RESEARCH ARTICLE

Physicochemical properties of starches extracted from bambara groundnut landraces

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The physicochemical properties of starches extracted from three bambara groundnut landraces, maroon, brown, and cream were studied. The amylose contents (31.5–34.6%) of the bambara starches were significantly different among the landraces. All the bambara starches exhibited an A-type crystalline pattern with an average relative crystallinity of 32%. The peak gelatinization temperature (approx. 73°C) of brown bambara starch was slightly low compared to maroon (approx. 78°C) and cream (approx. 76°C) bambara starch. The bambara starches showed substantially high proportion of resistant starch (71%) and similar predicted glycemic index (40.1) among landraces. Bambara starch can potentially be used as a thickening agent in food products and ingredient development.

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1 Introduction

The starch industry relies mainly on cereals such as corn as major sources of starch. Pulses including pea (*Pisum sativum*), cowpea (*Vigna unguiculata*), and bambara groundnuts (*Vigna subterranea*) are relatively good sources of starch (18–49%) [1–3]. These leguminous crops can play a role as alternative starch sources to the conventional cereal crops. Among pulses, pea starch has found some applications in the food and allied industries. However, traditional crops such as bambara have not been extensively researched and their application remains limited in the food industry.

Botanical origin, composition (*e.g.*, amylose content) and plant species may significantly influence the physicochemical properties of starch [4–7]. Potato starch granules appeared round or oval in shape with smooth surfaces compared to corn starch granules, which were irregular with many pores on the surface [8]. Gelatinization is a phase transition that occurs when heating starch in the presence of moisture. Gelatinization changes the starch structure from semi-crystalline to amorphous phase [10]. This process is frequent in food processing and therefore, has been extensively studied. Many studies reported a significant influence of amylose on starch melting temperature [9–11]. Joshi [10], studied the physicochemical properties of lentil starch with higher amylose content than corn and potato starches. The melting temperature of lentil starch was found to be intermediate between potato and corn starches [10]. However, some studies did not find any relationship between amylose content and gelatinization temperature [5, 12]. For instance, Chung [12], reported similar gelatinization temperatures for starches from three varieties of common bean differing in amylose contents. According to Jane [13], the fine structures of amylopectin play a significant role in gelatinization process and starch pasting. Pasting properties of starch such as peak viscosity, breakdown and final viscosity, have been found to vary greatly among potato cultivars [14]. Huang [15], also found some variations in the pasting properties of pulse starches. Cowpea starch with higher amount of long amylopectin chains showed higher peak and final viscosities compared to chickpea and yellow pea starches [15].

There is a growing interest in pulse starches because of their high resistant starch contents, which are known to have positive physiological effects [16, 17]. Previous studies

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Abbreviations: A_a, amorphous area; A_c, crystalline area; **HI**, hydrolysis index; **RC**, relative crystallinity

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related the digestibility of starch to crystalline patterns. A-type is associated with cereals such as corn starch, B-type with tubers such as potato starch and high amylose corn starch, while C-type (a mixture of A and B) is often associated with legume starches. According to Srichuwong [18], A-type starch are more susceptible to digestive enzymes than B and C-starches. Hoover and Sosulski [19], reported that approximately 74% of corn starch (A-type) was hydrolyzed compared to C-type dry bean starches (26–35%). The high proportion of resistant starch in pulse starches such as cowpea and peas have been attributed to their moderately high amylose content compared to cereal starches [1]. The United Nations General Assembly declared 2016 as the International Year of Pulses (A/RES/68/231). This is important in creating awareness on the importance of this category of crop in addressing food and nutrition challenges. By making this declaration, the United Nations hopes to position pulses as primary sources of protein and other essential nutrients such as dietary fiber and starch. More research is needed to tap into the potential of pulses grown in many parts of the world including Southern Africa.

