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(RESEARCH ARTICLE)



Physico-chemical properties and functional characteristics of Jack beans (*Canavalia ensiformis*) starch

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Abstract

Starch was isolated from Jackbeans (*Canavalia ensiformis*) and physico-chemical properties of native starch were determined. Moisture, ash, lipid, protein contents showed 11.3, 0.19,6.41% respectively while swelling and solubility profile showed two-stage swelling pattern with similar relaxation pattern between 60-70°C. The result of amylose and amylopectin content showed 20.2% and 79.8% and browning and charring temperatures 295 and 321°C respectively. Pasting property reduced with an increase in concentration from 0.150 g/100 ml to 2.500 g/100 ml with higher paste clarity at 2.500 g/100 g when compared with cassava starch. Fourier transform infrared spectroscopy study showed that JBS has definitive peaks around 2923,1637, and 1460 cm⁻¹ characteristics of starch granules while photomicrographs at different magnifications revealed uniform oval shape with an average size of 10-20 μm. Finally, X-ray diffractograms pattern gave stronger diffraction peaks around 15, 17, 18 and 23°.

Keywords: Starch; Jack beans; Canavalia ensiformis; physico-chemical properties; photomicrographs.

1. Introduction

Mankind has been predisposed to starch utilization as a cheap and good source of food and industrial raw materials which can easily obtained from plant tissues; leaves, roots, shoots, fruits, grains and stems [33],[34], [46]. Starch is one of the most abundant and renewable carbohydrate sources in the world, its supply annually increases to meet growing demands [28], [15]. Recently, the global utilization of starch in 2018 was estimated around 166 million metric tonnes of which majority coming from maize, cassava and other human feedstock. Ghana and Nigeria have been granted duty free quota to export 4 million of cassava chips to China [19].

Starch has been defined as a biodegradable polymer with well documented chemical features, thus making it a versatile natural resource for a number of applications in both food and non-food industrial sectors worldwide [12]. Their occurrence is in form of relatively dense granules which differ in shape and size (1-100µm), a feature that depends on the diversity of the botanical source [40]. It is grouped under the carbohydrate family made up of monosaccharide, oligosaccharides and polysaccharides [24]. The industrial applications of starch-based products dated back to the early Egyptians who developed it as an adhesive agent whereas their Greek counterparts used it in medical and pharmaceutical preparations. Such industrial applications include slight hydrolysis of native starch with vinegar to produce sweetener and ethanol. Gelatinization, pasting, retrogradation, and syneresis are the most important functional and physico-chemical properties of starch granules. Comprehensive study of these properties might help our understanding of the relationship between the structural and functional properties of starch. And also, help to determine further applications of starch and its relative flours [1].

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X-ray diffractometry can be used to study crystal structures at the atomic level [11]. Starch has a definite crystalline nature with well-ordered structure of the amylopectin molecules inside the granules. Its molecules exist as helices and these helices can have different packing arrangements therefore giving rise to different crystalline patterns [11]. X-ray diffraction pattern is also known to be the "fingerprint" of the crystal structure within starch granules molecule [5].The crystal structure of starch can be divided into three types, including A, B and C type [14],[39]. Of these A, B, and C-type are the crystal structures of natural starch [38]. All legume starches showed a characteristic C-type diffraction pattern, which actually consisted of a mixture of A and B-type crystalline structures [11]. Type A form of starch is mainly present in cereal starches, such as maize starch and wheat starch. Infrared spectroscopy is an analytical technique that is use to study functional groups of compounds including complex molecules such as starch. Infrared spectral of starch granules has been previously described by peaks near 3500, 3000, 1600, 1400, 1000, 800 and 500 cm⁻¹ [20]. Physico-chemical characteristics of starch determine its industrial utilization and applications. Starch majorly found several applications in the industries as a binder, pharmaceutical exipients, dispersing agent, etc. For instance, Clarity, stability and viscosity of starch pastes are very important characteristic of starches which determine their application in the food, textile, paper and the adhesive industry [26]. Starch clarity is a much desired property in the food industry particularly when the desired use is as a thickening agent [9].

