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Research article

Characterization of physicochemical properties of starches from improved cassava varieties grown in Zambia

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Abstract: Cassava starches processed from six different cassava varieties (Bangweulu, Katobamputa, Mweru, Kariba, Kampolombo and Chila) were assessed for variety effect on swelling, solubility, gelatinization, pasting and gel freeze-thaw stability properties. The swelling power was investigated using dispersion methods in water while gelatinization and pasting were determined using Differential Scanning Calorimetry and Rapid Visco Analyzer, respectively. The gel freeze-thaw stability was determined by syneresis method. The starch granules size of the cassava starches were in the range 1.17–22.22 μm. The swelling power and solubility index of starches were in the range of 2.22–15.63 g/g and 1.62–71.15%, respectively. Solubility index of starches correlated positively with amylose ($p < 0.0001$). Swelling powers of starches showed a weak negative correlation with resistant starch content. The onset (T_o), peak (T_p) and conclusion (T_c) gelatinization temperatures of cassava starches were ranged from 56.33–63.00 ℃, 62.00–71.29 ℃ and 69.10–77.12 ℃, respectively and varied among cassava varieties ($p < 0.05$). The pasting temperatures for starches were in the range of 64.54–70.54 ℃ and weak positively correlated with amylose ($r = 0.231$, $p < 0.001$). The peak viscosity (782.3–983.5 cP), breakdown viscosity (383.8–506.8 cP) and final viscosity (462.0–569.7 cP) varied ($p < 0.05$) among cassava varieties and exhibited negative correlation with amylose ($p < 0.05$, $p < 0.01$, and $p < 0.01$, respectively). The syneresis for the freeze-thaw and five freeze-thaw cycle storage were ranged from 0.00–29.11% and 0.00–42.40%, respectively, and varied ($p < 0.05$) among cassava varieties. The sources of variations in physicochemical properties among the cassava

varieties were due to differences in amylose, protein, lipid contents, and starch granule size distribution.

Keywords: amylase; cassava starch; freeze-thaw stability; gelatinization; pasting; proximate composition; resistant starch; swelling power

1. Introduction

The swelling of cassava starches in water is an important structural characteristics towards ascertaining the suitability toward processing and culinary applications of cassava starches and flours. Starches can undergo different stages of swelling from water absorption of amorphous regions of starch granules to the disintegration of the granules. Water absorption and heating can result into swelling of starch granules, disruption of hydrogen bonds, increase in granule sizes, and crystallite melting leading to separation of amylose-amylopectin, and exudation of amylose molecules [1,2]. Starches in excess water form dispersions. When dispersions are heated, swelling, starch granule gelatinization and solubilization occurs which influence the properties of both continuous and dispersed phases, and paste development. The properties of the paste and gels resulting from heating, freezing and thawing processes can be the basis of selecting cassava varieties for suitability of industrial applications. The factors influencing the dispersed phase are genetically inherent in cassava varieties and these include starches granule shape, size, starch granules composition (amylose/amylopectin ratio, lipids, protein and trace elements such as phosphorus, sodium and potassium) and molecular structures of amylopectin and amylose [3]. The concept of resistant starch content in the food system can be related to swelling power of starch granules. Park et al. [4] reported that swelling and rupture of the starch granular and melting of crystalline structures during gelatinization accelerated the digestion of native maize starch. Nevertheless, information on correlations between swelling and resistant starch is limited. The proteins and lipids are present in small amounts within the starch granules. The presence of these minor components associated with starch granules can affect functional properties of starches [5].

In most part of literature, swelling power of cassava starches were conducted at 1 % concentrations in distilled water using different, however, using different heating temperature of 90 ℃ [6], from 60 to 90 ℃ [7], 60 ℃ [8] and 70 ℃ [9]. Peak swelling values for cassava starch granules were reported to be in the range between 70 and 95 ℃ [10]. High swelling powers of starches at lower temperature 60 ℃ were characterized with high viscosities. However, an increase in shear rate will decrease the viscosities of starch paste due to shear thinning. Food quality can be associated with swelling and gelatinization of starch granules and susceptibility to enzymatic hydrolysis [11].

In Zambia, cassava crop is important for food security and is the most staple crop after maize [12]. The current national cassava strategy is focused on developing a viable cassava industry contributing to wealth creation and food security for improved livelihoods. Improvement of cassava through breeding is the Zambian Government's agricultural priority and the breeding objective is to produce high yielding, early bulking, pests and disease resistant varieties [13], including breeding for reduced cyanide content in cassava [14] to produce sweet varieties [15]. Thus, there are a number of cassava varieties that have been bred and officially released. However, there is limited on information on the composition, structural, functional and physicochemical properties of starches. This work was

undertaken to evaluate variety effects on swelling, solubility, gelatinization, and pasting, and freeze-thaw properties of cassava starches from local landraces and officially released improved cassava varieties cultivated in Zambia.

2. Materials and methods

2.1. Source of materials

The six cassava varieties (Bangweulu, Katobamputa, Mweru, Kariba, Kampolombo and Chila*)* were obtained from Mansa Root and Tuber Research Station, a branch of Zambian Agriculture Research Station (ZARI), Mansa District, Luapula Province, Zambia. The station is located 29 °00′E, 11 °30′E, and elevation of about 1200 m. The region receives rainfall (1000 and 1500 mm per year) and mean annual minimum temperature 10 °C and maximum temperature 31 °C. The cassava varieties were planted in a Completely Randomized Block Design in triplicates on a plot of 5 m with a plant spacing of 1m in January 2016 and were harvested at 18 months after planting (June 2017). The roots were collected from five cassava plants randomly selected from each block.

2.2. Native starch extraction

The extraction of starch was conducted using the method of Numfor and Walter Jr [16] with modification. The cassava roots were brought to the laboratory for analysis immediately after harvest. The fresh cassava roots were washed, peeled, chopped into small pieces and then pulverized in a blender. The pulp was suspended in potable water in the ration 1:10 (i.e., the volume of water 10 × the volume of pulp), and the well-stirred mixture was filtered using double cheesecloth. The collected filtrate was allowed to sediment, and after decanting of the supernatant, the sediment was washed six times. The resultant starch was washed using distilled water, and after decanting, the starch was oven-dried at less than 35 °C for 24 h. The starch yield was determined based on 400 g of peeled and blended cassava.

$$\text{starch yield (\%)} = \frac{\text{Mass of dried starch}}{400\ \text{g}} \times 100 \quad (1)$$

2.3. Proximate contents

2.3.1. Moisture content

The moisture content of the dried flour sample was determined in a triplicate according to AOAC [17] method 925.10 by drying of about 3.0 g sample at 105 °C overnight.

$$\text{Moisture content (\%)} = \frac{W_b - W_d}{W_b} \times 100 \quad (2)$$

where, W_b = weight of cassava starch before drying, W_d = weight of dried cassava starch.

2.3.2. Ash content

The starch ash content was determined according to AOAC [17] method 923.03 by taking about 3.0 g sample after carbonization and ignition at 500 ℃ for 6 h in the muffle furnace (Model 2-525, J M Ney furnace, Yucaipa, USA).

$$\text{Ash content (\%)} = \frac{W_b - W_a}{W_b} \times 100 \quad (3)$$

where, W_b = weight of cassava flour before ashing, W_a = weight of ash.

2.3.3. Determination of nitrogen content and crude protein

The crude protein content was determined as described in Nuwamanya et al. [18] using Dumas combustion method of nitrogen content analysis (Model FP-528, Leco Truspec, St Joseph Mi, USA) by taking about 0.3 g of sample and using the conversion factor % Protein = N% × 6.25.

2.3.4. Determination of crude lipid

The crude lipid content was determined using standard AOAC [17] method No of 920.39 by taking about 5 g of sample in a Soxhlet extraction unit (Soxhlet, Büchi, Switzerland) using petroleum ether as a solvent.

$$\text{Crude lipid content (\%)} = \frac{W_3 - W_2}{W_1} \times 100 \quad (4)$$

where: W_1 = Mass of a sample (g), W_2 = Mass of the Buchi fat beaker (g), W_3 = Mass of the Buchi fat beaker with extracted residue (g).

2.3.5. Determination of crude fiber

The crude fiber content was determined using AOAC [17] method No 962.09 after sequential digestion with 0.3 M H_2SO_4 and 0.25 M of sodium hydroxide (Sisco Research Laboratories, Maharashtra, India). Weighed 5 g sample (Wo) was boiled in 50 mL of 0.3 M H_2SO_4 under reflux for 30 min, followed by filtration (75 micron) under suction pressure. The residue was washed with hot distilled water to remove the acid. The residue was then boiled in 100 mL, 0.25M of sodium hydroxide under reflux for 30 min and filtered (75 micron) under suction. The insoluble was washed with hot distilled water to free the alkaline and quantitatively transferred to pre-ignited weighed ashing crucible (W_1). The insoluble was dried to the constant weight (W_2) in the oven at 100 ℃, 2 h, and then cooled in the desiccator. The sample was then carbonized in a blue Bunsen burner, ashed in a muffle furnace to subtract ash from the fiber, cooled in a desiccator and weighed (W_3).

$$\text{Crude fiber content (\%)} = \frac{(W_2 - W_3) - W_1}{W_o} \times 100 \quad (5)$$

where: W_o = weights of sample on dry matter basis, W_1 = weight of crucible, W_2 = weight of insoluble residue dried sample (100 ℃, 2 h) and W_3 = weight of ignited sample.

