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Assessing starch digestibility and carbohydrate quality of Ugandan maize varieties: implications for diet-related non-communicable diseases risk

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ABSTRACT

Low-carbohydrate-quality food consumption increases risk of the development of diet-associated non-communicable diseases (NCDs). Although maize is a major dietary carbohydrate source food consumed by 92% of households in Uganda, its carbohydrate quality is largely unknown. This study examined starch digestibility, carbohydrate quality, and the contents of associated intrinsic physico-chemical factors that influence starch digestibility for thirteen (13) maize varieties commonly consumed in Uganda. In general, the maize varieties examined had a carbohydrate-to-crude fiber ratio ranging from 5.28 to 10.15 and exhibited high glycemic indices in the range of 79.38 to 86.27, indicating low carbohydrate quality. The physico-chemical factors that influence starch digestibility varied significantly ($p < 0.05$) across varieties. Hierarchical cluster analysis grouped the varieties into four significantly different clusters based on the level of chemical factors that influence ($p < 0.05$) starch digestibility. Cluster composed of varieties DT MAX, Longe 5D, and WE3106 had the best matrix combination of physico-chemical characteristics associated with better starch resistance. This study has revealed that maize varieties consumed in Uganda have low carbohydrate quality, which suggests that their consumption is a risk factor for diet-associated non-communicable diseases in the country and surrounding countries. A concerted effort is necessary to improve the carbohydrate quality of the maize varieties.

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1. Introduction

Carbohydrates are the principal energy source in most societies throughout the world. They are generally present in foods in the form of starch, which is one of the three primary polysaccharides in nature and serves as a form of storage carbohydrate (Niaz et al., 2020). They are found in cereals, tubers, legumes, vegetables, fruit, seeds, leaves, and rhizomes (Bojarczuk et al., 2022) and provide 4 kcal/g of dietary energy, counting for 40–75% of total energy intake depending upon the population (Lal et al., 2021). However, throughout the past decades, there is debate on the amount and type of dietary carbohydrates required for health (Reynolds et al., 2019). This is because carbohydrates are increasingly being implicated in the epidemics of non-communicable diseases such as obesity, diabetes, and other metabolic diseases. This has led to the emergence of the concept of carbohydrate quality. This concept refers to the health-associated aspects of carbohydrates in foods. The carbohydrates quality helps identify carbohydrate-rich food sources and distinguish between those that would favor the development of chronic diseases and those that may contribute to prevent the diseases. Consumption of a significant amount of low-quality carbohydrates food disrupts the balance of hormones in the body which causes an excess of insulin and reduces the amount of energy burned by the body (Sievenpiper, 2020). Adversely, Reynolds et al. (2019)

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indicate that consuming high-quality carbohydrate foods can reduce the prevalence of non-communicable diseases by 15 up to 30%.

Several indices have been proposed for assessing the carbohydrate quality of food. These include glycemic index (GI), glycemic load, fiber, and whole grains (Hardy et al., 2020). The GI is a measure of the ability of the available carbohydrate in a food to increase blood glucose. High GI food consumption has been associated with poor glycemic control, leading to Type II diabetes (Bonsembiante et al., 2021). They contain a high proportion of carbohydrates that break down rapidly (rapidly digestible starch) and cause high postprandial glucose concentrations (Sluijs et al., 2010). In addition, high consumption of fiber has been associated with better glycemic control (Bonsembiante et al., 2021). Accordingly, carbohydrate-based diets with a low-glycemic index and higher amounts of fiber reduce glycemic and insulinemic responses and lower the risk of non-communicable diseases, mainly Type II diabetes (Nitzke et al., 2024; Pavithran et al., 2020). These are considered high-quality carbohydrate foods because of their health benefits. Their regular consumption assists in the management of weight loss, diabetes and lowers the risk of non-communicable diseases. Therefore, assessment of the carbohydrate quality of commonly consumed foods becomes an important strategy to monitor nutrition-related health risks for populations and assist in identifying, guiding, and promoting healthier food choices.

