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(RESEARCH ARTICLE)

Extraction, isolation, physicochemical characterization, phytochemical and proximate evaluation of pearl millet (*Pennisetum glaucum*) starch

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Abstract

Pearl millet (*Pennisetum glaucum*) is the most widely grown type of millet. Starch was extracted from its seeds and subjected to physicochemical characterization, proximate analysis and phytochemical screening in order to ascertain its properties and adjudge its suitability for use in various industries. Physicochemical characterization of the starch revealed that it had gelatinization temperature of 79 ^{0}C, foam and emulsion capacities of 4 and 20% respectively, pH of 6.88, water holding capacity of 28.345 ml, bulk and tapped densities of 0.526 g/cm3 and 0.667 g/cm3 respectively, browning temperature of 224.5 – 265.0 ^{0}C and charring temperature of 270.3 – 290.0 ^{0}C. The starch percentage solubility at 90 ^{0}C was 3.2 with a swelling power of 17.65. *Pennisetum glaucum* starch was discovered to be made up of 17.66% amylose and 82.34% amylopectin. Phytochemical screening of the starch showed the presence of saponins, terpernoids, carbohydrates and resins. Proximate analysis of the starch showed ash content of 0.18%, moisture content of 6.97%, crude lipid content of 5.99%, crude protein content of 3.46% and carbohydrate content of 83.4%. The various results from the characterization of the starch from *Pennisetum glaucum* showed that it is a potential source of pharmaceutical / industrial starch as its properties compare favourably with those of starch being used in the pharmaceutical industries.

Keywords: *Pennisetum glaucum*; physicochemical; proximate; starch; phytochemical; Pearl millet.

1. Introduction

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 [1]. Starch is the only qualitatively important digestible polysaccharide and has been regarded as nutritionally superior to low molecular weight carbohydrate or sugars [2]. Though starch is mainly used as food, it can also be readily converted chemically and biologically into many useful and diverse products such as paper, textiles, adhesive, beverages, confectionaries, pharmaceuticals and plastics [3].

Starch is a natural biodegradable biopolymer which has wide industrial applications. It is 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 availablity at low cost, high calorific value and inherent excellent physicochemical properties [4]. 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 use. The morphology and physicochemical characteristics of starch are typical of its biological origin hence, starch from each plant source will vary somewhat in appearance, composition and properties [1].

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Pearl millet, commonly known as bulrush millet with botanical name *Pennisetum glaucum* (L.) R. Br. Is a cultivated, small-grain, tropical cereal grass. It is locally called *bajra* in India, *gero* in Northern Nigeria (Hausa language), *hegni* in Niger (Djerma language), *sanyo* in Mali, *dukhon* in Sudan (Arabic), and *mahangu* in Namibia. Pearl millet is quantitatively the most important millet, with world annual production of about 14 million tons (Mt). It is cultivated mainly in the semiarid tropics, almost exclusively by subsistence and small-scale commercial farmers. Pearl millet is one of the most important millet species as it accounts for approximately half the total worldwide production of millets. It is mainly cultivated in India and Africa and is uniquely tolerant of hot and dry conditions [5]. The grain of pearl millet generally has higher fat and hence higher energy, higher protein content and better quality protein than most other cereal grains. It is a "high-energy" cereal that contains carbohydrates, protein and fat; rich in vitamins B and A, high in calcium, iron, and zinc, and also contains potassium, phosphorus, magnesium, zinc, copper, and manganese [6]. It has been almost exclusively a subsistence crop but today is becoming widely used in commercial small-scale food manufacture. Many traditional foods and beverages are produced from pearl millet, including couscous and flatbreads, doughs, porridges, gruels, nonalcoholic beverages, and beers. Recently, iron- and zinc-biofortified pearl millet has been developed for improved nutrition [7].

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 [8]. Thus, the aim of this research was to isolate and purify starch from Pearl millet (*Pennisetum glaucum*) seeds. The starch thus obtained was thereafter subjected to physicochemical characterization, phytochemical evaluation and proximate analysis to determine its suitability as a potential biomaterial for industrial application.

