Characterization and Comparison of Alkali Extracted Starches from Selected Cereals and Tubers

H. D. K. C. Wijesinghe¹ and K. D. P. P. Gunathilake¹*

¹Department of Food Science and Technology, Faculty of Livestock, Fisheries and Nutrition, Wayamba University of Sri Lanka, Makandura, Gonawila (NWP), Sri Lanka.

Authors’ contributions

This work was carried out in collaboration between both authors. Author HDKCW designed the study and performed the statistical analysis. Author HDKCW wrote the first draft of the manuscript and managed the analyses of the study. Author KDPPG managed the literature searches and wrote the protocol. Both authors read and approved the final manuscript.

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ABSTRACT

Aims: Starch is used in the food industry to impart functional properties and to modify food texture and consistency. In this study starches isolated from six sources using an alkali extraction method were evaluated for physicochemical properties.
Study Design: Complete randomized design.
Place and Duration of Study: Department of Food Science and Technology, Faculty of Livestock, Fisheries, & Nutrition, Wayamba University of Sri Lanka, Makandura, Gonawila (NWP), Sri Lanka between April 2017 and August 2017.
Methodology: Starches were isolated from white rice, foxtail millet, proso millet, cassava, sweet potato and all-purpose wheat flour. Proximate composition, mineral content, physical properties and microscopic characteristics were determined and they were compared.
Results: The level of starch extracted was within the range of 27.5-64.1% on a dry basis. Extracted starched contains about 99% carbohydrate in DW and less than 1% non-carbohydrate fraction (protein, fat, fiber and ash and minerals). The amylose content followed the order: proso millet > wheat > foxtail millet > rice > sweet potato > cassava. There was considerable variation in swelling

*Corresponding author: E-mail: kdppgunathilake@yahoo.com, kdppgunathilake@wyb.ac.lk;
factor, solubility, gelatinization temperature among all starches. Both swelling power and solubility had a positive relationship with temperature and the swelling power (at 90°C) followed the order: corn > cassava > foxtail millet > wheat > proso millet > rice > sweet potato. The gelatinization temperature of starches ranged from 62°C to 76°C.

**Conclusion:** The study would be helpful to better understand the chemical, physical and microscopic characteristics of these starches and the application of novel starches obtain from non-conventional sources which are foxtail millet, proso millet and sweet potato as a thickening agent and a substitute to other common starches in food.

**Keywords:** Foxtail millet; microscopic characteristics; physicochemical properties; proso millet; starch isolation.

### 1. INTRODUCTION

Starch is the second-largest biomass next to cellulose produced on earth. It is the major form of carbohydrate reserve in higher plants. It exists in a granular form and the starch granule is semi-crystalline and insoluble in water. Thus, a large amount of energy can be stored in a relatively small volume [1]. Today, the main sources of starch extraction are seeds, roots and tubers, primarily from maize, wheat, cassava, rice and potato [2]. Starch can easily be extracted with high purity, resulting in a white, tasteless and odorless powder. These good organoleptic properties make it an interesting resource for manifold applications, not only in human food and animal feed but also as feedstock for non-food industrial applications such as pulp and paper, adhesives and bioethanol [3]. Starch is a valuable ingredient in the food industry, it serves not only as a nutrient source for food but also as a thickener, a binding agent, a texturizer, a filler and a film-forming agent in the food industry [4, 5, 6].

Starches from different plant sources vary in their chemical and physical characteristics as well as their gelatinization properties [7]. A selection of starch varieties for different food products depends on starch functional properties such as gelatinization properties, solubility, viscosity, gel stability and retrogradation rate. According to Li [1], these functional properties are determined by the chemical structures of starch. For industrial purposes the main source of starch is maize. However, there are other potential sources of starches such as wheat, cassava, and rice and they possess the potential characteristics required for the industrial uses [8,9]. Being a tropical country, Sri Lanka has underutilized cereals and yams with a higher content of starches and may be possessing good physicochemical properties for industrial applications. Further, extraction of starches from these underutilized cereals may improve the value-adding options for underutilized crops and enhance our understanding and knowledge about physicochemical and functional properties of new starchy materials as well as their ability to replace conventional starch in the food industry [5]. Thus the application of non-conventional starch sources of cereals, roots and tubers in the food industry can be broadened which in turn may reduce the dependency on corn, wheat and cassava as the main sources of starch. In recent years, substantial efforts have been made to obtain starches from non-conventional sources and to study their functional, rheological and physicochemical properties. Foxtail millet and proso millet are some of the underutilized cereals grown in Sri Lanka. In the present study, the physicochemical properties of starches isolated from proso millet (Panicum miliaceum) and foxtail millet (Setaria italica) were studied and compared with other major starch sources such as wheat flour (Triticum spp.), rice (Oryza sativa), cassava (Manihot esculenta) and sweet potato (Ipomoea batatas).

