Isolation and Characterization Studies of *Moringa Oleifera* Root Starch as a Potential Pharmaceutical and Industrial Biomaterial

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Abstract

Moringa Oleifera Lam. (Moringaceae family) is a deciduous plant with tuber-like root. The tuber of the plant was isolated and examined for its starch constituent and physicochemical properties. The starch was isolated using 1 % w/v sodium metabisulphite solution. The starch obtained was found to be a pure white, crystalline, non-hygroscopic powder with a yield of about 16.31%. The starch percentage solubility at 95OC was 21.66 % with a swelling power of 18.07 and gelatinization temperature of 68OC. It has a browning temperature of 268.6 - 270.8OC, charring temperature of 284.5 - 295.7OC, water absorption capacity of 71.13 %, pH of 6.2, foam and emulsion capacities of 4 % and 7.5 % respectively. Phytochemical screening of the starch revealed the presence of carbohydrates, flavonoids, saponins and glycosides while the proximate analysis (in %) was found to be: fat - 14.3, ash - 0.143, crude fibre - BDL, protein - 6.125, moisture -12.76, and carbohydrates - 66.58. The paste clarity was determined at 580 nm as a function of the starch concentration. The amylose content was also determined using a colorimetric iodine affinity procedure. Generally. obtained from the physicochemical the values characterization of Moringa Oleifera Lam starch show that it has high potential for industrial application especially in the food, textile and pharmaceutical industries.

Keywords: *Moringa Oleifera* Lam, physicochemical properties, starch, phytochemical screening, paste clarity.

1. Introduction

Moringa Oleifera Lam. (Moringaceae) is one of the 14 species of the family moringaceae, native to India, Africa, Arabia, Southeast Asia, South America, and the Pacific and Caribbean Islands. Because *M. oleifera* has been naturalized in many tropic and sub-tropic regions worldwide, the plant is referred to by a number of names such as horseradish tree, drumstick tree, ben oil tree, miracle tree, and "Mother's Best Friend". *Moringa Oleifera* Lam. is commonly called ben oil tree and locally known as *Zogeli* among the Hausa speaking people of Nigeria. It is grown and widely cultivated in the northern part of Nigeria and many countries in tropical Africa. *M. oleifera* can be grown in a variety of soil conditions preferring well-drained sandy or loamy soil that is slightly alkaline. Almost every part of *M. oleifera* can be used for food and as a forage for livestock (Abdul, 2007).

Moringa tree was introduced to Africa from India at the turn of the twentieth century where it was to be used as a health supplement. It is traditionally used for the treatment of a number of ailments including as fomentation to relieve spasm, diarrhea, as diuretic and stimulant in paralytic affliction, epilepsy and hysteria. For centuries, people in many countries have used Moringa leaves as traditional medicine for common ailments (Muluvi et al ,1999).

Epidemiological studies have indicated that *M. oleifera* leaves are a good source of nutrition and exhibit anti-tumor, anti-inflammatory, anti-ulcer, a good source of vitamins, anti-atherosclerotic, immune booster, sperm booster and anti-convulsant activities. Clinical studies have begun to suggest that at least some of these claims are valid. With such great medicinal value being suggested by traditional medicine, further clinical testing is very much needed (Chumark et al, 2008).

Starch is one of the most abundant organic chemicals on earth. It is found in the leaves of green plants in the plastids where it is synthesized. It is also synthesized in the amyloplasts of seeds, grains, roots and tubers of most plants where it serves as the chemical storage form of energy (Afolayan *et al.*, 2012). Some lesser known and unconventional crops could be good sources of nutrients and starch and even have the potential of broadening the present narrow food base of the human species but the lack of data on the chemical composition and properties of such plants has limited the prospects for their utilization. (Viano *et al.*, 1995). Starches from different sources have been evaluated and use as an excellent binder and pharmaceutical excipients in either mucilage or dry powder form. There are many potential uses of starch industrially: unmodified starch can be used in the pharmaceutical, paper, mining and building industries. It can be modified and converted to starch derivatives (Omojola et al 2012). Excipients play an important role in dosage forms such as tablet, capsule, lotions, suspensions, syrups and ointments.

