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RESEARCH ARTICLE

Assessing variation in physicochemical, structural, and functional properties of root starches from novel Tanzanian cassava (*Manihot esculenta* Crantz.) landraces

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Cassava is an ideal "climate change" crop valued for its efficient production of root starch. Here, the physicochemical properties and functionality of starches isolated from six cassava landraces were explored to determine how they varied from each other and from those previously described, and how they may be potentially used as value-added foods and biomaterials. Among genotypes, the parameters assayed showed a narrower range of values compared to published data, perhaps indicating a local preference for a certain cassava-type. Dry matter (30–39%), amylose (11–19%), starch (74–80%), and reducing sugar contents (1–3%) differed most among samples ($p \leq 0.05$). Only one of the six genotypes differed in starch crystallinity (41.4%; while the data ranged from 36.0 to 37.9%), and mean starch granule particle size, (12.5 μm instead of 13.09–13.80 μm), while amylopectin glucan chain distribution and granule morphology were the same. In contrast, the starch functionality features measured: swelling power, solubility, syneresis, and digestibility differed among genotypes ($p \leq 0.05$). This was supported by partial least square discriminant analysis, which highlighted the divergence among the cassavas based on starch functionality. Using these data, suggestions for the targeted uses of these starches in diverse industries were proposed.

🖥 Additional supporting information may be found in the online version of this article at the publisher's web-site.

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Abbreviations: CLD, chain length distribution; **DigRaw,** digestibility of raw starch after 24 h incubation; **DM,** dry matter; **HPAEC,** high-performance anion-exchange chromatography; **PKT,** peak temperature; **PLS-DA,** partial least squares discriminant analysis; **PST,** pasting temperature; **PV,** pasting viscosity; **RC,** relative crystallinity; **RS,** reducing sugars; **RVA,** rapid viscosity analysis; **SOL70 /90,** solubility at 70°C/90°C; **SV,** setback viscosity; **SWPW 70/90,** swelling power at 70°C/90°C; **SY,** starch yield; **SynRf,** Syneresis at −20°C; **Syn re,** Syneresis at 4°C

1 Introduction

Cassava (*Manihot esculenta* Crantz) has been classified as an ideal 21st century "climate change" crop because it grows resiliently under adverse conditions, and has diverse uses as food, fuel, and polymer [1, 2]. This has led to a 60% increase in global production between 2000 and 2012 with further increases projected [2]. Cassava is valued primarily for its starch, which is an important source of calories for over 800 million people in Sub-Saharan Africa, Asia, and South

America, especially among food-insecure communities [1–4]. Cassava is also cultivated as a cash crop and there is a growing demand for the starch to be used as a raw material in various industries [3]. In many regions, cassava is cultivated instead of cereals because it is more productive and adaptable to environmental stresses, requires fewer agricultural inputs [2], and has a flexible production and harvesting window [5, 6]. In addition, compared with cereals, cassava starch is purer, more resistant to acid, and has unique pasting properties, which make it suitable for the production of paper and textiles, sweeteners, alcohol, and monosodium glutamate [5, 6].

The starch in cassava roots accumulates as round and irregularly shaped granules of approximately 13 μm [7]. Like most starches, it is composed of two large molecular weight glucan polymers called amylose and amylopectin, which occur in an approximate 20:80 ratio in non-mutant genotypes [7]. Both glucans are made up of chains of α-1,4 linked glucoses which are intermittently branched by α-1,6 glucosidic linkages [8]. There are branch points placed, on average, every 20 glucoses in amylopectin but only every ~4–100 glucoses in amylose [9]. The branching pattern in amylopectin is precise, so that clusters of glucan chains of 3–4 defined lengths are produced in an orderly array [10], which is essential for the formation of the semi-crystalline starch macromolecule [8, 11]. The organization of glucose molecules within the starch polymer, the glucan chain length distribution and amylose-to-amylopectin ratio, all influence granule morphology and size [8, 11, 12].

Starch functionality and hence its end-use is determined by its molecular structure [10, 13]. Starch is normally used after gelatinization and the resulting product is largely determined by the characteristics of the native granules [14]. Gelatinization causes a collapse of starch molecular order and to irreversible changes in water uptake, granule swelling, solubilization, viscosity, and, paste development [11]. These processes and the properties of the resulting gels and pastes formed during subsequent heating, freezing, and thawing, or after enzymatic hydrolysis, can indicate the suitability of starch for various applications [10, 13]. To monitor these changes, granule solubility, swelling, gelatinization, pasting, retrogradation, syneresis, and digestibility are often assessed [11]. These functional properties can sometimes be attributed to specific granule characteristics [8, 15, 16], but often, no associations are found because of the complex interplay of the factors that determine starch structure.

Because of the growing importance of cassava as a subsistence and cash crop, our aim was to characterize the starches from six understudied, famer-preferred landraces commonly grown in Eastern Tanzania (Fig. 1). Propagation of landraces aids ex situ conservation of locally adapted genetic resources [17], and documenting their organoleptic properties catalogs the natural diversity of cassavas in the region, which in turn, will help farmers, industrial end-users and breeders to select the most appropriate genotypes for their needs. Simple Sequence Repeat marker analysis indicated that there are distinct genetic subpopulations among African cassavas, with a "pivotal position for Tanzanian landraces" [18]. However, the starches from cassava cultivars adapted to grow in this region have yet to be studied. These genotypes may be distinct from those grown and analyzed in Asia, South America, and other parts of Africa [7, 18–20], and in addition, were not subjected to intense plant breeding and may synthesize starches with distinct profiles [21]. Such data could broaden our knowledge of starch structure-function. Here, we comprehensively investigated the compositional, structural, and functional properties of starches extracted from the Tanzanian cassava landraces, and used both correlation and multivariate analyses to understand the diversity of these starches and their possible end-uses (Fig. 1).