2.4. Amylose contents

The amylose content in cassava and wheat flour samples was determined by using a Megazyme amylose/amylopectin assay kit (K-AMYL 12/16 Megazyme International, Bray, Wicklow, Ireland). A 20 mg sample was dispersed by heating in dimethyl sulfoxide (Sisco Research Laboratories, Maharashtra, India). The lipids were removed by precipitating dispersed starch in ethanol using centrifuge (Avanti® J-26XPI, Beckman Coulter, Inc., Indianapolis, USA). After the dissolution of the precipitated starch sample in an acetate/salt solution (Sisco Research Laboratories, Maharashtra, India), amylopectin was selectively precipitated by the addition of lectin concanavalin A (Con A) and removed by centrifugation (Avanti® J-26XPI, Beckman Coulter, Inc., Indianapolis, USA). The amylose, in the supernatant, was enzymatically hydrolyzed by 0.1 mL amyloglucosidase/α-amylase enzyme system to glucose which was then treated with glucose oxidase/peroxidase (GOPOD) reagent and absorbance of color developed was measured by UV-Vis spectrophotometer (UV-1800PC, Shimadzu Corpor., Kyoto, Japan) at 510 nm. Total starch in a separate acetate/salt solution was hydrolyzed to D-glucose and was reacted with GOPOD reagent and its absorbance was measured similarly. The concentration of amylose in the starch sample was then estimated as the ratio of GOPOD absorbance of the supernatant at 510 nm of the Con A precipitated sample to that of the total starch sample.

$$\text{Amylose content (\%)} = \frac{\text{Absorbance (ConA Supernatant)}}{\text{Absorbance (Total Starch Aliquot)}} \times 66.8 \quad (6)$$

2.5. Resistant starch assay procedure

The resistant starch (RS) contents in cassava flours and starches were determined using starch assay kit (K-RSTAR 2/17, Megazyme International, Ireland) as starch components that resisted digestion by pancreatic α-amylase and amyloglucosidase (AMG). About 0.15 g sample was used. The non-resistant starch in the sample were hydrolyzed and solubilized by adding 4.0 mL of pancreatic α-amylase containing amyloglucosidase (AMG). Then, the mixture was incubated in water bath at 37 ℃ for 16 h. After removing the tubes from water bath, 4 mL ethanol (99% v/v) was added, stirred (vortex) and centrifuged (1500 g, 10 min). The supernatants were decanted into separate collecting tubes, and the pellet (sediment) re-suspended in 8 ml ethanol (50% v/v) and mixed, followed by centrifugation. The supernatant decanted were collected into the respective collecting tube. Suspension and centrifugation was repeated once more by carefully decanting the supernatant into the same collecting tubes. The tubes with pellet were inverted to drain off excess water. Then, 2 mL of 2 M KOH was added to dissolve RS under water bath with stirring. Sodium acetate buffer (8 mL of 12 M, pH 3.8) was added followed by 0.1 mL of AMG with mixing placing sample in water bath at 50 ℃ for 30 min. The contents were transferred to 100 mL volumetric flask (washing down contents using water) with mixing, and adjusted to 100 mL with distilled water followed by centrifugation. The supernatant of 0.1 mL aliquots were transferred into glass test tubes, to which 3 mL GOPOD reagent was added, and incubated at 50 ℃ for 20 min. Absorbance of the digested RS solution was measured at 510 nm against the blank. To measure non-RS, the combined supernatants were mixed, and 0.1 mL of diluted AMG solution in 100 mM sodium maleate buffer (pH 6.0) at 20 min at 50 ℃ water bath. To non-RS sample tube solutions, 3.0 mL GOPOD reagent was added, incubated (20 min at 50 ℃) and then the absorbance was measured at

510 nm similar as that for digested RS solution. The percent resistant starch (RS), Non-RS and total starch were calculated on dry weight basis as follows:

$$\mathrm{RS}(\%) = \Delta\mathrm{E} \times \frac{\mathrm{F}}{\mathrm{W}} \times 90 \tag{7}$$

$$\mathrm{Non-RS}\ (\%) = \Delta\mathrm{E} \times \frac{\mathrm{F}}{\mathrm{W}} \times 90 \tag{8}$$

$$\text{Total starch}\ (\%) = \mathrm{RS} + \mathrm{Non-RS} \tag{9}$$

where ΔE = absorbance (reaction) read against the reagent blank, F = conversion factor from absorbance to micrograms, W = dry weight of sample.

2.6. Determination of starch granule size distribution

The morphology of starch granules was studied using a Scanning Electron Microscope (SEM) as described in [19]. The double adhesive tape was cut into small piece were attached to a circular (10 mm diameter) specimen stub. The starch sample was splashed on the adhesive tape to form a film of finely distributed starch particles which were then sputter coated with gold using a sputter coater (Q150 ЄS, Quorum, West Sussex, UK). The prepared gold-coated samples were examined for granule microscopic morphologies using SEM (EVO LS15, ZEISS, Jena, Germany) set at a magnification of 1.00 KX with signal A at SEI, I Probe = 30 pA, and EHT = 5.00 kV. The microscopic starch granules image obtained from SEM were submitted to image analysis for starch granule size (diameter) estimation as shown in Figure 1 using Soft Imaging System GmbH (Olympus Soft Imaging Solutions, Munster, Germany). The measurement of diameter (granule size) was done on 130 granules per microscopic image replicated three times. Based on the size distribution of granule size, the diameter ranges (>20, 15–20, 10–15, 5–10, and 1–5 μm) were selected. The number of granules of specified size range was divided by a total number of granules to obtain starch granule size percent distribution.

$$\text{Starch granule size}\,(\%)\,\text{distribution} = \frac{\mathrm{N}_{\text{granule}}}{\mathrm{N}_{\text{sample}}} \tag{10}$$

where $\mathrm{N}_{\text{granule}}$ is the number of granule in a given specific size range, and $\mathrm{N}_{\text{sample}}$ is the total number of granules on the microscopic image.

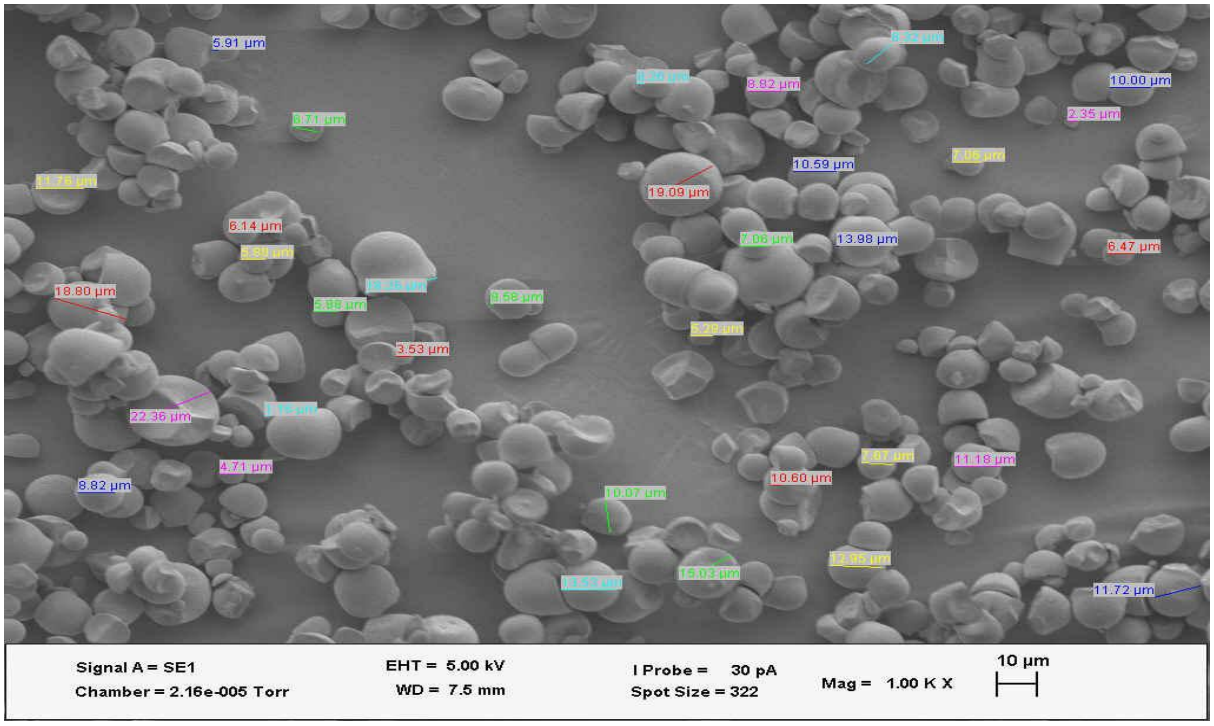

Figure 1. Starch granule size using Soft Imaging System GmbH.

2.7. Crystallinity

The X-ray diffraction pattern of starch powder samples was studied using analytical X-ray diffractometer equipped with photon counter as described in Huang et al. [20]. The X-ray diffractometer (D8, Bruker Corpor., Brucker, Germany) was equipped with a copper anode X-ray tube (Cu-K α-radiation, wavelength of 1.54060 nm) and position sensitive detector (LynxEye). Starch samples (1 g) were equilibrated in a 100% relative humidity chamber at room temperature for 24 h prior to the analysis. The diffractometer was operated at 40 mA and 45 kV, and the spectra were scanned over a diffraction angle (2θ) range of 5–40 ° at a step size of 0.1 ° and a count time of 2 s. The crystalline peak and total area of the diffractogram were measured. The percent crystallinity was calculated as the percentage of peak area to the total diffraction area.