### 2. MATERIALS AND METHODS

**2.1 Materials**

Dehulled seeds of foxtail millet (Setaria italica; variety ISC 480), proso millet (Panicum miliaceum; variety AC 254) and dehulled and polished rice (Oryza sativa; Bg 357) were supplied by Field Crops Research and Development Institute (FCRDI), Mahailluppallama, Sri Lanka. Commercial all-purpose wheat flour (Brand name- Prima), Cornflour (Brand name- Motha) were purchased from Cargills Food City, Dankotuwa, Sri Lanka and the fresh roots of cassava (Manihot esculenta; Kirikawadi) and sweet potato (Ipomoea batatas; Wariyapola-red) were obtained from the local market at Makandura area, Sri Lanka. All the chemicals used for the study were of analytical grade.
2.2 Flour Preparation

Flour extraction from selected sources was conducted following an established procedure mentioned in Alves [10]. The dehulled grains were cleaned by removing solid and other contaminants. For tubers, peeling, washing and slicing (~5 mm) were done. About 100 g of cereals/sliced tubers were dried at 40°C for about 30 hours in an oven (Model no: MEMMERT NLE 500) until they reached a constant weight. Subsequently, the dried grains/sliced tubers were milled into flour by using a laboratory scale grinder and sifted through a 300μm sieve. The flours were then packed into a sealed air tide polyethylene container and stored at -18ºC until used for further studies.

2.3 Isolation of Starch

The starch isolation was performed according to the method described by Correia and Beirão-da-Costa [11], with slight modifications. Briefly, the flour (120 g) was soaked in two volumes of 0.25% NaOH at 2-5°C for 24 h. The suspension was homogenized and screened through a muslin cloth and then 180μm sieve. The precipitate was screened successively in 63μm sieve. The mixture was centrifuged in a laboratory centrifuge (Model no: D-78532 Tuttlingen, Germany) at 800× g (4520 rpm) for 15 min. The mucilaginous layer was scraped away and the precipitate was then suspended in water. This last step was repeated twice. Isolated starch was dried for 48 hours at 45°C in the electrical drying oven. Then isolated starch was ground into a fine powder using a laboratory-scale grinder and sifted through a 300μm sieve. The isolated starches were weighed and determined the yield of starch on the dry weight basis. The starch was then packed into a sealed air tide polyethylene container and stored in a laboratory freezer.

2.4 Microscopic Characteristics of Starches

The granular shape of starches was examined following the method as described by Snyder [12]. A small drop of water was placed on one side of a standard microscope slide. About 5 mg of starch sample was transferred onto the water using a dissector needle. The starch was mixed thoroughly to disperse starch. A cover slip was placed over the suspension taking care to avoid entrapment of air bubbles. Excess water was wicked off with a small piece of tissue paper held at the edge of the cover slip to obtain a thin film. The granular shape was examined under a polarized light microscope at ×10×40 magnification. The micrographs were used to compare the morphology of the starch granules.

2.5 Determination of Proximate Composition

Moisture, protein, fat and ash contents in starches were determined by the method using the methods described in AOAC [13]. Crude fiber contents were determined by Weedy method using Fibertec™ M6 Fibre Analysis System. Total carbohydrate content was determined by subtracting the sum of the values of crude protein, crude fat and ash content (% dry weight basis) of the sample from 100 [14].

2.6 Amylose Content Determination

The total amylose content of starches was determined using the spectrophotometric method described by Hoover and Ratnayake [14].

2.7 Amylopectin Content Determination

Amylose and amylopectin contents were expressed relative to the total starch content.