2   Materials and methods

2.1   Materials

Cassava landraces were collected in February 2012 from the Eastern region (36.98° E 6.83°S) of Tanzania. They were:
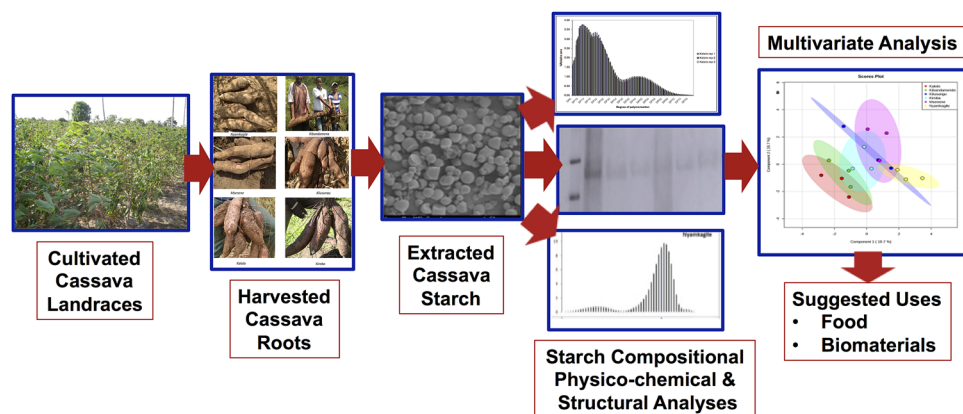
Figure 1. A scheme of the experimental overview and objectives.

Nyamkagile, Kiroba, Kalolo, Kibandameno, Kilusungu, and *Msenene* (Fig. S1). A completely random block design replicated three times using plot size of $5 \times 5\,m^2$ with spacing of $1 \times 1\,m^2$ was deployed. Roots were harvested 9 months after planting during the dry season. Three healthy plants were selected from each plot and harvested. Marketable roots were selected and immediately brought to the laboratory for analysis to avoid deterioration. Starch was obtained from the entire root.

2.2 Dry matter content

This was determined according to Benesi et al. [22], starting with exactly 200 g of grated fresh cassava root.

2.3 Starch extraction and purification

Cassava starch extraction was done according to Forsyth et al. [23] with slight modification. Ground cassava samples were treated as described [26], except that the filtrate was centrifuged twice in 100% (v/v) toluene and was left to dry overnight in a laminar hood at 37°C.

2.4 Starch content

The enzymatic method outlined in AOAC method 996-11 [24] with a slight modification was used. The starch flour (0.1 g) was placed in a glass test tube and mixed with 0.2 mL of 80% (v/v) ethanol. Then, 110 U of thermostable α amylase (from *Aspergillus oryzae*) diluted with 0.08 M phosphate buffer (pH 6) was added and the mixture was incubated in a boiling water bath for 6 min. Four milliliter of 200 mM acetate buffer (pH 4.5) and 3 U of amyloglucosidase (from *Aspergillus niger*) were added and the samples were incubated at 60°C for 3 h. The reaction was terminated by boiling for 30 min and the samples were filtered through Whatman no. 42 filter paper. The amount of glucose was determined by the glucose oxidase peroxidase (GOPOD; Megazyme™, Ireland) method.

2.5 Sugar content

Flour samples of 0.1 mg were boiled in 80% (v/v) ethanol to extract sugars, which were quantified by adding dinitrosalicyclic (DNS) according to Tanadul et al. [25].

2.6 Amylose/amylopectin content

Amylose was determined using an Amylose/amylopectin assay kit (K-AMYL, Megazyme™ International Ireland Ltd., Wicklow, Ireland) [25]. For each measurement, 20 mg of purified starch was used and instructions were followed exactly as outlined.

2.7 Phosphate content

Phosphate was determined at the DANR Analytical Lab (UC Davis) using the method of Prokopy [26].

2.8 Starch granule particle size distribution and morphology

At least 10 mg of purified starch was injected in the Microtec Analysette 22, laser scattering particle size distribution analyzer (Quebec, Canada). The frequency of granule size distribution was recorded [25]. The morphology and diameter of the cassava starch granule were estimated using Scanning Electron Micrographs (SEM) based on the scale bar provided on the SEM micrographs [27].

2.9 Relative crystallinity (%)

X-ray Diffractograms of purified starch samples were obtained using a Scintag XDS 2000 X-ray diffractometer as described [27]. The degree of crystallinity was quantitatively estimated by calculating the relative peak intensity as described by Wang et al. [28] with slight modifications. A smooth curve connected with the peak baseline was computer-plotted on diffraction. The upper diffraction area and the total area were measured using the image tool software (UTHSCSA, San Antonio, Texas). The ratio of upper area to total diffraction area was taken as the relative crystallinity (RC).

2.10 Branch chain length distribution of amylopectin by high-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD)

The method outlined by Bertoft was used [29]. Starch samples were debranched using 4 U isoamylase from *Pseudomonas* sp., (1000 U/mL, Megazyme™) and 2.88 U pullulanase M1 from *Klebsiella planticola*, (720 U/mL, Megazyme™) and analyzed by HPAEC-PAD (Dionex ICS-5000 system, Sunnyvale, CA) [29].

2.11 Swelling power and solubility

Swelling power and solubility of cassava starch suspended in deionized water and heated at 70 and 90°C were determined by the method previously described by Wang et al. [30].

2.12 Syneresis

Starch samples were suspended in deionized water (6% w/v) and were heated in a boiling water bath for 30 min with constant stirring. After cooling to room temperature, the

samples were stored at −20 and 4°C for 22 h, and later cooled in water bath (30°C) for 1.5 h. The samples were then centrifuged at 1050×*g* for 20 min. Syneresis at −20 and 4°C was determined as the amount of water released after centrifugation.

2.13 Rapid visco analysis (RVA)

A 8% (w/v) starch slurry was analyzed on an AR1000-N Rheometry (TA Instrument, New Castle, DE), following the method described by Thitisaksakul et al. [31]. RVA readings were done in triplicate for each biological replicate.

2.14 Starch digestibility

This was estimated using the method outlined by Salman et al. [32], with the following slight modifications. A mixture of porcine pancreatic amylase (Megazyme[TM]: 14 U) and amyloglucosidase (Megazyme[TM]; 0.33 U from *Aspergillus niger*) was incubated with 20 mg starch. An aliquot of 0.1 mL was collected at 30 min, 1, 2, 6, and 24 h and heated in a boiling water bath for 10 min. The amount of glucose produced was measured after incubating the aliquot with Megazyme[TM] GOPOD reagent for 20 min at 50°C according to the manufacturer's instructions (Megzyme[TM], Wickson, Ireland).

2.15 Statistical analysis

The data reported are an average of three independent biological replicates derived from roots originating from separate plants. The data were then subjected to Analysis of Variance (ANOVA) to determine statistical difference. Statistical analyses were determined to be different at a significance level of $p \leq 0.05$ or $p \leq 0.01$. When a statistical difference was found, mean separation was done using Tukey's multiple comparison test ($p \leq 0.05$). Pearson's correlation analysis (r) was done to elucidate the extent to which the variables analyzed were related (SPSS 16, IBM, CA). Multivariate analysis PLS-DA was performed using Metaboanalyst online tool (TMIC, Canada).