2.8. Swelling power and solubility

The swelling power and solubility patterns of cassava flour and starch granules were determined as described in Kusumayanti et al. [8] at different temperatures (50, 60, 70, 80 and 90 °C). Sample (0.5 g dry weight) was suspended in 20 mL water in centrifuge tube (50 mL) of known weight, heated for 30 min at specified temperature, swirling at every 5 min, centrifuged (8000 rpm for 20 min) using Beckman Coulter Centrifuge (Avant J-26 XPI, High Performance Centrifuge, USA), supernatant discarded and the sediment mass was measured as W_{sd}. The swelling power (g/g) was calculated as the ratio of W_{sd} to the original sample weight (W_s). The supernatant separated was collected on pre-weighed evaporating crucible dish, oven dried (105 °C for 12 h) and the dried

residue was weighed as W_d. The solubility was then expressed as a percentage of dried supernatant weight to the original sample weight (Ws).

$$\text{Swelling (g/g)} = \frac{W_{sd}}{W_{s\ dry\ basis}} \tag{11}$$

$$\text{Solubility (\%)} = \frac{W_d}{W_{s\ dry\ basis}} \times 100 \tag{12}$$

where W_{sd} = dried sediment mass, W_s = original sample weight, W_d = dried residue.

2.9. Starch gelatinization properties

The starch gelatinization properties (onset, peak and conclusion gelatinization temperature and enthalpy of gelatinization) were determined using differential scanning calorimetry (Perkin Elmer system (Model DSC7; Norwalk, CT, USA) as described in Huang et al. [20]. A starch sample (4 mg) was placed in aluminium pan and deionized water was added to obtain a starch to water ratio of 1:4 (w/w). The sample was sealed hermetically and equilibrated for 4 h. The prepared sample was then scanned in the heating program of 30 ℃ to 150 ℃ at the scanning rate of 10 ℃/min using nitrogen as a purging gas at the rate of 30 mL/min. Parameters analyzed from the DSC thermogram were gelatinization temperatures for onset (T_o), peak (T_p) and conclusion (T_c), and enthalpy of gelatinization (ΔH_{gel}).

2.10. Starch pasting properties

The starch pasting properties were determined using Rapid Visco Analyser (Model: RVA-4, Newport Scientific, Warriewood, Australia) as described in Colman et al. [21]. The starch samples (5 g dry basis) were suspended in 25 mL of distilled water. A heating and cooling cycle program was utilized. The samples were held at 50 ℃ for 1 min, followed by heating to 95 ℃ for 7.5 min at the heating rate of 6 ℃/min, holding at 95 ℃ for 5 min followed by cooling to 50 ℃ in 7.5 min and holding at 50 ℃ for 1 min. Parameters measured were pasting temperature (PT), peak viscosity (PV), hot paste viscosity at the end of the plateau (HPV), cooled paste viscosity (CPV) at 50 ℃, final viscosity (FV), breakdown viscosity (BD) as PV-HPV and setback viscosity (SB) as CPV-HPV.

2.11. Syneresis (freeze-thaw stability)

2.11.1. Syneresis after freezing

The syneresis of starches was determined as described in Morante et al. [22]. Starch gels were prepared by suspending starch samples in distilled water (5% w/v) and heated in boiling water bath for 30 min with constant stirring to ensure gelatinization of starch granules. After cooling to room temperature, 15 centrifuge tubes per starch gel variety were filled with approximately 6 g of gel (WG) and stored at −20 ℃ for five weeks. Every week (after every 7 days), three tubes were drawn out of the freezer and thawed for 90 min in a water bath at 30 ℃, followed by centrifugation of samples at 8000 rpm, 10 min using Beckman Coulter Centrifuge (Avant J-26 XPI, High Performance

Centrifuge, USA). After centrifugation, the mass of supernatant separated and weighted (WS). Syneresis was calculated using the following formula:

$$\text{Syneresis } (\%) = \frac{\text{WG}}{\text{WS}} \times 100 \quad (13)$$

where WG = weight of gel, WS = weight of supernatant.

2.11.2. Syneresis after consecutive freeze-thaw cycles

The syneresis after consecutive freeze-thaw cycles was determined as described in Morante et al. [22] and sample preparation and calculations were done as described above (2.11.1). Fifteen (15) centrifuge tubes per starch sample of each variety were filled with approximately 6 g of gel and stored at −20 ℃. Every seven days, the whole set of tubes were removed from the freezer and held at room temperature for 90 min in a water bath (30 ℃). Three random tubes were taken out and centrifuged (8000 rpm for 10 min; Avanti® J-26XPI), supernatant separated and weighed. The remaining tubes were frozen again for another freeze-thaw cycle.

2.12. Data analysis

A Completely Randomized Design comprising of two factors (variety and temperature) was used for swelling and solubility studies. One factor (variety) experiment was designed for resistant starch, gelatinization and pasting properties. A two factor (variety and frozen storage) experimental design was used for freeze-thaw stability. A triplicate data were analyzed using two-way ANOVA using GenStat 18th edition software. The mean differences were determined using Fisher's Least Significance Difference (LSD) test at the 5% significant level. Although $p < 0.05$ was generally used, $p < 0.01$ and $p < 0.001$ were used for some of the data to indicate greater significance of differences.

3. Results and discussions

3.1. Starch granule size

The starch granule size distribution is shown in Figure 2. The starch granule size was distributed in the range between 1.17 and 22.22 μm and significantly varied ($p < 0.05$) among the varieties. The trend in the distribution of starch granule sizes was 5–10 > 10–15 > 1–5 > 20–25 μm. This suggests that the largest and smallest proportional of starch granule sizes were in the range of 5–10 μm and 20–25 μm, respectively. Similar results were obtained by Mtunguja et al. [6] who reported the smallest starch granule size volume percent distribution in the range 25–48 μm and the largest in the range 12–25 μm. According to Lindeboom et al. [5], the granules size distribution obtained in this study can be classified as very small to medium size. Starch granule size influences water absorption, solubility, and swelling [23]. Small granules have a high surface area that can lead to high water absorption capacity [5]. Agnes et al. [24] reported small starch granule sizes exhibited higher solubility and increased water absorption capacity. Smaller starch granule size with a diameter similar to lipid micelles (approximately 2 μm) can be applied as fat mimetics [25].

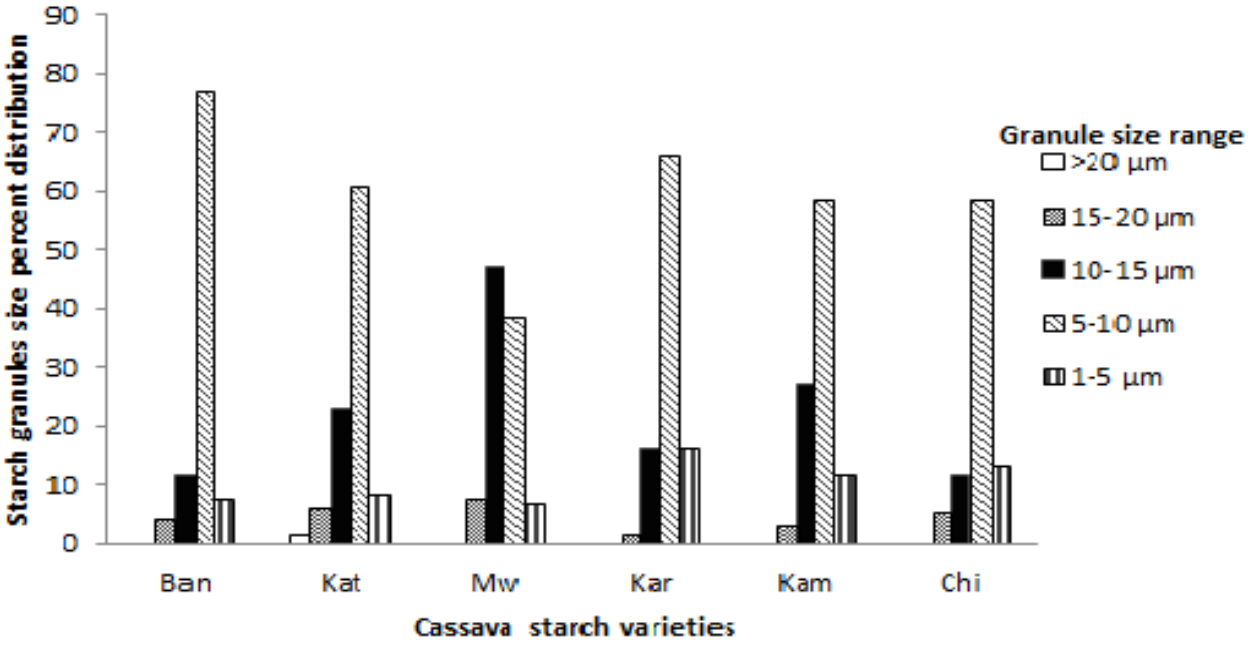

Figure 2. Starch granule size distribution for cassava starches from six different cassava varieties. Varieties Ban = Bangweulu, Kat = Katobamputa, Mw = Mweru, Kar = Kariba, Kam = Kampolombo, Chi = Chila.

