Starch Yield

Starch yield was calculated using equation 1;

$$\%\, Starch\ Yield = \frac{W_2}{W_1} \times 100 \qquad (1)$$

Where W_1 is the weight of the peels and W_2 is the weight of the starch

2.3.2. Determination of Moisture Content

The moisture content of the UPPS was determined by the gravimetric method. Briefly, 0.5g of the starch sample (W_s)

was measured into a previously dried and weighed crucible (W_c), and placed in an oven at 105°C for 3h. It was then cooled in a desiccator for 30 min. The weight of crucible and dried starch (W_{cs}) was noted. Percent moisture content was calculated using the difference in weight method as shown in Equation 2.

$$\%\ Moisture\ content\ = \frac{(W_{cs}-W_c)}{W_s} \times 100 \quad (2)$$

2.3.3. Determination of pH and Ash Content

The pH was determined using the method adopted by Zakpaa et al. [11], but with little modification. Here, 1g of the starch sample was weighed and mixed with 20 mL of distilled water in a 50mL beaker, stirred for 10 min and then filtered using a filter paper. The filtrate was tested for acidity and alkalinity using a Jenway 3520 model pH meter. The ash content was determined by incineration with a muffle furnace (LabTech, India) at 600°C by the Association of Official Analytical Chemists (AOAC) (1990) [12] standard method with little modification. Briefly, 3g of the starch sample (W_s) were weighed in a pre-weighed crucible (W_c) and placed in the furnace at 600°C for 3h, cooled in a desiccator and re-weighed (W_{cr}) to the nearest decimal from which weight of residue (W_r) was calculated. Ash content was calculated as shown in Equation 3.

$$Ash\ Content\ (\%) = \frac{W_r}{W_s} \times\ 100 \quad (3)$$

2.3.4. Determination of Water Binding Capacity (WBC)

Water binding capacity of the starch was estimated by the method of Medcalf and Gilles [13] with slight modification. In brief, in a 15ml centrifuge tube, 0.5g of the starch sample was dissolved in 10ml distilled water. The sample suspension was shaken for 10min and then centrifuged using Powerspin^{TM} centrifuge (Model C8624) at 2500rpm for 20min. The supernatant was decanted from the wet starch, drained for 5min and the wet starch weighed. The water binding capacity was then calculated thus;

$$\%\ WBC\ = \frac{Waterbound}{sample\ weight} \times\ 100 \quad (4)$$

2.3.5. Determination of Fat Binding Capacity (FBC)

Same procedure as in WBC but power cooking vegetable oil used instead. Fat binding capacity was calculated as shown below:

$$\%\ FBC\ = \frac{Fatbound}{sample\ weight} \times 100 \quad (5)$$

2.3.6. Determination of Bulk Density (BD)

2.5g of the starch sample was measured into 10mL graduated cylinder and dropped three times on the laboratory table from a height. The volume reading (V_b) was taken from the calibrations on the graduated cylinder and bulk density calculated using equation 6;

$$\rho_b\ = \frac{M}{V_b} \quad (6)$$

Where ρ_b is the bulk density, M is the mass in grams of the starch and V_b is the volume reading in ml.

2.3.7. Determination of Tapped Density (TD)

The 10ml graduated cylinder with the starch (2.5g) in section 2.3.7 was tapped (more than 200 taps) on the laboratory table until there was no further diminution of the sample level i.e. until a constant volume is observed. Volume reading was taken and tapped density calculated using equation 7;

$$\rho_t = \frac{M}{V_t} \quad (7)$$

Where ρ_t is the tapped density, M is the mass in grams of the starch and V_t is the volume reading in ml.