Starch is also one of the most widely used biomaterial in the food, textile, cosmetics, plastics, adhesives, paper and pharmaceutical industries. The diverse industrial usage of starch is based on its availability at low cost, high calorific value

and inherent excellent physicochemical properties (Omojola *et al.*, 2010). The versatility of starch in industrial applications is clearly defined by its physicochemical properties; therefore, a thorough evaluation of the necessary parameters is important in elucidating its industrial uses.

The morphology and physicochemical characteristics of starch are typical of its biological origin hence starch from each plant source will vary somewhat in appearance, biochemical composition and properties. As a result of the competing demands for starch as food, pharmaceutical and industrial uses coupled with the need to attain self sufficiency in starch production, there is a need to find other high yield sources different from cassava, maize and potato (Gebre – Mariam *et al.*, 2006).

Starch extracted from various locally available tubers, rhizomes and fruits have been studied by many researchers and find application in several industries. Little or no work appears to have been done on the isolation and physicochemical characterization of starch from *Moringa Oleifera root*. The purpose of this study is to extract, characterize and provide data on the various physicochemical characterization and nutritional composition of *Moringa Oleifera* starch as a new starch feedstock for industrial use which can reduce the burden on other starch sources such as cassava, corn, yam, potatoes and other complex carbohydrates. And also to provide an inherent nutritional benefits for various industrial products that use starch as one of the raw materials.

2. Materials and Methods

2.1 Sampling

Two years old *M. oleifera* root was harvested from medicinal and Botanical gardens located in Sheda Science and Technology Complex (SHESTCO) near Kwali, Abuja (8o21'N; 6o25'E). All other analytical grade reagents were obtained from Chemistry Advanced Laboratory, Sheda Science and Technology Complex, Abuja, Nigeria.

2.2 Starch Isolation

The fresh roots were peeled and washed. Peeled tubers (0.944 kg) were chopped into small pieces and soaked in sodium metabisulphite solution (2 L 1 % w/v) at room temperature (28 $^{\circ}$ C). Thereafter, the pieces of tuber were removed and wet milled into a slurry using a grater. The paste was dispersed in a large volume of 1 % sodium metabisulphite and filtered through muslin cloth. The supernatant was carefully decanted and the mucilage scraped off. The process was repeated for three times with the mucilage on the starch scraped continuously until a pure starch was obtained. The resulting starch was dried in the sun and further dried at 60 $^{\circ}$ C in a hot air oven, pulverized, weighed and stored in sample bottles for analysis.

3. Determination of certain physicochemical properties

3.1 Swelling power

The method described by Afolayan *et al* (2012) was used to determine the swelling power. The starch sample (0.1 g) was weighed into a test tube and 10 ml of distilled

water was added. The mixture was heated in a water bath at a temperature of 50 $^{\circ}$ C for 30 min with continuous shaking. In the end, the test tube was centrifuged at 1500 rpm for 20 min in order to facilitate the removal of the supernatant which was carefully decanted and weight of the starch paste taken. The swelling power was calculated as follows:

Swelling power = <u>Weight of starch paste</u> Weight of dry starch sample

3.2 Solubility Profile

Solubility index was determined over a temperature range of 50 $^{\rm O}$ C – 100 $^{\rm O}$ C as follows: Starch sample (0.5 g) was added to 10 ml distilled water in a test tube. This was subjected to heating in a water bath with a starting temperature of 50 $^{\rm O}$ C for 30 min. It was then centrifuged at 1500 rpm for 30 min. 5 ml of the supernatant was decanted and dried to constant weight. The solubility was expressed as the percentage (%) by weight of dissolved starch from heated solution.

3.3 Gelatinization temperature

This was evaluated using the method of Attama *et al* (2003). The starch sample (1 g) was put in a 20 ml beaker and 10 ml of distilled water was added. The dispersion was heated on a hot plate. The gelatinization temperature was then read with a thermometer suspended in the starch slurry.