Cereals, mainly maize, are the most commonly consumed starch foods and are considered a staple food crop for many people in Africa. Maize accounts for nearly half of the calories consumed in East Africa (Macauley, 2015). In Kenya, maize and its products are the staple food of the majority of the population, with an annual per capita consumption of approximately 77 kg (Koskei et al., 2020). In Uganda, it is the staple food and provides over 40% of the calories consumed per capita in both rural and urban areas (Wacoo et al., 2018). The flour is used for porridges, soups, and paste preparation by over 92% of households (Wacoo et al., 2018). Several studies have demonstrated that processing methods, such as refining, grinding and boiling, substantially increase the starch digestibility and glycemic response of maize product (Caballero-Rothar et al., 2022; Chauhan & Singh, 2020, 2021; Ge et al., 2023). Consequently, frequent consumption of highly processed maize products raises public health concerns due to their potential impact on blood glucose levels and associated non-communicable disease risk. Despite this high consumption of maize, its carbohydrate quality assessment in the literature is limited. The existing literature focused on the improvement of the starch quality through several methods, mainly for maize isolated starch (Asare et al., 2021) and waxy maize starch (Chang et al., 2020). However, a more comprehensive analysis of carbohydrate quality parameters of maize consumed in the region is missing to the best of our knowledge. In addition, varietal effects on carbohydrate quality analysis are rarely considered.

Uganda is used as the case study in the present study for various reasons. According to the National Agricultural Research Organization (NARO) in the country, several varieties of maize are available and consumed by the Ugandan population. These varieties are promoted based on their yield, drought-tolerant varieties, resistance to major biotic stresses, responsiveness to inputs, and good nitrogen use (Simtowe et al., 2019). In addition, the country supplies huge quantities of maize grains to all the East African countries. Despite the high level of maize production and consumption in Uganda, to the extent that it exports excess to neighboring East African countries, and the importance of carbohydrate quality in starch food assessment, there are limited scientific reports on the carbohydrate quality of these maize varieties consumed in the region. Therefore, the present study investigated the starch digestibility and carbohydrate quality of the maize grains commonly consumed in Uganda and East African countries. The aim of the study was to understand factors that affect starch digestibility and identify maize varieties that could help prevent diet-related non-communicable disease risks based on their starch digestibility.

2. Materials and methods

2.1. Materials

Up to 86 varieties of maize are reported available in Uganda and are exported/imported to/from other East African countries (NARO, 2022). Out of these, thirteen (13) varieties are commonly grown and consumed in the country. All 13 varieties have been considered for the present study (Supplementary material 1). To accurately reflect the forms of maize currently consumed by households, the maize grain

samples were collected directly from farmers under natural field conditions, to account for the influence of environmental conditions on key maize grain characteristics such as starch, protein, fat, and fiber contents (Mekonnen et al., 2023), which affect its digestibility. This approach was used to allow the samples to capture the genuine effects of natural environmental conditions on maize grain development and nutrient composition, and to ensure they reflect the natural characteristics as commonly consumed by households. Each maize variety was collected from at least three farmers in the regions where they were most grown, following the guidance of the seed companies (the most sold maize grain varieties in their respective region).

Maize samples were collected then from farmers' fields across three agroecological zones of Uganda, specifically Central, Western, and Northern regions where farmers cultivated under natural conditions and apply standard agronomic practices until harvest. Maize varieties DK777 and DK9089 (both East African varieties) were collected from the Central agroecological zones (Masaka district with Latitude: −0.32574, Longitude: 31.7338), while Longe 5D, UH5053, and UH5355 were collected from the Western agroecological zones (Masindi district with Latitude: 1.68024, Longitude: 31.7131), and KH 500 43 A (East African seed), Longe 5, Longe 10H, MM3, UH5051, UH5354, DT Max, and WE3106 bought from Northern agroecological zones (Lira district with Latitude: 02.3333; Longitude: 33.1000, and Gulu district with Latitude: 2.76667, Longitude: 32.3056). The Central Uganda agroecological zones experiences a tropical climate with average temperatures generally range from 20.8 °C to 29.5 °C, annual rainfall typically falls between 700 and 950 mm, and soil pH between 5.6 and 6.5 (Mwangu et al., 2025; Nsubuga & Rautenbach, 2018). In Western Uganda agroecological zones, temperatures ranged from 15 °C to 30°Cwith the hottest months being January to March, the rainfall is a bimodal has a pattern varying between 800–1600 mm, and soils pH ranges between 5.5 to 6.5, which is moderately acidic and suitable for a wide range of crops, including maize (Mulinde et al., 2022; Nsubuga & Rautenbach, 2018). In Northern Uganda agroecological zones, temperatures ranged from 21.5 to 30.2 °C, the rainfall is a unimodal regime, varying between 650 and 850 mm with most rain falling during April to November (Atube et al., 2022). The soil has a pH range between 5.5 and 6.8. All the maize grains samples were sampled from the harvested stock from December 2022 to February 2023.