3 Results and discussion

3.1 Cassava dry matter content and chemical composition

Table 1 contains various chemical and compositional properties of the cassava starches studied. Attempts were made to compare the data generated here with published studies, with the caveat that differences in root developmental age, growth conditions, and analytical techniques used between studies, may introduce undesirable variables that

Table 1. Physicochemical composition of cassava landraces and their purified starches[a]

Cultivars	Dry matter (%)	Starch content (%) dry weight	Total reducing sugars (%)	Amylose content (%)	Mean particle size (µm)	Granule size volume percent distribution (%)			Relative crystallinity (RC) (%)
						Small (<12 µm)	Medium (12–25 µm)	Large (25–48 µm)	
Nyamkagile	33.6 ± 0.4[b]	80.3 ± 0.4[b]	1.03 ± 0.2[a]	19.4 ± 0.4[c]	13.33[b]	46.19[a]	52.04[a]	1.76[a]	37.9 ± 1.1[a]
Kibandameno	39.5 ± 0.6[d]	80.0 ± 0.5[b]	1.43 ± 0.1[ab]	11.9 ± 0.5[a]	12.50[a]	49.87[a]	49.14[a]	0.96[a]	41.4 ± 0.8[b]
Kilusungu	30.6 ± 0.5[a]	77.1 ± 1.5[ab]	2.12 ± 0.7[ab]	19.2 ± 0.3[c]	13.21[b]	42.58[a]	57.37[a]	0.00[a]	37.0 ± 0.4[a]
Msenene	33.4 ± 0.4[b]	78.4 ± 1.5[ab]	1.75 ± 0.4[ab]	17.1 ± 0.3[b]	13.78[b]	42.02[a]	55.36[a]	2.62[a]	37.4 ± 0.4[a]
Kalolo	30.8 ± 0.8[a]	74.3 ± 1.5[a]	3.10 ± 1.03[b]	16.9 ± 0.3[b]	13.09[b]	44.05[a]	55.96[a]	0.02[a]	36.0 ± 0.3[a]
Kiroba	36.7 ± 0.5[c]	80.2 ± 0.8[b]	1.96 ± 0.2[ab]	17.2 ± 0.4[b]	13.43[b]	42.33[a]	57.32[a]	0.37[a]	36.1 ± 0.5[a]

Values with different letters in the column differ significantly (*p*-value ≤ 0.05).
[a] Mean ± SEM for three independent biological replicates.

mask genotypic effects [10, 33]. Among the genotypes, dry matter and amylose content varied most, followed by starch content, and reducing sugars, while mean particle size and relative crystallinity showed the least variation (Table 1).

3.1.1 Dry matter content

Cassava landraces tend to have a lower dry matter (DM) content, lower starch and higher reducing sugars compared to the more advanced clones [7]. In an extensive screen of cassava germplasm, the 3272 landraces examined had an average dry matter of 32.8% while the values for the 772 improved clones was higher i.e., 36.7% [7]. Among our genotypes the lowest value was 30.6% for *Kilusungu* and the highest was 39.5% for *Kibandameno* (Table 1) with the average dry matter equaling 34.1%, which is intermediate between the landraces and improved clones studied by Sanchez et al. [7].

3.1.2 Starch content and reducing sugars

Starch varied from 74.3 to 80.3% (Table 1), which is a much smaller range, compared to 70.4–89.9% reported by Nuwamanya et al. [20] for Ugandan cassava cultivars [20]. The average starch content of our genotypes was 78.3%, lower than the 84.5% reported for both landraces and improved genotypes surveyed by Sanchez et al. [7]. The reducing sugar in our analysis was also higher (average 1.89%) than that of the landraces and the improved clones (1.25 and 1.56%, respectively) in that study [7].

The average DM, starch, and sugar contents of our genotypes are typical of cassavas that have not been subjected to extensive scientific breeding. *Kibandameno* was the exception as it had the highest DM and starch contents and therefore, may have faced greater selection for high yield. In comparison, *Kilusungu* and *Kalolo* accumulated the lowest starch and DM and had high free sugar content (Table 1). These are characteristics of unimproved clones, which tend to divert more resources for stress responsive mechanisms, than for storage in organs of perennation [21].

3.1.3 Amylose content

The proportion of starch accumulated as amylose has a profound effect on starch functionality [34], however Sanchez et al. showed that there was no significant difference in average amylose content among landraces and improved germplasm [7]. Amylose content analyzed using Concanavalin A among landraces in this study ranged from 11.9% (*Kibandameno)* to 19.4% (*Nyamkagile* and *Kilusungu*) (Table 1). These values are lower and less varied than the 16.96–28.8 % amylose reported by Mweta et al. [35] for Malawian cassavas [35] and the 15.2–26.5%

reported in the global analysis by Sanchez et al. [7]; however, differences in analytical methods can lead to such discrepancies [33]. In our hands, using an amperometric titration that detects iodine binding to solubilized starch, we found amylose content to range from 14.1 to 16.4% with no significant difference among genotypes (data not shown). This differed from the data collected using the Concanavalin A method in Table 1. SDS-PAGE analysis of starch granule intrinsic proteins of these starches indicated that there was a higher level of a 63 kD protein presumed to be granule bound starch synthase I (GBSSI; which makes amylose), extracted from *Nyamkagile*, which has an amylose content of 19%, while the level was lower in *Kibandameno* of amylose content 11.9% (data not shown). The amount of GBSSI protein extracted from starch varies proportionally to the amylose content of that starch [25] and is supportive of there being some variation in amylose indicated by the Concanavalin A. The discrepancy in values however urges caution in interpreting amylose content in these genotypes.

3.1.4 Phosphorous content

This element is present in root and tuber starches as a phosphate monoester [36]; however phosphorous content in our starches was too low to be quantified spectrophotometrically. A similar result was also reported by Peroni et al. [34] and may be explained by the fact that the phosphorous in cassava starch is not bound to amylopectin, and is thus easily washed out during purification [37].

3.2 Starch granule size distribution and morphology

Differences in granule size distribution may influence starch functionality [38], and were examined by scanning electron microscopy (SEM) (Fig. S2) and laser diffractometry (Table 1). There was no apparent difference in granule size and morphology among the cassava landraces studied. Most of the granules were round, but a few polygonal granules were observed by SEM, similar to those seen in other studies [34, 35]. A distinct small and large granule size class was observed by SEM, which was also previously reported by Gumul et al. [39].

