Assessment of starch (es) from the seeds of *Artocarpus altilis* using varied extraction methods

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DOI: 10.29322/IJSRP.11.02.2021.p11089
http://dx.doi.org/10.29322/IJSRP.11.02.2021.p11089

Abstract- The study is aimed at extracting starch from *Artocarpos altilis* seeds and the isolation effected from the seeds by solvent extraction method using aqueous and alkali (0.1NNaOH) medium respectively. The starch extracted was, evaluated for its physical, physicochemical properties, proximate and mineral compositions. The aqueous-extracted starch had a yield of 9.69% while the yield from alkaline-extract was 11.15%. The identification tests carried out on the starch (Molisch and Iodine test) confirms the presence of carbohydrate and starch. The starch obtained from both medium appeared powdery but that from the aqueous-extracted was less powdery and more crystalline although milkish-white in color while the alkaline-extract was brownish and appeared very amorphous with high content of fines. Proximate analysis indicates high carbohydrate content (77.5%) and potassium as the highest mineral content in the starch.

Index Terms- *Artocarpus altilis* seed, starch, hydrolysis, extraction, assessment

I. Introduction

Starch is a natural polysaccharide widely found in different parts of plants ranging from the fruits, seeds, tubers, and roots where it could be stored and serve as a source of energy and carbon hydrate reserve in the plant. The synthesis of starch comprises of biochemical pathways that involve glucose production in plant cells via the process of photosynthesis [1].

Starch is widespread, abundantly available, less costly, degradable, pollution free and renewable and can be largely found in various sources of crops as in cassava, potato, maize, wheat with smaller amounts from rice sorghum, sweet potatoes, arrowroot, beans, and recently micro algae [2].

Starches obtained from cassava and sorghum is useful as food whereas that from maize, potato and wheat are, used for food and other purposes that might not be food related. The starch is an abundant source of energy in human diet and consumed as bread and pasta products and its digestion can readily take place in the gastro intestinal tract (GIT), with little quantity fermented by bacteria in the colon.

Apart from the carbohydrate constituents, starch also consists of associated molecules such as waxy substances causing it to possess high crystallinity and requiring high energy for gelatinization. It may, also contain lipids as well as proteins and these molecules could originate from the granule surface and might increase the functionality of the starch and as well influence certain qualities such as hardness. Certain starch (es) also may contain important mineral constituents such as magnesium, phosphorus, potassium, sodium, calcium etc. and of notable importance is that they can occur in the form of monophosphate esters, phospholipids and in organic phosphates [3].

Naturally, major constituent of starch includes two types of molecules named as amylopectin and amylose. The amylose is basically a linear molecule having a molecular weight between 105 to 106 composed of linear chain of α-D-glucose units joined together by α-1,4-glycosidic bonds and because of hydrogen bonding, amylose could acquire spiral structure that contains six glucose units per turn. Amylopectin on the other hand is associated with molecular weight of several millions and has many branched polymers formed by anhydro-glucose units linked α-(1, 4) and additionally with 2-4% α-(1,6) linked branches [4].

Amylose as revealed by several studies, contain branches, which could differ from one plant species to another. The amount of amylose present in the granule significantly affects the physicochemical and functional properties of the starch. The characteristic blue-violet color, which appears when starch is treated with iodine, is as a result of the formation of the amylose-iodine complex and this color on test is sufficient enough to detect even minute amounts of starch present in solution.

Amylopectin molecule on the other hand consist of a helical structure, which could be disrupted by the branching of the chain and therefore, instead of the blue-violet color as observed with amylose, in the presence of iodine, amylopectin produces a less reddish brown color [4].