2. Material and methods

Materials

Pearl millet seeds were obtained from Daffo, Bokkos LGA of Plateau State Nigeria and were identified at Federal college of forestry, Jos, Plateau state as *Pennisetum glaucum*. Corn starch (BP) and other analytical grade reagents were obtained from Chemistry Advanced Research Centre, Sheda Science and Technology Complex, Abuja, Nigeria.

2.1. Starch Isolation

The pearl millet seeds were picked to separate dirts and washed. The separated seeds (0.95 kg) were washed and soaked in sodium metabisulphite solution (2 L 1 % w/v) overnight at room temperature (27 ^{0}C). Thereafter, the hydrated seeds were removed and wet milled into a slurry using a laboratory blender. The paste was dispersed in a large volume of 1 % sodium metabisulphite solution and filtered several times through a muslin cloth. The suspension was centrifuged at 3500 rpm for 10 mins to facilitate the removal of dirts. 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 ^{0}C in a hot air oven, pulverized, weighed and stored in sample bottles until required for analysis.

2.2. Determination of Physicochemical Properties

2.3. Swelling Power

The method described by Afolayan *et al* (2014) was used to determine the swelling power with slight modifications [9]. 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 ^{0}C for 30 mins with continuous shaking. In the end, the test tube was centrifuged at 1500 rpm for 20 mins 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:

$$\text{Swelling power} = \frac{\text{Weight of starch paste}}{\text{Weight of dry starch sample}}$$

This was carried out over a temperature range of 50 ^{0}C – 95 ^{0}C.

2.4. Solubility Index

The method described by Afolayan *et al* (2014) was also used to determine the solubility index with slight modifications [9]. 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 ^{0}C for 30 mins. It was then centrifuged at 1500 rpm for another 30 mins. 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. This was carried out over a temperature range of 50 ^{0}C – 95 ^{0}C.

2.5. Gelatinization Temperature

This was evaluated using the method of Attama *et al,* (2003) [10]. 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.

2.6. Foam Capacity

The method of Omojola *et al* (2010) was used with slight modifications [4]. 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 cylinder and the volume recorded after 30 s. The foam capacity was expressed as the percent increase in volume.

2.7. Emulsion Capacity

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 mins. 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.

2.8. Browning and Charring Temperature

The method of Builders *et al* (2001) was used with slight modifications [11]. 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.

2.9. 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.

2.10. Water Holding Capacity

The method described by Omojola *et al* (2010) was used to determine the water holding capacity [4]. 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.

2.11. Bulk and Tapped Density

The bulk density of the starch was determined using the method described by Narayana and Narasinga Rao (1984) with slight modifications [12]. Starch powder (50g) was poured into a 250 cm^3 calibrated measuring cylinder by means of a short – stemmed glass funnel. The volume occupied by the starch was noted to determine the bulk density.

$$\text{Bulk density (g/cm}^3\text{)} = \frac{\text{Weight of sample}}{\text{Volume occupied}}$$

For the tapped density determination, the cylinder was tapped continuously using a ruler until a constant volume was obtained.

2.12. Amylose and Amylopectin Content

Starch sample (0.1g) and standard were weighed into separate test tubes. To these test tubes, 1 cm^3 of 95% ethanol and 9 cm^3 1 mol dm^{-3} NaOH were carefully added. The test tubes were covered with foil paper and mixed thoroughly. The samples were heated for 10 mins in a boiling water bath to gelatinize the starch and cooled very well. The suspensions were diluted 10 times. An aliquot of 0.5 cm^3 of the extract was used for analysis where 0.1 cm^3 of acetic acid solution was added, followed by the addition of 0.2 cm^3 of iodine solution. This was made up to 10 cm^3 with 9.2 cm^3 of distilled water. The solution was left for 20 mins for colour development, vortexed and read at 620 nm [13].

$$\% \text{ Amylose content} = \frac{\% \text{ Amylose of standard X Absorbance of sample}}{\text{Absorbance of standard}}$$

2.13. Proximate Analysis

Moisture, crude protein, crude lipid, ash and carbohydrates was determined according to AOAC (1990) [14]. All the Samples analyzed were done in triplicates.