Jack bean is one of African underutilized leguminous crops and scientifically known as *Canavalia ensiformis* which belong to the family of Leguminosae, the sub-family Papilionoideae has about 480 genera and 12,000 species distributed throughout the world [10]. Jack beans can mildly toxic and copious consumption must be avoided [14, [47]. The high demand for starch for various industrial and food applications have recently experienced a hike in its market price in the face of the country's attempt to attain national self-sufficiency in starch production for various industrial applications and national food security. And the use of food crops (such as cassava, maize, potatoes, etc) highly suitable for human consumption as an industrial raw material for non-food consumption is frowned upon by some stakeholders. Hence, there is need for alternative sources for production of industrial grade starch from non-food sources especially from under-utilized plant such as Jack beans with a view to meet technological needs of today.

2. Methodology

2.1. Sampling

Matured seed of Jack beans (*Canavalia ensiformis*) was harvested from Auchi area of Edo State where it was planted for ornamental purposes. The seeds were de-husked following separation of dirt and puffs and thereafter sun dried and kept in a zip lock bag for isolation of starch granules.

2.2. Starch Isolation

A starch granule was isolated from Jack beans using the methods previously described by Awokoya *et al* [14]. Briefly, 1 kg of Jack beans plus 5 g of sodium metabisulphite was soaked in 10 L distilled water for 72 hours after which seed coat was removed and washed then macerated using laboratory blender (master cheff). Sufficient quantity of distilled water was used to reconstitute starch slurry in a white clean plastic container and using muslin cloth, mucilage was separated from starch after allowed the mixture to stand and settled for 1 hour. Starch juice was washed repeatedly with distilled water and air-dried in tray and later oven dried between 60 °C to 105 °C (carbrolite -England) respectively and allow to cool and weighed to determine percentage yield. The dried starch granules were grounded gently in a laboratory grounder and packed in transparent double zip lock bag for analysis [14].

2.3. Proximate analysis

The proximate composition of starch sample was determined using the standard methods of AOAC [8]. Air-oven method was used to determine the moisture content of the sample. The protein was determined by micro-Kjeldahl method with conversion factor of 6.25. The ash content was determined using a muffle furnace at 550°C for 4 hours until constant weight is obtained for the ash. Soxhlet extraction method using petroleum spirit was used to extract the fat content. The crude fibre was determined according to above standard method. The carbohydrate content was obtained by difference. The analyses were done in triplicates and the average taken as the candidate values.

2.4. Swelling power (SP)

Swelling power (SP) and water solubility index (WSI) determination were carried out in the temperature range 50 °C-100°C at 10 °C interval using the method previously described by Oshodi and Ekkperigin, [30]. Briefly, 0.1 g of native starch sample was weighed into pre-weighed test tubes and 10 ml of distilled water was added. The mixture was heated in a thermo started water bath with shaker at 50 °C for 30 minutes the mixture was cooled and centrifuged at 1500 rpm

for 20 minutes the supernatant was carefully decanted (kept for WSI) and weight of the starch paste taken. This process was repeated for 60, 70, 80, 90and 100 °C and weight of respective starch paste taken. The swelling power and solubility index was expressed as follow:

$$Swelling power = \frac{Weight of starch paste}{Weight of dry starch sample}$$
$$WSI = \frac{Weight of dissolved solids in supernatant}{Weight of dry solid} \times 100$$

2.5. Amylose and amylopectin content

Weigh into a 100 ml volumetric flask and 1 ml of 99 % (v/v) ethanol and 9 ml 1 M NaoH were carefully added and securely covered. The sample solution were heated at 80°C for 10 min in boiling water to gelatinize and later allowed to cool then made up to the mark with distilled water and shaken thoroughly. 5 ml of aliquot was pipetted into another 100 ml volumetric flask and 1.0 ml of 1 M acetic acid and 2.0 ml of iodine solution was added. The flask was topped up to the mark with distilled water. The absorbance of each solution and blank were recorded using a UV/visible spectrophotometer at 620 nm (Cecil 7500 - British). The amylose content was calculated as follow:

Amylose content (%) = 3.06 × absorbance x 20, Amylopectin (%) = 100 - % amylose content [22]

2.6. Bulk density and tapped density

The bulk density of starch sample was determined by pouring the starch powders at an angle of 45° through a funnel into a 50 ml glass measuring cylinder. The weight of starch powder and the volume it occupied was noted. The tapped density was determined by tapping the measuring cylinder containing powder on to a wooden surface from a height of 3cm at a rate of 2 tapping per second. The tapings were continued until a constant volume (300 tapings) was obtained for the starch samples. Tapped density was measured as the mass/tap volume of sample after tapping as elaborated below [31].