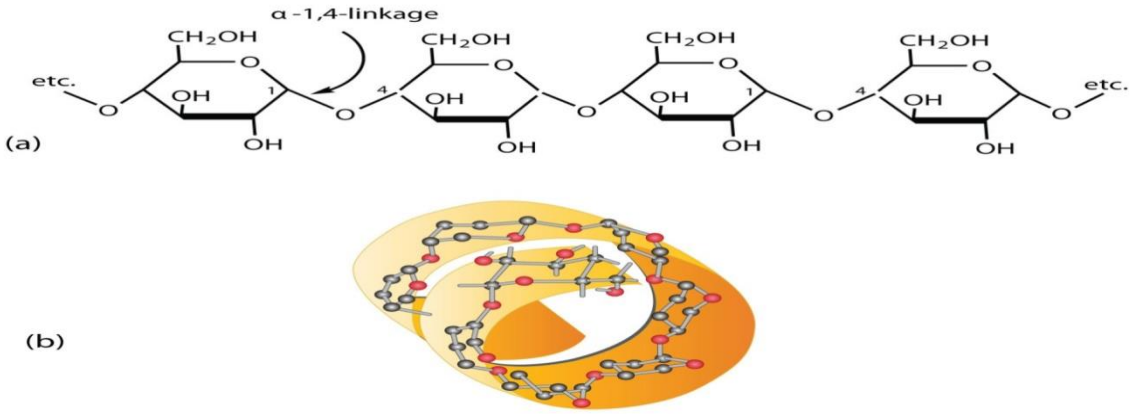

Figure 1.*0*a Linear chain of α-D-glucose units of amylose joined together by α-1,4-glycosidic bonds. **Figure 1.0***b* Spiral structure of amylose due to hydrogen bonding containing six glucose units per turn.

Artocarpus altilis Plant

This plant has, been known from ages as useful for its therapeutic properties. The genus Artocarpus comprises of about 50 species and is widely distributed in tropical and sub-tropical regions. The generic name of the species comes from the Greek words 'artos' meaning bread and 'karpos' meaning fruit hence commonly called breadfruits and belongs to the family Moraceae. It is widely distributed and a tropical fruit tree which produces fruit from march through june and july to september. The fruits are, known to be traditionally rich in starch and hence a great source of carbohydrate with low fat content [5].

Fig1. Leaves and fruits of *Artocarpus altilis*

Fig 2. Seeds of *A. altilis*

A altilis trees are very large, evergreen and about 18 meters tall. The leaves are thick and leathery with a dark green color on the dorsal side, which often appears to be glossy. It bears multitude of flower, which is monoecious. The breadfruit also comes with the seeded type common in south western pacific and the seedless types, which are common in many places.

The seeds are brown in color, shiny, round, ovoid in shape and irregularly compressed with little or no endosperm but, no period of dormancy and can germinate immediately hence cannot be dried or stored for planting.

Artocapus altilis could be cultivated from the hot humid and tropical low land areas although rain plays an important role in the rate of growth of the fruit hence requires a rainfall of fairly equal distribution. The plant grows best in equatorial lowlands and occasionally found in the highlands but the production and quality of the fruits decreases in cooler conditions. The soil conditions required for the proper growth of the plant are sandy or loamy soil with essentially good drainage. The plant grows best in hot temperature of 21-32°C and requires a soil that is neutral to alkaline and with a pH of 6.1 to 7.4 [6].

Studies from extracts and metabolites from leaves, stem, fruit and bark of *A. altilis* has confirmed it to contain numerous beneficial biologically active compounds including, flavonoids, lecithin, cryobenzofuranoids and stilbenoids. Such compounds could be useful for various biological activities especially against, bacterial, tubercular, viral, fungal, platelet, arthritic, cytotoxicity and as tyrosinase inhibitory agent while nutritional compositions of the seeds have been identified to include, water, protein, carbohydrates, fat, calcium, phosphorus, iron, niacin, moracin, dihydromorin, artocarpesin etc [7].

The aim of the study is to extract and isolate starch from the seeds of *Artocapus altilis* using aqueous and alkaline (0.1N sodium hydroxide) medium, then access the quality and efficacy of the extracted starch as possible excipient in pharmaceutical solid dosage formulation.