2.3.8. Determination of Particle (True) Density (PD)

Specific gravity method was used to determine the true density of the starch sample, and xylene (WharefeDale Lab, England) was used as the displacement fluid. The pycnometer (specific gravity bottle- 50ml) was washed and rinsed with distilled water, dried and weighed (W_b). Xylene was poured into the pycnometer up to the brim, and the bottle lid was used to displace overflowing xylene. The xylene was transferred into a 100ml volumetric cylinder, and the volume of xylene (V) that filled the empty pycnometer was read from the calibrations on the cylinder. The pycnometer was again cleaned and dried. Some quantity of starch sample was poured into the pycnometer and weighed (W_{bs}). The bottle with starch was filled with xylene (not to brim), stirred with a glass rod and allowed to stand for 10min. More xylene was poured into the bottle up to the brim (and lid used again to displace overflowing liquid) and weighed (W_{bsx}). Calculations were made as shown below;

$$\text{Weight of starch sample, } W_s = W_{bs} - W_b \quad (8)$$

$$\text{Weight of added xylene, } W_x = W_{bsx} - W_{bs} \quad (9)$$

$$\text{Volume of added xylene, } V_x\ = \frac{W_x}{\rho_x} \quad (10)$$

$$\text{Volume of starch, } V_s = V - V_x \quad (11)$$

Where ρ_x is the density of xylene (g/cm^3)

Therefore, particle density of UPPS was calculated as;

$$\rho_p\ = \frac{W_s}{V_s} \quad (12)$$

2.3.9. Determination of Starch Dispersibility and Some Flow Properties of the Native Starch

Starch dispersibility was determined according to the method of Kulkarni et al. [14] with little modifications. 1g of the starch sample was measured into a 10ml measuring cylinder, and distilled water was added up to the 10ml mark. The set up was stirred and allowed to stand for 3h. The volume of the sediment was read from the cylinder subtracted from 10, multiplied by 100 and expressed as percentage dispersibility. Also, some flow properties of the native starch such as Carr's (compressibility) Index and Hausner's Ratio were determined from the values of bulk,

tapped density and true density using the formula shown below;

$$Carr's\ Index\ (C.I)\ =\ 100\ \left(1\ -\ \frac{\rho_b}{\rho_t}\right) \quad (13)$$

$$Hausner's\ Ratio\ (H)\ =\ \frac{\rho_t}{\rho_b} \quad (14)$$

2.3.10. Determination of Swelling Power and Solubility Index

Swelling power and solubility indices were determined using the method of Leach et al. [15] with some modifications. Briefly, in a weighed 15ml centrifuge tube, 0.2g of starch was added and distilled was added to the 10ml mark. Starch suspension was stirred for 10min and later heated in a water bath for 30min at temperatures 60°C, 70°C, 80°C and 90°C with constant stirring to avoid fragmentation. The tube was removed, wiped dry on the outside and cooled to room temperature. Centrifugation was done at 2500rpm for 15min using a G. Bosch Centrifuge (800D). 5ml of the supernatant was transferred into a weighed crucible and dried from which the weight of the soluble was obtained. The percentage swelling power and solubility were calculated as shown in equations 13 and 14 respectively.

$$swelling\ power\ =\ \frac{weight\ of\ paste\ (g)}{weight\ of\ starch\ (g)} \quad (15)$$

$$\%\ solubility\ index\ =\ \frac{weight\ of\ soluble}{weight\ of\ starch}\ \times\ 100 \quad (16)$$

2.4. Morphology Study Using Scanning Electron Microscopy

A little quantity of starch was placed on a double adhesive sticker placed in a sputter coater machine for 5 sec to give the samples a conductive property. The starch sample was imaged using 15kv at different magnifications with MODEL -PHENOM ProX Scanning Electron Microscope.

3. Results and Discussion

3.1. Physico-chemical Analysis of Unripe Plantain Peel Starch

Table 1 presents the proximate composition of the unripe plantain peel starch. The starch yield was low compared to the value of 3% reported by Happi-Emaga et al. [7]. Comparison of this value to the values of the rich sources of starch (cassava, corn, wheat, etc.) shows a small yield. The value does not take anything away from the potentials of unripe plantain peel as a source of starch considering that the peels are a waste while the sources are food; hence competing with food consumption. Also, the low yield may be attributed to the wet milling method of isolating the starch granule. Starch content in unripe plantain peels has been reported to be 38% (380g/kg) [10], 39.3% (393g/kg) [16] and 40% [17]. Hence, wet milling might not be the best method for UPPS starch extraction. A better yield may be possible if dry milling method, enzymatic process or simple engineered methods are employed.

Table 1. *Proximate analysis of the unripe plantain peel starch.*

Proximate Analysis	Starch yield (%)	Moisture content (%)	Ash content (%)	pH at 25°C
Value	1.86*	12.60*	0.47*	7.46*

*Mean values of duplicate analysis for the parameters used.