Bambara groundnut (*V. subterranea*) is a starch-rich (22–45%) leguminous crop [2, 20, 21], grown in many parts of Africa including Southern Africa. The bambara plant is highly drought tolerant and well adapted to the changing climate. Previous studies reported significant variations in amylose contents (21–35%) of bambara starch depending on source and cultivar [2, 20, 22]. Other studies on the characterization of bambara starch revealed different results on crystalline patterns [2, 20, 21]. Some authors reported the C-type pattern for bambara starch [2, 21] while others found the A-type crystallinity [20, 22]. Differences in crystalline patterns of bambara starches may be associated with the origin of the grains and variety. Many varieties of bambara are grown in Southern Africa. Bambara landraces dominate the production areas and these varieties are grown by farmers mainly for subsistence. To facilitate the utilization of bambara landraces, knowledge of the physicochemical properties of their starch component may be important. Hence, this study investigated the physicochemical properties of starches extracted from three bambara groundnut landraces.

2 Materials and methods

2.1 Materials

Three types of bambara groundnut landraces harvested from Markathini farm station Jozini, South Africa, were used in this study. Bambara grains were differentiated by their grain coat colors as maroon, brown, and cream. All chemicals and solvents used were laboratory grade. Glucose oxidase and peroxidase assay kit (No. GAGO-20), amyloglucosidase (No. 7095), alpha-amylase (No. 7545), guar gum (No. 4129), potato and corn starch were purchased from Sigma–Adrich (St. Louis, MO).

2.2 Preparation of bambara flour and starch extraction

Bambara flour was prepared according to the method of Sirivongpaisal [22] with slight modifications. Briefly, bambara grains were dehulled, dried, ground into flours, and sieved (sieve aperture size: 355 μm). Starch was then extracted as reported by Oyeyinka [2]. The yield of starch was calculated as a ratio of dried starch to bambara flour. Starch samples were stored at 4°C until analyzed.

2.3 Microscopy

Starch granule morphology was examined using a scanning electron microscope (EVO 15 HD) with an accelerating potential of 4 KV. Briefly, a thin layer of the starch granule was mounted on the aluminium specimen holder with double-sided tape. Starch sample was coated with a thin film of gold for 2 min with a thickness of about 30 nm [23]. Average starch granule size was determined from the diameter of individual granules ($N = 40$) on the basis of the scale bar provided on the captured scanning electron micrographs [24].

2.4 Amylose contents

Amylose contents of the starches were determined by iodine binding method [25].

2.5 X-ray diffraction

X-ray diffraction pattern of bambara starch was done as described by Oyeyinka [2]. The relative crystallinity (RC) of the starch was calculated using Eq. (1)

$$\mathrm{RC}(\%) = \frac{100A_c}{(A_c + A_a)} \tag{1}$$

where A_c is the crystalline area and A_a is the amorphous area on the X-ray diffractogram.

2.6 Fourier transform infrared spectroscopy

Fourier transform infrared spectroscopy (FTIR) spectra of the extracted starches were obtained using a spectrometer (Varian 800 Series) following the method reported by Oyeyinka [2] Spectra were obtained in the transmittance mode with 64 scans from 400 to 4000 cm^{-1}.

2.7 Differential scanning calorimetry

Thermal properties of bambara starches were determined using a differential scanning calorimetric (SDT Q600, USA) coupled with a thermal analysis data station and data recording software. Starch samples were prepared in a ratio of 1:3 with distilled water in an aluminium DSC pan. The pans were allowed to equilibrate at 25°C for 12 h prior to DSC analysis. Samples were scanned from 10 to 120°C each with an interval heating rate of 10°C/min. An empty pan was used as reference for all measurements.

2.8 Swelling power

Swelling power of bambara starch was determined as described by Oyeyinka [2]. Briefly, 1% starch suspension in water was heated for 30 min at temperatures ranging from 50 to 90°C with constant stirring. The suspension was centrifuged (Centrifuge model: Ependorf 5810R, Germany) at $3400 \times g$ for 20 min and the supernatant discarded. Swelling power was obtained by weighing the residue after centrifugation and dividing by original weight of starch on dry weight basis.