II. MATERIALS AND METHODS

MATERIALS

0.1N sodium hydroxide, distilled water, methanol, concentrated hydrochloric acid, iodine solution, perchloric acid, 0.1N Hydrochloric acid, fresh seeds of *Artocarpus altilis* (Mbiama market, Nigeria), milling machine,), pH meter, sieves (test sieve

ASTM E11 BODY 316L MESH S-STEEL/RF, Germany 500µm, 1mm, 2mm),

METHODS OF STARCH EXTRACTION

PROCUREMENT, SORTING AND REMOVAL OF *Artocarpus altilis* SEEDS EXOCARP

Fresh seeds of *A. altilis* were, procured from Mbiama market, Rivers State, Nigeria, identified and authenticated while the procured seeds were, sorted for the best grades, isolated and weighed. The exocarp of the seeds was, removed using suitable knife, washed, weighed, and stored in a dry place

MILLING AND SOAKING OF SEEDS

The washed seeds was milled, divided into two equal parts, and weighed. Part A was, dispersed in 0.1N NaOH and part B in distilled water for 12 hours at atmospheric temperature with intermittent stirring after which the slurry was filtered using suitable muslin cloth and the sediment washed with water thrice.

PRECIPITATION AND ISOLATION OF STARCH

The filtrate obtained was, precipitated over night at atmospheric temperature after which the supernatant was, discarded and the crude starch obtained, was washed with distilled water thrice to obtain the starch cake, which was dried at 40°C for 24 hours using a tray dryer. The dried starch was later milled, packed in an airtight container and stored at room temperature for further use.

STARCH YIELD

The yield, of the starch extracted was calculated using the formula:

$$\text{Starch yield (\%)} = \frac{\text{Weight of peeled seeds (g)}}{\text{Weight of starch portion (g)}} \quad \ldots\ldots\ldots\ldots \text{(eqn1)}$$

MOISTURE CONTENT DETERMINATION

Moisture content was, determined by oven drying method where 1.5 g of well-mixed sample was accurately weighed in clean, dried crucible (W). The crucible was placed in an oven at 100-105°C for 6-12 h until a constant weight was obtained. It was, then removed and placed in a desiccator for 30 min to cool. After cooling, the sample was, weighed again and weight recorded as (W_2). The percent moisture content was, calculated using the formula:

$$\text{\% Moisture} = \frac{W - W_2}{W} \times 100 \quad \ldots\ldots\ldots\ldots \text{(eqn2)}$$

Where: W = initial weight of crucible + sample, W_2 = final weight of crucible + sample

Note: moisture free samples were, used for further analysis.

DETERMINATION OF ASH

Clean empty crucible was placed in a muffle furnace at 600°C for an hour, cooled in a desiccator and then weight of empty crucible was noted (W). One gram of each of the sample was placed in the crucible and weight noted (W_2). The sample was, ignited over a bunsen burner with the help of blowpipe, until it is charred. The crucible was, later placed in a muffle furnace at 550°C for 2-4 h. The appearance of, grey white ash indicate complete oxidation of all organic matter in the sample. After ashing the furnace was, switched off and the crucible cooled and reweighed (W_3). Percent ash was, calculated using the formula:

$$\text{\% Ash} = W2 - \frac{W3}{W2} * 100 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \text{(eqn 3)}$$

Where: W_2 = weight of sample, W_2-W_3= difference in wt. of ash

DETERMINATION OF CRUDE PROTEIN: Protein in the sample was determined following Kjeldahl method. The samples were digested by heating with concentrated sulphuric acid (H_2SO_4) in the presence of the digestion mixture. The mixture was then made alkaline while ammonium sulphate thus formed, released ammonia which was collected in 2% boric acid solution and titrated against standard HCl. Total protein was calculated by multiplying the amount of nitrogen with appropriate factor (6.25) and the amount of protein was thus determined.