The laser diffractometer provided a better estimate of starch granule size distribution (Table 1, Fig. S3). Mean granule size ranged from 12.5 to 13.8 μm, which is smaller than the variation (11.3–15.7 μm) found in four Sri Lankan cultivars [19, 40]. *Kibandameno* was the only cultivar that varied and had the smallest mean particle size (12.5 μm).

3.3 Starch crystallinity

X-ray diffractometry was used to estimate the crystallinity of cassava starches (Table 1). Cassava starch may be of the A- or

C_A-type crystallinity [33]. The diffraction pattern (Fig. 2) showed prominent peaks at $2\Theta = 15.2$, 23.4, and a doublet at 17.2 and 18.2, which is typical of the type A crystallinity [20, 32, 41, 42]. The relative crystallinity (RC) ranged from 36.1 to 41.4%, which is similar, but slightly higher than the values reported for Thai cassava, which averaged 35.8% [41]. *Kibandameno* was the only genotype that differed significantly having the highest value of 41.4% (Table 1). Interestingly this genotype had the lowest amylose (11.9%) consistent with the negative correlation between starch crystallinity and amylose content observed in many starches [43].

3.4 Branch chain length distribution (CLD) of amylopectin

Differences in glucan chain length distribution (CLD) can influence starch properties. For example, starches with a high proportion of very short glucan chains (degree of polymerization; $DP = 6–9$) of amylopectin may interfere with the normal crystalline order of starch [44], while longer chains ($DP > 18$) may provide greater stability [19, 45].

There are relatively few reports of amylopectin branch CLD in unprocessed cassava starch, therefore the amylopectin chain lengths were classified into four classes and the frequency of occurrence of each class was determined

as done by Franco et al. [46] for comparability (Table S1, Fig. 3). There was no significant difference in amylopectin CLDs among the landraces [30, 47]. Chains of DP 13–24 (medium chains) occurred with greatest frequency (38.6–38.9%), while long chain lengths with DP 25–36 were of the lowest frequency (18.1–18.6%) for all sample analyzed ($p \leq 0.05$).

As noted, there are no extensive published data on the CLDs of different cassava starches. The cumulative studies cited in Charoenkul et al., suggested that cassava CLD is bimodal with two peaks that occur from DP 10–15 (Peak I) and DP 36–44 (Peak II), respectively, and a shoulder at approximately 16–22 DP [47]. These may represent the A, $B_2 + B_3$, and B_1 chains of amylopectin, respectively [47]. In our work, Peak I occurred at 12 DP, Peak II at 44 DP and the shoulder at 20 DP, respectively (Fig. 3). Our data for the short chains were similar to the DP 12 for Brazilian cassava starch [46] and DP 11–12 for Thai cassava starch [47]. The DP of the longer chains were more varied among the published studies. It ranged from DP 43.3–44.7 for our landraces, which is lower than the DP 47 reported by Franco et al. [46] and the values for our samples were narrower compared to the DP 40–44 published by Charoenkul et al. [47]. No significant differences in CLD were observed among starches of cassava genotypes analyzed in those other studies.
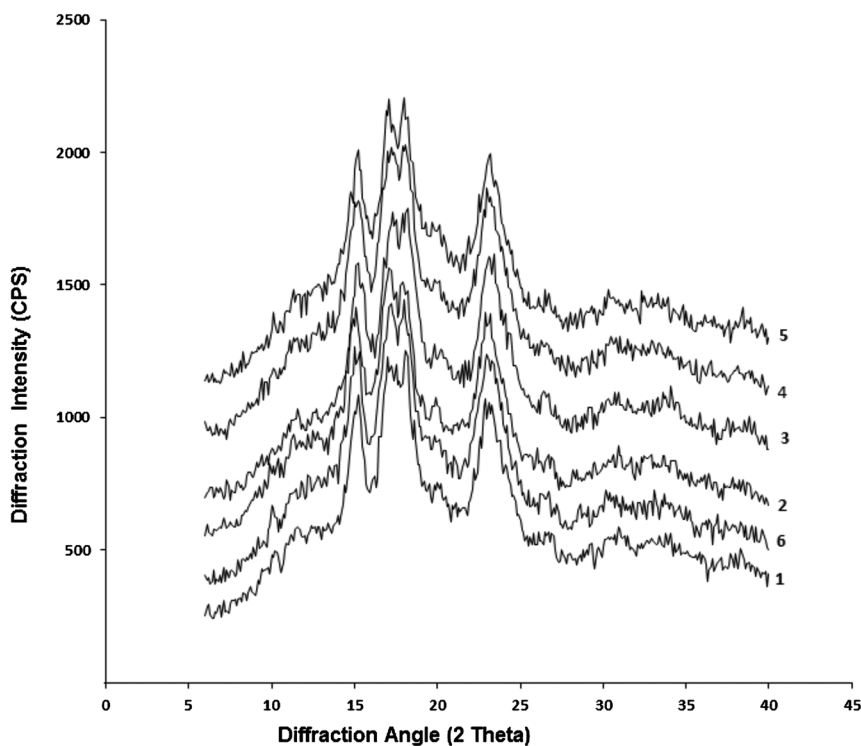
Figure 2. Wide-angle X-ray diffraction patterns of cassava starch. The graph was offset for clarity and the scan signal shown after $6°2\theta$. There was no peak at $5.6°2\theta$ which would indicate a C_A-type crystallinity 1, *Nyamkagile*; 2, *Kibandameno*; 3, *Kilusungu*; 4, *Msenene*; 5, *Kalolo*; and 6, *Kiroba*.

Table 2. Swelling power, water solubility, syneresis, and pasting properties of starches from cassava landraces[a]