3.2. Crystallinity

The percentage crystallinity of starches was in the range 31.06–33.40% (Table 1) and exhibited insignificant ($p < 0.05$) differences among the cassava varieties. Similar percentage crystallinity values were reported: 28.9–37.4% [26] and 35–40% [22]. However, the differences in crystallinity could be due to variation in amylose content. Amylose-free cassava starch (waxy) had crystallinity 49% [22,27]. In a related study, Cheetham and Tao [28] reported that the degree of crystallinity of free-amylose corn starch was 41.8% while high amylose content (84%) exhibited 17.2% crystallinity. Also, crystallinity was reported to be influenced by water content, as crystallinity increased with an increase in hydration [22,28]. The starch granules structural crystallinity depending on their botanical source, have been classified into three types of crystallinity patterns: A (Bragg angle 2θ at about 15.3 °, 17.1 °, 18.2 °, and 23.5 °), B (Bragg angle 2θ at about 5.6 °, 14.4 °, 17.2 °, 22.2 °, and 24.0 °) and C (Bragg angle 2θ at approximately 5.6 °, 15.3 °, 17.3 °, and 23.5 °). In the current study, all the starches exhibited prominent crystalline peaks (2θ-scale) at around 15 ° and 23 °, and unresolved double peak at 17 °, 18 °2θ (Figure 3). This suggests that the cassava starches fell in the range of type A crystallinity, a characteristic feature of regular to waxy starches. A similar range of XRD on cassava starch was obtained by Lemos et al. [29].

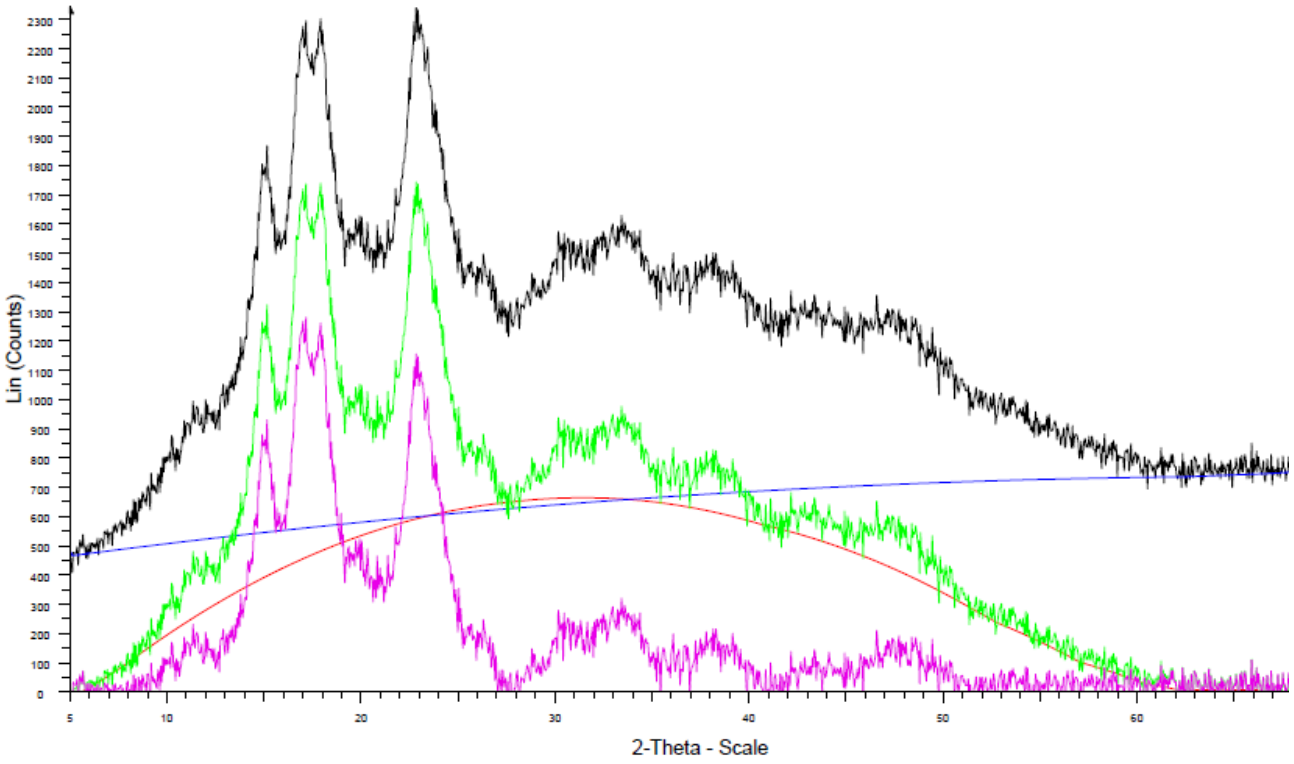

Figure 3. XRD (2θ-Scale) crystalline curves (replicated three times) for cassava starch extracted from Bangweulu.

3.3. Proximate composition analysis of starch granules

The moisture contents ranged between 5.50% and 6.91% and significantly varied ($p < 0.05$) among the cassava varieties (Table 1). Moisture content influences the storage stability of starches. The recommended moisture level for storing commercial starches is 10–12%. The moisture contents >12% encourages microbial contamination and induces degradative biochemical reactions leading to spoilage of starches during storage [30]. The starch with very low moisture contents is recommended for moisture conditioning for processing specifications requiring high moisture contents [31]. Moisture contents lower than 10% is specified for incorporation into low-density polyethylene matrix in the production of biodegradable products [32]. The protein contents of cassava starches were in the range of 0.37–0.61%. The protein content of cassava starches were previously reported as 0.13–0.17% [33], 0.55% [34], 0.34% [35], 0.27% [26] and 0.26% [36]. The lipid content of cassava starches ranged between 0.03 and 0.17% and varied ($p < 0.05$) among the cassava varieties. The lipid values from previous studies were reported 0.37% [33], 0.79% [37], and 1.00% [38]. The fiber contents were in the range of 0.33–0.46%. These results were higher than fiber contents (0.20–0.23%) [33], and lower than fiber content (1.50%) reported by Eke-Ejiofor [37]. The ash contents were in the range of 0.14–0.23%. Previous studies reported ash contents in the range of 0.36–0.37% [33] and 0.12–0.23% [26]. The starch granular surface features such as surface pores [5] including crevices can accumulate non-starch components such as inorganic matter which can contribute to the variation of ash content.

Table 1. Moisture, protein, lipid, fiber and ash contents, and percent crystallinity of cassava starches from six different varieties.

Variety	Moisture (%)	Protein (%)	Lipid (%)	Fiber (%)	Ash (%)	Crystallinity (%)
Bangweulu	5.98(0.26)^{bc}	0.47(0.13)^{a}	0.10(0.01)^{a}	0.41(0.35)^{a}	0.14(0.02)^{b}	32.77(0.88)^{a}
Katobamputa	6.56(0.61)^{ab}	0.48(0.06)^{a}	0.17(0.06)^{ab}	0.33(0.28)^{a}	0.23(0.01)^{a}	32.84(0.71)^{a}
Mweru	6.91(0.27)^{a}	0.37(0.02)^{a}	0.15(0.02)^{bc}	0.15(0.15)^{a}	0.23(0.04)^{a}	33.34(0.55)^{a}
Kariba	5.51(0.13)^{c}	0.40(0.02)^{ab}	0.09(0.05)^{c}	0.11(0.19)^{a}	0.22(0.01)^{a}	31.50(1.87)^{a}
Kampolombo	6.39(0.19)^{ab}	0.49(0.08)^{ab}	0.028(0.01)^{cd}	0.46(0.32)^{a}	0.14(0.02)^{b}	31.06(1.26)^{a}
Chila	6.54(0.49)^{ab}	0.61(0.08)^{b}	0.034(0.02)^{d}	0.38(0.52)^{a}	0.19(0.02)^{ab}	32.59(1.85)^{a}

Note: All values are means of three replications. Data in the parenthesis are the standard deviations. Within the same column, the values with different letters are significantly different at $p < 0.05$ by LSD test.

3.4. Amylose content

The amylose contents in cassava starches were in the range of 16.04–26.95% (Table 2). The amylose content in Katobamputa was significantly different ($p < 0.05$) from other cassava varieties. Similar amylose content in the cassava starches have been reported: 19.50–20.30% [39], 22.60 ± 1.30 [40], 17.06–25.72% [41], 11.9–19.4% [6], 21.0–22.5% [33], 26.73% [37], 22% [42], 19.2% [43] and 26.85% [44]. The differences in amylose contents from those reported previously could be due to variations in genotype [45,46] and differences in methods of analysis. The amylose content is the basis of classifying starches into waxy, semi-waxy, normal/regular and high-amylose types when amylose content is 0–2%, 3–15% 15–35%, and >40% of the total starch, respectively [39,47,48]. In the current study, the cassava starch varieties can be classified as normal or regular starches. Amylose content showed weak negative correlation with starch granule size ($r = -0.024$, $p < 0.0001$). A similar was observed by Charles et al. [49], who reported that cassava starch varieties with smaller granule dimensions contained higher amylose contents. This could be due to reduced interference from fiber contents. Smaller granule size had smaller fiber contents ($r = 0.164$, $p < 0.001$). The amylose contents exhibited weak negative correlation with ash content ($r = -0306$, $p < 0.05$) and lipids ($r = -0.101$, $p < 0.001$). This suggests that high amylose content starch granules had low lipid and ash contents. Lipids bind amylose molecules to form an amylose-lipid complex that competes with iodine to form a complex. Boonpo and Kungwankunakorn [50] reported that defatted cassava starch exhibited higher absorbance of the amylose-iodine complex. The high amylose contents can increase resistant starches level [51].

Table 2. Percentage amylose, resistant starch (RS), Non-RS (digested starch) and total starch contents from six different cassava varieties.

