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Comparison of Granule Size Distribution of Starches and Biochemical Profile of Flour From Selected Cassava (*Manihot esculenta* Crantz) Genotypes

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Research Article

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ABSTRACT

The present study assessed the granule morphology and biochemical properties of starches and flours derived from storage roots of ten cassava varieties. All examined cassava starch samples exhibited smooth surfaces, with few truncated shapes, and predominantly spherical to oval shapes. Granule size distribution varied across the varieties, ranging from 19.83 µm to 34.11 µm. Significant differences were observed in the biochemical profile: Starch content (24.02% to 38.19%), total carbohydrate (237.4 mg/g to 338.9 mg/g), amylose (15.37% to 19.69%), and dry matter contents (33.45% to 41.69%) of the cassava flours. However, amylopectin content (ranging from 79.75% to 84.25%) showed no significant variance among the evaluated cassava genotypes. These findings underscore the substantial impact of genotypic variability on granule size distribution of cassava starches and the biochemical characteristics of the flours. Such variations hold implications for determining the utility of different cassava varieties in the food industry and for breeding programs aimed at enhancing cassava starch and flour quality.

Keywords: Cassava; Starch; Flour; Granule size distribution; Biochemical profile

INTRODUCTION

Cassava stands as a crucial crop for food security and industrial purposes, serving as a staple food in Africa, Asia, and South America for both human consumption and livestock feed. Additionally, it provides a valuable biomass for various industrial applications. Among the lucrative products obtained from cassava are flour and starch [1,2]. The cassava storage root is rich in energy due to its high carbohydrate content, primarily comprised of starch, which constitutes 80%-90% of the root's dry weight. Extracting the starch or processing it into flour is among the most effective methods to harness the potential of the cassava storage root, making it suitable for both food production and industrial applications [3-5].

Starch is composed of two types of α-glucans: amylose and amylopectin, with amylose accounting for 17% and amylopectin for 83%. The proportion of amylose, a key quality indicator of starch, plays a crucial role in determining its diverse properties and, consequently, its suitability for different end-users. This aspect has been extensively investigated, as documented in studies conducted by Liang, et al. and Zhang, et al. [6,7].

Several factors influence cassava starch production and the biochemical composition of cassava storage roots. These factors encompass environmental conditions, such as different growth locations producing significant variations in biochemical properties and starch yield despite the cultivation of the same cassava variety, as well as harvest timing, genetic diversity, among others [8,9].

Extensive research spanning numerous decades has delved deeply into the physical, morphological, biochemical, and chemical properties of cassava-derived starch and flour, yielding a wealth of published studies [10]. In contrast to starches derived from other starchy crops like corn, rice, potato, and wheat, our understanding of cassava starch remains relatively limited. Therefore, we conducted an investigation into the starch granule morphology, granule size distribution, and biochemical profile of storage roots from ten cassava varieties harvested at 12 months after planting, in Tamil Nadu, India. This study offers valuable insights into the correlation between cassava genotypes and the properties of cassava starch and flour. Such insights could prove beneficial for food processors utilizing cassava starch and flour and for development of improved cassava varieties by cassava researchers.

MATERIALS AND METHODS

Plant materials

Ten cassava genotypes grown in Tamil Nadu India, namely: YTP-1, YTP-2, H-226, H-165, TME-419, UMUCAS-36, Mulluvadi-1 and white Thailand were used for the study. TME-419 and UMUCAS-36 cassava varieties were obtained from the National Root Crops Research Institute, Umudike Nigeria, while the other eight varieties were obtained from Tapioca and Castor Research Station, Yethapur, Tamil Nadu State, India.

Sample preparation

The cassava storage roots were harvested from the field after 12 months of planting and promptly transported to the laboratory. Upon arrival, they were washed under running tap water, followed by peeling and rinsing with distilled water to ensure cleanliness. Subsequently, samples from the distal, middle and apical sections of the pulp were cut into small cubes and subjected to drying in a hot-air oven set at 45°C for 48 hours. After drying, the samples were finely milled into flour using an electric blender (Prestige Hero Mixer, India). The resulting flour samples were then sealed in airtight plastic bags and stored at room temperature (25 °C - 28 °C) for subsequent analyses.

