jopat 10 2005 6 - 12

Journal Of Phytomedicine
And Therapeutics

# Extraction and characterization of *Voandezia subterranean (L)* (earth pea) starch a potential pharmaceutical excipient

Ohwoavworhua F.O. [1]*, Adeola J.I. [2] and Kunle O.O. [1]

[1]*Department of Pharmaceutical Technology and Raw Materials Development,*
[2]*Department of Medicinal Plant Research and Traditional Medicine,*
*National Institute for Pharmaceutical Research and Development (NIPRD)*
*Idu Industrial Area, P. M. P. 21, Garki, Abuja, NIGERIA*

**Abstract**

Earth pea starch (EPS) obtained from *Voandezia subterranea,* Papilionacea has been characterized. The starch was extracted from the underground seed and then defatted. The starch was dried in the oven at 60$^0$C. Corn starch B.P was used as a reference standard. Particle size, amylose/amylopectin ratio, paste clarity, tapped and true densities, charring, browning and gelatinization temperatures, moisture content, hydration capacity, rate of water penetration , moisture sorption capacity and elemental analysis are characteristic properties investigated. The study revealed that the starch compares favourably with official requirements as specified in the British Pharmacopoeia and the Handbook of Pharmaceutical Excipients for starches.

Keywords: *Voandezia subterranea;* starch; characterisation

## Introduction

Starch is widely used in the production of pharmaceuticals. This is because of its relative inertness, wide availability, low cost and its functional nature. Its uses are mainly due to its adhesive, thickening, gelling, swelling and film forming properties.

Pharmaceutical grade starch can be obtained from various sources depending on the ease of extraction, abundance of the material in any particular location as well as cost. Nigeria has many native plant species which can serve as sources for pharmaceutical grade starch. Some of these have been investigated and found to be suitable (1- 6).

*Voandezia subterranean* (L), Papilionaceae, commonly known as earth pea nut or bambara groundnut is a annual plant widely cultivated in many parts of Nigeria. It is closely related to ground nut (*Arachis hypogaea*). The fresh or dried nut is widely eaten boiled or roasted as a snack in various parts of Nigeria. It has a starch content of between 47-59% (7).

The chemical composition and physical characteristics of a starch which affects its performance in pharmaceutical formulations [3] are essentially dependent on its biological origin [8]. It is therefore essential to study the physicochemical characteristics of a starch before its use in pharmaceuticals. There is no report in literature of the physiochemical evaluation of earth pea starch. This study is therefore designed to determine the physicochemical properties of earth pea starch which might influence its role in pharmaceutical formulations. These properties were compared to those of corn starch BP.

* Author for Correspondence (e-mail: frankohwo@yahoo.com)

## Materials and methods

*Materials*

EPS was extracted as described below from the seeds of *V. subterranean* purchased from Jiwa, Abuja, Nigeria. The seeds were authenticated by Mr. Ohaeri (late), taxonomist of the National Institute for Pharmaceutical Research and Development (NIPRD), Abuja, Nigeria. Sodium metabisulphite (Sigma Chemicals Ltd., St. Louis, USA); petroleum ether analytical grade (May and Baker, England), corn starch BP (BDH Chemicals Ltd., Poole, UK). All other materials used were either analytical or reagent grade.

*Methods*

*Preparation of earth pea starch*

The dried seeds were chopped into small bits and the seed coats separated from the endocarp by winnowing. The endocarp was then milled and 1.4 kg weight of the powder was steeped in large quantities of 0.07% w/w solution of sodium metabisulphite (to generate sulphur dioxide required to prevent discoloration of the starch product by oxidative enzymes) for 4 hours, after which the supernatant was decanted. The sedimented starch was repeatedly washed with sodium metabisulphite solution until the suspension became clear. The material was then passed through fine muslin cloth to remove cell debris. The starch was collected and dried in an oven at 60 $^{0}$C for 12 hours.

*Defatting process*

The method of Perrin and Armerago (9) was used. 20.0 g of the starch sample was dispersed in 500 ml of water. The slurry obtained was shaken with about 700 ml of petroleum ether and left for 2 days with 5 hours of continuous shaking each day. The organic (upper) phase containing the fatty substances was separated from the aqueous phase after the second day. The starch sediment in the aqueous phase was washed several times with distilled water, dried in a hot air oven at 65 $^{0}$C for 4 hours, reduced to fine powder and stored in an air-tight jar. The defatted starch was subjected to the following tests.