Cultivars	Swelling power (g/g)		Water Solubility (%)		Syneresis (%)		Pasting properties				
	70°C	90°C	70°C	90°C	4°C	−20°C	Pasting temperature (°C)	Peak viscosity (Pa.s)	Peak temperature (°C)	Breakdown viscosity (Pa.s)	Setback viscosity (Pa.s)
Nyamkagile	8.9 ± 0.8ᵃ	13.5 ± 0.2ᵃ	3.0 ± 0.3ᵃ	6.0 ± 0.3ᵃᵇ	23.0 ± 1.6ᵃ	57.7 ± 2.3ᶜ	69.6 ± 0.6ᵃ	0.64 ± 0.10ᵃ	89.9 ± 0.7ᵃ	0.30 ± 0.05ᵃ	0.21 ± 0.04ᵃ
Kibandameno	10.3 ± 0.6ᵃᵇᶜ	14.2 ± 0.3ᵃ	3.0 ± 0.5ᵃ	7.4 ± 0.1ᵇᶜ	26.8 ± 1.6ᵇ	38.3 ± 3.3ᵃᵇ	67.5 ± 0.9ᵃ	0.72 ± 0.10ᵃ	80.5 ± 4.6ᵃ	0.46 ± 0.09ᵃ	0.29 ± 0.04ᵃ
Kilusungu	12.3 ± 0.4ᶜ	16.3 ± 0.8ᵇ	2.8 ± 0.5ᵃ	5.0 ± 0.3ᵃ	21.5 ± 1.8ᵃᵇ	38.3 ± 1.7ᵃᵇ	66.4 ± 0.3ᵃ	0.77 ± 0.20ᵃ	76.6 ± 2.6ᵃᵇ	0.57 ± 0.09ᵃ	0.28 ± 0.08ᵃᵇ
Msenene	11.7 ± 0.3ᵇᶜ	16.0 ± 0.9ᵇ	2.1 ± 0.4ᵃ	7.9 ± 0.7ᶜ	16.7 ± 1.7ᵃ	31.7 ± 1.7ᵃ	66.7 ± 0.9ᵃ	0.54 ± 0.10ᵃ	76.8 ± 3.4ᵃᵇ	0.44 ± 0.06ᵃ	0.11 ± 0.01ᵃᵇ
Kalolo	9.5 ± 0.3ᵃᵇ	15.7 ± 0.3ᵇ	3.9 ± 0.6ᵃ	8.5 ± 0.3ᶜ	38.3 ± 1.7ᶜ	48.3 ± 2.8ᵇᶜ	67.7 ± 0.4ᵃ	0.64 ± 0.10ᵃ	88.1 ± 0.5ᵃ	0.36 ± 0.02ᵃ	0.28 ± 0.05ᵃ
Kiroba	11 ± 0.3ᵃᵇᶜ	14.5 ± 0.5ᵃᵇ	3.3 ± 0.03ᵃ	5.3 ± 0.2ᵃ	28.7 ± 0.7ᵇ	36.7 ± 2.0ᵃ	67. ± 0.6ᵃ	0.68 ± 0.04ᵃ	85.1 ± 1.3ᵃ	0.31 ± 0.06ᵃ	0.39 ± 0.03ᵃ

Values with different letters in the column differ significantly (p-value ≤ 0.05).
[a] Mean ± SEM for three independent biological replicates.

3.5 Swelling power, solubility, and syneresis

Starch swelling power (SP) and solubility depend on the capacity of the starch molecule to hold water through hydrogen bonding during gelatinization. They both measure the strength of interaction between water molecules and glucan chains [48]. As thermal energy increases, the bonds between glucan chains relax and the granules absorb water and swell [49]. There were differences in SP among the cassava landraces in this study, attributed to disparities in bonding forces within the starch granule [11]. *Nyamkagile* had the lowest SP at both temperatures (Table 3), which indicates strong forces holding the granules, thereby enabling them to resist swelling. Low SP in this cultivar may be due to its higher amylose content (19.4%; Table 1) which impedes granule expansion [19]. *Kilusungu* and *Kiroba* had the lowest solubility at 90°C (Table 3), indicative of strong intragranular organization, as more energy is required to relax these molecular forces [30].

Syneresis is the tendency of a starch gel to release water when subjected to repeated cycles of freezing and thawing during storage, and is an undesirable property for most applications. Assaying this component is important if a starch is to be used in refrigerated (4°C) or frozen (−20°C) food products [50]. There was a significant difference (Table 3) ($p \leq 0.05$) in syneresis among the landraces. *Msenene* had the lowest syneresis at both storage temperatures, indicating its suitability for low temperature applications. The higher levels of amylose accumulated in *Nyamkagile* may influence the higher syneresis observed at −20°C (Tables 1 and 3) while in *Msenene,* low syneresis may be due to the slightly lower amylose content (17.1%) observed although other factors are likely.

3.6 Starch pasting properties

Pasting properties were examined using rapid visco-analysis (Table 2). Starch pasted at temperatures between 66.4 and 69.6°C, similar to those found in Brazilian (67.4 ± 0.2°C) [34] and Thai (67–70°C) [42] cassavas. In the 3272 cassava landraces surveyed by Sanchez et al., pasting values varied from 58–71°C, but among the cassava cultivars the range was 60–69.7°C [7]. Our values aligned closely with those of improved clones and may have been an attribute that faced stronger selection pressure due to human preference [51]. We found no significant difference in pasting temperature among cultivars, which is in accordance with data reported by Charoenkul et al. for 12 Thai cassavas, among which 11 were highly bred varieties with different cooked root textures (i.e., mealiness and firmness) [42]. This may mean that the uniformity in the DP of the long chain fraction among different regional cassavas (see Section 3.4) led to similar pasting properties.
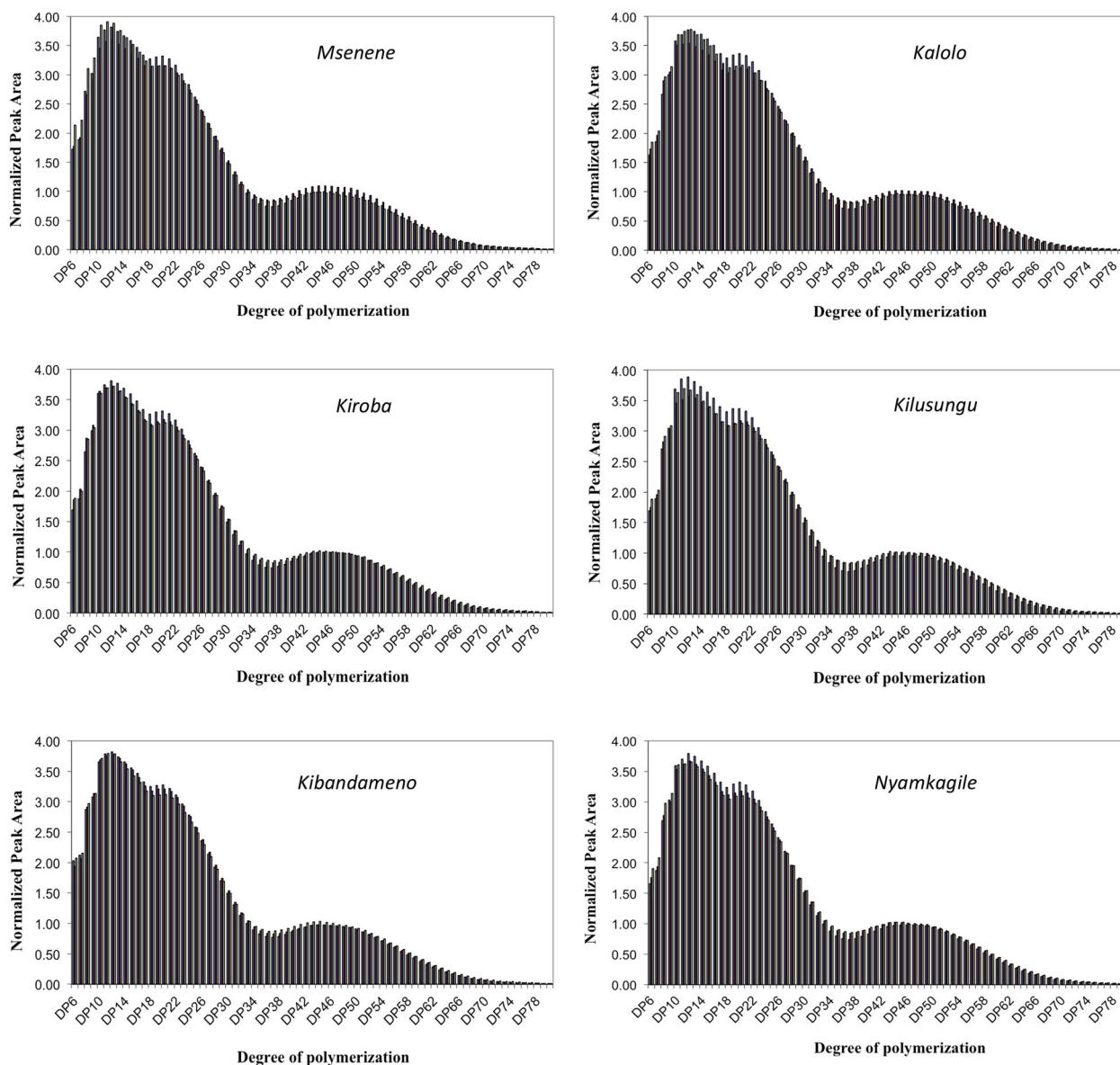
Figure 3. Branch-chain length distributions of debranched amylopectin of isolated starches from cassava landraces analyzed by HPAEC-PAD. Values were derived from three biological replicates, where each replicate constituted one root. Data from each biological replicate is indicated on the respective graphs.