Bulk density = $\frac{Weight of starch granule}{Volume occupied}$ Tapped = $\frac{Weight of starch granule}{Volume occupied after tapping}$

2.7. Foaming Capacity

Starch sample (1.000g) were homogenized in 50 ml distilled water using a Variwhirl vortex mixer for 5 minutes. The homogenate was poured into a 100 ml measuring cylinder and the volume recorded after 30 seconds. The foam capacity was expressed as the percent increase in volume [31].

2.8. Browning and charring temperature

The Afolayan *et al.,* [3] was used. Some of the starch sample was put into a capillary tube, the browning and charring temperatures were recorded using a melting point apparatus with (model Electrothermal 9100).

2.9. Paste clarity

Paste clarity was determined by obtaining accurate concentrations of the starch slurry between 0.15 - 2.5 % w/v were made in different boiling tubes and heated in a water bath for 30 minutes. The transmittance was determined at 580 nm using a UV/visible double beam spectrophotometer (Cecil 7500) with cassava starch as the reference standard [3].

2.10. Gelation Properties

Gelation studies were investigated by Awokoya *et al.* [5] with slight modification samples of starch (0.1, 0.2, 0.3, 0.4, 0.5, and 0.6 g) were combined with 5 ml portions of distilled water in test tubes and blended by a Variwhirl mixer (Model A901, salver chem. Chicago, IL, USA) for 5 minutes. The test tubes were then heated for 30 min at 80°C in a water bath, followed by rapid cooling under running cold tap water. The test tubes were further cooled and held at 6 °C for 2 hours. The lowest gelation concentration of the native and modified starch samples was determined as that concentration when the sample did not fall down or slip from the inverted test tube.

2.11. Determination of pH

A 20 % w/v dispersion of the sample was shaken in water for 5 minutes and the pH value recorded using a pH meter (Hanna instrument) that was previously calibrated with buffer 4,7 and 10 [31].

3. Instrumental Characterization

3.1. Fourier Transform Infrared Spectroscopy

Infrared spectra of JB starch was recorded on FTIR spectrophotometer (FT-IR-Nicolet is5 Fourier transform infrared spectrophotometer Thermo Fisher Scientific, USA) with a view to determine the functional groups present in JBS. The sample was done neat by placing the starch sample between interferometer and detector. The interferograms were Fourier transformed to produce various spectra between 4,000 to 500 cm⁻¹ (mid-infrared region) at a resolution of 0.44 cm⁻¹. [27].This analysis was carried out in Chemistry Advanced Research Centre, Sheda Science and Technology Complex, Abuja.

3.2. Scanning Electron Microscopy

A scanning electron microscope (SEM) model EVO/ MA 10 was used to determine morphological properties of the JB starch granule based on the method earlier proposed by [23] The granule morphology which includes particle size at 200X, 500X, and 1000X magnifications, shape, surface area properties, and type of granular starch. The sample was imaged with the secondary electron detector at an accelerating voltage of 20 kV, probe current of 200PA and variable pressure of 50 Pa. This analysis was carried out in Chemistry Advanced Research Centre, Sheda Science and Technology Complex, Abuja.

3.3. X-ray Diffractometry

The degree of crystalinity of the JBS was quantitatively estimated using the method previously described by (Cheetham *et al.*[13] and using an X-ray diffractometer (Empyrean X'pert Pro of PanAnalytical, Holland) operating in the 2 θ modes using a copper anode X-ray tube. A standard polycrystalline silicon powder was used to calibrate the equipment. The starch powder samples were packed tightly into the circular aluminum sample holders that were previously incubated in the chamber at 100% RH for 24 hours. JBS was exposed to the X-ray beam generator running continuously at 45 kV and 40 mA. The scanning region of the diffraction angle ranged from $2\theta = 9$ to 75° at an increment of 0.50 with a count time of 1.5s and count step of 0.026°; the rotary speed of the sample holder was 30 min-1. Radiation was detected with a proportional detector. A smooth curve, which connected peak baselines, was computer-plotted on the diffractograms. Duplicate measurements were made at ambient temperature.

3.4. Statistical Analysis

Data obtained were subjected to One-way analysis of variance (ANOVA), and significant means were separated using New Duncan's Multiple range test at p = 0.05.

3.5. Starch purity and percentage yield

Jack bean starch was found to be a white, crystalline, non- hygroscopic powder with a yield of about 21 %. It exhibited no pasting or gelling properties when heated at 100 °C for 30 min. The JB starch shown a similar thermal behavior with an earlier work on legume starch [15]. The pH value of 6.50 recorded was within the pH range of 3 – 9 obtained for most starches used in the pharmaceutical, cosmetics and food industries [15].