*Microscopy*

A small quantity of each of the starches was mounted in a drop of glycerol on a glass slide and covered with a slip. The particle size was determined with a microscope (Olympus) equipped with micrometer using 40 X magnification.

*Estimation of amylose/amylopectin ratio*

The amylose/amylopectin content was determined using the method of Onah and Bristol (6). The method essentially involves weighing 2.0 g each of the starches and suspending in 50 ml of water, which was then added with stiring to boiling butanol: water mixture (1:9). Butyl and amylacohol (1:1) was added to the suspension which was then allowed to cool in a cold water bath. The mother liquor was decanted and the microcrystalline precipitate formed was washed repeatedly with butanol saturated with water, dried in oven and weighed. Amylopectin was similarly precipitated with excess methanol from the first supernatant.

*Paste clarity*

This was determined as described by Attama and Adikwu (4). Graded aqueous concentrations of EPS and corn starch pastes ranging from 0.5 to 2.5% w/v were prepared. The percentage of light transmitted at 580 nm was determined for each paste using UV-visible spectrophotometer (Shimadzu, Tokyo, Japan). The percentage of light transmitted was plotted against the starch paste concentration for the two starches.

*Particle density*
The densities of the starch powders were determined by the liquid displacement method using petroleum ether as the immersion fluid (10).

*pH determination*
The pH of 20% w/v slurry of each of the starches was determined using a pH meter (Corning, Model 10, England).

*Acidity*
The BP (1993) method was used.

*Gelatinization, browning and charring temperatures*
A 0.29 % w/w starch suspension in water contained in a suitable vessel was heated in a thermostated water bath at 40 $^{0}$C. The temperature was gradually raised by about 2 $^{0}$C/min. Visual estimated of the portion of particles that lost their polarization cross were made using polarizing microscope (Vicker Instrument, England) (12). The browning and charring temperatures were determined in the Electro-thermal melting point apparatus (Electrothermal Engineering Ltd., Southend, England).

*Elemental analysis*
One gram of each of the starch powders was taken and reduced to ash in furnace. The residue was mixed with concentrated nitric acid and further ashed. The residue was dissolved in water and made up to 100 ml. The elements present and their concentrations were determined by means of atomic absorption/flame emission spectrophotometer (AA 680 Shimadzu).

*Moisture content*
About 5.0 g of each starch powder sample was placed in a moisture balance (OHAUS MB 200, USA) at 110 $^{0}$C for 3 hours. The percent moisture was determined.

*Hydration capacity*
The method of Odusote and Nasipuri (13) was used. 1 g sample of starch was placed in a tarred 20 ml stopper centrifuge tube. 10 ml of water was added and shaken vigorously for two minutes. It was allowed to stand for ten minutes during which it was mixed by inverting the tube three times at the end of 5 and 10 minutes. The sample was then centrifuged for 10 minutes at 1000 rpm and the aqueous supernatant carefully removed. The tube with the sediment was re-weighed. The experiments were carried out at about 37 $^{0}$C. The hydration capacity was calculated as the ratio of the weight of the sediment to the initial weight of dry powder.

*Redispersion time*
Ten mls of water was carefully added to the sediment obtained from the determination of hydration capacity. The tubes were then shaken gently with the same intensity until the sedimented starch was completely redispersed. The time taken for the sediment to redisperse was taken as the redispersion time.

*Rate of water penetration*
The method of Chukwu and Okorie (14) was used with slight modification. 4 g of each of the starch powder was carefully introduced into separate clean calibrated dry perspex tubes of internal diameter 5.0 mm. The tubes were tapped on a padded platform until there was no more decrease in powder volume. They were then simultaneously immersed into a Petri dish containing water. The tubes were supported on a retort stand. The rate of penetration of water

into the powder columns was determined by measuring the time taken by the aqueous medium to ascend varying heights for a total time interval of 40 minutes. The procedure was carried out two times and the mean value noted.

*Moisture sorption capacity*
The method of Odusote and Nasipuri (13) was used. 10 g each of the dried starch, accurately weighed was evenly distributed in a Petri dish. The samples were exposed to atmosphere of 98% relative humidity (saturated solution of Lead nitrate at room temperature) in a desiccator. At various time intervals, the weight gain by the samples was recorded and the percentage of water sorbed was calculated from the weight difference.

*Statistical analysis*
The data were analysed statistically by the student t-test (level of significance 0.05) using Excel 2000 Windows™ (Microsoft Corporation).