3.4 Water holding capacity

The method of Omojola *et al* (2010) was used with slight modifications. The starch sample (5 % w/v) was dispersed in a pre-weighed centrifuge tube. The tube was agitated in a vortex mixer for 2 min. The supernatant was then discarded and the weight of the tube and hydrated sample taken. The weight was calculated and expressed as the weight of water bound by 100 g dry starch.

3.5 Amylose content

Amylose content determination was carried out using a colorimetric iodine affinity procedure. The starch sample (0.1 g) standard was weighed into a test tube. To the test tube was added carefully 1 ml of 95 % ethanol and 9 ml 1 mol / dm3 NaOH. The tube was covered with foil, thoroughly mixed and heated for 10 min in boiling water both to gelatinize the starch thereafter it was cooled very well. The suspension was diluted 10 times. An aliquot of 0.5 ml of the extract was used for analysis where 0.1 ml of acetic acid solution was added, followed by the addition of 0.2 ml of iodine solution. This was made up to 10 ml mark with distilled water. The solution was left for 20 min for colour development, and absorbance was read at 620 nm.

3.6 Foam capacity

The method of Omojola *et al* (2010) was used with slight modifications. Starch sample (1 g) was homogenized in 50 ml distilled water using a vortex mixer (vortex 2 Genie set at shake 8) for 5 minutes. The homogenate was poured into a 100 ml measuring

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cylinder and the volume recorded after 30 s. The foam capacity was expressed as the percent increase in volume.

3.7 Emulsion capacity

The method of Omojola *et al* (2010) was again used also with slight modifications. Sample (1 g) was dispersed in 5 ml distilled water using a vortex mixer for 30 seconds. After complete dispersion, 5 ml vegetable oil (groundnut oil) was added gradually and the mixing continued for another 30 s. The suspension was centrifuged at 1600 rpm for 5 min. The volume of oil separated from the sample was read directly from the tube. Emulsion capacity is the amount of oil emulsified and held per gram of sample.

3.8 Browning and charring temperature

The method of Builders *et al* (2001) was used. Some of the starch sample was put into a capillary tube, the browning and charring temperatures were determined using a melting point apparatus with model Electrothermal 9100.

3.9 Paste clarity

This was determined spectrophotometrically. 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 spectrophotometer with cassava starch as the reference standard (Builders *et al*, 2001).

3.10 Proximate analysis

Moisture, protein, fat, ash, crude fibre and carbohydrates was determined according to AOAC (2000). All the Samples analyzed were done in triplicates. Moisture content was determined by accurately weighing 4 g of the sample and drying in an oven at 105 $^{\circ}$ C to a constant weight, the percentage weight loss was determined. Fat was assayed by extracting the sample for 24 hours with petroleum ether (boiling point range 40 $^{\circ}$ C to 60 $^{\circ}$ C) in a soxhlet extractor. Ash content was determined by incinerating 4g of the pulp in a furnace (Carbolite-RHF 1600) for 4 hours at 550 $^{\circ}$ C, the percentage ash content was determined. The protein content of the starch was determined using kjeldahl method. Total energy or gross energy was calculated from proximate analysis results using N.R.C. method (Isong, E.U & U.I. Idiong,1997).

3.11 Titratable acidity

2g of the starch sample was suspended in 20ml of distilled water and titrated with 0.1 M NaoH solution using phenolphthalein indicator. The result was expressed in percentage.

3.12 pH

A 20 % w/v dispersion of the sample was shaken in water for 5 minutes and the pH was determined using a pH meter.

3.13 Phytochemical screening

Preliminary phytochemical screening of the starch extracted was done according to the procedures described by Sofowora (1993).

All the above parameters were determined in triplicates and the mean and standard deviations were recorded.

4. Results

Table 1 shows some physicochemical properties of the starch while the proximate analysis is shown in Table 2. The swelling and solubility patterns for the sample are given in Figures 1 and 2 and the paste clarity is shown in Figure 3.

Parameters	Results
Appearance/colour	Clear white
Odour	Odourless
% Yield	16.31
Bulk density (g/cm ³)	0.435
Tap density (g/cm^3)	0.589
Charring temperature (°C)	284.5-295.7
Browning temperature (°C)	268.6-270.8
Gelatinization temperature (°C)	64
Emulsion capacity %	7.5
Foam capacity %	4
Amylose content (mg/l)	0.532
Titratable acidity (TTA) %	5
pH	6.23

Table 1: Results of Pysico-chemical Properties of Moringa Starch.