3.6. Proximate Analysis of Jack bean starch

Proximate analysis result as presented in table 1 below indicated that JBS has moisture (11.2%), ash (6.41%), lipid (0.91%), crude protein (2.28%), and carbohydrate (79.2%) and metabolizabele energy (431.7 kcal/100g). Low moisture content is required for safe storage as high moisture content can lead to microbial damage and subsequent deterioration in starch quality. [6], [10] Ash content represents mineral composition present as minor component such, calcium, sodium, potassium and phosphorus [26]. Lipid content revealed that JB starch has low value and result is comparable with starch isolated from other leguminous seed and other feedstock sources [25],[3]. High lipid content in starch sample will compromise starch appearance and functional properties. Lipid reacts with phosphorus in starch to form phospholipids which retards water penetration into the starch granular matrix. Thereby affecting its rheological properties such as swelling power, solubility profile paste clarity and viscosity [45].High crude protein in starch sample is regarded as a contaminant and thus it is very important to remove mucilage from starch since the later constitute

protein fat and other unwanted components [31]. The value obtained for crude protein (2.280 %) was similar to previous work on starch from various sources including legumes [5], [25]. Where 0.400% and 5.000% were recorded for starch isolated from bambarra nut and cowpea respectively. The result of crude fibre (0%) suggests good starch processing and in agreement with previous work done on starch from various sources [5], [20]. Carbohydrate content and metabolizable energy as presented on table 1 revealed that JB starch has (79.2 %) and metabolizable energy (430.1 kcal/100g). The values obtained were similar to previous work done on starch isolated from other sources [6]. Minor components of native starches vary according to their botanical origin; the legume starch contains more lipids and proteins than root and tuber starches [2].

Table 1 The result of Proximate Analysis and physicochemical properties.

Parameters (%)	Results		
Moisture	11.3 ^a ±0.11		
Lipid content	6.41 ^a ±0.23		
Ash content	0.19 ^a ±0.32		
Crude fibre	0.00 ± 0.00		
Protein content	2.28 ^a ±0.19		
Carbohydrate	79.2 ^a ±0.01		
WHC (%)	97.4 ^a ±0.01		
OAC (%)	2 2.0 ^a ±0.01		
F C (%)	10.2 ^a ±0.10		
B T (°C)	295 ^b ±0.50		
CT (°C)	321 ^b ±0.50		
G T (ºC)	83.0 ^b ±0.50		
рН	6.50 ^a ±0.10		
TA (%)	0.05 ^a ±0.00		
TD g/cm ³	0.96 ^a ±0.00		
BD	0.80 ^a ±0.00		
Amylose (%)	20.20		
Amylopectin (%)	79.80		

3.7. Oil absorption capacity and water holding capacity (OAC& WHC)

Certain industrial food preparations require proper oil emulsification and water holding capacity for food stability and good qualities such as colour, taste, and texture. Therefore, an oil absorption and water holding properties are very important functional properties of native and modified starch. The result of oil absorption and water holding capacity of JB starch were 22.0 and 97.4% as presented in table 1 above and it was observed that that the result obtained is comparable with previous work done on starch isolated from other legume . For instance, previous values reported values between 33.8-34.3% while lkegwu *et al. [23]* recorded higher values, ranging from 69.04-78.68% for OAC against starch isolated from different cassava cultivar.

3.8. Gelatinization Temperature (GT)

The result of GT as displayed on table 1 above revealed that 83°C was recorded and the value obtained is in agreement with previous work earlier reported on starch isolated from other sources including legumes. However, Afolayan *et al.*, 2012 reported 72°C against starch isolated from *Anchomanes difformis* while Ikegwu *et al.* [12] recorded values ranging from 61.0-107.75°C for starch isolated from different cassava cultivar. A low gelatinization temperature property is require for lower operational cost of maintaining production plant in the starch industries [32].

3.9. Browning and Charring Temperature (B&CT)

From table 1 above, the values obtained for browning and charring temperatures of JB starch was between 281-283 ^oC and 320-321^oC respectively as against 254.6-262.8 ^oC and 282.9-289.9 ^oC as earlier reported by Omojola *et al.* [32] for Icacina starch. While Afolayan *et al.*, [6] also recorded 257-268.2 ^oC for browning temperature and 281.4-291.6 ^oC for charring temperature for *Anchomanes difformis* starch. The retrogradation properties of starches are indirectly

influenced by the structural arrangement of starch chains, which in turn, influence the extent of granule, break down during gelatinization. [37]. Starch with high charring and browning temperature is require to withstand plant thermodynamic conditions and to retain food colour and other physico-chemical properties.