2.9 Pasting properties

The pasting properties of bambara starch were examined using a Rapid Visco-Analyzer (Newport Scientific Australia) according to standard method provided by the instrument manufacturer. Briefly, starch (2.8 g) was weighed into the test canister containing 25 mL of distilled water. The mixture was agitated by mixing manually before inserting the canister into the instrument. Starch was stirred at 960 rpm for 10 s before the shear input was decreased and held constant at 160 rpm during the subsequent heating and cooling cycles.

2.10 In vitro digestibility

Digestibility of bambara starch was done as reported by Naidoo [23]. The glucose assay kit was used to analyze the glucose content of the starch fractions. Nutritional starch fractions based on digestibility were: RDS represents the portion of starch that was hydrolyzed within 20 min of incubation, SDS represents the starch hydrolyzed between 20 and 120 min while RS was estimated as the starch not digested after 120 min of incubation. The predicted glycemic index was estimated using Eq. (2) as previously described [26]:

$$\mathrm{GI} = 39.71 + 0.549\mathrm{HI} \qquad (2)$$

where HI is the hydrolysis index.

2.11 Statistical analysis

Starch samples were prepared in duplicate and analyses were run at least in triplicate. Data were analyzed using analysis of variance (ANOVA) and means were compared using Fischer's least significant difference test ($p < 0.05$).

3 Results and discussion

3.1 Starch yield, morphology, and amylose contents

The starch yield was significantly different among bambara landraces. Maroon bambara gave a slightly high starch yield (35%) compared to brown and cream, which were similar (approx. 29%) (Table 1). The starch yields observed in this study are within the range reported in the literature for pulse starches including bambara [1, 2, 21, 22].

The majority of bambara starch granules appeared oval with a few granules round and irregular in shape (Fig. 1). All starch granules were smooth with no fissures. These granules were moderately large in size with diameter ranging from 24 to 29 μm (Table 1). The shape and size of bambara starch granules are in agreement with previous reports on pulse starches extracted from pea [7, 27], cowpea [3], and bambara varieties obtained elsewhere [2, 20].

Starches extracted from bambara landraces showed significantly different amylose contents (approx. 32–35%) (Table 1), which compared favourably with literature [1, 2, 20, 22]. A similarly high level of amylose contents (approx. 33–36%) have been reported for peas grown in China [7]. With the exception of high amylose maize starches, pulse starches have relatively high amylose contents compared to cereal and tuber starches. The amylose contents of bambara starches from this study are higher than those reported for normal corn (24.8–25.1%) and potato (14.9–23.2%) starches [10, 28].

Table 1. Yield, granule size, amylose contents, relative crystallinity, swelling power, and thermal properties of bambara starches

	Bambara landraces		
Parameters	Maroon	Brown	Cream
Yield (%)	$35.0^a \pm 0.1$	$28.0^b \pm 0.1$	$29.0^b \pm 0.2$
Granule size (μm)	$27^a \pm 4.7$	$24^a \pm 4.5$	$29^a \pm 5.5$
Amylose content (%)	$31.5^b \pm 0.4$	$34.6^a \pm 0.2$	$32.9^b \pm 0.2$
Relative crystallinity (%)	$33.0^a \pm 0.5$	$30.5^b \pm 1.0$	$32.5^a \pm 0.1$
Swelling power (g/g)			
50°C	$1.39^a \pm 0.02$	$1.34^a \pm 0.05$	$1.25^a \pm 0.11$
60°C	$1.55^a \pm 0.21$	$1.39^a \pm 0.04$	$1.27^a \pm 0.09$
70°C	$2.82^a \pm 0.07$	$2.75^a \pm 0.07$	$2.70^a \pm 0.14$
80°C	$11.55^a \pm 0.31$	$12.45^a \pm 0.21$	$11.90^a \pm 0.61$
90°C	$15.72^b \pm 0.16$	$16.31^a \pm 0.07$	$15.45^a \pm 0.92$
Thermal parameters			
Onset temperature (°C)	$72.0^a \pm 0.2$	$68.3^b \pm 0.3$	$70.9^a \pm 0.2$
Peak temperature (°C)	$77.5^a \pm 0.1$	$73.1^b \pm 0.4$	$76.4^a \pm 1.0$
Conclusion temperature (°C)	$84.4^a \pm 0.1$	$77.4^b \pm 0.1$	$82.5^a \pm 0.1$
Enthalpy of gelatinization ($J\,g^{-1}$)	$12.9^b \pm 0.1$	$13.6^a \pm 0.2$	$12.7^b \pm 0.1$