Amylopectin content (%) = % Total starch content - % amylose content

2.8 Determination of Mineral Content

The analysis of phosphorous was done using the method described by Varvel and Peterson, [15] and the absorbance was measured using a UV-Visible spectrophotometer (Model no: SP- 3000 Plus). Calcium, Magnesium, Zinc, Copper, Iron, Manganese and Aluminum concentrations in starch samples were analyzed by using iCE 3000 series Thermo Scientific Atomic Absorption Spectrometer.

2.9 pH of Starch

The method reported by Benesi et al. (2004) as cited in Nand [16] was used for pH determination. Approximately five grams of starch sample was added to 20 ml of distilled water in a beaker. The contents were stirred for 5 min. Starch was allowed to settle and the pH of the water phase was measured using a calibrated pH meter.
2.10 Particle Size Analysis

The geometric mean diameter and particle size distribution of the flour samples were determined by the sieving method as described in Patva [17]. The particle size of isolated starches was determined by sieving method. Each empty sieve was accurately weighed. The sieves were set as such, sieve with the pour size that largest diameter was on the top and placed on sieve shaker. Accurately 100 g of starch was weighed and put into the top sieve. The lid was placed on top and tightens clamps. Then the shaker was started to sieve and shaken for 10 minutes and weighed at 5-minute intervals thereafter. Sieving was completed when the weight on the smallest sieve containing starch changes less than 0.2% of total sample weight for 5 minutes. Then the starch left on each sieve and amount of starch sieved from final sieve were accurately weighed. Calculate the percentage of the weight of each sieve. Then the geometric mean diameter of analyzed starches was determined using the following equation.

\[
\text{Geometric mean diameter of the particles by mass (mm)} = \log^{-1}\left[ \frac{\sum(W_i \log d_i)}{\sum W_i} \right]
\]

Where, \(W_i\) is the mass on the \(i\)th sieve (g), \(n\) is the number of sieves, and \(d_i\) is the nominal sieve aperture size of the \(i\)th sieve (mm).

2.11 Gelatinization Temperature

The gelatinization temperature of starch was determined by the method described by Linus [18]. A 20 ml of 0.29% W/V suspension of the sample in water in a 25 mL beaker was warmed in a water bath at 40°C. The solution was thoroughly mixed and prepares a smear from it. The smear was observed under the mid-power of the microscope. The temperature was then gradually raised while mixing continuously and measuring temperature. Then after every ~2°C a sample was withdrawn and observed under a light microscope until the starch is fully gelatinized. The temperature when starch granule begins to lose their structure was recorded as gelatinization temperature.

2.12 Swelling Power and Solubility

Swelling power (SP) and solubility index (SOL) were determined using a modification of the method of Leach et al. (1959) as cited in [19].

2.13 Statistical Analysis

Significant differences between the results were calculated by analysis of the Least Significant Difference (LSD) with the help of SAS software. Differences at \(P < 0.05\) were considered to be significant. Results were expressed as mean ± SD (Standard Deviation). Values were the average of triplicate experiments.

3. RESULTS AND DISCUSSION

Table 1 shows the percentage yield of flour and starches obtained from studied starch sources. Flour extraction from cereals and tubers lies in the range 38.88–83.40% at 12% moisture level. Compared to tubers, cereals have given the higher amount of flour due to higher moisture content in tubers than cereals. Sweet potato yielded a relatively higher amount of flour than cassava. The yield of flour of all starches was significantly different (\(P=.05\)) to each other. In this study commercially available corn flour and wheat flour were used. The starch yield depends on the starch content of the plant source, variety of the plant, starch granular size, presence of soluble and resistant starch and extraction method. The yield of starch of all sources ranged from 27.49 to 64.10% on a dry basis and visually the resultant dried starches had an attractive appearance. Higher starch amount yielded from corn flour (70.57 %) and it was significantly higher (\(P=.05\)) than others. Proso millet, foxtail millet and sweet potato yielded starches from their flours about 52.82%, 27.49% and 40.77% respectively. However, the yield of both cassava and proso millet were not were not statically significant different (\(P=.05\)). According to Mistry [20], alkali concentration significantly affected the starch yield and the lower starch yield at lower alkali concentration can be observed due to lower solubility and dispersibility of glutelin proteins present in the flour type. Furthermore, high levels of hydration and low density of the damaged starch granules increased the viscosity of the slurry of flour and lead to difficulties in the screening operations and therefore lower level of starch yield can be observed [20]. However, in the present study, the aim was to evaluate a method of starch extraction which is relatively simple and might readily be adapted to larger scale preparation conditions.
3.1 Determination of Microscopic Characteristics of Starches