3.7 Starch digestibility

The percentage of raw and gelatinized starches digested in vitro by amylase and amyloglucosidase are shown in Fig. 4. The digestibility of raw starches varied among cultivars ($p \leq 0.001$). *Kibandameno* had the highest digestibility at almost all time points, while *Nyamkagile* and *Kilusungu* had the lowest ($p \leq 0.05$, Fig. 4). It is worth noting that *Kibandameno* starch had the highest proportion of small granules (12 µm), the highest crystallinity (41%) and the lowest amylose content (11%; by the Concanavalin A method), characteristics that collectively can lead to high digestibility (Table 1 and Fig. 4) [25, 52]. In contrast,

Nyamkagile and *Kilusungu* had the highest amylose content of all genotypes (~19%; Table 1), which may be a factor retarding enzyme hydrolysis [52].

The rate of raw starch digestion was then calculated over the initial (0.5–2 h), middle (2–6 h), and late (6–24 h) stages of hydrolysis (Fig. S4). When digestion conditions are the same, rates are determined by starch structure and physical chemical properties [25]. Rates are faster during the beginning stages of digestion, but then slow down over time [52]. Even if the proportion of starch digested as a single time point varies among genotypes, there should be similar reaction kinetics because of the resemblance in granule crystalline ultrastructure [55]. This was borne out in our

Table 3. Pearson's correlation coefficient (*r*) for physicochemical and functional properties of cassava starch.

	AC	SC	RS	DM	SWP	SOL	SN4	SN20	DIG	PST	PV	PKT	BV	SV
SC	0.36													
RS	0.06	0.01												
DM	−0.30	0.2	0.28											
SWP	0.19	0.01	0.52*	0.36										
SOL	−0.57**	−0.6	0.40	0.41	0.41									
SY4	−0.25	0.23	0.34	0.23	0.01	0.40								
SY20	0.07	−0.35	−0.17	−0.49*	−0.39	−0.31	0.24							
DIG	**−0.73****	0.14	0.04	0.31	−0.16	0.36	0.26	−0.21						
PST	0.12	−0.02	−0.41	−0.52*	**−0.72****	−0.45*	0.15	**0.63****	−0.12					
PV	0.01	−0.38	0.52*	0.26	0.31	−0.15	0.08	−0.01	0.07	−0.28				
PKT	0.11	0.15	−0.13	−0.37	−0.55*	−0.23	0.47*	**0.712****	−0.04	**0.81****	−0.33			
BV	−0.03	−0.27	0.31	0.41	**0.66****	0.16	−0.20	−0.39	−0.12	**−0.7****	**0.68****	**−0.82****		
SV	−0.10	−0.28	0.46*	0.19	−0.13	−0.10	0.36	0.13	0.43	0.08	**0.61****	0.19	−0.03	
RC	**−0.62****	**−0.72****	−0.29	−0.20	−0.39	0.10	−0.25	0.16	0.24	0.23	0.04	−0.12	0.06	0

AC, amylose content; SC, starch content dry base; RS, reducing sugars; DM, dry matter; SWP, swelling power; SOL, solubility; SN4, syneresis at 4°C; SN20, syneresis at −20°C; DIG, digestibility; PST, pasting temp; PV, peak viscosity; PKT, peak temp; BV, breakdown viscosity; SV, setback viscosity; RC, relative crystallinity.

* $p \leq 0.05$.
** $p \leq 0.01$.

Values in bold are statistically significant.

analysis (Fig. S4). While *Kibandameno* had the highest percentage of starch hydrolyzed at most time points (Fig. 4), it had the same digestion rate over the first 2 h as *Kilusungu* and *Msenene*, and the same rate as *Kalolo* and *Kiroba* over the next 4 h ($p \leq 0.05$; Fig. S4). In the final stages of digestion, the rates were expected to be similar among genotypes because the reaction should be at saturation, however it was higher in *Kiroba*, indicating perhaps, differences in the structure of the slowly digestible starch fraction [52]. These data highlight that different starches can be delineated based on structural and organizational characteristics.