Percent crude protein content of the sample was, calculated using the formula:

% crude protein = 6.25* x %N (* correction factor)……. (eqn4)

$$\%N = \frac{(S-B) \times N \times 0.014 \times D \times 100}{\text{Weight of the sample} \times V} \quad \ldots\ldots\ldots\ldots \text{(eqn5)}$$

Where: S = sample titration reading, B = blank titration reading, N = normality of HCl

D = dilution factor of sample after digestion, V = volume taken for distillation

0.014 = milli-equivalent weight of Nitrogen

DETERMINATION OF CRUDE FAT

Crude fats were, determined by process of extraction involving ether extraction method using soxhlet apparatus. The percent (%) crude fat was determined using the formula

$$\frac{W_e}{W_S} * 100 \quad \ldots\ldots\ldots\ldots \text{(eqn6)}$$

Where: W_e= weight of ether extract, W_s= weight of sample

DETERMINATION OF CRUDE FIBER

A moisture free and ether extracted sample of crude fiber made of cellulose was first digested with dilute H_2SO_4 and then with dilute KOH solution. The undigested residue collected after the digestion was ignited and loss in weight after ignition was recorded as crude fiber calculated as:

$$\text{\% Crude fiber} = \frac{W_1 - W_2}{Wo} \times 100 \quad \ldots\ldots\ldots\ldots \text{(eqn7)}$$

Where W_O = initial sample weight, W_1 = weight of sample after digestion and W_2 = weight of digested sample after drying in muffle furnace.

ELEMENTAL ANALYSIS

DETERMINATION OF IRON (Fe), LEAD (Pb), ZINC (Zn) AND MAGNESIUM (Mg)

The digested sample was analyzed for mineral content by Atomic Absorption Spectrophotometer (Hitachi model 170-10) in Analytical Concept ltd, Elelenwo, Port-Harcourt. Different electrode lamp was used for each mineral and in the technique adopted, the atoms of an element are vaporized and atomized in the flame where they absorb light at a characteristic wavelength. The source of the light is a hollow cathode lamp, made up of the same element that has to be determined. The lamp produces, radiation, at an appropriate wavelength, which, while passed through the flame is absorbed by the free atoms of the sample. The absorbed energy is, measured, by a photo-detector read-out system

and the amount of energy absorbed is proportional to the concentration of the element in the sample.

For determination of magnesium (Mg), further dilution of the original solution (0.5ml) was, done with sufficient distilled water used to make up to 100 ml. The dilution factor for all minerals was 100 and the concentrations of minerals is recorded in terms of parts per million (ppm) often converted to milligram per gram.

DETERMINATION OF SODIUM (Na) AND POTASSIUM (K)

Analysis for the element is, done by method of flame photometry as it measures the emission of radiant energy as the element return to their ground state after their excitation by the high temperature of the flame more over since degree of emission is related to the concentration of the element in the solution.

PARTICLE SIZE DETERMINATION

A modified method, was adopted in determining the particle size [8]. Here 10g of the powdered starch sample was shaken through 2mm, 1mm, 500µm, 250µm, 125µm, 63µm, 45µm sieve sizes on a mechanical sieve shaker for 5minutes. After agitation, weight of starch residue on the sieve was recorded and used to, determine the percentage, retention and fines of the powder. The ease of passage of the starch particles in each of the mesh is an indication of the particle size.

CHEMICAL TESTS FOR *Artocarpus altilis* SEED STARCH

MOLISCH TEST

0.1g quantity of the starch powder was, placed in a clean test tube and 2drops of Molisch's reagent (freshly prepared) was, introduced into the tube. 1.0ml of concentrated sulfuric acid was, gradually added to the side of the tube to form a layer below the aqueous solution and the results obtained recorded accordingly.

IODINE TEST

0.1g of the dried powder extracted from the varied medium was, separately placed a test tube, to which 1ml of 0.2N iodine was, added and observed for color changes.

ORGANOLEPTIC PROPERTIES OF THE EXTRACTED STARCH GRADES

The extracted starches were observed for physical properties such as color, odor, taste and texture.