The optical micrographs of the starches are given in Fig. 1 (A, B, C, D, E, H). All of the starch granules seem to consist of both simple and compound granules and packed in a slightly different matrix. The native starches of studied sources consisted of a mixed population of large, medium and small granules. Wheat starch granule (A) shapes include symmetrical spheres and asymmetrical spheres. The rice starch granules (B) were ellipsoidal to irregular or cubical and similar observations were also reported in [21]. Proso millet starch granules (C) were spherical as well as polygonal in shape. The foxtail millet starch granules (D) had irregular shapes, which varied from oval, round to polygonal in shape and granules are larger than proso millet starch granules. The cassava starch granules (E) appeared as spherical and similar observations were seen in [16]. The shapes of the sweet potato starch granules (F) varied from polygonal to round/bell shapes and this is in agreement with previous reports on sweet potato starch granules as mention in [22].

3.2 Chemical Characteristics of Starches

The proximate composition of the different starches is shown in Table 2. The moisture content of studied starches was varied among sources and this could be attributed to the variation in the extent of drying of the starches. Moisture content falls within the moisture level (<20%) recommended for commercial starches as mentioned in Soni [23]. It was observed that the moisture content of starches is below 13%, which is recommended for safe storage in most starch producing countries [24]. Table 2 shows tuber starches contained a higher percentage of moisture compared to cereal starches. The protein contents in starch were very low and most of the proteins in the sources have removed during the extraction process. The protein content of the starches was ranged from 0.43 to 0.71% on a dry basis. Lower protein contents were also reported in starches extracted from root tubers of purple, yellow and white sweet potatoes [25]. The highest value was observed from rice whereas sweet potato had the lowest. There was no statistical difference (P=0.05) in the protein content of both cassava...
Fig. 1. Optical micrographs of starch granules from selected sources at ×400 magnification

and corn starches. The protein content of starches followed the order: wheat > foxtail millet > rice > proso millet > cassava > sweet potato. Studied tubers showed significantly lower protein level compared with that of cereals. Low protein content may be an indication of the absence of endosperm protein which could affect the purity and crystallinity of the starch and as a result
could adversely affect the physicochemical properties of the starches. The fat and fiber content of the starches was very low and it is in the range of 0.10-0.14%.

The total ash content of the starches ranged from 0.25 to 0.47% and the highest value was observed in cassava starch and the lowest from corn. Significantly lower cereal starches contain lower ash content. Higher mineral content was observed in cereal starches than tuber starches studied. Ash is the inorganic residue remaining after the water and organic matter has been removed by heating in the presence of oxidizing agents, which provides a measure of the total amount of minerals within a food. It has been suggested that higher ash contents of starch are a result of the presence of material commonly referred to as "fine fiber" [14]. The term fine fiber designates a highly hydrated material originating in the cell wall structures normally surrounding the starch granules. Higher ash content was indicated the lower yield of starch due to the higher fine fiber content. Generally, starch ashes are mainly composed of phosphorous, sodium, potassium, magnesium and calcium [26,7]. The ash content is an estimate of the total mineral content of the starch. Even though, the values for all the starches were low. The low ash content is an indication of the good quality of the starches because high mineral content is sometimes used to retard the growth of certain microorganisms [14]. The carbohydrate content of each starch is determined by difference on a dry basis and results of the analysis are given in Table 2. The alkaline steeping method is considered as one of the best methods of extraction high purity of starch [27]. Another popular method is the enzymatic method. All studied starches contained carbohydrate within the range of 85-89% and the highest values were observed in rice, foxtail millet and corn whereas the lowest carbohydrate content in cassava starch.