Gelatinization increases starch susceptibility to enzyme attack, as a result of destruction of starch's crystalline structure [8, 16]. Gelatinized samples were rapidly degraded (approximately threefold faster), especially in the initial stages (30 min, 1 and 2 h) compared with the raw starches (Fig. 4). The A-type crystalline structure of cassava starch may increase its susceptibility to enzyme hydrolysis when gelatinized, compared to other roots [40, 41, 53]. Cassava digestibility of ∼91% after 24 h have been reported [54], which is within the range of our samples (85–95%; Fig. 4). There were fewer differences among the starches analyzed compared with data from the raw starches, however after 6 and 24 h *Kibandameno* and *Kiroba* were digested to a greater extent than the other landraces (Fig. 4). Gelatinization may have made the starches more uniform, enabling similar kinetics by the degradation enzymes used.

3.8 Correlation among the physicochemical and functional properties of cassava starches

Pearson's correlation coefficients were calculated to determine the relationship among the different starch parameters (Table 3). Although there were few or no significant differences among cultivars for some starch features, small variations may nonetheless affect some aspects of starch functionality. This could be reflected in significant correlations ($p \leq 0.01$) among data, which in some instances may be causal (Table 3).

3.8.1 Starch physiochemical properties

Amylose content was inversely proportional to starch solubility, digestibility, and RC. Wickramasinghe et al. also reported a negative correlation between amylose content and digestibility [40]. However, amylose did not correlate with either swelling or pasting properties as have been reported by others [20, 30, 35, 41]. This might have been due to the narrow range of amylose content data in our landraces since mutant cassavas with low (0%) and high (30%) amylose showed correlation with these parameters [55, 56]. Except for *Kibandameno*, our analysis also did not reveal a general correlation between starch granule particle size with digestibility and amylose content as has been previously shown for
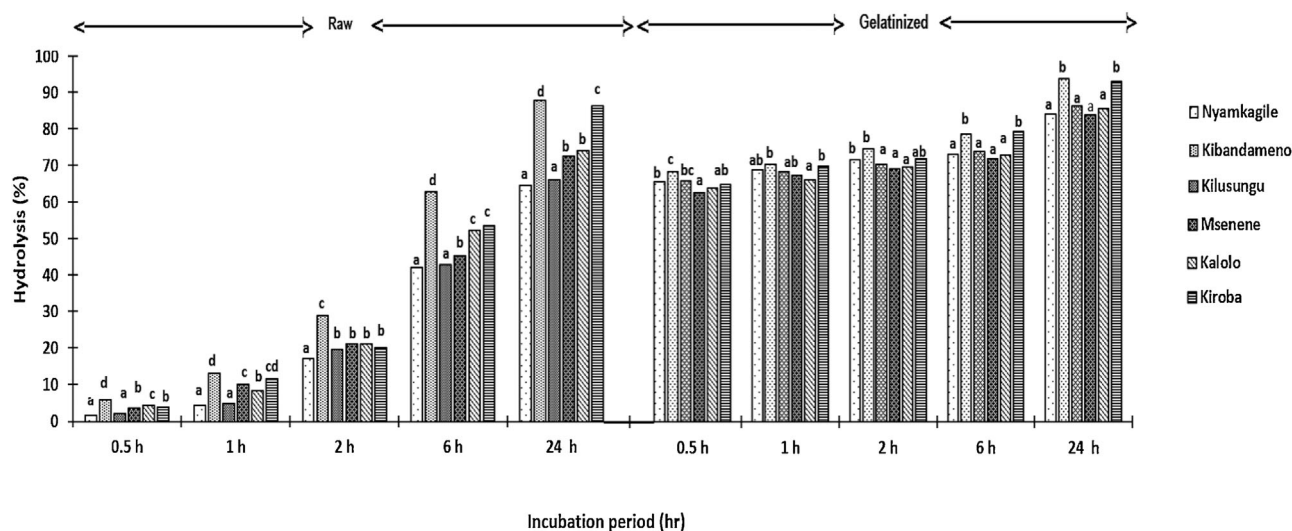
Figure 4. Enzymatic digestibility of raw and gelatinized cassava starch at different time intervals: 30 min, 1, 2, 6, and 24 h. Bars with different letters within the same time point, implies there is significantly difference ($p \leq 0.05$) in digestibility among landraces.

cassava starch [15, 16]. However, analyses of starches from different botanical sources [41, 57], also showed no correlation between digestibility and starch particle size, which is consistent with our finding. Starch content was negatively correlated with RC, but it is difficult to draw significant biological meaning from this relationship.

3.8.2 Starch functional properties

Swelling power negatively correlated with pasting temperature and peak temperature, but positively correlated with breakdown viscosity (Table 3). Peak temperature was also positively associated with the syneresis at −4 and −20°C. *Msenene* and *Kilusungu* had the highest swelling power and lowest peak temperature compared to other cultivars (Table 3). Low peak temperature is an indication that the starch granules swell best at the lowest temperature before their physical breakdown. Furthermore, starch granules from these landraces have the ability to absorb more water compared to other cultivars [12]. Significant interactions among pasting properties have also been observed by others [58].

3.9 Multivariate analysis

To gain insight on the extent of variance among cassava landraces based on starch properties, the data in this study were subjected to partial least squares-discriminant analysis (PLS-DA). PLS-DA is a supervised multivariate technique that maximizes differences among groups by minimizing within group variation [59]. The PLS-DA score plot (Fig. 5A) of all of the data showed clear separation among some landraces, indicative of differences in starch properties.

Based on their coordinates on the plot *Kalolo* and *Nyamkagile* were most disparate among the genotypes, *Kibandameno* could be distinguished from the remaining genotypes (although there was some overlap), while *Kilusungu*, *Msenene*, and *Kiroba* clustered together (Fig. 4C). PLS-DA loading scores (Fig. 5B) explained the separation seen on the PLS-DA scores plot. Values close to the origin have a smaller impact on the score plot pattern, whereas those further away make a bigger contribution [60]. Reducing sugars, set back viscosity and to a lesser extent solubility at 90°C were the primary determinants of the PLS-DA score plot.

When only starch functionality data was considered, there was greater separation among the landraces. *Nyamkagile* was the most disparate and *Kalolo* and *Msenene* were also distinct (Fig. 5C). In contrast, *Kilusungu* shared features with three other genotypes suggesting core commonality in starch functional. Setback viscosity, solubility at 90°C and syneresis at −20°C were most important in defining the coordinates of each genotype relative to each other (Fig. 5D). Although *Kibandameno* was most different in starch structure and composition compared with other cultivars (Table 1), these features did not lead to a corresponding disparity in starch functionality (Fig. 5C), highlighting the lack of congruency in starch structure-function relations.