Variety	Amylose	RS	Non-RS	Total starch
Bangweulu	22.22(2.78)^{ab}	2.81(0.62)^{cd}	78.33(6.84)^{b}	81.13(7.03)^{b}
Katobamputa	26.95(2.30)^{b}	4.14(1.04)^{e}	66.47(2.05)^{a}	70.6(1.76)^{a}
Mweru	17.95(8.02)^{a}	1.12(0.42)^{a}	68.11(5.82)^{a}	69.23(5.84)^{a}
Kariba	18.47(7.30)^{a}	1.22(0.64)^{a}	66.14(10.22)^{a}	67.36(10.28)^{a}
Kampolombo	16.15(3.88)^{a}	1.40(0.46)^{ab}	69.51(2.13)^{a}	70.91(2.42)^{a}
Chila	20.83(0.45)^{ab}	1.68(0.05)^{abc}	69.07(5.39)^{a}	70.74(5.44)^{a}

Note: All values are means of three replications. Data in the parenthesis are the standard deviations. Within the same column, the values with different letters are significantly different at $p < 0.05$ by LSD test.

3.5. Resistant starch (RS)

The RS for cassava starches were in the range 1.12–4.14% (Table 2) and varied ($p < 0.05$) among varieties. The RS contents in cassava starch were reported in the range 5.99–6.01% [33], 2.2–4.5% RS [52] and 9.69% RS [53], 0.19–2.21% [54]. Aprianita et al. [55] reported RS in starches (10.4 ± 1.21%) and flours (19.3 ± 3.80%). High levels of RS in the range 5.00–19.6% were recorded in cassava flour samples [46]. The differences in RS could be due to variations in amylose contents and non-starch contents (protein, fiber, and lipid). The RS positively weak correlated with amylose content ($r = 0.214$, $p < 0.001$). This suggests that high amylose starches had high RS content. This is in agreement with Mtunguja et al. [6] who reported that amylose content was inversely proportional to starch digestibility. RS weak positively correlated with protein ($r = 0.171$, $p < 0.001$), fiber ($r = 0.195$, $p < 0.0001$) and lipid ($r = 0.555$, $p < 0.05$). This suggests that higher non-starch contents in starches may possibly increase RS content. The non-starch components hiders enzyme access to catalyse the hydrolysis of glucoside bonds and also might probably compete for available water [56,57] which probably limited enzymatic hydrolysis of starch.

3.6. Non-resistant starch (Non-RS)

The non-RS (digestible starch) for starches were in the range 66.14–78.33%. The non-RS content of cassava starches have been reported, 44.4–72.1% [55], 70–80% [6], and 75.00–94.80% [54]. The differences in Non-RS content could be attributed to variations in ultra-molecular starch granule structure differences, digestibility time and form of starch (raw or gelatinized). A related study by Park et al. [4] on maize starch reported that swelling, and rupture of the starch granular and crystalline structures during gelatinization accelerated the digestion of native starch. Thus gelatinized forms are likely to record higher digestibility rates than raw native forms. Mtunguja et al. [6] reported that differences in α-amylase hydrolysis rate between raw and gelatinized starch were more prominent in the digestibility period of 1–6 h, after which digestibility time (16–24 h) did not exhibit significant differences in rate of enzymatic hydrolysis between raw and gelatinized starch. In the current study, the amylase digestibility incubation was based on 16 h the period in which the form of starch is not likely to influence the rate of α-amylase hydrolysis.

3.7. Starch contents

The total starch content (dry weight) of cassava starches were in the range 67.36–81.13%. The variety of Bangweulu was significantly ($p < 0.05$) different from other cassava starch varieties. Starch content in cassava was reported in the range 87.8–89.2% [9], 77.4% [55], 74.3–80.3% [6] and 70.4–89.9% [26]. The differences in starch contents could be due to differences in genotype. Mtunguja et al. [45] who reported that the genotype had a major influence on the variability of starch contents while effects due to variation in environmental factors were insignificant. Similarly, Mejía-Agüero et al. [46] screened and compared starch content among twenty-five cassava cultivars planted and harvested simultaneously in a single plantation, and observed significant differences in starch contents due to inter-cultivar variability.

3.8. Swelling power and solubility of starches

Table 3 shows the swelling capacities of starches. The average swelling powers in the heating temperature range of 50–90 ℃ were recorded in the range 2.22–15.63 g/g and exhibited two swelling peaks. The peak swelling power observed at 60 ℃ for Kampolombo and Chila is an indication that these varieties have the capacity to swell at low temperature. The highest and lowest peak swelling power at 70 ℃ were recorded in Bangweulu and Katobamputa, respectively. Swelling powers of cassava starches were reported, 10.80 g/g [58], 8.9–16.3 g/g [6], 5.62–20.79 g/g [26] and 3.3–18 g/g [10]. The differences in swelling power could be ascribed to variations in amylose and non-starch contents. The swelling power showed weak negative correlation with amylose content ($r = -0.038$, $p < 0.0001$) and protein content ($r = -0.080$, $p < 0.001$). This suggests that higher swelling starches had lower amylose and protein contents. Similarly, Mtunguja et al. [6] reported that low swelling power of cassava starches was due to higher amylose contents. The results are also is in agreement with Sánchez et al. [59] who reported the highest swelling power (49.7–51.0 g/g) for waxy cassava starches. Furthermore, the protein compounds are known to restrict swelling of starch granules [60]. The protein molecules may increase hydrophobicity leading to reduced uptake of water which may result in decreased swelling of starches granule [61]. The swelling power of cassava starches showed a weak negative correlation with resistant starch ($r = -0.072$, $p < 0.0001$) and weak positive correlation with digestible starch ($r = 0.026$, $p < 0.0001$). This suggests that high swelling starches had lower resistant starches and showed high susceptibility to amylolytic digestion. The intact molecular structure restricts the accessibility of amylases [62]. Swelling disrupts the double helical structure of starch granules and increased interaction of hydroxyl and water molecules renders the swollen granules susceptible to digestive enzymes.

Table 3. Swelling powers (g/g) of cassava starches in the temperature range of 50 ℃ to 90 ℃ from six different cassava varieties.

Variety	50 ℃	60 ℃	70 ℃	80 ℃	90 ℃
Bangweulu	2.22(0.20)^{a}	10.36(0.33)^{hijk}	15.63(3.30)^{m}	8.30(0.50)^{fgh}	3.58(0.43)^{abc}
Katobamputa	2.49(0.16)^{ab}	9.56(0.21)^{hij}	12.69(2.96)^{kl}	6.67(0.68)^{defg}	3.70(0.55)^{abc}
Mweru	2.23(0.07)^{a}	9.04(1.28)^{ghi}	14.66(2.69)^{lm}	6.79(1.48)^{efg}	6.73(2.96)^{efg}
Kariba	2.31(0.09)^{a}	11.50(0.96)^{ijk}	12.48(1.72)^{kl}	5.52(0.76)^{cde}	6.28(3.19)^{def}
Kampolombo	2.44(0.09)^{a}	12.53(0.74)^{kl}	11.24(0.50)^{ijk}	4.93(0.18)^{bcde}	4.23(0.14)^{abcd}
Chila	2.32(0.08)^{a}	11.73(0.51)^{jk}	11.26(0.41)^{ijk}	6.28(2.00)^{def}	5.30(2.59)^{cde}

Note: All values are means of three replications. Data in the parenthesis are the standard deviations. Within the same column, the values with different letters are significantly different at $p < 0.05$ by LSD test.

The swelling power of starches showed two-stage process, an initial slow swelling in the heating temperature range 50–60 ℃ with insignificant variations among cassava varieties followed by a significant increase in the range 60–70 ℃. This is in agreement with Akinwale et al. [63] and Demiate and Kotovicz [64] who reported a two-stage swelling process of starches. During the initial heating stages, hydrogen bonding within starch granules forming a complex with lipids and entanglement with amylopectin branches and proteins might have restricted swelling. However, an increase in temperature above 60 ℃, water penetrates into the crystalline region of starch granules causes disruption of hydrogen bonding, crystalline melting and an increased swelling [18]. The decrease in the swelling is indicative of increased solubilization of starch molecules. Starch granules

swell to the peak value, after which the swollen granules disintegrate to release the soluble materials including amylose molecules [64]. Thus, the decreased swelling power and increased solubility index were observed in the temperature range of 70 to 90 ℃.

3.9. Solubility of starches

The solubility index values of cassava starches were in the range of 1.62–71.15% (Table 4). The solubility index values of starches were reported, 10.0–46.7% [58]. There was a weak negative correlation between swelling power and solubility of cassava starches ($r = -0.302$, $p < 0.01$). This suggests that a decrease in swelling probably due to leaching of some starch molecules out of the granules particularly after peak values led to increased solubility. The solubility of starches showed a weak positive correlation with amylose content ($r = 0.051$, $p < 0.001$). This suggests that higher solubility starches had higher amylose contents. Starch granules swell to the peak value, after which the swollen granules disintegrate to release the soluble materials including amylose molecules [64,65]. The decrease in solubility index after peak values could be attributed to the pasting phenomenon of amylose molecules and some amylopectin molecules entanglement through increased gel junction zones and partial aggregation of double-helices formations in the continuous phase that occurs on cooling leads to the formation of starches gel [66].

Table 4. Solubility (%) of cassava starches in the temperature range of 50 ℃ to 90 ℃ from six different cassava varieties.

Variety	50 ℃	60 ℃	70 ℃	80 ℃	90 ℃
Bangweuru	2.61(1.13)^{a}	9.15(1.15)^{abc}	13.53(1.71)^{bc}	32.11(0.87)^{e}	71.15(10.05)^{kl}
Katobamputa	1.62(0.55)^{a}	4.61(1.19)^{ab}	24.08(2.89)^{de}	42.06(6.39)^{fg}	75.79(6.61)^{l}
Mweru	2.59(1.08)^{a}	5.31(2.33)^{ab}	18.19(8.85)^{cd}	33.84(5.18)^{ef}	57.36(16.28)^{hi}
Kariba	3.27(1.13)^{a}	10.54(1.26)^{abc}	10.41(3.06)^{abc}	68.13(6.67)^{jkl}	56.92(9.26)^{hi}
Kampolombo	1.97(0.02)^{a}	10.56(2.48)^{abc}	7.78(3.06)^{ab}	63.41(2.57)^{ijk}	59.95(4.88)^{hij}
Chila	5.91(3.37)^{ab}	7.85(0.15)^{ab}	8.55(1.08)^{abc}	53.83(13.78)^{hi}	51.25(10.61)^{gh}

Note: All values are means of three replications. Data in the parenthesis are the standard deviations. Within the same column, the values with different letters are significantly different at $p < 0.05$ by LSD test.