Estimation of starch content

The starch content of the cassava flours was determined using the anthrone method, following the protocol outlined by Landhäusser, et al. [11]. Initially, a solution of anthrone was prepared by dissolving 2 grams of anthrone in 1 Liter of concentrated sulfuric acid (H_2SO_4). A standard stock solution was then created by dissolving 100 micrograms of glucose in 1 mL of water, from which 10 mL was dispensed and diluted to 100 mL with water to form a working standard. Next, a 100 mg sample of cassava flour was dissolved in 20 mL of 80% ethanol and centrifuged for five minutes at 2000 rpm to separate the supernatants. This process was repeated twice to ensure thorough extraction of soluble sugars. The residue after drying was dissolved in 1 mL of 52% perchloric acid ($HClO_4$) and heated in a boiling water bath for 20 minutes. Neutralization was achieved using solid sodium carbonate (Na_2CO_3), followed by the addition of 1 mL of ethanol and centrifugation at 2000 rpm for 5 minutes. The resulting supernatant was combined with the previously extracted soluble sugars and diluted to 10 mL with distilled water. Subsequently, 0.2 mL of the solution was transferred to a test tube and diluted to 1 mL with distilled water. Then, 4 mL of anthrone reagent was added to the test tube, and the optical density was measured at 630 nm using a UV spectrophotometer.

Determination of amylose and amylopectin

The amylose content of the cassava flour samples was determined using the iodine method as outlined by Rosado-Souza, et al. with appropriate adjustments [12]. Initially, 50 milligrams of cassava flour sample were placed in a glass test tube, followed by the addition of three drops of 80% alcohol and 5 mL of 1 N NaOH. The mixture was then transferred to a 50 mL standard flask, boiled for 15 minutes, cooled for two minutes, and the volume was adjusted to 50 mL with water. Then, 0.5 mL of the sample extract was pipetted into a new test tube, to which 2.0 mL of water was added. Subsequently, 2 drops of 1% phenolphthalein were introduced, resulting in a pink coloration of the extract. To neutralize the solution, a few drops of 0.1 N HCl were added while shaking until the pink colour disappeared. Following this, 1 mL of iodine reagent was added, and the volume was adjusted to 10 mL with water. The optical density was then measured at 600 nm using a UV spectrophotometer. The amylose content of the solution was calculated using the provided equation, while the amylopectin content was determined by subtracting the amylose values from the total starch values

$$Amylose\ \%\ (w/v) = \frac{Absorbance\ (Concentration\ A\ Supernantatnt)}{Absorbance\ (Total\ Starch\ Aliquot)}\ x\ \frac{6.15}{9.2}\ x\ 100$$

$$= \frac{Absorbance\ (Concentration\ A\ Supernantatnt)}{Absorbance\ (Total\ Starch\ Aliquot)}\ x\ 66.8$$

6.15 and 9.2 are dilution factors for Concentration A and total starch extracts respectively.

Determination of dry matter content

The dry matter content was assessed using the method outlined by Kashala-Abotnes, et al. [13]. Clean, peeled cassava storage roots weighing approximately 200 g ± 0.5 g each were randomly selected from three plants of each cassava genotype in the field. These roots were diced into small cubes and combined, then dried in triplicate in a hot air oven at 50°C until a constant weight was achieved, typically taking around 72 hours. The dry matter content was determined by calculating the difference between the initial mass before drying and the mass loss after drying.

Starch extraction for analysis of starch granule size and morphology

The washed cassava storage root pulp was diced into small cubes and processed in an electric blender with a root-to-cold water ratio of 1:1 to achieve a smooth mixture. Subsequently, the mixture was filtered through a voile-type fabric sieve (100 μm) and collected in a plastic bowl. The slurry was washed with cold water to extract the starch.

Afterward, the filtrate was left undisturbed at room temperature for 12 hours to allow for starch decantation. Following decantation, the supernatant was discarded, and the settled starch at the bottom of the bowl was transferred to aluminum trays. The starch was then dried in a hot air oven at 50°C for 24 hours. Once dried, the starch was carefully ground into a fine powder using a mortar and pestle. Finally, the starch was packaged in airtight plastic bags and stored at room temperature for subsequent analysis.