Foreign matter, dirt, and damaged grains were removed, and the grains were sorted and cleaned. For the laboratory analyses, the maize grains were milled under laboratory conditions using a laboratory blender 8010EG (Model HGBTWTG4, Torrington, CT) and sieved through a 100 µm sieve to have a similar granulometry of whole maize grain flour sold in the local market. The flour was packed in paper bags and stored in a well-ventilated room for 10–15 weeks.

The megazyme for digestible and resistant starch (K-DSTRS) was purchased from Megazyme (Megazyme, Wicklow, Ireland). The enzymes used in the experiments were purchased from Sigma-Aldrich, Co. (St. Louis, MO). All chemicals were molecular and analytical grade and purchased from Merck KGaA (Darmstadt, Germany).

2.2. Determination of starch digestibility of the maize varieties studied

Starch digestibility procedure was conducted following the simulated in vitro gastro-intestinal digestion according to the method of Englyst et al. (1992) and using the Megazyme procedure with some modification (Neogen cooperation, 2023). Before the analysis, two enzyme-working solutions were prepared: Pancreatic α-amylase/amyloglucosidase (PAA/AMG) solution and amyloglucosidase (AMG) solution. PAA (40 KU/g) and AMG (17 KU/g) solution was prepared by adding 0.5 g of PAA/AMG powder mixture to 25 mL of sodium maleate buffer, 50 mM, pH 6.0. The PAA/AMG solution was stirred on a magnetic stirrer for 5 min and stored on ice until use. The AMG solution was prepared by adding 1 mL of the content of Amyloglucosidase (3300 U/mL) to 30 mL of sodium acetate buffer pH 4.5. The solution was magnetically stirred for 5 min and stored at 4 °C until use. The reagent was prepared immediately before use.

Maize sample flour (1 g) was placed into 50 mL polypropylene screw-cap tubes and mixed with 1.0 mL of 95% (v/v) Ethanol and 35 mL of Sodium maleate buffer, 50 mM, pH 6.0. The mixture was submersed in a magnetic stirrer water bath (Wagtech OLS, Model 200, Germany) to equilibrate at 37 °C for 5 min at 170 rpm. Then, 5 mL of PAA/AMG enzyme solution prepared as stated above was added, and the mixture was then incubated in a water bath (Wagtech OLS Model 200, Germany) at 37 °C with agitation (170 rpm) for 180 min. Successive aliquots (1.0 mL) of the hydrolysate were taken after 0, 20, 60, 90, 120 and 180 min

and mixed with 20 mL of 80% ethanol to inactivate the enzyme. A 2.0 mL sample of each solution was picked and centrifuged at 6000 rpm for 10 min using a Centurion Scientific Benchtop centrifuge (Model 1.C2015, UK). After centrifuging, a duplicate aliquot (0.1 mL) was picked, and 0.1 mL of AMG enzyme solution prepared below was added and well mixed. The mixture was incubated at 50 °C for 30 min and mixed with 3.0 mL of freshly prepared GOPOD Reagent (light yellow).

Reagent blank solutions were prepared by mixing 0.2 mL of sodium acetate buffer, pH 4.5, with 3.0 mL of GOPOD reagent, while the D-glucose standards were prepared by mixing 100 µg of D-glucose with 0.1 mL sodium acetate buffer, 100 mM, pH 4.5 and 3.0 mL of GOPOD reagent. The sample was mixed with GOPOD, the reagent blank and D-glucose standards prepared as described below and incubated at 50 °C for 20 min for color development. The absorbances of the sample and glucose standards were read at 510 nm against the reagent blank using the spectrophotometer (Jenway 6305 UV/Visible, Cadmus, UK). The percentages of rapidly digestible starch (RDS) within 20 min and slowly digestible starch (SDS) between 20 and 120 min were calculated using the Megazyme formula (K-DSTRS), accessed from the Megazyme website (www.megazyme.com) as shown in Equation 1.