Table 5. Correlation coefficients of swelling, solubility, amylose, protein, lipid, fiber, resistant starch, and digested starch.

Parameter	Swelling	Solubility	Amylose	Protein	Lipid	Fiber	RS	DS
Cassava starches								
Swelling	1							
Solubility	−0.302	1						
Amylose	−0.038	0.051	1					
Protein	−0.080	0.028	−0.075	1				
Lipid	0.031	0.010	0.435	−0.394	1			
Fibre	0.015	0.019	0.049	0.498	−0.277	1		
RS	−0.072	0.037	0.656	0.149	0.481	0.156	1	
DS	0.026	−0.042	0.121	0.103	−0.136	0.232	0.173	1

Note: RS = Resistant starches, DS = Digestible starch.

3.10. Gelatinization properties

3.10.1. Onset temperature

The onset gelatinization (T_o) of cassava starches were in the range of 56.33–63 ℃ (Table 6) and varied ($p < 0.05$) among cassava varieties. The T_o of cassava starches were reported, 64.3 ℃ (Ai and Jane 2015) and 63.70 ℃ [67]. The differences in T_o of starches could be ascribed to variations in amylose and lipid contents. The T_o of cassava starches positively correlated with amylose content (r = 0.530, $p < 0.05$) and showed weak negative correlation with swelling power (r = −0.090, $p < 0.0001$). This suggests that high amylose starches inhibited granule swelling resulting in low swelling power and resistant to gelatinization. This observation did not agree with Morante et al. [22] who reported that waxy cassava starch had high gelatinization temperatures. Nevertheless, it should be noted that all the cassava starches in the present study were classified as regular/normal starches (16–26% amylose contents), and exhibited type A crystallinity (polymorph) of the X-ray diffraction pattern. In a related study, normal rice and high-amylose maize starches V and VII exhibited high gelatinization temperatures due to A-polymorph. The double helical structure of A-polymorph which is densely packed with about 8 water molecules in a unit cell exhibit more molecular compact than B-polymorph with 36 water molecules in a unit cell [68,69]. In the current study, A-polymorph obtained in all six cassava starches can be suggested to have contributed to a linear relationship between amylose and gelatinization temperatures. Increased gelatinization temperatures resulting from increased amylose content were attributed to competing action between starch granule and amylose for water molecules [70]. The T_o of cassava starches correlated positively with protein content (r = 0.390, $p < 0.01$), lipid (r = 0.100, $p < 0.001$) and fiber contents (r = 0.386, $p < 0.01$). This suggests that starches with high protein, lipid and fiber contents had high T_o. High levels of lipids were reported to lower starch granule susceptibility to gelatinization. The presence of amylose-lipid complex inhibits gelatinization of starch granules [49]. Lipids may affect the diffusion of water into the starch granules, and their presences on starch granules can retard gelatinization. Li et al. [71] reported that defatted starch resulted in decreased gelatinization temperature. The protein and starch granules compete for water molecules [60] which probably results in inhibited swelling and increased gelatinization temperature. There was a positive correlation between the solubility index and T_o implying that solubilization of solutes increased linearly with gelatinization temperature.

Table 6. Gelatinization properties of starches from six different cassava varieties.

	T_o	T_p	T_c	Enthalpy (J/g)
Variety	Starch	Starch	Starch	Starch
Bangweulu	56.56(1.89)^{a}	63.23(1.66)^{a}	70.8(4.23)^{ab}	13.57(0.45)^{c}
Katobamputa	58.33(2.51)^{ab}	70.93(0.81)^{b}	77(2.00)^{cd}	14.1(1.01)^{c}
Mweru	61(2.64)^{bc}	70.67(2.08)^{b}	76.67(1.52)^{cd}	10.67(1.25)^{b}
Kariba	63(1.00)^{cd}	71.29(1.58)^{bc}	77.12(2.10)^{cd}	14.7(0.26)^{c}
Kampolombo	56.33(1.52)^{a}	62(1.00)^{a}	71.1(4.74)^{ab}	13.73(2.19)^{c}
Chila	57.31(2.84)^{cd}	64.73(1.27)^{a}	69.1(4.00)^{a}	11.13(0.90)^{b}

Note: To = onset, Tp = peak and Tc = Conclusion gelatinization temperatures. All values are means of three replications. Data in the parenthesis are the standard deviations. Within the same column, the values with different letters are significantly different at $p < 0.05$ by LSD test.

Table 7. Correlation coefficient swelling, solubility, amylose, gelatinization, pasting properties and granule size of starch.

Parameter	Swelling	Solubility	Amylose	To	Tp	Tc	Ent	PV	FV	PT	GS
Swelling	1										
Solubility	−0.302	1									
Amylose	−0.038	0.051	1								
To	−0.090	0.047	0.530	1							
Tp	−0.127	0.074	0.360	0.686	1						
Tc	−0.061	0.040	0.292	0.653	0.846	1					
Ent	−0.079	0.068	0.261	0.321	0.586	0.508	1				
PV	0.018	−0.027	−0.561	−0.677	−0.425	−0.418	0.316	1			
FV	−0.003	−0.023	−0.383	−0.330	−0.409	−0.425	0.103	0.640	1		
PT	0.025	−0.019	0.231	−0.304	0.028	−0.052	0.132	0.178	0.0742	1	
GS	0.066	−0.052	−0.155	0.275	0.132	0.247	0.197	0.227	−0.369	−0.366	1

Note: To = onset, Tp = peak, Tc = Conclusion gelatinization temperatures, Ent = Enthalpy, PV = Pasting viscosity, FV = Final viscosity, PT=Pasting temperature, GS = Starch granule size.

Table 8. Correlation coefficient protein, lipid, fiber, gelatinization, pasting properties of starch and granule size of starch.

Parameter	Protein	Lipid	Fiber	To	Tp	Tc	Ent	PV	FV	PT	GS
Protein	1										
Lipid	−0.394	1									
Fiber	0.498	−0.277	1								
To	0.390	0.100	0.386	1							
Tp	0.360	0.084	0.220	0.686	1						
Tc	0.146	0.268	0.264	0.653	0.846	1					
Ent	−0.194	0.008	0.097	0.321	0.586	0.508	1				
PV	0.005	−0.453	−0.172	−0.677	−0.425	−0.418	0.316	1			
FV	0.162	−0.559	0.130	−0.330	−0.409	−0.425	0.103	0.640	1		
PT	−0.109	0.063	−0.188	−0.304	0.028	−0.052	0.132	0.178	0.0742	1	
GS	0.243	0.019	0.164	0.275	0.132	0.247	0.197	−0.227	−0.369	−0.366	1

Note: To = onset gelatinization, Tp = peak gelatinization, Tc = Conclusion gelatinization temperature, Ent = Enthalpy, PV = Pasting viscosity, FV = Final viscosity, PT = Pasting temperature, GS = Starch granule size.

3.10.2. Peak gelatinization temperature

The peak gelatinization temperature (T_p) of cassava starches were in the range of 62.00–71.29 ℃ and varied ($p < 0.05$) among cassava varieties. The T_p for cassava starches were reported, 65.5 ℃ [72], 68.69 ℃ [67], 61.2–69.9 ℃ [22] and 68.3 ℃ [73]. The differences among cassava starch varieties could be due to variations in amylose, protein and lipid contents. The T_p showed weak positive correlations with amylose content (r = 0.360, $p < 0.01$), protein (r = 0.360, $p < 0.01$) and lipid contents (r = 0.084, $p < 0.0001$). This implies that cassava starches with high amylose content had high T_p. The high T_p were recorded in waxy cassava starches [22]. Amylopectin chain length degree of polymerization has been reported as the major factor for discriminating gelatinization transition

temperatures. In a related study on wheat starch, amylopectin chain lengths were reported to influence gelatinization temperature as starches with high short amylopectin chain length (degree of polymerization less than 12) exhibited lower gelatinization temperature than long chain [74] justifying that amylose content is not the only factor that influences gelatinization temperature. Jane et al. [75] cautioned that starch gelatinization could be influenced by many external factors such growing and processing conditions, and asserted that chemically extracted starches exhibited higher gelatinization temperatures than starch extracted using mild-chemical and enzymatic methods. There was a weak negative correlation between T_p and swelling powers for starches ($r = -0.127$, $p < 0.001$). Similar correlations were observed by Mtunguja et al. [6], who reported negative coefficients between peak gelatinization temperature and swelling power of cassava starches. This suggests that low swelling starches had high T_p.

3.10.3. Conclusion gelatinization temperature

The conclusion gelatinization temperatures (T_c) of cassava starches ranged from 69.10–77.12 ℃ and varied ($p < 0.05$) among cassava varieties. Similar T_c for cassava starches were reported, 78.9 ℃ [72] 66.7–75.1 ℃ [22] and 74.8 ℃ [73]. The variation of T_c among the cassava starch varieties could be due to differences in amylose, protein and lipid contents, and possibly amylopectin chain length differences.