Starch granule size analysis

Starch was extracted from fresh cassava storage roots, and the analysis of starch granule size was conducted using a particle size analyzer (Horiba Scientific India). Initially, one milligram of the starch sample was placed at the bottom of a glass test tube, followed by the addition of 10 mL of distilled water. The mixture was homogenized using a vortex mixer, and subsequently subjected to three cycles in a water bath Sonificator. Finally, the mean diameter of the samples was determined using the particle size analyzer.

Analysis of starch granule morphology by scanning electron microscope

To examine the starch granules within the cassava starch samples, a Scanning Electron Microscope (SEM FEI, Czech Republic, Model: Quanta 250) was utilized. The samples were carefully spread out onto a conductive carbon tape with double-sided adhesive, which was then positioned on a stub and placed into the sample chamber of the scanning electron microscope. Once the high vacuum condition was achieved, the filament was activated, and parameters such as electron beam, intensity, spot size, voltage, and emission current were adjusted accordingly. Subsequently, images were captured and recorded for analysis.

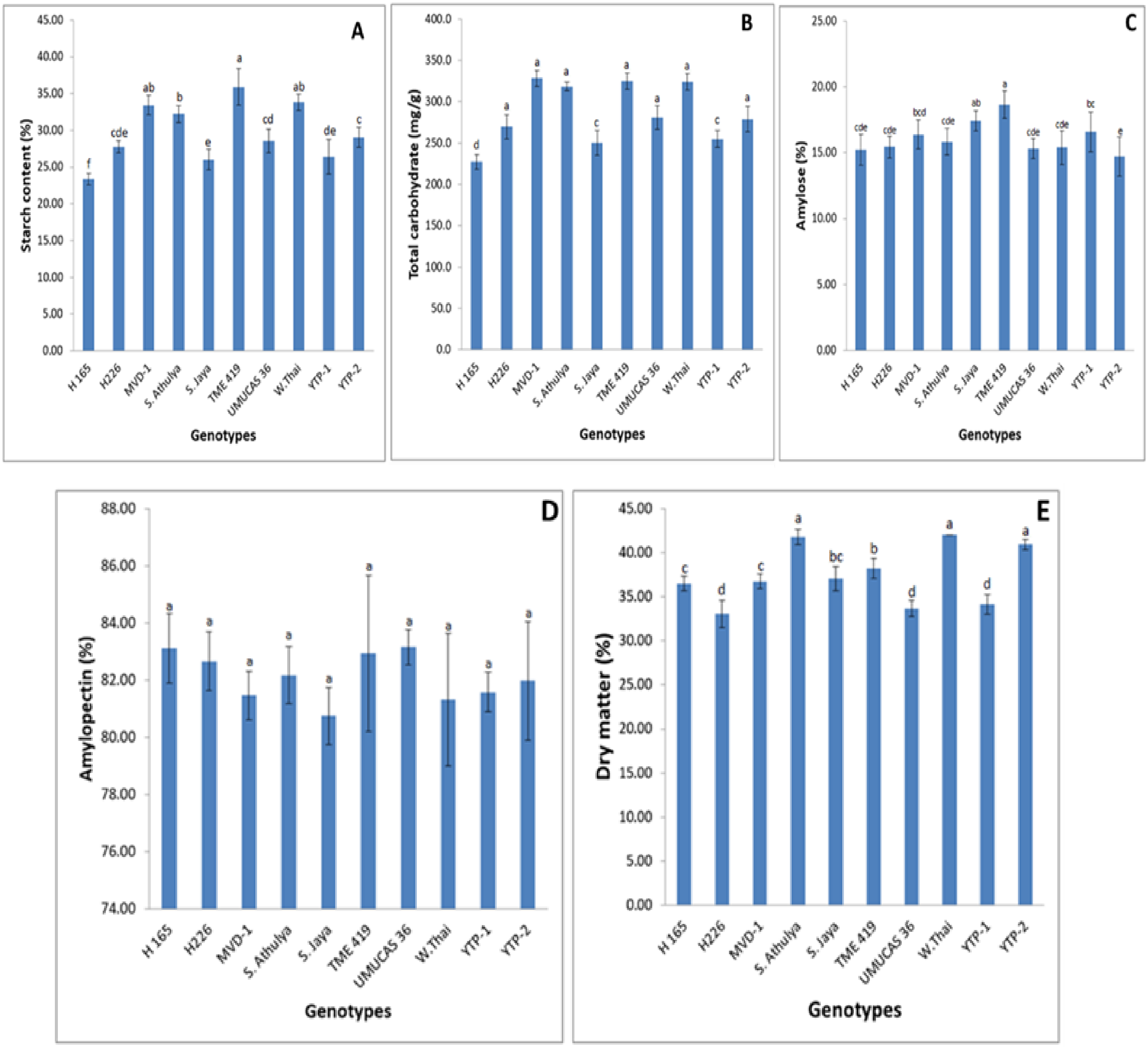
Statistical analysis

The results obtained were statistically analyzed through Analysis of Variance (ANOVA) using R 4.3.0 software 2021. Differences between means indicated with error bars were compared using the Least Significance Difference (LSD) test at p=0.05 using the Agricolae R package.

RESULTS

The biochemical profile is presented in Figure 1. TME-419 variety recorded the highest starch content (38.19%) followed by White Thailand (34.95%) and MVD-1 (34.49%) genotype, while H-165 variety was observed to have the lowest starch content (24.02%) (Figure 1A). The total carbohydrate content varied among the cassava varieties, MVD-1 (338.6 mg/g) was observed to have the highest carbohydrate content, while H-165 (237.4 mg/g) recorded the lowest carbohydrate content (Figure 1B). TME-419 genotype recorded the highest amylose content (19.69%), while