4 Potential targeted uses of the starches from the cassava landraces

Using the data accumulated in this study we attempted to identify appropriate uses of the starch from each genotype. *Msenene* and *Kilusungu* starches had high swelling power, which makes them potentially suitable for use as thickeners
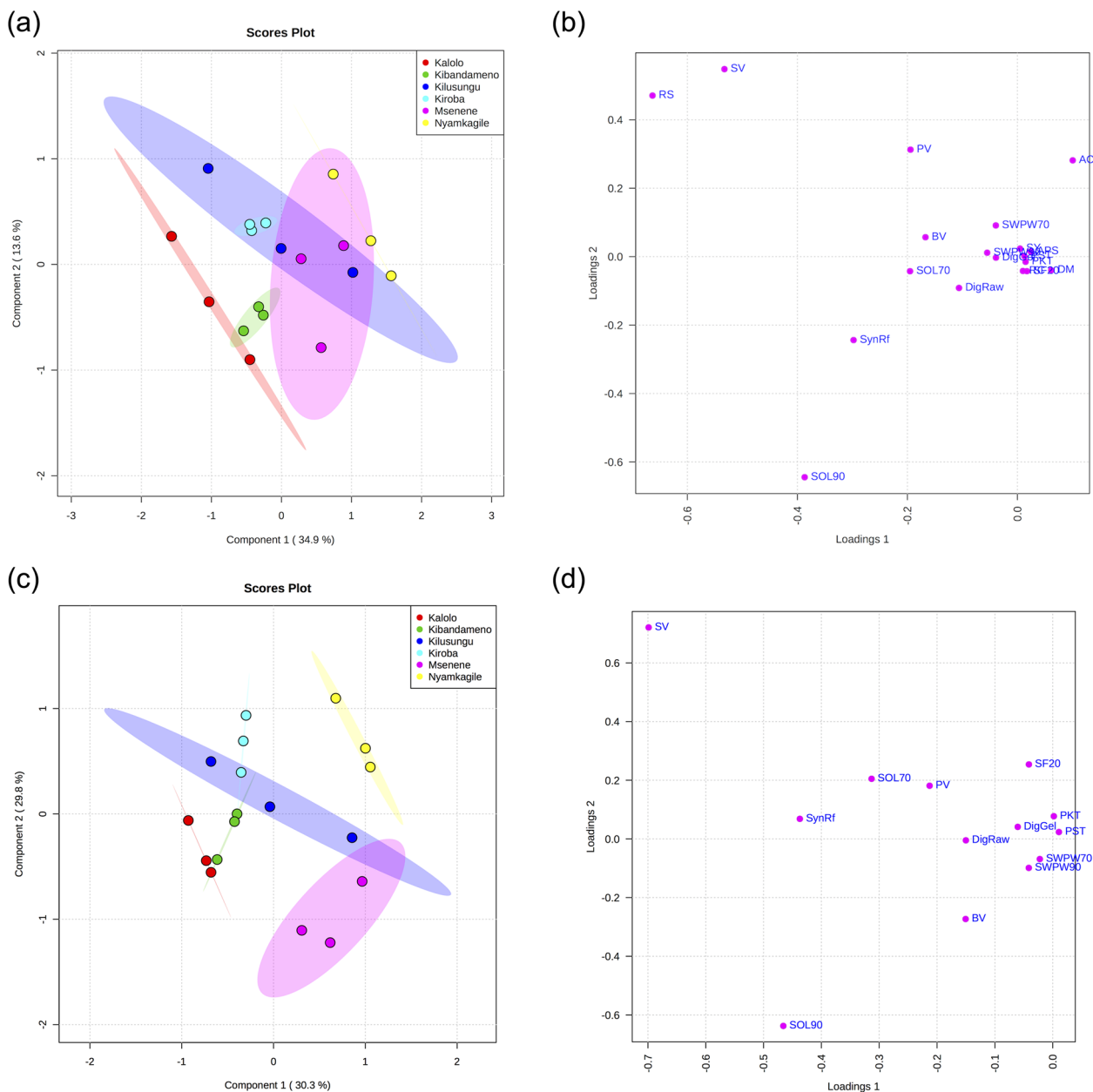
Figure 5. Partial least square-discriminant analysis (PLS-DA) of cassava starch functionality. (A) PLS-DA score plot showing the separation of cassava landraces using all data collected in this study. (B) PLS DA loading scores showing the parameters that contributed to the separation of cassava landrace seen in the score plot. (C) Starch functionality PLS-DA score plot showing the separation of cassava landraces using functionality data only. (D) Loading scores for the score plot in 4C. Key is as follows: AC, amylose content; APS, average particle size; BV, breakdown viscosity; DigGel, digestibility of gelatinized starch after 24 h incubation; DM, dry matter; PKT, peak temperature; PST, pasting temperature; PV, pasting viscosity; RC, relative crystallinity; RS, reducing sugars; SOL70, solubility at 70°C; SOL90, solubility at 90°C; SV, set back viscosity; SWPW 70, swelling power at 70°C; SWPW 90, swelling power at 90°C; SY, Starch yield; Dig Raw, digestibility of raw starch after 24 h incubation; SynRf, syneresis at −20°C, Syn re, syneresis at 4°C.

and binding agents for food and non-food uses. *Msenene* also had a relatively low setback viscosity after cooling, and low syneresis, ($p > 0.05$), desirable properties in starches used as gelling agents and thickeners in refrigerated and frozen food products. Similarly, *Kilusungu* had the highest peak viscosity, and a low pasting and peak temperature ($p > 0.05$). This may

indicate a potential difficulty that may occur when mixing this starch paste, as there may be a resultant high viscous load. *Kibandameno* starch had the highest enzyme digestibility and lowest particle size distribution ($p \leq 0.05$) compared to other starches, this makes the cultivar suitable for making glucose syrup, adjuncts in breweries

(fermentation stock), low fiber feed, and sweeteners. *Nyamkagile* ($p \leq 0.05$) had the lowest digestibility and may find applications in food for individuals wishing to manage their glycemic index such as diabetic and overweight patients.

5   Conclusions

The landraces explored here showed a narrower range of values for most starch parameters compared with cassavas in other studies. Dry matter, starch, and sugar content data suggest that these cassavas were simultaneously selected for yield and perhaps also for resistance to stress [7, 18, 21]. Although there was great similarity among starch properties measured, the genotypes could be distinguished from each other by PLS-DA. Differences in starch swelling power, solubility, syneresis, and digestibility were observed during this analysis, indicating that these genotypes could be targeted for use in different food and non-food industries even though they were not always statistically significant ($p > 0.05$).

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