## Results and discussion

*Physicochemical Properties*
The percentage yield of earth pea starch after defatting is about 58.5% with a moisture content of 11.5%.

The observed shape of corn starch is consistent with those that have been reported in literature for corn starch (6). The earth pea starch granules are predominantly angular and oblong in shape and mostly simple, while those of corn starch are mainly round and polyhedral. EPS helium occurs as single, double or triple-rayed cleft, mostly occurring as centric while few others are eccentric. Few faintly marked concentric striations were visible on the large sized granules. Granular size ranged from 7-43-65 μm as compared to 4-12-21 μm for corn starch.

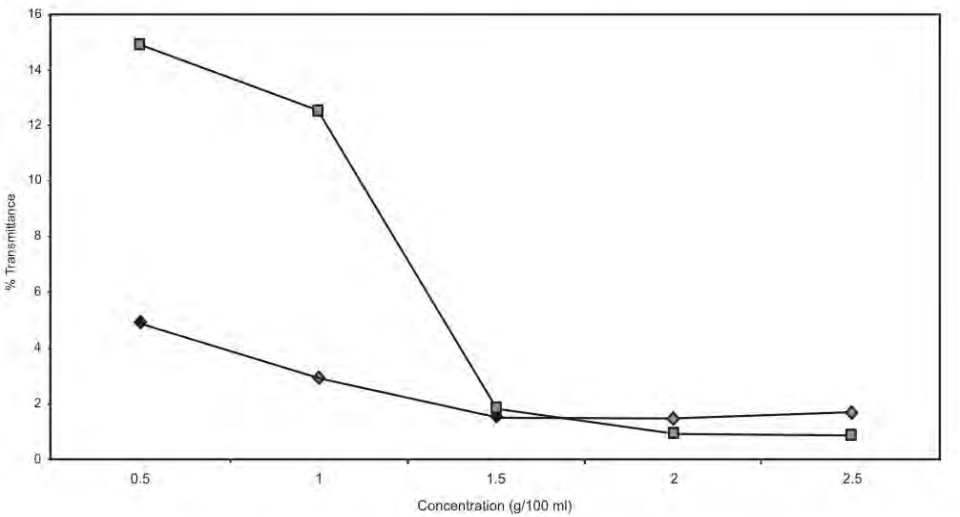


Fig. 1: Percent Transmission/Conc. (g/100 ml). A Measure of Paste Clarity of the Starch Samples

The result of the paste clarity of the starches is shown in Figure 1. At concentrations below 1.5% w/v, EPS had significantly higher transmittance than corn starch since waxy starches (high amylopectin) are known to have higher paste clarity than non-waxy ones (4). The relatively higher amylopectin content of EPS compared to corn starch could account for the observed result. The large granule size of the EPS could also have contributed to the observed result (4). However, at the higher concentrations (greater than 1.5% w/v), there was no significant difference in their transmittance.

Table 1: Characteristic properties of earth pea and corn starches of relevance to pharmaceutical excipients (Mean & Standard Deviation, n =3)

| S/No. | Parameters | Earth Pea Starch | Corn Starch |
|---|---|---|---|
| 1. | Shape of granules | Angular/oblong | Angular/round/polyhedral |
| 2. | Diameter of granules | (7-43-. | (4-12-. |
| 3. | Amylose/Amylopectin | 6:94 | 15:85 |
| 4. | True density (g/ml) | 1.39 ± 0.046 | 1.42 ± 0.216 |
| 5. | Bulk density (g/ml) | 0.634 ± 0.016 | 0.634 ± 0.016 |
| 6. | Tapped density (g/ml) | 0.75 ± 0.000 | 0.714 ± 0.000 |
| 7. | Percent compressibility (%) | 15.47 | 11.20 |
| 8. | Porosity ($\varepsilon = 1-D_b/D_t$) | 0.5438 | 0.5535 |
| 9. | pH (20% w/v slurry) | 5.0 | 7.4 |
| 10. | Acidity (millieq gm) | $3.9 \times 10^{-3}$ | $2.3 \times 10^{-3}$ |
| 11. | Gelatinization temperature ($^0$C) | 54 - 63 | 63 - 74 |
| 12. | Browning temperature ($^0$C) | 216.5 - 257.5 | 223.5 - 230.8 |
| 13. | Charring temperature ($^0$C) | 253.5 - 257.5 | 256.5 - 260.5 |
| 14. | Moisture content (%) | 11.5 | 14.5 |
| 15. | Hydration capacity | 2.36 ± 0.035 | 1.72 ± 0.027 |
| 16. | Redispersion time (mins) | 6.33 ± 0.577 | 3.37 ± 0.577 |

The pH of both starches were within the acceptable (15) range of 4.5 to 8 (Table 1). The acidic nature of EPS might make it more suitable for use in tableting since starches are known to hydrolyse mainly at alkaline pH values. Caution however, need to be exercised when it is to be used with alkaline drugs.