YTP-2 (15.37%) had the lowest amylose content (Figure 1C). The highest amylopectin content was observed in H-165 (84.25%) variety, whereas S. Jaya (79.75 %) had the lowest amylopectin content (Figure 1D), and no significant difference was observed in the amylopectin content across the ten cassava genotypes. Variety gave the highest dry matter content at 41.69%, while H-226 recorded the lowest dry matter content at 33.45% (Figure 1E).

Figure 1 (A-E). Comparison of the starch, total carbohydrate, amylose, amylopectin and dry matter contents of the cassava genotypes. The data are Mean ± SE of three independent experiments. The lowercase letters represent significant difference. Bars with the same letters are not significantly different, while the bars with different letters are significantly different at p=0.05.



In the analysis of variance, the mean squares due to genotype were highly significant at P<0.0001 for total carbohydrate and dry matter, while starch content was observed to be significantly different at P<0.01, and amylose content was observed to have significant difference at P<0.05, among the evaluated cassava varieties. All the mean sum of squares, including starch content, total carbohydrate, amylose content, and dry matter content was observed to be significant, except amylopectin content (Table 1). This implies that there is the existence of variation among the cassava flours and variability among the cassava varieties.

Correlation analysis for the biochemical properties is presented in Table 2. It was observed that amylose and amylopectin contents had significant positive relationship between each other. Dry matter content had a positive association with starch content. Total carbohydrate content exhibited a significant direct relationship with dry matter content and a relatively strong positive correspondence with starch content.

Table 1. Analysis of variance of the biochemical profile of the ten cassava varieties.

SV	Df	Starch (%)	TC (mg/g)	Amylose (%)	Amp (%)	DM (%)
Rep	2	4.221	804.27	7.0726	19802	4.4129
G	9	48.668	3936.50	4.2787	20154	32.9042
Error	18	2.17	65.83	0.6226	20308	0.6218