The mineral compositions of the studied starches are presented in Table 3. The phosphorus content of all starches was ranged from 9.41 to 55.13 mg/100g. The highest value was obtained from rice and the lowest from wheat. Phosphorus content of rice, foxtail millet and proso millet was higher than root starches. According to [26], phosphorous in cereal exist as phosphate groups which are bounded to the amylopectin molecules and confer a polyelectrolyte nature to the chains. This ionic nature allows starch dispersions to develop high viscosity [26]. In the same way, Bergthaller [28] have proposed that the thickening capacity of starch is associated with the high content of phosphate ester groups. Root starches contain very low amounts of phosphorus and mostly in the phosphate monoester forms [29]. The higher phosphate content of starch is also the responsible parameter of high viscosity developed by these starch dispersions. Significantly higher calcium content was observed in starches of wheat, rice and foxtail millet than other studied starches.

3.3 Amylose and Amylopectin Content of Isolated Starch

Starch consists of two major forms of polysaccharides such as amylose and amylopectin. Amylose is essentially a linear α-1,4 glucan with some molecules having α(1,6). Amylopectin is a branched molecule found in starch and the linear chain, the D-glucopyranose units are connected by α-1,4 linkages with 5-6% of its bonds being α-1,6 branch linkages. Amylose and amylopectin content in starches plays a significant role in influencing the functional properties of starches. Amylose and amylopectin content varied significantly (P<0.05) among the starches studied with values ranging from 17.08 to 27.83% and 72.16 to 82.91%, respectively (Table 4). According to Oduro [30], the differences in amylose content of these studied starches may be due to genotypic differences, environmental factors and starch processing methods. Interestingly, among the starches studied, proso millet showed the highest amylose content similar to commercial corn starches while the lowest was found in cassava starch. Cassava starch contains about 17% amylose and similar results were also found in Wang [31]. High amylose containing starches are characterized by their high gelling strength which is indicating their usefulness in the production of pasta, sweets and bread-like products [32]. The amylose content affects gelatinization and retrogradation properties, swelling power and enzymatic susceptibility of starches [33]. Therefore, it is quite important to quantify the amylose content to determine the usability of the starches.

3.4 pH of Isolated Starch

The pH values of the starches are shown in Table 4. The pH values of studied starches were close to neutrality. However, the pH of commercial corn starch was within the acidic range (4.38). The higher pH values can increase the effect of the degree of ionization which has a
significant effect in the hydration behavior of starches to permit the interaction between water molecules and amylopectin and amylose chains [34]. According to [16], the paste clarity of starches increased at very low pH and which decreased sharply towards high pH and in very acidic solutions, negatively charged phosphate groups are neutralized, and the ionization of hydroxyl groups is suppressed. Therefore, lysophospholipid may complex with amylose chains that contain only electropositive nitrogen and the presence of Coulombic repulsion between these positive nitrogen on adjacent amylose chains decrease the compactness of the amorphous region, thus increasing the transmission [35].

3.5 Gelatinization Temperature

Gelatinization temperature is the temperature of the initial point that the starches lose their granule structure. The results in Table 4 showed that the corn starch has the highest gelatinization temperature than all other selected starches. Gelatinization temperatures of starches were ranged from 50°C to 76°C. Proso millet has the lowest gelatinization temperature and foxtail millet has the highest among the studied sources and though significantly lower compared with the gelatinization temperature of the commercial corn starch. The gelatinization property of starch is a determining factor in its functionality in food applications.

3.6 Particle Size Distribution Analysis

The particle size distribution of isolated starches is presented in Fig. 2. According to the analysis most of the particle size of wheat, rice, foxtail millet, proso millet, cassava and sweet potato were at 0.180-0.063 mm, 0.106-0.063 mm, 0.180-0.063 mm, 0.180-0.106 mm, 0.063-0.038 mm and 0.106-0.038 mm range respectively. The geometric mean diameters of isolated starches are shown in Fig. 3. It presented the geometric mean diameter of wheat, rice, foxtail millet, proso millet, cassava and sweet potato are 107 µm, 77 µm, 99 µm, 121 µm, 78 µm, 93 µm and 73 µm respectively. The particle size of starch is one of the most important characteristics, which may influence other physicochemical properties such as swelling power, paste clarity, and water-binding capacity, among others [36].