3.10. Amylose and Amylopectin content

The amylose and amylopectin content of starch granule determine the functional, physico-chemical morphological and crystalinity characteristics of starch granule [25], [23]. The result of amylose and amylopectin content of JB starch were 20.2 % and 79.8 % as shown on table 1. The outcome of the result was comparable with previous values recorded against some starches isolated from legumes [27]. And besides, Awokoya *et al.*, [5] recorded 25.45 % for amylose content and 74.55 % for amylopectin content for starch isolated cocoyam.

3.11. Swelling Power and Solubility Profile

Starch has been classified as a moderately hygroscopic material [16]. The extent of hydration and swelling depends on the accessibility of the hydroxyl groups in the starch to the water, and it has been suggested that the amorphous regions are responsible for the reversible swelling of starch upon the absorption of water [36],[17]. The semi-crystalline structure of the starch granules is responsible for their low solubility even at high temperatures [16]. Results of the effect of temperature on swelling power and solubility of JBS were presented in Table 2 and 3 below respectively. The results indicate that both swelling power and solubility were temperature dependent, values increased with increase in temperature. This suggests that increase in temperature enhanced penetration of water into the starch granules samples as thermodynamic mobility of particles increased as temperature increased, thereby facilitating penetration of water into the granules [5],[1]. Previously, it was also reported that swelling power capacity increase with increasing temperatures for all legume starches as raising temperature weakened the intragranular binding forces of both native and chemically modified starches, thereby enhancing leaching of granular particles which led to increased solubility. Maximal swelling capacity and solubility in all cases was observed at 95 °C. The starch samples showed a two-stage swelling pattern as previously been reported for other sources previously [3]. This type of swelling has been noted to be typical swelling pattern of legume starch [33]. Relaxation occurred between 60-70 °C and at 95 °C. This development is in agreement with the previous result recorded for legume starch [39, [6].

Table 2 The results of swelling profile of JB starch

Temp.(°C)	50	60	70	80	90	95
JBS	1.41 a ±0.04	3.04b±0.003	2.31 a ±0.22	2.67b±0.12	12.0 b ±0.02	14.3 b ±0.07

Table 3 The result of Solubility profile for JB starch

Temp.(°C)	50	60	70	80	90	95
JBS	1.60 a ±0.51	1.00 a ±0.01	1.00 a ±0.12	1.00 a ±0.11	1.90 a ±0.51	3.60 a ±0.23

3.12. Paste Clarity

When a starch granule is heated in excess water, it leads to further granule swelling, additional leaching of soluble components and total disruption of granule. This process results in the formation of a viscous starch paste [26]. The viscosity of starch, as a food component is a vital factor for consideration in its applicability to food systems.

Table 4 The result of Paste Clarity of Jack bean starch and cassava starch

Conc.	NSA	TNS	CS A	CST
0.150	0.35a±0.01	2.85 ±01	0.35 a±0.02	2.83 a±0.00
0.500	0.69a±0.05	1.44 a ±0.02	0.87 a±0.12	1.13 a±0.00
1.000	0.96a±0.01	1.03 a ±0.01	1.16 a±0.34	0.85 a±0.00
1.500	1.12a±0.01	0.88 a ±0.02	1.28 a±0.44	0.77 a±0.00
2.500	1.41a±0.01	0.70 a ±0.10	1.47 a±0.00	0.68 a±0.00

Test.*NSA =Native starch Absorbance, TNS =Transmittance Native starch, CSA=Cassava starch absorbance, CST= Cassava starch transmittance, Conc.: Concentration

The result of pasting property of JBS is presented in Table 4 below and it was revealed that pasting clarity reduced with an increase in concentration from 0.150 g/100 ml to 2.500 g/100 ml . JBS has higher paste clarity at 2.500 g/100 g when compared with cassava starch. This result is in agreement with previous work done on other starch from different sources such *moringa oleifera* starch ([20] tacca citrate), anchomanes starch [6].