MICROSCOPIC ANALYSIS

A little quantity of starch powder was, mounted in a visible-light microscope, and viewed at x 40 lens.

pH DETERMINATION: pH values of 1.0%w/v starch suspension was measured using a digital pH meter.

TESTS FOR THE FUNCTIONAL PROPERTIES OF *Artocarpus altilis* SEED STARCH

HYDRATION CAPACITY

A 1g (W_0) of the seed starch was placed in a centrifuge tube and the weight (W_1) noted then hydrated with 10ml of distilled water. The tube was, shaken for 5mins then allowed to stand for another 15mins before centrifuging at 3000rpm for 10mins. The supernatant was decanted and the weight of the powder after water uptake and centrifugation was determined (W_2) [9]. Water-binding capacity was, calculated as:

$$\text{Hydration capacity} = \frac{W_2 - W_1}{W_1 - W_0} \quad \text{.....(eqn 8)}$$

Where:

W_2-W_1= Weight of bound water, W_1-W_0 = Weight of tube, W_0 = weight of starch

SWELLING INDEX

The swelling capacity was determined by filling a 100ml graduated cylinder to the 10ml mark with the powdered sample while distilled water was, added to make the volume up to 100ml. The top of the graduated cylinder was covered then content mixed and allowed to stand for 12hours after which the volume occupied by the sample was, recorded.

CHARACTERIZATION OF EXTRACTED STARCH(ES)

BULK DENSITY AND TAPPED DENSITIES

A 20g quantity (Wp) of starch powder was gently introduced through a short stemmed glass funnel into a 250ml graduated cylinder. The volume occupied by the powder was taken as Vb. The powder was tapped on a padded tabletop from a height of about 7mm until no further change in volume was observed and the volume (Vt) was taken as the tapped volume. The bulk and tapped density was computed using the formula

$$Bd = \frac{Wp}{Vb} \quad \text{…… (eqn 9)}$$

$$Td = \frac{Wp}{Vt} \quad \text{…… (eqn 10)}$$

Where Bd is the bulk density, Td is the tapped density, Wp is weight of powder, Vp is volume of powder and Vt is the tapped volume.

HAUSNERS RATIO (H_R) AND CARR'S COMPRESSIBLITY INDEX

The result obtained was used to calculate for the Hausner's ratio and the Carr's Compressibility Index using the formula:

$$H_R = \frac{\text{tapped density}}{\text{bulk density}} \quad \text{..... (eqn 11)}$$

$$CI = \frac{\text{Tapped density} - \text{bulk density}}{\text{Tapped density}} \quad \text{..... eqn 12)}$$

ANGLE OF REPOSE: The static angle of repose (θ) was measured according to the fixed funnel and free standing cone method and the tangent of the angle of repose calculated using the equation

$$\tan\theta = 2h/D \quad \text{……(eqn 13)}$$

Were h is the height of the heap of powder and D is the diameter of the base of the powder heap.

FLOW RATE

Using the flow through the hopper method, 30g of each batch granule was measured, allowed to pass through the orifice, and the time taken to pass through was recorded. The flow rate was determined using the relation;

$$\text{Quantity of granules (g) per unit time (second)} = \frac{m(g)}{t(sec)} \quad \text{……(eqn 14)}$$

III. RESULTS

PERCENTAGE YIELD OF STARCH

Weight of peeled seeds for each extract =6.1kg
Weight of water-extracted starch = 0.591kg
Weight of alkaline-extracted starch = 0.680kg

Aqueous-extract starch yield = 9.69%
Alkaline-extract starch yield = 11.15%

TABLE 1: PRELIMINARY CONFIRMATORY TEST FOR STARCH

TESTS	OBSERVATION	INFERENCE
Molisch test:	Violet color at the junction of the two layers	Carbohydrate present.
Iodine test:	Blue-black coloration	Starch present

TABLE 2: PROPERTIES CHARACTERIZATION OF THE STARCH
ORGANOLEPTIC PROPERTIES

ORGANOLEPTIC PROPERTY	WATER-EXTRACT	ALKALINE-EXTRACT
color	milkish-white	brownish
odour	perculiar	perculiar
texture	powdery	finely powdery