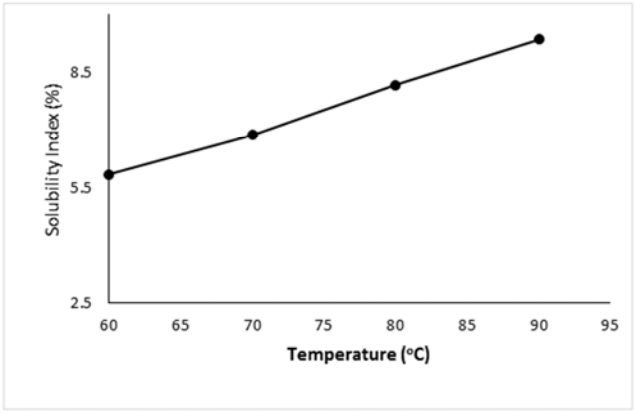

Figure 2. *Effect of Temperature on Solubility Index of UPP Starch.*

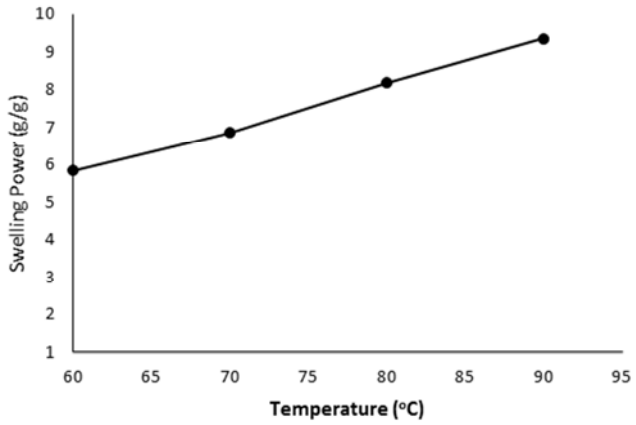

Figure 3. *Effect of Temperature on the Swelling Power of UPP Starch.*

The pH (alkaline) of the UPPS is quite high and did not fall within the acceptable range of 4.7 to 5.3 [18] for an excellent quality starch. The value is also greater than 4.82 reported by Zakpaa et al. [11] for plantain pulp starch. However, the pH value falls within the acceptable range (3-9) for starches used in the pharmaceutical, cosmetics and food industries [19]. Also, it has been reported that high pH starches have increased solubility because of increased hydrophilic characters of the starch at these pH values [20], hence at pH within the value reported, UPPS starch may exhibit such increased solubility.

The value obtained for the moisture content of the starch was within the acceptable range (10-13.5%) [18] for high-quality starch giving an indication of good shelf life. High moisture content also suggests improved food quality and standards for food applications. The value also compares favourably with those reported by researchers for other sources of starch. The ash content depicts the presence of little traces of mineral elements and inorganic salts and

indicates a low level of surface contamination. The ash value for UPPS could mean the presence in excessive amounts of mineral elements like sodium, iron, calcium, potassium and magnesium as reported by Ighodaro [9]. The ash content is greater than 0.2% which is recommended for a high-quality starch [18] but lower than 0.6% specified by British Pharmacopeia [21] for starches used in pharmaceutical industries. Hence, UPPS could find efficient use in the pharmaceutical industry. Kiin-Kabari et.al. [22] reported values of 0.05%, 0.29% and 0.45% for pulp starches from different varieties of Nigerian plantain.

Table 2. *Functional and some flow properties of the unripe plantain peel starch.*

Property	B. D (g/ml)	T. D (g/ml)	P. D (g/ml)	WBC (g/g)	FBC (g/g)	Dispersibility (%)	C. I (%)	H
Value	0.46	0.56	1.18	1.66	1.38	84.00	17.86	1.22

The mean value of duplicate analysis used.