Mean ± SD. Mean with different superscript letters along a row are significantly different ($p < 0.05$).

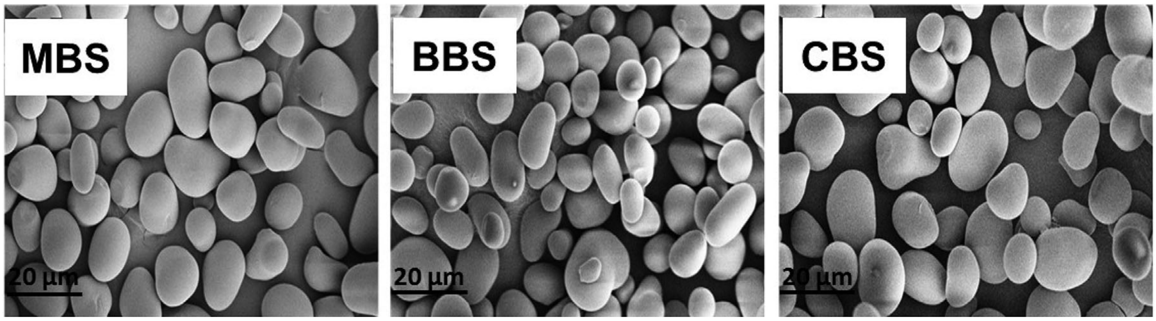

Figure 1. Micrographs of starches extracted from bambara groundnut landraces. Magnification, ×1500. MBS, maroon bambara starch; BBS, brown bambara starch; CBS, cream bambara starch.

Among bambara landraces, brown bambara starch had a slightly higher amylose content (approx. 35%) than maroon and cream bambara starches. Previous studies on bambara similarly found some minor variations in amylose contents of starches extracted from bambara varieties grown in Cameroon [20] and Thailand [22]. The amylose content of different starches may vary with botanical source, growth location, and genotypic differences. Adebooye and Singh [3], also reported variation in the amylose contents of starches extracted from two cowpea varieties grown in the same location.

3.2 XRD

All bambara starches exhibited strong peaks at 15° (2θ), a doublet at 17° and 18° (2θ), and a single peak at 23° (2θ) (Fig. 2), suggesting the A-type crystallinity pattern. Similarly, Kaptso [20] and Sirivongpaisal [22] reported the A-type pattern for starch extracted bambara groundnut grown in Cameroon and Thailand, respectively. However, other studies on the structure of bambara starch observed the C-type pattern typical of most pulse starches [2, 21]. It is possible to observe differences in crystallinity patterns of starch from the same species. These variations may depend on growing conditions and cultivar differences. For instance, both the A and C-type pattern were reported for some varieties of mung bean starch [29, 30] and Mexican yam bean starch [31].

The relative crystallinity of brown bambara starch was slightly lower than those extracted from maroon and cream bambara (Table 1). This may be explained by its slightly high amylose content (Table 1). Since the side chains of amylopectin forms the crystalline structure in starches, the relative crystallinity should be inversely related to the amylose content [32].