3.10.4. Enthalpy of gelatinization

The enthalpy of gelatinization of cassava starches ranged from 10.67–14.10 J/g and varied among the cassava varieties. Similar results for cassava starch varieties were reported, 13.1–15.1 J/g [72], 14.70 J/g [73] and 9.8–14.2 J/g [22]. The differences in enthalpy of gelatinization could be due to variations in amylose contents and starch granule crystalline levels. The enthalpy of gelatinization positively correlated with amylose contents ($r = 0.268$, $p < 0.001$). This suggests, in part, that amylose structures were more organized and would require high energy to break hydrogen bonding. Furthermore, amylopectin structures could also influence the enthalpy of gelatinization since they are the core for starch granules crystallinity which relates to enthalpy of gelatinization.

3.11. Pasting properties

3.11.1. Pasting temperature

The pasting temperature for starches ranged from 64.54–70.54 ℃ (Table 9). Similar pasting temperatures for cassava starches were reported in other studies: 66.4–69.6 ℃ [6], 63.7–71.7 ℃ [22], 67.9–74.4 ℃ [76] and 62.0–68.0 ℃ [77]. The differences in pasting temperatures among the starches could be due to variations in amylose contents and starch granule sizes. The pasting temperature showed weak positive correlation ($r = 0.23$) with amylose content ($p < 0.001$) suggesting that high amylose starches had high pasting temperatures. Katobamputa had high amylose content and yielded higher pasting temperatures. The pasting temperature negatively correlated with granule size ($r = -0.369$, $p < 0.001$) implying that starches with high pasting temperature had lower granule sizes. In a related study, potato starches with large granule size fraction showed low pasting temperature [78].

3.12. Peak viscosity

The peak viscosity values of cassava starches were in the range of 782.3–983.5 cP and varied ($p < 0.05$) among the cassava varieties. The peak viscosity exhibited negative correlation with amylose content ($r = -0.561$, $p < 0.05$). This suggests that starches with high amylose content had low peak viscosity. Katobamputa recorded the highest amylose content and lowest peak viscosity. This is in agreement with Morante et al. [22] who reported that waxy cassava starches recorded higher peak viscosities than normal amylose containing starches. In a study on wheat starch, peak viscosity exhibited a negative correlation with amylose content [79]. Similarly, native starches from waxy maize and waxy rice showed higher peak viscosities than normal amylose containing starches [73]. There was a negative correlation between peak viscosity and lipid content ($r = -0.453$, $p < 0.05$). The amylose-lipid complexes form entanglements with amylopectin structure restricting swelling of starch granules, and subsequently decreasing the peak viscosity.

3.13. Breakdown viscosity

The breakdown viscosity values of cassava starches were in the range of 383.8–506.8 cP and varied among cassava varieties ($p < 0.05$). Breakdown viscosity negatively correlated with amylose content ($p < 0.01$). This suggests that high breakdown viscosity occurred in starches with low amylose content. The breakdown viscosity exhibited a positive correlation with peak viscosity for both starches ($r = 0.924$, $p < 0.05$). This is in agreement with Charles et al. [49] who reported that high peak viscosities with their major breakdown values were due to low levels of amylose and failure to re-associate with amylopectin and reinforce the molecular network within the granule. The lowest breakdown value displayed in Bangweulu starches could suggest resistance against shear disruption/dissolution of starch granules.

3.14. Final viscosity

The final viscosity values of cassava starches were in the range 462.0–569.7 cP and varied among the varieties. The differences in final viscosity among the varieties could be due to variations in amylose and lipid contents. The final viscosity negatively correlated with amylose ($r = -0.383$, $p < 0.01$) and lipid ($r = -0.559$, $p < 0.05$) contents. This suggests that starches with low amylose content were associated with high final viscosities. The lipid-amylose complexes tend to restrict swelling, and thus affecting the viscosity. In a related study on wheat, the defatted wheat starch exhibited high viscosities [80]. The final viscosity positively correlated with peak viscosity ($r = 0.640$, $p < 0.05$) but final viscosity values were significantly lower ($p < 0.05$) than peak viscosity values. The cold paste viscosities of cassava starches were low due to significant starch granules breakdown on shearing tinning of the paste and weakness to build high viscosity during cooling [75,81].

3.15. Setback viscosity

The setback viscosity of cassava starches was in the range 278.1–487.0 cP and varied ($p < 0.05$) among the varieties. The differences in setback viscosity among varieties could be attributed to variations in amylose contents and its gelation capacities. There was a negative correlation between

setback viscosity and amylose content (r = −0.432, p <0.01). This suggests that starches with high amylose content had low setback viscosity values. This is in agreement with Morante et al. [22] who reported higher setback viscosity values in cassava waxy starches than normal starches. This observation could be attributed to the failure of short chains to form double helices and therefore accountable for less organized granular structure [82]. Starches with high setback viscosity during cooling can possibly result into high rate of starches retrogradation. The paste (viscoelastic gel) is majorly an interaction of water with biphasic system characteristic of both amylopectin enriched swollen granules (dispersed) and amylose network (continuous) phases [83]. The tendency of amylose molecules to crystallize causes phase separation between polymer and water, and this is likely to result in increased setback values.

Table 9. Pasting properties of starch and flour from six cassava varieties.

Variety	PT	PV	BV	FV	SV
Bangweulu	65.56(1.56)^{a}	790.4(94.99)^{bc}	383.8(66.21)^{b}	514.3(113.47)^{abc}	278.1(33.1)^{bc}
Katobamputa	70.54(0.56)^{b}	782.3(17.09)^{b}	397.1(23.35)^{b}	462(29.14)^{ab}	337.3(21.77)^{b}
Mweru	65.36(1.07)^{a}	963.5(37.57)^{def}	486.8(66.52)^{c}	498.7(20.01)^{abc}	487(35.09)^{a}
Kariba	65.21(1.48)^{a}	960.2(52.63)^{def}	475.8(24.77)^{c}	523.3(52.29)^{bc}	455.5(49.11)^{a}
Kampolombo	65.20(2.53)^{a}	966.2(57.39)^{ef}	484.5(12.31)^{c}	521.5(25.34)^{bc}	463.5(39.14)^{a}
Chila	64.75(1.66)^{a}	983.5(76.10)^{f}	506.8(44.88)^{c}	569.7(19.63)^{c}	431.8(57.92)^{a}

Note: PT = Pasting temperature, PV = Pasting viscosity, BV = Breakdown viscosity, FV = Final viscosity, SV = Setback viscosity. All values are means of three replications. Data in the parenthesis are the standard deviations. Within the same column, the values with different letters are significantly different at $p < 0.05$ by LSD test.

3.16. Freeze-thaw stability and syneresis

3.16.1. Syneresis at −20 ℃ freezing storage

The syneresis of cassava starch gels were in the range 0.00–29.11% throughout the five weeks storage at −20 ℃ and varied ($p < 0.05$) among varieties (Table 10). The waxy cassava starch (zero amylose starch) was reported to have zero syneresis throughout five weeks freeze storage at −20 ℃ [22]. Other studies reported that waxy cassava starch gel had no syneresis after 5 weeks of storage at −20 ℃ [59]. Mtunguja et al. [45] reported syneresis of cassava starches in the range 31.7–57.7% and attributed the lowest syneresis value to lower amylose content (17.1%). During cooling of starch gels, amylose tendency to crystallize causes phase separation which results in loss of gel structure leading to the formation of water zones. The water zones transform into ice crystals during freezing and upon thawing the ice crystals transforms into the water leading to phase separation from the food system.

Table 10. Syneresis in freezing storage at −20 ℃ for six different cassava starch varieties.

Variety	% Syneresis				
	Week 1	Week 2	Week 3	Week 4	Week 5
Bangweulu	10.76(13.66)^{abc}	18.07(8.76)^{cd}	2.05(2.89)^{ab}	1.26(1.74)^{ab}	1.38(1.73)^{ab}
Katobamputa	2.45(2.45)^{ab}	18.07(5.51)^{cd}	29.11(6.83)^{d}	18.32(9.72)^{cd}	19.69(12.95)^{cd}
Mweru	12.55(12.91)^{bc}	20.01(5.25)^{cd}	11.45(14.00)^{abc}	4.62(1.30)^{ab}	17.15(10.17)^{c}
Kariba	0.06(0.09)^{a}	9.37(6.11)^{abc}	0.85(0.14)^{ab}	11.71(16.93)^{abc}	4.02(4.35)^{ab}
Kampolombo	0.03(0.05)^{a}	4.47(2.23)^{ab}	0.00(0.00)^{a}	5.23(4.51)^{ab}	0.00(0.00)^{a}
Chila	0.00(0.00)^{a}	3.58(2.31)^{ab}	0.26(0.45)^{a}	2.27(2.54)^{ab}	0.00(0.00)^{a}

Note: All values are means of three replications. Data in the parenthesis are the standard deviations. Within the same column, the values with different letters are significantly different at $p < 0.05$ by LSD test.

3.16.2. Freeze-thaw cycles freezing storage at −20 ℃

The syneresis in the five freeze-thaw cycles ranged from 0.40–42.50% (Table 11) and varied significantly ($p < 0.05$) among the varieties. The variety Katobamputa recorded the highest syneresis throughout freeze-thaw cycles. The high level of amylose content in Katobamputa could be the reason for high syneresis. The syneresis for five freeze-thaw cycles in cassava starches was reported, 0.00–0.40% [22] and 50–67% [84]. Some of these results were higher than syneresis values reported in the current study.

Table 11. Syneresis in storage freezing at −20 ℃ for five weeks freeze-thaw cycles for six different cassava starch varieties.