$$RDS\ or\ SDS = \frac{\Delta A\,x\,F\,x\,EV}{W\,x\,0.0189} \tag{1}$$

With 0.71 ± 0.24 For RDS (ΔA^{RDS}), absorbance reaction read against the reaction blank after 20 min. For SDS (ΔA^{SDS}) absorbance reaction reads against the reaction blank after 120 min minus ΔA^{RDS}. *F* the conversion from absorbance to µg (the absorbance obtained for 100 µg of D-glucose in the GOPOD reaction is determined) [F = 100 (µg of D-glucose) divided by the GOPOD absorbance for this 100 µg of D-glucose]. *EV is* the extraction volume (41 mL), and *W* is the weight of the sample analyzed (1 g).

Before determining the RS content and estimating glycemic index, the total starch contents of maize flour and white bread sampleswere determined. The total starch content of maize samples was analyzed using the sedimentation method of Goñi et al. (1996) as described by Dhillon et al. (2018). A 0.1 g maize flour sample was weighed in a 50 mL plastic Falcon tube, and 0.2 mL aqueous ethanol (80%, v/v) was added and vortexed to ensure dispersion. The mixture was allowed to decant, and the ethanol was removed, and the process was repeated twice to ensure the extraction of reducing sugars from the sample. Then, 2 mL of 2 M Potassium hydroxide solution was added and stirred with a magnetic bar (5 × 15mm) for 20 min. After this stage, 8 mL of 1 M sodium acetate solution (pH 3.8) was added, followed by 3 mL thermostable α-amylase (0.5%). The mixture was incubated at 95 °C for 10 min in a magnetic stirrer water bath (Wagtech OLS, Model 200, Germany). Amyloglucosidase (0.1 mL) was added, and the mixture was incubated at 50 °C for 30 min and cooled to room temperature. The volume of the solution was adjusted with deionized water to 50 mL, and centrifuged at 4000 × g, for 10 min using a Centurion Scientific Benchtop centrifuge (Model 1.C2015, UK). The sample (0.1 mL) was collected in a 10 mL glass tube to determine the glucose content following the phenol–sulphuric acid method of Dubois et al. (1956) as applied by Dhillon et al. (2018) and read with the spectrophotometer (Jenway 6305 UV/Visible, Cadmus, UK) at 490 nm. The soluble starch solution was used as the standard solution for curve building. The percentage of total starch was calculated using a multiplying factor of 0.9 after obtaining the glucose content.

The resistant starch (RS) in the products was calculated then according to the formula applied by Cahyana et al. (2019) as follows 0.64 ± 0.29 With TS the total starch in each maize flour.

2.3. Determination of carbohydrate quality of the maize varieties studied

In this study, carbohydrate quality was determined in terms of estimated glycemic index and the carbohydrate-to-fiber ratio.

2.3.1. Determination of estimated glycemic index

The estimated glycemic index was determined according to the hydrolysis index (HI) of the starch and the linear model of Goñi et al. (1996) applied by Caballero-Rothar et al. (2022). The hydrolysis index (HI) of the starch was derived from the in vitro starch digestibility procedure as applied by Yaman

et al. (2019). According to Costantini et al. (2024), in vitro starch methods mimicking in vivo human digestion and is simple, reliable, accurate, a fast alternative, and cost-effective to predict and estimate the glycemic response to a food and can be largely used in the evaluation of the predicted GI in starchy products. During the process, starch is completely converted to D-glucose, and the rate of starch hydrolysis can be quantified indirectly. Therefore, the starch hydrolysis fractions determined during the in vitro starch digestibility within 0, 20, 60, 90, 120 and 180 min were considered for the determination of HI. HI was determined by dividing the area under the curve (AUC) of the starch digestograms for each variety by the AUC of a reference sample, fresh white bread containing the average starch content of the samples (Supplementary Figure S1). AUC was determined using the trapezoid method of different time intervals (Pautong et al., 2022). Due to the linearity between hydrolysis index (HI) and glycemic index (GI), predicted GI (pGI) was estimated by using the equation 2 of Goñi et al. (1996).