3.3 FTIR

Bambara displayed characteristic FTIR bands associated with starch which were similar among landraces. All starches showed complex vibrations in the region below 800 cm^{-1} because of the skeletal vibration of the glucose pyranose ring. A broad band in the region of 3000–3600 was observed with peak at approximately 3431 cm^{-1} (Fig. 3). This peak could be attributed to OH stretching [33, 34]. Similar FTIR band patterns were reported for bambara starch [2]. Maroon bambara starch showed lower peak intensities than did brown and cream bambara in the C–H stretching region of 2800–3000 cm^{-1}. These differences in peak intensities could be linked to variations in amylose contents [2, 35]. Other peaks were observed around 1650 cm^{-1}, which could

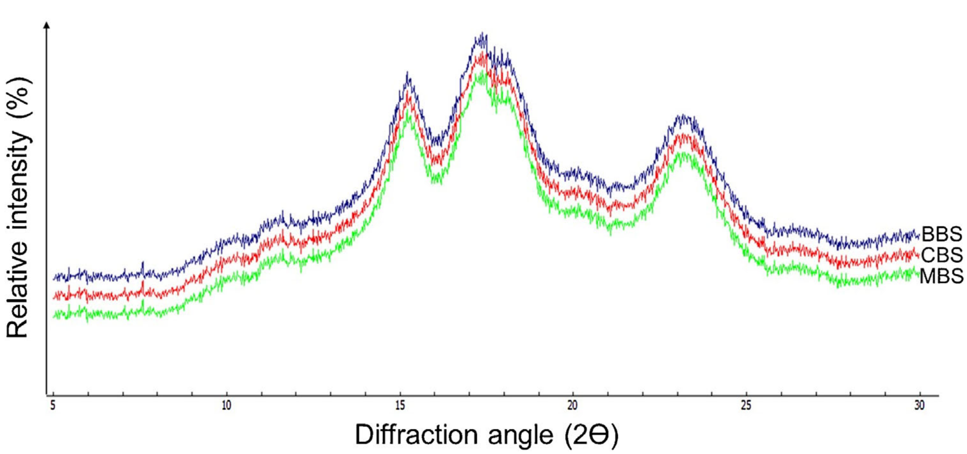

Figure 2. Diffractograms of starches extracted from bambara groundnut landraces. MBS, maroon bambara starch; BBS, brown bambara starch; CBS, cream bambara starch.

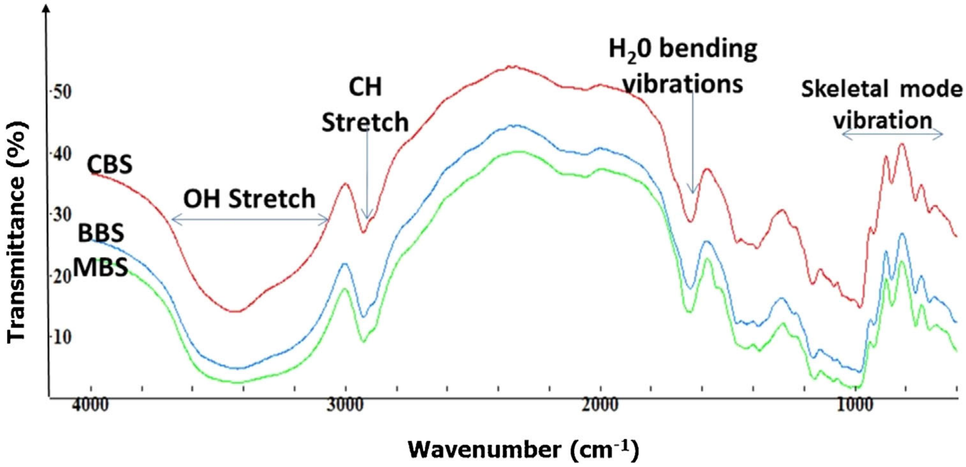

Figure 3. FTIR spectra of starches extracted from bambara groundnut landraces. MBS, maroon bambara starch; BBS, brown bambara starch; CBS, cream bambara starch.

be attributed to bending vibrations of H_2O absorbed in the amorphous regions of starch [35–37]. Kizil [35], also observed peaks in the same region (1650 cm^{-1}) for potato, corn, and wheat starches.