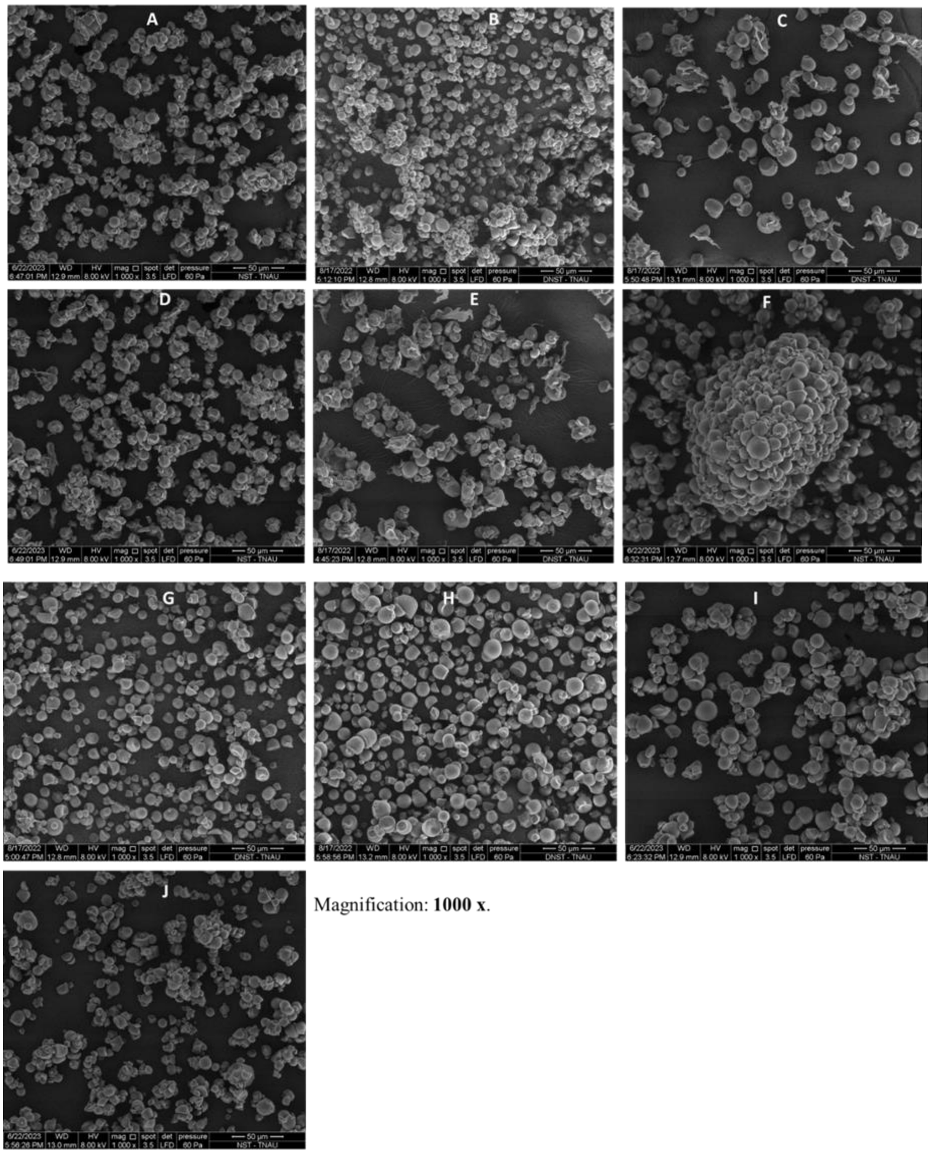
Note: Rep: Replication, G: Genotype, Df: Degree of Freedom, SV: Source of Variance, TC: Total Carbohydrate, Amp: Amylopectin, DM: Dry Matter.

Table 2. Correlation analysis of the biochemical properties evaluated in the cassava storage roots.

Biochemical properties		1	2	3	4	5
Amylopectin (%)	1	1	-	-	-	-
Amylose (%)	2	0.46	1	-	-	-
Dry matter (%)	3	-0.03	-0.01	1	-	-
Starch (%)	4	0.17	0.3	0.49	1	-
Total carbohydrate (mg/g)	5	0.22	0.29	0.51	0.91	1

Starch morphology was observed by scanning electron microscopy studies. The ten cassava genotypes recorded different starch granule sizes at different radial locations ranging from 19.8 µm to 34.1 µm. The electron micrographs revealed that the starch granules exhibited a smooth surface devoid of any discernible holes and cracks, primarily adopting a spherical or oval shape with minimal irregularities (Figures 2A-2J). The variations observed in the starch granule size are shown in Figure 3. MVD-1 cassava variety was observed to have the largest starch granule size (34.11 µm), followed by TME-419 (31.87) while H-165 variety recorded the lowest starch granule size (19.93 µm).