2.14. Phytochemical Screening

Preliminary phytochemical screening of the starch extracted was done according to the procedures described by Sofowora (1993) [15].

3. Results and discussion

The starch obtained was found to be a brilliant white, crystalline, non- hygroscopic powder with no smell and a yield of about 32%. The yield is considered to be very appreciable especially when compared with starches from other sources such as cassava and corn. Table 1 shows the results of the physicochemical properties and proximate analysis of pearl millet starch. The swelling profile and solubility profile are shown in Figures 1 and 2 respectively. The phytochemical result of finger millet starch showed the presence of saponins, terpernoids, carbohydrates and resins.

Table 1 Physicochemical Properties & Proximate Analysis of Pearl Millet Starch

PARAMETER	**VALUE**
Gelatinization temperature	79 ^{0}C
Foam capacity	4%
Browning temperature	224.5 – 265.0 ^{0}C
Charring temperature	270.3 – 290.0 ^{0}C
pH	6.88
Water holding capacity	28.345 ml
Bulk density	0.526 g/cm^{3}
Tapped density	0.667 g/cm^{3}
Emulsion capacity	20%
Ash content	0.18%
Moisture content	6.97%
Crude lipid	5.99%
Crude protein	3.46%
Carbohydrate	83.4%
Amylose content	17.66%
Amylopectin content	82.34% **79°C**