Table 2: Elemental constituents earth pea and corn starch powder

| ELEMENT | % Elemental Constituent In 1.0 G Sample | |
|---|---|---|
| | Earth Pea Starch | Corn Starch (bp) |
| Iron | 0 | 0 |
| Manganese | 0 | 0 |
| Copper | 0 | 0 |
| Zinc | 0 | 0 |
| Magnesium | 0.003 | 0.003 |
| Lead | 0 | 0 |
| Calcium | 0.0025 | 0.007 |
| Sn | 0 | 0 |
| Sodium | 0.0070 | 0.0056 |
| Potassium | 0.0059 | 0.0113 |

**LEGENDS**

Figure 1: Paste Clarity; (□) Earth Pea Starch (EPS), (◇) Corn Starch (CS)

Figure 2: Rate of Water Penetration; (▩) Earth Pea Starch (EPS), (□) Corn Starch (CS)

Figure 3: Moisture Sorption Profile; (□) Earth Pea Starch (EPS), (◇) Corn Starch (CS)

The results of elemental analysis (Table 2) show that the starches met official (BP) standards. EPS had comparable elemental composition to the official corn starch. This indicates that it can be used in pharmaceutical formulations. More importantly, heavy metals such as Lead and manganese whose presence are undesirable in pharmaceutical raw materials were completely absent.

*Powder properties*

Although corn starch had a higher range of water penetration than EPS, this was not statistically significant (Figure 2). This indicates that both starches might have similar mechanism of action as disintegrants in tablet formulations.

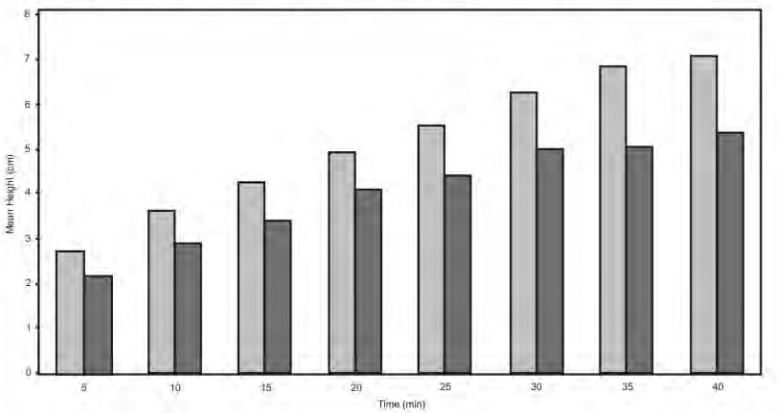


Fig. 2: Mean Height (cm)/Time (min). Study of Rate of Water Penetration through columns of the Selected Starches

The observed rapid penetration of water in corn starch could be due to the formation of a large system of capillaries because of its smaller sized grains. The higher porosity of corn starch relative to EPS (Table 1) supports this.

Knowledge of moisture sorption of starches is necessary where controlled powder flow or compaction is critical. Moisture modifies the flow and mechanical properties of many powders including starches (8). The moisture sorption profiles of EPS and corn starch equilibrated at 98% relative humidity were similar (Figure 3) with the total amount of moisture taken up by corn starch being higher. This observation could be due to the large surface area of the smaller size corn starch as compared to EPS (Table 1). The difference was however, not statistically significant.