3.7 Swelling Power and Solubility

Swelling power and solubility values for the isolated starches are presented in Fig. 4. The swelling power (at 90°C) followed the order: corn > cassava > foxtail millet > wheat > proso millet > rice > sweet potato. The swelling power of foxtail and proso millet and solubility of rice and proso

### Table 3. Mineral content of starches

| Starch source | P (mg per 100 g) | Cu (mg per 100 g) | Al (mg per 100 g) | Zn (mg per 100 g) | Ca (mg per 100 g) | Fe (mg per 100 g) | Mn (mg per 100 g) |
|---------------|-----------------|------------------|------------------|------------------|------------------|------------------|------------------|
| Wheat         | 9.41            | 1.06             | 9.95             | 0.28             | 41.82            | 6.05             | 0.28             |
| Rice          | 55.13           | 1.25             | 23.27            | 1.41             | 32.24            | 6.45             | 0.56             |
| Foxtail millet| 28.22           | 1.51             | 20.45            | 0.16             | 7.49             | 6.90             | 0.24             |
| Proso millet  | 33.84           | 1.59             | 20.50            | 1.39             | 21.55            | 7.01             | 0.28             |
| Cassava       | 10.12           | 1.54             | 21.82            | 1.57             | 6.80             | 6.40             | 0.32             |
| Sweet potato  | 12.50           | 1.50             | 23.62            | 0.25             | 6.34             | 6.51             | 0.42             |

Average values of three measurements (For n=3 ± SD). All data reported on a dry basis

### Table 4. Amylose, amylopectine content, ph and gelatinization of starches

| Starch        | Amylose Content (mg per 100 g) | Amylopectin Content (mg per 100 g) | pH | Geletinization Temp. (°C) | Finish Start |
|---------------|--------------------------------|------------------------------------|----|--------------------------|-------------|
| Wheat         | 25.67±0.32ab                  | 74.32±0.32c                      | 6.33±0.56 | 52 | 60                       |
| Rice          | 22.44±0.35c                   | 77.56±0.35c                      | 7.58±0.63 | 54 | 74                       |
| Proso millet  | 27.56±0.56a                   | 72.44±0.56d                      | 7.86±0.82 | 50 | 62                       |
| Foxtail       | 23.85±0.84c                   | 76.14±0.84c                      | 7.21±0.69 | 62 | 72                       |
| Cassava       | 17.08±0.13d                   | 82.91±0.13a                      | 6.81±0.92 | 59 | 70                       |
| Sweet potato  | 18.58±0.25d                   | 81.41±0.25a                      | 7.33±0.52 | 60 | 72                       |
| Corn          | 27.83±0.40a                   | 72.16±0.40d                      | 4.38±0.71 | 64 | 76                       |

Average values of three measurements (n=3 ± SD). All data reported on a dry basis. Values followed by the same letter in each column are not significantly different (p < 0.05) by LSD test. pH values measured at 29°C
Millet are not significantly different ($P=.05$). Sweet potato had the lowest swelling power and highest in cassava. All selected starches were lower than the swelling power of commercial corn. The swelling power of starches is often related to their protein and starch contents. Higher protein content in flour may cause the starch granules to be embedded within a stiff protein matrix, which subsequently limits the access of the starch to water and restricts the swelling power. In addition to protein content, a higher concentration of phosphorous may increase hydration and swelling power by weakening the extent of bonding within the crystalline domain [36]. Furthermore, the amylopectin is primarily responsible for granule swelling, thus higher amyllose content would reduce the swelling factor of starch [37].
Fig. 4. Swelling power and solubility of starches at 90°C

4. CONCLUSION

The study showed that the starches extracted from selected sources exhibited differences in properties such as starch content, proximate composition, amylose, swelling power, solubility and gelatinization properties. Cereal starches contain a higher level of lipid and protein while tuber starches contain higher levels of moisture and fiber comparatively. The amylose content was high in proso and foxtail millets comparatively. Proso millet showed the highest gelatinization temperature whereas foxtail millet showed lowest comparatively. The study would be helpful to better understand the chemical, physical and microscopic characteristics of these starches and the application of novel starches obtain from non-conventional sources which are foxtail millet, proso millet and sweet potato as a thickening agent and a substitute to other common starches in food. Also, provide useful information for the further utilization of starches from food sources including two underutilized cereals for food and nonfood industries.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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