$$pGI = 39.71 + (0.549 * \mathrm{HI}) \tag{2}$$

2.3.2. Determination of the carbohydrate-to-fiber ratio

Before the carbohydrate-fiber ratio could be determined, the proximate composition of the maize samples was analyzed in order to reveal the carbohydrate content by the difference method. The ash content was determined using the gravimetric method of AOAC (2005). Fat content was determined by the Soxhlet method using a solvent extraction system (Soxtec® Avanti 2050 Auto System, Foss Tecator AB, Höganas, Sweden) and petroleum ether as solvent according to AOAC (2005). The micro-Kjeldahl AOAC (2015) method applied by Afolayan et al. (2022) was used for Nitrogen determination using the Kjeldahl digester (VELP Scientifica, Model A00000176, Italy) and the Kjeldahl distiller (VELP Scientifica, Model F30200140, Italy). As most proteins contain 16% N, the conversion factor of 6.25 (100/16 = 6.25) was used to convert percent Nitrogen to percent of crude protein. The carbohydrate (CHO dry weight) content was determined by a differential method according to the equation 3 below.

$$\textbf{Carbohydrate}(\%, \textbf{db}) = \textbf{100}\% - \textbf{protein}(\%, \textbf{db}) - \textbf{lipid}(\%, \textbf{db}) - \textbf{ash}(\%, \textbf{db}) \tag{3}$$

Following the determination of the carbohydrate content, the Weende method which is based on the solubilization of non-cellulosic compounds by sulfuric acid and potassium hydroxide solutions was used for crude fibre determination as described by Musembi et al. (2024). The crude fiber was extracted using a fiber analyzer (Velp Scientifica, F30520200, Italy) by solubilizing non-cellulosic substances using 1.25% sulfuric acid and 1.25% sodium hydroxide. In this instance, a 1 g sample and oven-dried glass crucibles were weighed and placed in a fiber analyzer. Sulfuric acid 1.25% was added to the 150 mL mark, followed by 5 drops of octan-1-ol (antifoam). The samples were boiled for 30 min at 100 °C after being prepared up to the point of boiling using the fiber analyzer. After draining the sulfuric acid, samples were washed 3 times in hot deionized water (30 mL per wash). The procedure above was repeated, but sulfuric acid 1.25% was replaced by 1.25% solution of sodium hydroxide and boiled for 30 min. After draining the sodium hydroxide, the samples were washed 3 times with 30 mL of hot deionized water (100 °C) and once with cold deionized water (20 °C) to cool the crucibles. Thereafter, the samples were washed 3 times with 25 mL of acetone, oven-dried at 105 °C for an hour, desiccated, and weighed. This was followed by 3 h of ashing in a muffle furnace at 550 °C and reweighing. Equation 4 was used to calculate the percent of crude fiber.

$$\textbf{Crude fiber}(\%) = \frac{\textbf{Oven dried sample weight} - \textbf{Ashed sample weight}}{\textbf{Fresh sample weight}} * \textbf{100} \tag{4}$$

However, the crude fiber analysis using the Weende method determines solely cellulosic compounds such as lignin, hemicellulose, and pectin, the insoluble fiber content (da-Silva & Walter, 2012). As the maize grains have an average 92.6% (91.75–93.45%) of insoluble fiber which is typically determined through the Weende method and 7.4% of soluble fiber (Lasek et al., 2020; Picolli da Silva & de Lourdes

Santorio Ciocca, 2005), the dietary fiber representing by both soluble and insoluble fiber was estimated using equation 5 and used for carbohydrate to estimated dietary-fiber ratio determination.

$$\textbf{Estimated dietary fiber}(\%) = \textbf{Crude fiber} * \textbf{1.074} \quad (5)$$

2.4. Determination of factors affecting starch digestibility

Starch digestibility is intricately influenced by the composition and interaction of macronutrients and bioactive compounds within food matrices. Studies have shown that a consistent inverse relationship between protein, fat, phenolic compounds and amylose content and the starch digestibility. Proteins form complexes with starch, which hinder hydrolysis enzymatic to the starch and thus slowing glucose release (Bello-Perez & Flores-Silva, 2023). Additionally, certain amino acids stimulate insulin secretion, further reducing glycemic response and thus decrease starch digestibility (Rytz et al., 2019). Fats may encapsulate starch granules and form amylose-lipid complexes, limiting enzyme access and slowing digestion. Otherwise, polyphenols, known for their enzyme-inhibitory properties, can bind to starch or digestive enzymes, thereby decreasing starch hydrolysis (Kwaśny et al., 2022). In addition, foods rich in amylose, such as specific cultivars of rice, wheat, maize typically exhibit lower GI values attributed to the tightly packed, linear configuration of amylose molecules, which impedes enzymatic access and facilitates the formation of resistant starch fractions during digestion (Iqbal et al., 2021). Dietary fibre and crude fibre reduce starch digestibility by creating physical barriers that limit enzyme access and slow hydrolysis (He et al., 2023). Conversely, foods with higher total carbohydrate and total starch content often show high starch digestibility as more substrate is available for enzymatic hydrolysis, leading to greater glucose release and higher postprandial blood sugar responses (Tuaño et al., 2021).