3.4 DSC

Bambara starches showed slight differences in onset gelatinization temperature (T_o), peak gelatinization temperature (T_p), conclusion gelatinization temperature (T_c), and gelatinization enthalpy (ΔH_{gel}) (Fig. 4 and Table 1). The T_p of bambara starches varied between 73 and 78°C (Fig. 4), which is in agreement with previous reports on bambara [20–22]. However, the peak gelatinization temperatures of bambara starches were high in comparison with values reported for corn and potato starches, respectively [10, 28]. This could be attributed to differences in amylose contents of bambara (Table 1), corn (approx. 25%), and potato (14.9–23.2%) starches [10, 28]. However, Liu [7] found significantly reduced peak gelatinization temperatures for pea starches, which had comparable levels of amylose contents to bambara starches (Table 1). These findings suggests that amylose is not the only factor that could affect starch gelatinization. According to some reports, the melting temperature of starch could depend on the distribution of amylopectin short chains rather than the proportion of amylose to amylopectin [38].

The gelatinization enthalpy (ΔH_{gel}) of bambara starches were not very different (approx. 13 J/g) (Fig. 4). Kaptso [20], also observed slight differences in ΔH_{gel} for starch extracted from two bambara varieties grown elsewhere. However, higher ΔH_{gel} values up to 25.2 J/g have been reported for bambara starch by some authors [21]. Variation in ΔH_{gel} values may be attributed to differences in the extent of interactions between the double helices forming the crystalline region of the respective starches [39].

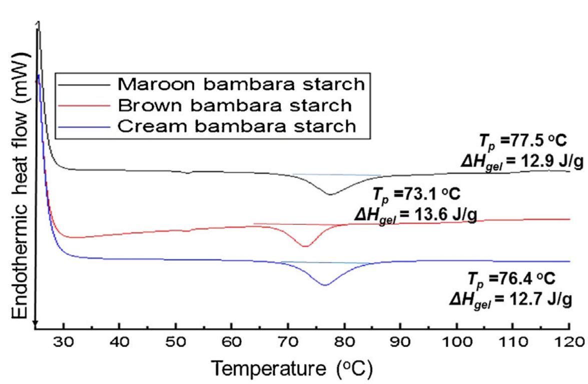

Figure 4. Typical thermograms of starches extracted from bambara groundnut landraces.

3.5 Swelling power

Bambara starches showed similar swelling behavior. The swelling power of bambara starches progressively increased at a temperature range of 70–90°C (Table 1). The rapid increase in swelling power at temperatures above 70°C have been attributed to melting of starch crystallites, which confirms gelatinization [19]. Sirivongpaisal [22], reported slightly high swelling power for bambara starches, which could be attributed to their low amylose content (approx. 22%) compared to the studied landraces (Table 1). Amylose has been suggested to restrict starch swelling behavior [23]. According to Tester and Morrison [40], swelling of starch is primarily a function of amylopectin while amylose and lipids act as a diluent. However, in some instances, starches with significantly high amylose content did not show restricted swelling [4, 5, 23]. Factors such as the molecular structure of amylopectin and the magnitude of interaction within the amorphous and crystalline regions have also been suggested to influence starch swelling [41].