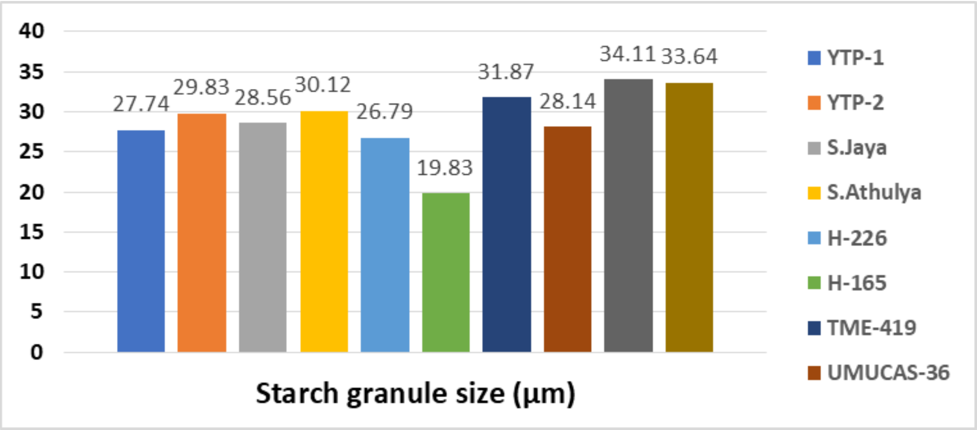
Figure 2 (A-J). Scanning electron micrography of starch granule morphology of the cassava genotypes at harvest.



Magnification: **1000 x**.

Starch granule morphology of the cassava varieties (A): YTP-1 (B): white Thailand cassava genotype. (C): H-165 (D): H-226 (E): S. Jaya (F): TME-419 (G) YTP-2 (H): MVD-1. (I): UMUCAS-36 (J): S. Athulya.

Figure 3. Starch granule size distribution of the cassava genotypes at harvest. **NOTE:** (■) YTP-1; (■) YTP-2; (■) S. Jaya; (■) S. Athulya; (■) H-226; (■) H-165; (■) TME-419; (■) UMUCAS



DISCUSSION

We describe a comparative evaluation of granule morphology and biochemical properties of starches and flours processed from the storage roots of ten different cassava varieties. Variation was observed in the starch content, total carbohydrate, amylose, amylopectin, and dry matter contents in all the cassava genotypes. Most of the values we obtained in the biochemical profile of the cassava flours (Figure 1) closely align with previous studies on various cassava cultivars, where variations were also reported in amylose content ranging from 15.9% to 22.4% and amylopectin content 82.3 to 85.7% [14]. Pérez, et al. found starch content ranging from 28.65% to 42.13% and amylose content between 15.2% to 26.5% [15]. Breuninger, et al. observed starch content ranging from 15 to 33% and amylose content between 15.8% to 22.5% [16]. Rolland-Sabaté, et al. reported amylose content ranging from 16.8 to 21.5% and starch content ranging from 24% to 35% [17]. Also, in previous study by Rui, et al. the cassava landrace: Thukkuvella 6 was reported to possess 15.2% amylose content and 19.35% starch content, while Kavaram Kutty cassava genotype was reported to have starch content of 27.36 % [18].

Total carbohydrate content between the range of 83.55% and 96.95%, was documented in previous study by Dudu, et al. and Tambo et al. respectivel [19,20]. Also, Nilusha, et al. recorded the total carbohydrate content of 75. 99 % to 93.13 %, which were higher than the values we obtained in the ten cassava genotypes (23.7% to 33.9 %) [21].

It was noted that starch content, total carbohydrate, amylose and dry matter contents exhibited strong association with genotype (Table 1). This shows the existence of variability among the casava varieties used in the flour production. In the correlation analysis (Table 2), we observed that amylose content, dry matter content, and total carbohydrate had direct relationship with starch content. Shadrack, et al. documented similar results, indicating a positive correlation between starch content, amylose content, and the distribution of starch granule sizes across different cassava varieties [1]. Additionally, Hasmadi, et al. reported a positive relationship between starch, amylose, and carbohydrate contents [22].

The dry matter content recorded by Nuwamanya, et al. ranged between 30% to 38 % in 21 different cassava varieties, also another report of the dry matter content of 34.61% to 42.31 % in flours derived from different improved cassava varieties, which are similar to our finding (33.45%-41.69 %) [23]. In cassava, the dry matter content of the root is primarily composed of starch, making variations in dry matter content pivotal in determining the suitability of a variety for various applications [24]. Improved cassava varieties hold promise for both industrial and food-based uses, with high adoption rates reflecting farmers' preference for varieties with elevated dry matter content. The impact of increased dry matter content on industrial applications of cassava is particularly notable due to the significant contribution of starch, which can constitute up to 90% of the dry matter in cassava roots, and its consequential influence on starch properties [23].