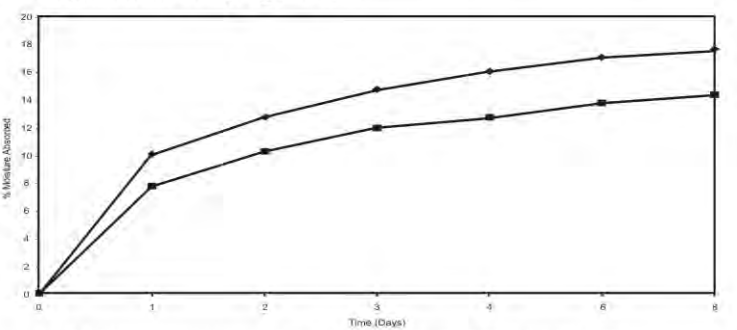


Fig. 3: Percent Moisture Absorbed/Time (days). A Moisture Sorption Profile of Selected Starches exposed to 95% Relative Humidity

It is assumed that the hydration of a starch represents the water absorbed by the granule and/or the granular surface. The hydration capacity values for EPS were approximately twice that of corn starch though it had lower surface area (Table 1). The implication for the high hydration capacity values for EPS is that faster rate of disintegration due to swelling should be expected from tablets containing it as disintegrant compared to those containing corn starch. Adebayo and Itiola (16) however, cautioned that hydration capacity values should not be taken as an absolute indicative index of disintegrant powder.

## Conclusion

The earth pea starch has been observed to have fundamental, derived and physicochemical properties which could favour its use as a pharmaceutical excipient. While its hydration capacity is superior to official corn starch, its other fundamental properties like rate of water penetration and moisture sorption capacity were statistically indistinguishable from corn starch. The nature and level of elemental constituents of EPS are similar to those of corn starch BP; indicating that the process adopted for its extraction appears to be suitable for obtaining pharmaceutical grade starch from earth peanut (*Voandezia subterranean*). The study revealed that the starch compares favourably with corn starch BP.
Further studies are being carried out on its use as an excipient in tablet formulation.

## References


(1) Nasipuri, R. N. (1975). Evaluation of cassava starch as tablet binder and disintegrant. J. Medical and Pharm. Marketing, 4, 17.

(2) Opakunle, W. O. and Jaiyeoba, K. T. (1978). Glidant properties of cassava and yam starches. Manf. Chem. & Aer. News, 49, 77.

(3) Kunle, O. O. and Bangudu, A. B. (1990). The effect of some starches on the properties of promethazine tablets. Pharm. World J., 12 (7), 26- 31.

(4) Attama, A. A. and Adikwu, M. U. (1999). Physicochemical properties of starch derived from *Tacca involucrate*. Nig. J. Nat. Prod. and Med., 3, 71 73.

(5) Adebayo, A. S. and Itiola, O. A. (1998). Evaluation of breadfruit and cocoyam starches as exo-disintegrants in a paracetamol tablet formulation. Pharm. Pharmacol. Commun, 4 : 385 389.

(6) Onah, J. O. and Bristol, D. O. (1999). Studies on the physicochemical properties of starches from *Cajanus cajan* and *digitaria exilis*. J. Pharm. Res. Dev., 4, 73-78.

(7) Dalziel, J. M. (ed) (1937). The useful plants of West Tropical Africa; The Crown Agent for the Colonies, London.

(8) Gebre-Mariam, T. and Schmidit, P. C. (1996). Isolation and physicochemical properties of Enset starch. Starch/Starke, 48, 208 214.

(9) Perrin, D. W. and Armerago, D. (1993). Common physical techniques used in purification. In: Purification of Laboratory Chemicals.

(10) Kings, R. E. (1980). Remington's Pharmaceutical Sciences Author Osol, 16th ed, Merck Publication, Pennsylvania, 1553.

(11) The British Pharmacopoeia (1993). HMSO Press, London, 1, 399

(12) Okafor, I. S., Udeala, O. K. and Onyechi, J. O (1991). A comparative study of modified starches in direct compression tableting some physicochemical properties of the modified starches. 1st NAAP Scientific Conference Proceeding, 111-115.

(13) Odusote, M. O. and Nasipuri, R. N. (1987). Correction between some properties of starches and the disintegration behaviour of tablets. Nig. J. Pharmacy, 18 (3), 28 31.

(14) Chukwu, A. and Okorie, O. (1992). Penetration rate of water into some tablet disintegrants and subsequent disintegration of diazepam tablets, 2nd NAAP Scientific Conference Proceeding.

(15) The Handbook of Pharmaceutical Excipients (1986). American Pharmaceutical Association, U.S.A and the Pharmaceutical Society of Great Britain, England.

(16) Adebayo, .A.S. and Itiola, O. A. (1998). Properties of starches obtained from *Cocasia esculenta* and *Artocarpus communis*. Nig. J. Nat. Prod and Med., 2, 29-33.