Variety	Syneresis				
	Cycle 1	Cycle 2	Cycle 3	Cycle 4	Cycle 5
Bangweulu	0.4(0.72)^{a}	27.3(11.76)$^{d–g}$	4.2(4.27)^{abc}	0.00(0.00)^{a}	2.00(1.74)^{ab}
Katobamputa	42.5(7.04)^{g}	29.4(24.84)^{efg}	37.4(13.08)^{fg}	41.7(6.67)^{g}	30.3(25.19)^{efg}
Mweru	15.1(15.06)$^{a–e}$	12.8(7.28)$^{a–e}$	6.1(3.27)^{abc}	4.7(4.44)^{abc}	21.7(30.86)$^{c–f}$
Kariba	4.3(3.15)^{abc}	14.4(4.08)$^{a–e}$	7.8(8.93)^{abc}	10.5(15.86)$^{a-d}$	1.4(1.23)^{ab}
Kampolombo	0.8(0.98)^{a}	18.9(2.05)$^{b–e}$	2.6(2.20)^{ab}	2.2(1.23)^{ab}	2.00(1.19)^{ab}
Chila	3.3(1.72)^{ab}	41.6(18.09)^{g}	2.8(2.93)^{ab}	2.2(2.93)^{ab}	14.7(11.27)$^{a–e}$

Note: All values are means of three replications. Data in the parenthesis are the standard deviations. Within the same column, the values with different letters are significantly different at $p < 0.05$ by LSD test.

4. Multivariate analysis

The principal component analysis (Figure 4) was conducted on gelatinization and pasting properties of starches to determine the differences among the cassava varieties. The axes of T_o, T_p and T_c associated closely in the same direction, and there was no variety which was significantly differentiated by high gelatinization temperatures. The varieties, Kariba and Mweru were located on the lower axes of granule size, and T_o, T_p and T_c. This suggests that Kariba and Mweru were significantly distinguished ($p < 0.05$) by small granule sizes and high gelatinization temperatures. Furthermore, Mweru was distinguished by high pasting temperature. Amylose and final viscosity clustered on the same axis but in the inverse direction. Also, amylose associated with peak and

breakdown viscosities in the opposite direction. This indicates that amylose had a significant negating effect on the final viscosity and negatively impacted peak viscosity and breakdown viscosities. The variety Bangweulu was disparate by low amylose content and high viscosities. Katobamputa clustered towards the axis of setback viscosity and was the only variety located close to the coordinates of the axis of amylose. Katobamputa was significantly distinct by high setback viscosity. The swelling and solubility at peak values closely associated together and did not cause differences among the cassava varieties. This suggests that all varieties showed similar ($p > 0.05$) response to swelling and solubility.

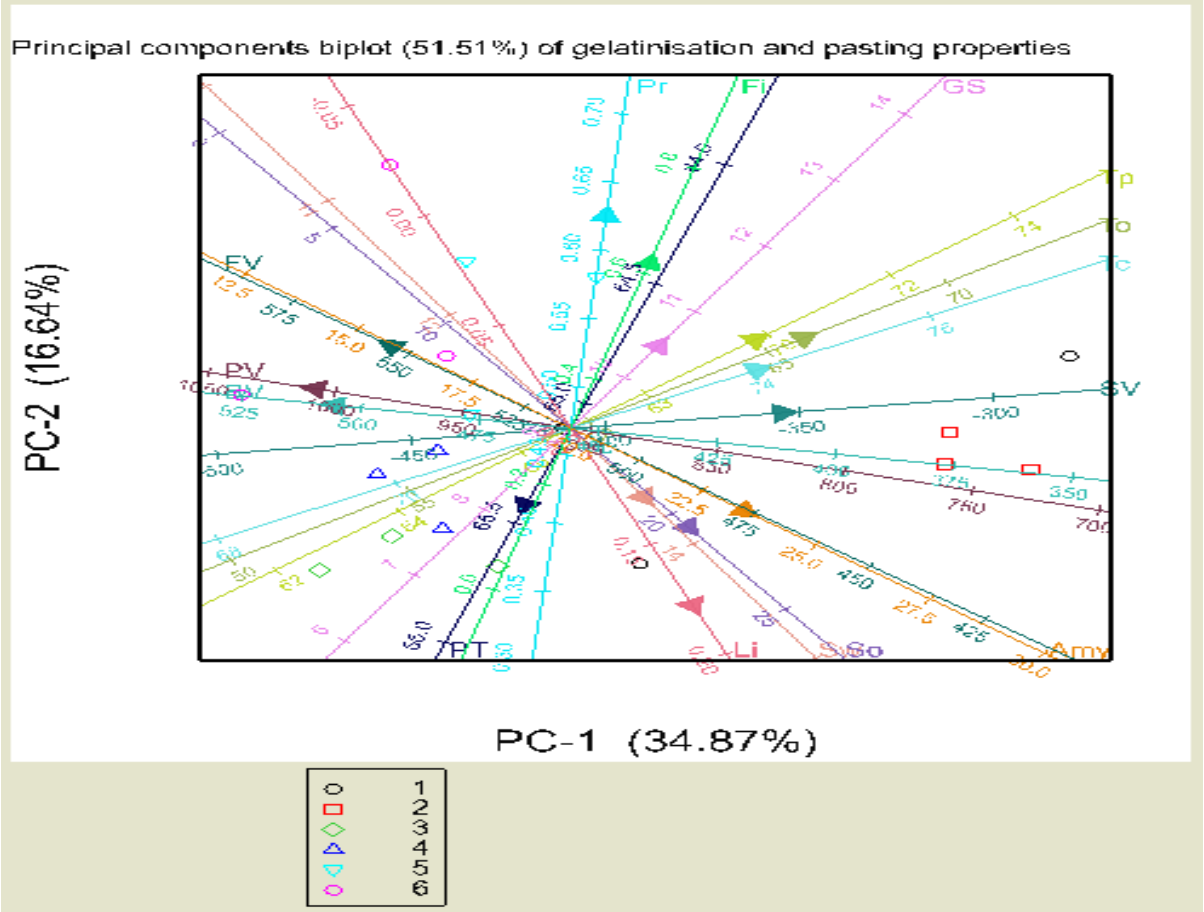

Figure 4. Principal component biplot of gelatinization and pasting properties of starches from different six cassava starches. Variety 1 = *Bangweulu*, 2 = *Katobamputa*, 3 = *Mweru*, 4 = *Kariba*, 5 = *Kampolombo*, 6 = *Chila*. To = Onset, Tp = Peak, and Tc = Conclusion gelatinization temper temperatures, GS = Granule size, PT = Pasting temperature, PV = Peak viscosity, BV = Breakdown viscosity, SV = Setback viscosity, FV = Final viscosity. Pr = Protein, Li = Lipid, Fi = Fiber, Amy = Amylose.

5. Potential application of cassava starches

Quality of food products is influenced by swelling powers of starches [8,85]. Thus swelling power can be used to ascertain the optimal temperatures for cooking (gelatinization) and the use of starch and flours in various dietary applications. The starches of Chila and Kampolombo exhibited

the capacity to swell and gelatinize at low-temperature range (60–74 ℃) and paste in the range of 70–74 ℃. Some starches molecules leached and solubilized at 80 ℃. These properties are indicative of the formation of viscosities with low energy requirements during cooking (8–14 J/g). This demonstrates the potential use of cassava starches/flours as an inclusion ingredient into wheat flour for bread making and in formulation and development of near-instant porridge products. Efforts to combat protein-energy malnutrition in Zambia identify dietary protein-energy rich porridge products for children. However, given the high cost of energy requirements for cooking, such efforts must seek energy serving food materials which can justify the role of starches derived from Chila and Kampolombo for their capacity to get gelatinized at low temperature. This characteristic is consistent with the requirements for instant porridges [86].

The starches of Bangweulu and Mweru exhibited high solubilization of their molecules and probably starches in these varieties are more susceptible towards amylolytic enzymes [87]. High swelling powers were associated with the high digestibility of starches [33]. This characteristic is desirable in the brewing and starch liquefaction industry. Some of the most important factors for efficient conversion of starch into fermentable sugars are temperature program of the mashing and solubilization processes. The mashing temperatures were reported in the 48–72 ℃, and effective enzymatic hydrolysis was reported to occur after the starch has been solubilized [88,89]. Therefore the higher solubility values obtained in this study suggest starches can find relevance for use as adjunct materials in the brewing industry, local liquefied beverages such as Maheu and Munkoyo drink, and other local traditional sweet beers in Zambia. The swelling properties of starches in this study have potential application in soup, cream, salad, and sauce products since the starches were able to absorb water and swell almost 18 times the original volume [90].

6. Conclusions

The amylose, protein and lipid contents were the sources of variations evidenced in different peak swelling, solubility, gelatinization, and viscosity values. High amylose variety (Katobamputa) had high resistant starch content and showed restricted swelling. The peak swelling of starches Chila and Kampolombo at 60 and 70 ℃ in is indicative of early gelatinization, rapid solubilization, and high amylolytic susceptibility which suggest potential application of cassava starches in food such as instant pudding, pie filling, cake frosting and soups, and in the manufacture of syrups such as glucose and fructose. It is worth noting that based on amylose contents, the starches in the current study were classified as regular or normal starches. Therefore the inter-cultivar variations were not highly significant. The cold paste viscosity was generally two times lower than peak viscosity, an indication of significant ruptures of swollen starch granules. The breakdown and final viscosities were negatively influenced by amylose content. The freeze-thaw (syneresis) values were within an acceptable range to suggest the application of cassava starches and flours in frozen food systems. The significant breakdown viscosities could be stabilized potentially through the chemical and physical modification of starches, and blending with other commercial starches and flours.

Conflict of interest

The authors declare no conflict of interest.

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