Table 2 illustrates the functional and some flow properties of the native starch. The functional properties of water and fat binding is important in food formulations and processing. They influence the quality and texture of food products. WBC stabilises food products against the effect of syneresis which sometimes occurs during retorting and freezing [23]. FBC describes the tendency of starch to absorb and retain fat by capillary attraction. FBC is important in food applications like sausage and dairy foods production since it describes the suitability of the starch in facilitating enhancement in flavour and mouth feel of such food products. The values for the water binding capacity and fat binding capacity of the UPPS starch are as presented in Table 2. Talisman et.al [24] reported WBC value of 13.5g/g for plantain peel starch. The very low value obtained in this study may be due to the species and plant origin. Dispersibility measures the reconstitution of starch in water. A highly dispersible starch will exhibit better reconstitution [14]. The value obtained for dispersibility shows better reconstitution property of unripe plantain peel starch. The value is higher than values reported by Ashogbon & Akintayo [25] for cereal (75.1 & 80.02%) and leguminous (72.1 & 74.2%) starches.

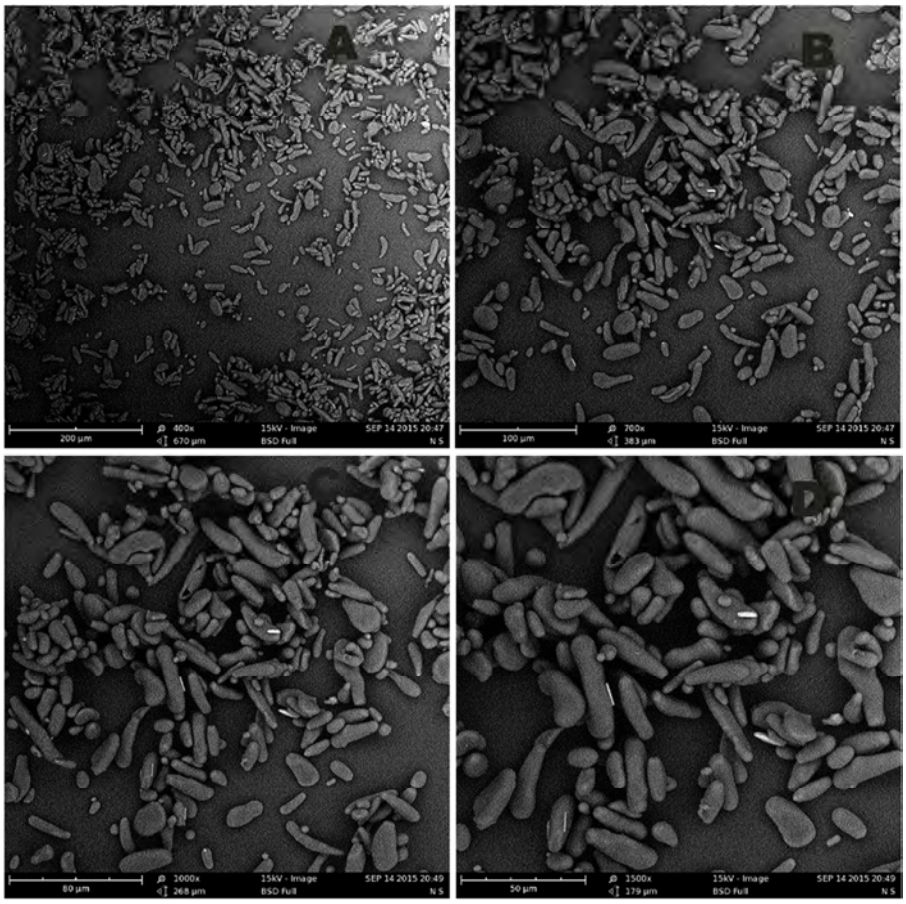

Figure 4. *SEM Images of Native Unripe Plantain Peel Starch at (A) 400X (B) 700X (C) 1000X and (D) 1500X.*

Bulk density is an important property in industrial applications of starch. In the pharmaceutical industry, it describes the packing behaviour of a starch powder during various unit operations of tabletting such as die to fill, mixing, granulation and compression [26]. Bulk density is defined as the mass divided by the total volume occupied by

the starch powder. The total volume includes the volume of the air entrapped between the particles. The tapped density is an increased bulk density obtained after mechanically tapping the container containing the powder sample. The values of bulk, tapped and particle density of UPPS are given in Table 2. These parameters are used in the analysis of some flow properties of the starch powder.