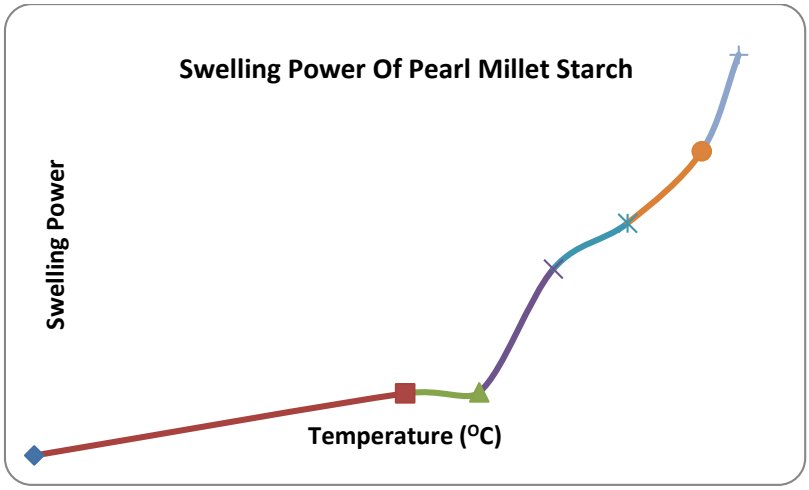

Figure 1 Swelling Power of Pearl Millet Starch

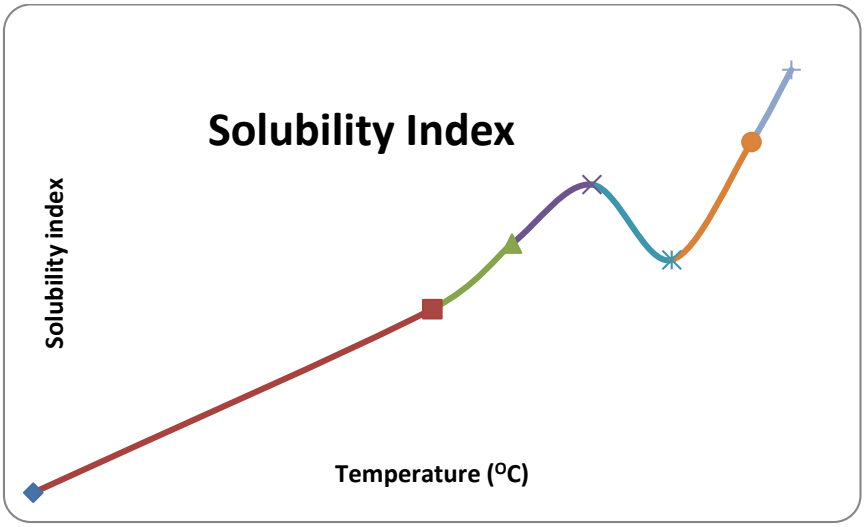

Figure 2 Solubility Index of Pearl Millet Starch

3.1. Chemical Composition

Starch isolated from seeds of *Pennisetum glaucum* was brilliant white, crystalline and non – hygroscopic which is characteristic of pure starch. It also had no smell and had a yield of 32% which is quite high. It had a gelatinization temperature of 79 ^{O}C which falls within the range of gelatinization temperatures commonly observed for starches. The foam capacity of 4% and emulsion capacity of 20% are also as been reported generally for starches. [1] Browning and charring temperatures of starches indicates the temperature to which it can be heated without browning and charring. For pearl millet starch, its browning temperature is quite lower than that reported for maize starch, tiger nut starch and others but can still find application in industries that use starch at very high temperatures while the charring temperature is very much comparable with other starches [16, 17]. The water absorption capacity of 28.345 ml in 100 g of sample is very low when compared with earlier results reported for ginger, tiger nut, *icacina trichantha*, *anchomanes difformis* and maize starch [18]. The variation in water absorption capacity could be due to different proportion of crystalline and amorphous regions within the granule. This could have an effect on the swelling capacity of the starch also. The bulk and tapped densities are in conformity with the findings of Shihii *et al* as reported and falls within the range indicated for maize starch [19]. This shows that the starch can be compressed well. Proximate analysis of the starch indicates that its contents are very much comparable with that of cassava, maize and other starches and within the range earlier reported [20]. The high carbohydrate content translates to the high starch yield gotten. The phytochemical screening of pearl millet starch showed that it contained saponins, carbohydrates, terpenoids and resins. The presence of saponins translates to the appreciable foam capacity recorded. The starch was also observed to have quite high amylose content which may not make it a good choice food for diabetics and other health conscious individuals [3].

3.2. Swelling and Solubility

The swelling and solubility profiles of pearl millet starch over a temperature range of 50 – 95 ^{0}C are illustrated in Figures 1 and 2. The profiles show a general trend of increase with increase in temperature for the starch although a slightly two – stage swelling pattern can be observed. This is an indication of the water absorption characteristic of the granules during heating. The swelling curve for the starch demonstrated temperature relaxation between 50 – 60 ^{0}C and also a little at 90 ^{0}C just like it was reported for several other starch [4]. There is first, a slight decrease from 50 – 60 ^{0}C followed by an increase up till 80 ^{0}C then a slight level off and then another rapid increase from 90 ^{0}C. This pattern has been attributed to two sets of internal bonding forces that relax at different temperatures [17]. The swelling power is quite appreciable; higher than tiger nut, ginger and anchomanes starches but lower than maize starch. Increase in swelling power is indicative of suitability of a starch being used as a disintegrant in the pharmaceutical industry [21], hence pearl millet starch can be effectively used as a disintegrant in the formulation of tablets. The solubility profile for the starch shows an initial decrease from 50 – 70 ^{0}C and thereafter an increase in solubility with temperature rise.

4. Conclusion

Pennisetum glaucum starch has been extracted and some of its physicochemical properties analyzed. These properties compare favourably with other starches as gotten from literature. The study has therefore shown that *Pennisetum glaucum* is a good source of starch and is therefore a potential biomaterial for industrial use especially if its physicochemical properties are improved upon to meet specific industrial grade. This 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.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare that they have no conflict of interest.

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