Therefore, for the purpose of the present study, carbohydrate, lipid, protein, fiber, total starch, amylose, amylopectin, and total phenolic compounds were analyzed as factors affecting starch digestibility (Cai et al., 2015). Carbohydrate, lipid, protein, fiber and total starch contents were determined according to the procedure described in sections 2.2 and 2.3.2. This section, therefore, focuses exclusively on the determination of amylose, amylopectin, and phenolic compounds.

2.4.1. Determination of amylose and amylopectin

Amylose content in each maize variety was determined using the iodine calorimetry method applied by Babatola et al. (2021). Prior to the amylose determination, the maize starch was initially extracted from the maize flour using the method proposed by Nuwamanya et al. (2024). A hundred grams of the maize flour was mixed with clean distilled water, and the slurry was filtered through a muslin cloth. The starch was allowed to settle, and the supernatant was decanted. The remaining starch was washed with water two times and then dried in an air-forced oven at 40 °C for 48 h. A sample of maize starch (0.1 g) was mixed with 95% ethanol (1 ml) to remove the fat and avoid under-estimation of the amylose (Neogen Corporation, 2024), and mixed with 1 N of NaOH (9.2 mL). The mix sample was heated in a magnetic stirrer water bath (Wagtech OLS, Model 200, Germany) at 100 °C for 10 min and allowed to cool. About 0.5 mL of this mixture was pipetted and added to 0.1 mL of acetic acid solution (1 N), and 0.2 mL of iodine solution containing (0.2% I2 and 2% KI, w/v) in distilled water. The mixture was made up to 10 mL with distilled water and allowed to stand for color development and read with the spectrophotometer (Jenway 6305 UV/Visible, Cadmus, UK) at 620 nm. The potato amylose was prepared in 1, 5, 10, 20 and 35% in ethanol and NaOH (1 N) and used as a standard amylose solution. A sample procedure applied to the samples was applied to the standard and the absorbance read at 620 nm using the spectrophotometer (Jenway 6305 UV/Visible, Cadmus, UK). A standard curve was constructed and used for the calculation of amylose content in the maize flour samples. Amylopectin content was calculated by subtracting amylose content from 100.

2.4.2. Total phenolic extraction and determination

Total phenolic compounds were determined using a modified colorimetric method applied by Kayodé et al. (2007). Total phenolic compounds were extracted from 50 mg of maize sample in 1.5 mL of HCl/methanol (1% v/v) for 1 h under continuous stirring at room temperature. The mixture was centrifuged at 5000 × g

for 10 min using a Centurion Scientific Benchtop centrifuge (Model 1.C2015, UK), after which the supernatant was removed and analyzed. The pellet was re-extracted, and the supernatants were pooled. The extracts (300 µL) were mixed with 4.2 mL of distilled water, 0.75 mL of Folin–Ciocalteu's reagent (Sigma Chemical), and 0.75 mL of sodium carbonate solution (20% w/v). After incubation for 30 min at 37 °C, the optical density was measured at 760 nm using a spectrophotometer (Jenway 6305 UV/Visible, Cadmus, UK). Blanks were freshly prepared, in which Folin–Ciocalteu's reagent was replaced by water to correct interfering compounds. Gallic acid was used as standard, and calibrated at t 0, 50, 100, 200, 300 and 500 µg/mL using HCl/methanol 1% (v/v). The curve was generated and maize flour phenolic compounds were estimated. The results have been expressed as gallic acid equivalent (GAE) per 100 g dry weight of samples.