The flow properties of starch particles determine their suitability for use as direct compression excipients. Carr's Index and Hausner's Ratio give indications of the flowability of a starch powder. A Carr Index lower than 16% is a sign of excellent flowability, values between 16-18 suggests good flowability while values above 25% suggest poor flowability. Hausner's Ratio less than 1.25 represents good flowability. Depending on the values measured by these indicators, powders can be classified as "excellent", "good", "fair", "passable" or "poor" flowing materials. Carr's index of the native starch was lower than 18% while Hausner's ratio was less than 1.25 hence suggesting that the flowability of the starch is good. It should also be noted that there is a slight difference between the bulk and tapped density of the native starch, suggesting the existence of less interparticle interactions and hence giving credence to an excellent flowability property of the native starch. UPPS can be utilised as tablet excipients (either as a binder, diluent or disintegrant).

Figures 2 and 3 display the results of the swelling power and solubility index of the starches at various temperatures respectively. The results show that both swelling power and solubility indices are temperature dependent, and values increased with increase in temperature. As temperature increases, starch molecules become thermodynamically activated, and the resulting increase in granular mobility enhances penetration of water into the amorphous region of the starch granule. The increase in temperature facilitates dissolution and improved swelling capacities. The S. P and S. I of the pulp starch at 85°C has been reported to be 9.48g/g, 10.16g/g, 10.10 and 7.47%, 3.55%, 5.02% respectively of three different varieties of Nigerian plantain [22].

3.2. Morphological Properties of Unripe Plantain Peel Starch

The results of the Scanning Electron Microscopy of the unripe plantain peel starch are given in Figure 4. The images were presented at different magnifications (400X, 700X, 100X and 1500X). The morphology of the native starch enunciated smooth, irregular, rod-like and elongated shapes of the plantain peel starch granules. Talisma et al. [24] made a similar investigation and reported that the granules are smooth and elongated filaments with irregular and spheroid shapes. Comparison of the morphology of the unripe plantain peel starch granule and other sources of starch are presented in Table 3.

Table 3. *Morphology of starch granules from different sources.*

Starch Source	Granule Shape	Reference
Unripe plantain peel starch	Smooth, irregular, rod-like, elongated	(Talisma et.al., [24]; Present study)
Plantain pulp	Smooth, irregular, spheroid-shaped	(Talisma et al.., [24]
Corn (Wild type)	Angular	(Singh et.al, [27]; Alcazar-Alay &Meireles, [28]
Corn (High Amylose)	Angular and Polygonal	(Ellis et.al, [29]; Alcazar-Alay &Meireles,[28]
Cassava	Flake shape and irregular	(Falade & Akingbala, [30]; Alcazar-Alay & Meireles.[28]
Potato	Smooth-surfaced, oval and irregular	(Singh et.al, [27]; Alcazar-Alay &Meireles, [28]
Sweet Potato	Polygonal	(Jane et.al, [31]; Alcazar-Alay &Meireles,[28]
Rice (Wild type)	Angular, Polygonal	(Hoover et.al, [32]; Alcazar-Alay &Meireles, [28]
Sorghum (wild type)	Polygonal, dented and round	(Wankhede et.al., [33]; Alcazar-Alay &Meireles,[28]
Wheat (wild type)	Spherical and lenticular	(Singh et.al, [27]; Alcazar-Alay &Meireles, [28]
Barley (wild type)	A-type: Disc-shape B-type: lenticular	(Ellis et.al, [29]; Alcazar-Alay &Meireles,[28]
TaccaInvolucrata	Oval and polyhedral	(Nwokocha et.al., [34]

4. Conclusions

Starch was isolated from the peels of unripe plantain (*Musa Paradisiaca*) and the properties studied. The properties were comparable to the properties of the pulp starch and some sources of starch. Unripe plantain peels can be exploited as a useful source of starch for industrial applications especially as compression excipients in the pharmaceutical industry. However, isolation methods like enzymatic processes and dry milling are recommended to improve the starch yield.

Acknowledgements

The authors are grateful to Mr Isa Yakubu of the Department of Chemistry, Ahmadu Bello University Zaria, Nigeria for the SEM analysis. The authors also appreciate Mr Nti Simon of Soil Science Laboratory, Federal University of Technology, Owerri, Nigeria for helping with some of the apparatus used in this research work.

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