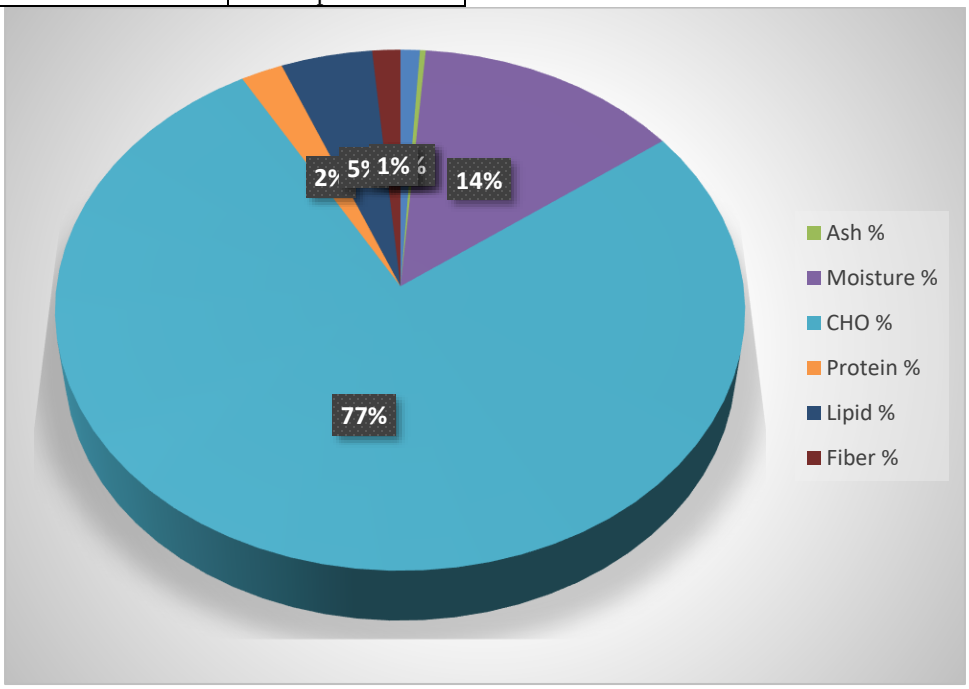

FIG 1: PROXIMATE ANALYSIS OF BREADFRUIT SEED STARCH

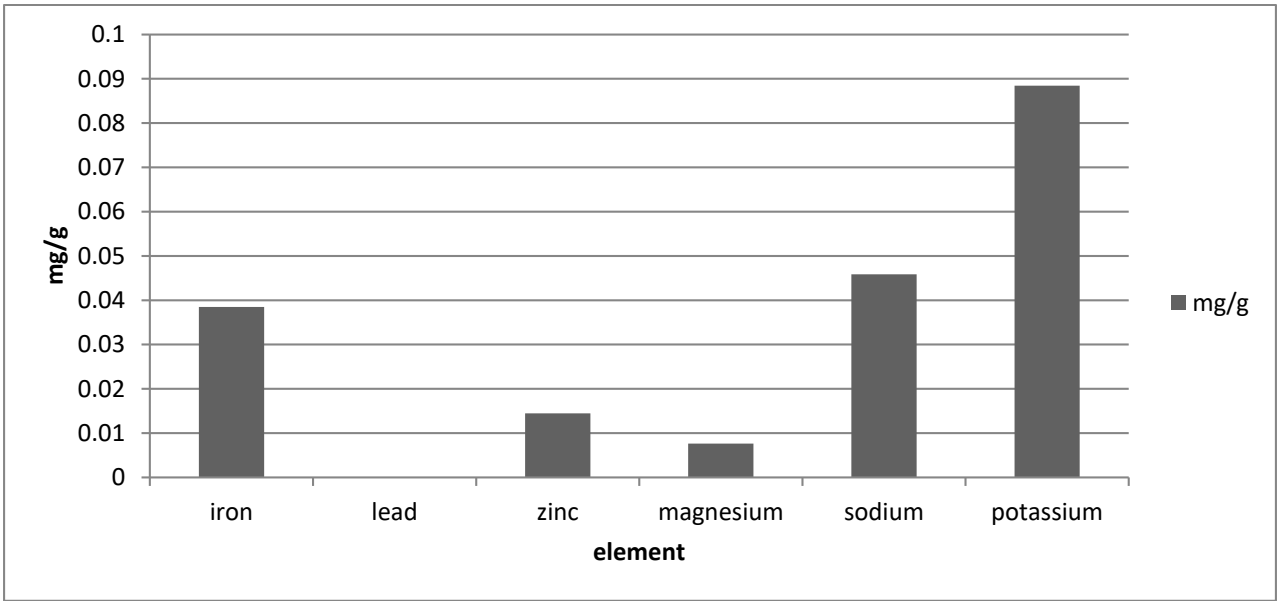

FIG 2: ELEMENTAL ANALYSIS OF *Artocarpus altilis* SEED STARCH

Sieve NO	Sieve size	Weight of sieve (g)	Weight of sieve + starch (g)	W_{AS} (mg)	% W_{AS} retained	Cumulative %	% fine	W_{WS} (mg)	% retained	Cumulative %	%fine
10.00	2.00mm	357.00	357.19	0.19	1.90	1.90	98.10	0.01	0.1	0.1	99.9
18.00	1.00.mm	310.00	310.35	0.35	3.50	5.40	94.60	0.1	1	1.1	98.9
35.00	500.00µm	282.00	284.35	2.35	23.50	28.90	71.10	2.086	20.86	21.96	78.04
60.00	250.00µm	272.00	278.10	6.10	61.00	89.90	10.00	6.25	62.5	84.46	15.54
120.00	125.00µm	266.00	267.00	1.00	10.00	99.90	0.10	1.444	14.44	98.9	1.1
230.00	63.00µm	256.00	256.01	0.01	0.10	100.00	0.00	0.11	1.1	100	0
325.00	45.00µm	256.00	256.00	0.00	0.00	100.00	0.00	0	0	100	0

TABLE 3: Particle size analysis of alkaline and aqueous extracted *Artocarpus altilis* starch

W_{AS} = weight of alkaline extracted starch, W_{WS} = weight of water extracted starch

Table 4: Result of functional properties of extracted *Artocarpus altilis* starch(es)

PROPERTY	Sw	Sa
MELTING POINT	240-243^{o}C	238-240^{o}C
pH	7.1	7.2
SWELLING INDEX	10.170	8.026
WATER-BINDING CAPACITY	104.5	81.5

Sw= Water extract starch; Sa= Alkaline extract starch;

Sample	Bulk density(g/ml)	Tapped density (g/ml)	Hausners' ratio	Carr's index	Angle of repose (θ)
Corn starch	0.4143 ± 0.0214	0.5454 ± 0.0043	1.318 ± 0.0502	24.05 ± 2.9518	32.3 ± 0.3606
Water-extract starch	0.4415 ± 0.0147	0.6254± 0.0196	1.4168 ± 0.0093	29.4166 ± 0.4666	35.5667 ± 1.8230
Alkaline extract starch	0.4444 ± 0.0099	0.5949± 0.0027	1.3398 ± 0.0910	25.1353 ± 5.0156	35.6667 ± 0.9292