3.6 Pasting

Bambara starches showed similar pasting profile curves for the three landraces (Fig. 5). The pasting temperature of

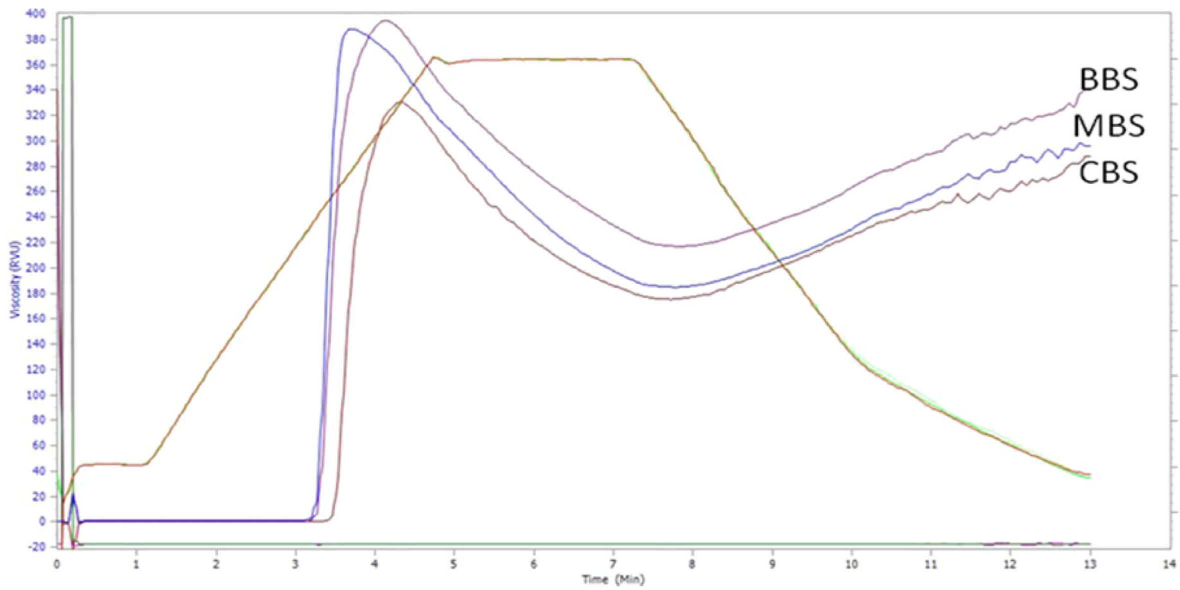

Figure 5. Typical pasting curves of starches extarcted from bambara groundnut landraces. MBS, maroon bambara starch; BBS, brown bambara starch; CBS, cream bambara starch.

bambara starches varied between 77.6 and 80.4°C (Table 2). These values are within the range reported in the literature for bambara [22, 42, 43] and cowpea starch [3]. In comparison with other crops, the pasting temperatures of bambara starches are intermediate between the values reported for potato starch (66.2–68.6°C) [10, 44] and normal corn starch (77–88°C) [10, 44]. This is in agreement with previous report on lentil starch [10]. Starch extracted from cream bambara showed the lowest peak viscosity compared to brown and maroon bambara starches, which were similar (Table 2). The peak viscosity, also referred to as "swelling peak" may be influenced by starch composition, structure, and the presence of other minor components of starch such as lipids [40]. Starches with high amylose contents would show low peak viscosity due to restricted swelling of starch granules [45]. However, in this study, the variation in peak viscosities did not seem to show any inverse correlation with amylose contents of starches. Previous studies similarly found that differences in amylose contents were not sufficient to explain the variation in peak viscosity of starch [3–5, 46]. Huang [15], associated the presence of a high proportion of long amylopectin chains in cowpea starch with its high peak viscosity compared to those of chickpea and yellow pea starches. Thus, the variation in peak viscosity of bambara starches could possibly be linked to differences in the chain length of amylose [47, 48] and amylopectin components of these starches [13, 15]. Furthermore, among the studied landraces, brown bambara starch showed the highest setback (127.1 RVU) and final (339.5 RVU) viscosities. This may be associated with its significantly higher amylose content compared to maroon and cream bambara starches (Table 1). The re-association of starch as double helices upon cooling is responsible for the setback viscosity of starches and the extent of this re-association during the cooling phase is closely related to the amylose contents of bambara starches. The final pasting viscosity results of bambara landraces suggest that their starches can be potentially used as thickening agents in food application.