Table 2: Results of Proximate Analysis of Moringa Starch.

Parameters	Results
Moisture (%)	12.76 ±0.24
Protein (%)	6.125 ±0.12
Fat (%)	14.39 ± 0.23
Ash (%)	14.39 ±0.23
Crude fibre (%)	BDL
Carbohydrate (%)	66.58 ±0.00
Metabolizable Energy kcal/g	462.93±0.00

BDL – Below detectable limits











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Figure 3

5. Discussion

Chemical Composition: Moringa starch was isolated from freshly harvested young root of Moriga Oleifera plant. The starch obtained was found to be a pure white, crystalline, non- hygroscopic powder with a yield of about 16.31 %. The yield is considered to be low especially when compared with starches from Icacina trichantha (76.8 %) and cassava.

The pH measurement shows that Moringa starch is very close to that of corn starch (Omojola *et al*, 2010) and comparable with the previous pH values reported for tuber starches (Coursey and Rasper, 1967) and within the pH range of 3 - 9 obtained for most starches used in the pharmaceutical, cosmetics and food industries.

Proximate analysis of the starch sample shows that its contents are comparable with that of cassava starch and within the range earlier reported (Adejumo *et a*l, 2011). Moringa starch contains 6.125 percent of crude protein compare to other earlier reported starch from corn, cassava, *Icacina trichantha (0.44%)* and anchomanes difformis (0.66%). The protein composition (6.125%) will be an additional benefit both as a binder and as a distegrant in pharmaceutical industries. The foam and emulsion capacities are little higher compare with other sources like *anchomanes difformis, Icacina trichantha* and corn. Moringa starch have negligible amount of crude fibre when compared with corn Starch. Moringa Starch have a good swelling profile, Solubility profile, and emulsion capacity.

Swelling and Solubility: The swelling and solubility profiles of moringa starch over a temperature range of 50 - 100 OC are shown in Figures 1 and 2. The profiles show a general trend of increase with increase in temperature for the starch. This is an indication of the water absorption characteristic of the granules during heating. Like other sources - anchomanes and Icacina straches which shows temperature relaxation between 50 - 60 °C and 90 °C as reported by Omojola et al, 2012. The swelling power is slightly lower than that of cassava starch but higher than that of corn starch. Increase in swelling power is indicative of suitability of a starch being used as a disintegrant in the pharmaceutical industry (Chowdary *et al*, 2011), hence moringa starch starch can be used as a disintegrant in the formulation of tablets. Also high swelling power results into high digestibility and ability to use starch in a range of dietary applications (Nuwamanya *et al*, 2010)).

The browning and charring temperatures indicates the temperature to which starch can be heated without changing colour or charring. This is observed to be quite high for moringa starch and quite higher than the reported values for some other starches - Icacina and anchomanes (Builders *et al*, 2001, Omojola *et al*, 2012, Afolayan *et al*, 2012). This shows that the starch can even be heated to a higher temperature without changing colour or charring. This quality will make it a preferable starch in industries that use starch at higher temperatures. The starch was also observed to have a very low amylose content which makes it a good choice food for diabetics and other health conscious individuals (Agbo *et al*, 2010). The phytochemical screening of the starch shows that it contains flavonoids, Saponins, carbohydrates and cardiac glycosides.

The above technical data and information confirms the applicability of Moringa starch in the industries that use starch as raw materials.

Conclusion

Some physicochemical properties of *Moringa Oleifera* starch have been examined and these properties compare favourably with other starches. The study has therefore shown that *Moringa Oleifera starch* will be a good source of starch and a biomaterial for industrial uses. Therefore it is a potential source of industrial starch. Moringa starch will help to reduce the burden on starch from other well known sources such as corn, potato and cassava and make starch available at low cost. *Moringa Oleifera* starch can also provide additional nutritional values to some of industrial products that use starch as a biomaterial.

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