2.5. Statistical analysis

All the analysis were carried out in triplicate except the starch digestibility carried out in duplicate. The average values and standard deviation values (Mean ± SD) were calculated. One-way analysis of variance (ANOVA) was used to determine the significant difference in estimated parameters among the various varieties and the difference between the clusters. Tukey's multiple comparison method was used as a post hoc test in case of any significant difference. A significant difference level of 5% was used. Multivariate analyses were performed, specifically a Principal Component Analysis (PCA) and Hierarchical Cluster Analysis to assess the relationship between the varieties. PCA was computed to find out the established quality traits within the varieties using the composition parameters and the FactoMineR package. The similarity of the maize samples was classified using the plug-in Heat Map with Dendrogram, a major statistical method for finding homogeneous groups of cases based on the measured characteristics (Zhang et al., 2017). The Ward minimum variance and Euclidean distance method was used, and the agglomeration coefficient (AGNES) and cophenetic correlation were computed for the dendrogram validation. All the analyses were performed using R software version 4.2.2.

3. Results

3.1. In vitro starch digestibility of maize grains

Starch digestibility was determined in terms of rapidly digestible starch (RDS), slowly digestible starch (SDS), and resistant starch (RS) proportion in the maize samples. RDS, SDS and RS varied significantly ($p < 0.05$) within the maize varieties (Table 1). RDS fraction of the maize ranged between 27.80% and 39.12% for UH5354 and DT Max maize varieties, respectively, whereas RS content, hydrolyzed after 120 min, ranged from 9.90% in DK777 to 16.68% in UH5354. The combined content of slow digestible starch (SDS) and resistant starch (RS), referred as SDS+RS, expected to play an important role in the carbohydrate quality of maize, ranged from 61% to 72%. Overall, the sample DT Max exhibited the highest RDS and thus, the lowest SDS+RS content, while the sample UH5355 had the lowest RDS and the highest SDS+RS (Table 1).

Table 1. Starch digestibility fraction of 13 maize grains sampled from Uganda.

Maize varieties	RDS (%)	SDS (%)	RS (%)	SDS + RS (%)
DK777	$36.46^{ab} \pm 0.05$	$53.65^{ab} \pm 0.54$	$9.90^{c} \pm 1.29$	$63.54^{bcd} \pm 0.74$
DK9089	$37.28^{ab} \pm 1.43$	$49.38^{ab} \pm 1.29$	$13.34^{abc} \pm 1.97$	$62.72^{bcd} \pm 1.69$
DT Max	$39.12^{a} \pm 2.64$	$46.13^{b} \pm 2.53$	$14.75^{abc} \pm 2.74$	$60.88^{d} \pm 0.40$
KH 500 43A	$29.98^{bc} \pm 1.27$	$55.85^{ab} \pm 0.57$	$14.17^{abc} \pm 1.03$	$70.02^{abc} \pm 1.60$
Longe 10H	$32.91^{abc} \pm 2.80$	$54.43^{ab} \pm 2.53$	$12.66^{abc} \pm 1.62$	$67.09^{abcd} \pm 0.90$
Longe 5	$39.11^{a} \pm 0.24$	$46.80^{b} \pm 1.87$	$14.09^{abc} \pm 0.01$	$60.89^{d} \pm 1.87$
Longe 5D	$38.02^{ab} \pm 1.31$	$46.78^{b} \pm 1.36$	$15.19^{ab} \pm 0.03$	$61.98^{cd} \pm 1.38$
MM3	$35.43^{abc} \pm 3.46$	$50.41^{ab} \pm 3.62$	$14.16^{abc} \pm 0.50$	$64.57^{abcd} \pm 4.14$
UH5051	$29.67^{bc} \pm 2.40$	$58.77^{a} \pm 2.13$	$11.56^{bc} \pm 0.56$	$70.33^{ab} \pm 1.56$
UH5053	$33.59^{abc} \pm 0.80$	$55.76^{ab} \pm 0.29$	$10.65^{bc} \pm 0.37$	$66.41^{abcd} \pm 0.66$
UH5354	$32.58^{abc} \pm 0.60$	$55.29^{ab} \pm 0.80$	$12.12^{abc} \pm 0.24$	$67.42^{abcd} \pm 1.03$
UH5355	$27.80^{c} \pm 4.02$	$55.53^{ab} \pm 6.03$	$16.68^{a} \pm 1.21$	$72.19^{a} \pm 4.82$
WE3106	$32.58^{abc} \pm 1.81$	$54.40^{ab} \pm 2.21$	$13.02^{abc} \pm 0.40$	$67.42^{abcd} \pm 1.81$
***p* Values**	***0.001***	***0.002***	***0.004***	***0.001***

The values indicate the means of duplicates ± *SD*. Means with different letters in the same column differ significantly ($p \leq 0.05$). RDS: Rapid Digestible Starch; SDS: Slow Digestible Starch; RS: Resistant Starch.