Table 2. Pasting properties of starches extracted from bambara groundnut landraces

	Bambara landraces		
Parameter	Maroon	Brown	Cream
Peak viscosity (RVU)	$383.1^{a} \pm 1.3$	$385.6^{a} \pm 1.5$	$329.3^{b} \pm 0.6$
Trough viscosity (RVU)	$184.5^{b} \pm 0.4$	$212.4^{a} \pm 1.0$	$174.3^{b} \pm 1.2$
Breakdown viscosity (RVU)	$198.7^{a} \pm 1.1$	$173.2^{b} \pm 0.9$	$155.0^{c} \pm 1.9$
Final viscosity (RVU)	$289.4^{b} \pm 0.4$	$339.5^{a} \pm 1.4$	$286.6^{b} \pm 1.2$
Setback viscosity (RVU)	$104.9^{b} \pm 0.4$	$127.1^{a} \pm 0.2$	$112.3^{ab} \pm 0.0$
Pasting temperature (°C)	$77.6^{b} \pm 0.1$	$77.6^{b} \pm 0.1$	$80.4^{a} \pm 0.5$
Peak time (min)	$3.7^{c} \pm 0.1$	$4.1^{b} \pm 0.0$	$4.4^{a} \pm 0.1$

Mean ± SD. Mean with different superscript letters along a row are significantly different ($p < 0.05$).

3.7 In vitro digestibility

The nutritional starch fractions were similar among bambara landraces (Table 3). Rapidly digestible starch and slowly digestible starch fractions were about 12 and 16% of the total starch fractions, respectively. However, bambara starches contained substantial amount of resistant starch fractions (69.7–72.6%). This result is in agreement with previous reports on native legume starches [4, 5, 7, 49].

Table 3. Nutritional starch fractions and predicted glycemic index of starches extracted from bambara groundnut landraces

Starch	RDS (%)	SDS (%)	RS (%)	GI
Maroon bambara	$12.3^{b} \pm 0.1$	$16.3^{a} \pm 0.2$	$71.4^{a} \pm 0.1$	$40.1^{a} \pm 0.1$
Brown bambara	$13.3^{a} \pm 0.1$	$16.9^{a} \pm 0.1$	$69.7^{b} \pm 0.1$	$40.1^{a} \pm 0.2$
Cream bambara	$11.5^{b} \pm 0.2$	$15.9^{a} \pm 0.2$	$72.6^{a} \pm 0.1$	$40.1^{a} \pm 0.1$

Mean ± SD. Mean with different superscript letters along a column are significantly different ($p < 0.05$).
RDS, rapidly digestible starch, SDS, slowly digestible starch, RS, resistant starch, GI, glycemic index.

Further, the resistant starch contents of bambara starches were substantially higher than the values reported for native corn starches (5–20%) [50–52]. Cereal starches have relatively low amylose contents compared to pulse starches including bambara starch (Table 1). From previous reports, pulse starches with high amylose contents have been found to show high resistance to digestive enzymes [19]. The double helical structure of amylose is presumably not accessible to the amylase enzyme [23]. The proportion of resistant starch and predicted glycemic index (approx. 40) of starches from bambara landraces compare favorably with reports on common bean and pigeon pea starches [5, 32, 49].

4 Conclusions

Bambara groundnuts starch consists mainly of oval shaped granules. The amylose contents of bambara starches are significantly different among landraces. All starches exhibit the A-type crystalline pattern. Brown bambara starch displays significantly low peak gelatinization temperature, which may be due to its high amylose content compared to cream and marron bambara landraces. However, bambara starches show no inverse correlation of peak viscosities with their amylose contents. Bambara starches contain considerable amount of resistant starch fractions. Starch extracted from bambara groundnut landraces can potentially be used as thickening agents in food product and ingredient development.

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The authors declare that there is no conflict of interest.

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