Table 5: PHYSICOTECHNICAL PROPERTIES OF THE VARIOUS STARCH

IV. DISCUSSION

Starch extraction methods as proposed by Waziri *et al* 2007, involve three successive phases including, an anatomical fragmentation, cell breakage and finally separation/purification processes. After undergoing the processes, the percentage yield obtained, showed higher starch content from the alkaline-extracted starch (11.15%) as compared to the water-extracted starch (9.69%) and from the result of yield obtained it could generally be said that *Artocarpus altilis* plant seed has high content of starch with proposed minimum average of about 10% starch being obtained from 6.1kg of seeds. Preliminary confirmatory tests carried out on the extracted starch confirmed the presence of carbohydrates and starch using the Molisch reagent and iodine solution respectively. The extracted starch (es), observed for texture, odour and color, was seen to vary in color, with variation in texture but had peculiar smell especially as the water-extracted starch appears milkish white, while the alkaline-extracted starch is brownish and this could be because of intensive alkaline hydrolysis leaving high ash content which may be associated with greater sample impurity. The starches extracted from both medium are powdery but lack sufficient binding action based on outcome of their water holding capacity hence could preferably be useful as disintegrant and not as binders in pharmaceutical tablet formulation. Starch from alkaline medium appears powdery high percentage fine and this implies large presence of amorphous although in mixture with crystalline particles. Since decrease in particle size up to certain extent, causes an increase in compressibility and plastic deformation of granules, improved tablet stability could be achieved the use aqueous extracted starch as excipients in anticipated tablet formulation especially upon application of wet granulation method of tablet formulation. That formed as a result of alkaline hydrolysis consist of very high percentage of fines and this might become over hydrated and could form clogs which might lack pore spaces if wet granulation is adopted with an eventual effect on powder flow, ultimate tablet outcome and stability. Based on this therefore, the alkaline extracted *A. altilis* seed starch will seem more suitable as a disintegrant in direct compression tablet formulation.

Proximate analysis of the breadfruit seed starch gave various percentage of important molecular constituents such as carbohydrate 77.80%, protein 2.17%, lipid 4.75%, moisture 13.80% content, fiber 1.46% and ash value 0.30% amongst all as analyzed. Carbohydrates had the highest percentage hence terming the starch component of the *Artocarpus altilis* seeds to be carbohydrate dominant although alkali hydrolysed starch might tend to produce starch low in proteins and lipids. During the extraction process, differences in relation to the amylose content between the alkaline and aqueous extraction could have occurred may be because of lixiviation or degradation of amylose during the extraction process. The high amylose content could be attributed to the fact that alkaline hydrolysis attacks the crystalline region of the extracted starch and this could influence the changes in crystalline quality and nature of powder formed. This could also cause increase diffusion of ions like sodium more so as amylose plays crucial role in reducing starch crystallinity.

Elemental analysis showed an inconsequential amount of lead (0.00005mg/g) which is a pointer to safety of consumption of this seed starch. Potassium ion was found to be of the highest concentration (0.08845mg/g), followed by sodium (0.04585mg/g), iron (0.03850mg/g), zinc (0.01445mg/g) and magnesium (0.00765mg/g). Potassium plays vital role in keeping the hearth beat regular, muscles working right and it is an electrolyte paramount in the regulation of body fluids and blood levels in the body.

The melting point of a substance indicates the temperature at which a change of state from solid to liquid could occur hence important technique for monitoring purity of materials and the values obtained indicates similarity of melting points across the extracted starches. The pH of the extracted starch was observed to be 7.1 and 7.2 for the water-extracted and the alkaline-extracted starch (es) respectively and this depicts the starch to be rather neutral and does not require preliminary acidification or basification. The swelling index an indication of the water retention capacity as well as the hydration capacity that of the water absorption of the extracted starches were low depicting the extracted starch as in- diffusible hence may be suitable as excipients in suspension formulation aided by a suspending agent or useful as disintegrants and as direct compression excipient in tablet formulation.

V. CONCLUSION

The extracted *A.altilis* seed could be most useful as disintegrant in direct compression tablet formulation especially the alkaline hydrolysed starch while the aqueous extracted starch with its sparing crystalline nature might be suitable as a disintegrant adopting wet granulation method.

In all, both lacks, a suitable binding ability hence might not be useful as a binder in solid dosage form formulation but could

probably be of relevance as a disintegrant and agent in suspension drug delivery system.

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