Starch content serves as a crucial factor in identifying desirable genotypes within the cassava species [22]. Starches with high levels of amylose exhibit reduced enzymatic digestibility, leading to a range of nutritional and physiological benefits. These advantages encompass heightened dietary fiber intake, enhanced glycaemic regulation, and decreased caloric intake [25,26].

The composition of amylose and amylopectin varies based on the botanical origin and genetic background of starch sources. Lipids, such as phospholipids, interact with amylose to form single helical complexes, enhancing the stability of starch molecules and reducing their water-binding capacity [27]. The amylose to amylopectin ratio, the length of α-glucan chains, and the degree of branching in amylopectin collectively determine the structure and functionalities of starch granules across different plant species. Amylose content significantly influences the biochemical and functional properties of starch, allowing it to be tailored for specific applications [28].

As observed through scanning electron microscopy, the surface morphology of the starch granules appeared smooth, predominantly spherical or rounded, with some exhibiting an oval shape, and occasionally displaying truncated forms (Figure 2). Notably, the cassava starch granules showed no signs of perforation. The observed morphology is consistent with starch granules extracted from related tuber crops such as sweet potato and yam. Similarly, the scanning electron microscopy images of cassava starch documented in previous research by Vasconcelos, et al. also depicted granules with spherical, oval-shaped, smooth surfaces, and occasional truncated features, across the evaluated cassava varieties [29]. Starch molecules are synthesized within the starch granules, which exhibit diverse sizes, shapes, and distributions depending on their botanical origins within the plant tissues. The properties of starch granules, such as amylose and amylopectin composition, as well as crystallinity, play a significant role in determining the functional attributes of starch [30].

There was variation in granule size of the cassava starches at different radial locations, ranging from 19.8 µm to 34.1 µm (Figure 3). These findings were in agreement with previous reports by Udoro, et al. and Vasconcelos, et al. [29,31]. However, Nuwamanya, et al. recorded smaller starch granule sizes in the range of 12.74 µm to 15.73 µm among the five cassava varieties evaluated, and Albert, et al. documented starch granule dimensions range of 12.9 to 17.2 micrometers [14,23]. Starch granules are classified into three main types: A, B, and C, according to the granule sizes. Type A granule (>15µm), type B granules (5 µm-15 µm), and type C granules (<5µm). Our observations demonstrated that the cassava varieties studied possess type A starch granules. Each granule type has distinct properties that make them suitable for various applications [7,32,33]. Type a starch granules are favored in baking applications where rapid starch gelatinization is desired, such as in cakes, muffins, and breads [34].

Cassava flour is usually employed as composite flour blends for production of instant flours, baking of short biscuits, Choco cookies, reengining, pandan cookies, and Sponge Rolls, gluten-free pasta, to give a soft/tender/crispy texture [35-37]. It is important to note that although cassava flour is predominantly composed of starch, disparities in properties such as starch content, dry matter, amylose, and total carbohydrate levels between flour and starch may lead to distinct differences in their characteristics [38,39]. Numerous studies have explored the properties of cassava root starches, both in their native and modified states, particularly focusing on white-flesh variants [40-42]. Consequently, the selection of specific cassava variants in food production or as a source of industrial raw material should take into account their inherent biochemical traits.

CONCLUSION

Cassava starch and flour are valuable commodities derived from cassava storage roots, with superior characteristics such as clear appearance, minimal impurities, resistance to retro gradation, and excellent texture, making them highly versatile for both culinary and industrial purposes. Our research sheds light on the correlation between cassava variety and the properties of its starch and flour. Varieties with high levels of amylose (TME-419, S. Jaya, YTP-1), high starch content (TME-419, MVD-1, W. Thai), and sizable starch granules (MVD-1, TME-419) as observed in the study exhibit exceptional suitability for food and industrial applications. Furthermore, these findings underscore the potential of such varieties in breeding programs aimed at enhancing nutritional quality of cassava cultivars.

CONFLICTS OF INTEREST

No conflict of interest was declared by the authors.

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