3.13. FTIR Spectroscopy

The spectrum of JB starch as presented in figure 1 below shown broad peak 3000-3200 cm⁻¹ and 2923.55 cm⁻¹ correspond to -OH and -CH stretching respectively while the peaks at 1637.80 and 1460.29cm⁻¹ were attributed to the bending modes of H–C–H, C–H and O–H [28].Peaks around 900-950 cm⁻¹ connote vibration of the glycosidic linkage [42] A distinctive peak was aroused at 1640.81 cm⁻¹ which may be due to the presence of the water molecules bounded to the starch granules why peaks around 1458.98 and 1376.85 cm⁻¹ arise due to scissoring vibration of CH₂ [18].

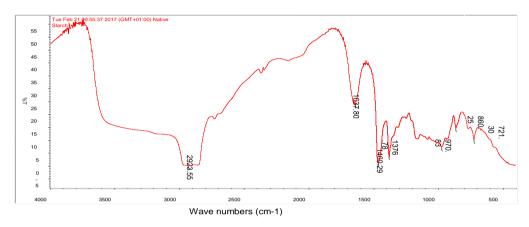


Figure 1 Infrared spectrum of Jack bean starch

3.14. X-ray diffractometry analysis

The XRD pattern gave stronger diffraction peaks around 15, 17, 18 and 23° as displayed in figure 2 below and the diffractograms follow similar crystalinity pattern (type C) of leguminous starch indicating type-C crystalline structure. Comparing diffractograms with previous studies done on leguminous starch it was observed JBS has a typical C-type crystalline patterns with other leguminous starch as previously reported [17] with major peaks at 2 theta =15°,17°,24°; 15°,17°23°; 15°,17°23°, 15°,17°23°, 31°& 15°,17°,24° respectively.

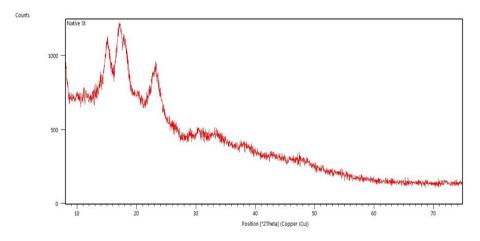


Figure 2 X-ray powder diffractograms of Native starch of Jack bean

3.15. Granule Morphology

Photomicrographs of JB starch at various magnifications (X202,X502 and X1000) as displayed in Figure 3-5 below indicate that starch granules are generally small in size ($20 \mu m$) with a uniform oval shape, free from dirt as the surface is smooth and granular with no fragments. It was also observed that the granules are free from dirt and disruption indicating good processing and handling. The starch granules have an average size of 10-20 μm with granules evenly

packed and not clustering. This is in agreement with previous starch work isolated from other legume [12]. Evenly spread or packed have been reported to have varied utilization in food and pharmaceutical industries [32], [1] .The result of EDX revealed elemental composition qualitatively present in the starch samples which are majorly carbon and oxygen [40].

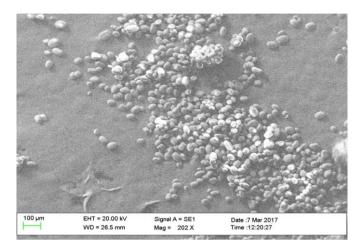


Figure 3 The Scanning electron Micrograph of JB Starch at x202 magnification scanning electron.

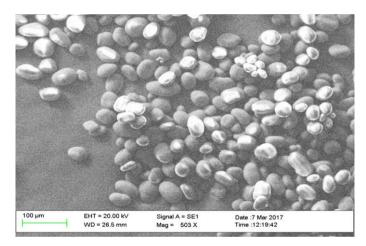


Figure 4 The Scanning electron Micrograph of JB Starch at x 1000 magnification scanning electron

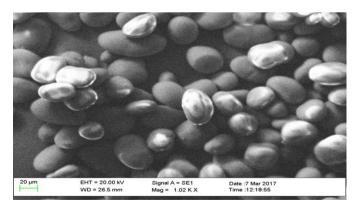


Figure 5 Scanning electron micrograph of JB starch at x1000 magnification scanning electron.

4. Conclusion

This study revealed that a useful potential source of industrial grade starch was isolated from Jack beans (*Canavalia ensiformis*) which do not have direct competition with human food. The physico-chemical properties of starch revealed

that jack bean starch can compete favourably with starch isolated from other established sources such as cassava and corn. Instrumental characteristics revealed that the starch molecules architecture and granular integrity were intact after isolation and also have resemblance with starch isolated from other sources. The chemical properties jack bean starch as revealed in this study appears to be a good candidate for industrial grade starch.

Compliance with ethical standards

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Disclosure of conflict of interest

The Authors declare no conflict of interest.

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