3.2. Carbohydrate quality of maize grains

The carbohydrate quality of the maize samples was estimated using two parameters: carbohydrate to crude fiber ratio and estimated glycemic index (Table 2). The carbohydrate-to-crude fiber ratios of the maize varieties varied significantly ($p < 0.05$) among the different maize varieties. The carbohydrate-crude fiber ratio ranged from 5.28 to 10.15, and the lowest ratio was observed for the MM3 variety, while the highest value was found for WE3106. Seven varieties, namely DK777, DK9089, DT Max, KH 500 43 A, Longe 5D, UH5051, and MM3, had a ratio between five and eight. The varieties Longe 5, Longe 10H, UH5354, UH5053, and UH5355 have a ratio higher than 8 but less than 10. The varieties DK777, DK9089 and DT Max have almost similar ratios, which varied between 5.37 and 5.70. The maize varieties Longe 10H, UH5053, UH5354 and UH5355 have a ratio varied between 8.21 and 8.56, almost similar ($p > 0.005$). However, the carbohydrate-to-dietary fiber ratio ranged from 4.85 to 9.38, with MM3 and DK777 exhibiting ratios below 5, while all other maize varieties, except WE3106, which had ratios between 5 and 8. On the other hand, the glycemic index of the samples varied significantly according to the varieties. All the maize samples were found to have high glycemic indices, ranging from 79.38 to 86.27. The varieties DK777, DK9089, and DT Max, which expressed the lowest carbohydrate-crude fiber ratio, also had low estimated glycemic index values. On the other hand, the variety WE3106, which had the highest carbohydrate-crude fibre ratio, did not have, in contrast, the highest estimated glycemic index values (81.14).

3.3. Factors affecting the starch digestibility

Proximate composition, total starch, amylose and amylopectin, and phenolic compounds contents were analyzed as factors affecting the starch digestibility of maize (Table 3). Only the fat and ash contents were similar ($p > 0.05$) across all varieties. All those analyzed parameters varied significantly ($p < 0.05$) within the varieties, and the trend of the variation differed depending on the parameter. The lowest protein, fiber, carbohydrate, and total starch for 100 g dry weight sample were found for longe 10H (7.21), WE3106 (7.66), MM3 (70.38) and DK777 (63.01), respectively. The variety UH5053 expressed the lowest amylose content of 20.47% (dw) while KH 500 43 A had the lowest phenolic compounds of 180.25 GAE/100g dw.

3.4. Relationship between maize varieties and starch digestibility factors

Principal Component Analysis (PCA) was employed to classify the 13 maize varieties based on the factors that impact their starch digestibility (Figure 1). The first and the second principal components (PCs) explained 45.47% and 21.12% of the variance, respectively (Supplementary Figure S2). Thus, these two

Table 2. Variation in the carbohydrate-fiber ratio, carbohydrate to estimated dietary fiber and glycemic indices of 13 maize grains sampled from Uganda and consumed in East Africa.

Maize varieties	Crude fiber (g/100g dw)	Ratio of carbohydrate to crude fiber	Estimated dietary fiber (g/100g dw)	Ratio of carbohydrate to estimated dietary fiber*	Resistance starch (%)	Estimated glycemic index (%)	ESI classification
DK777	$13.39^{a} \pm 0.46$	$5.37^{de} \pm 0.24$	$14.38^{a} \pm 0.05$	$4.93^{cd} \pm 0.22$	$9.90^{c} \pm 1.29$	$80.02^{ef} \pm 0.87$	High GI
DK9089	$13.09^{a} \pm 0.90$	$5.52^{de} \pm 0.46$	$14.06^{a} \pm 0.97$	$5.07^{cd} \pm 0.43$	$13.34^{abc} \pm 1.97$	$81.37^{cdef} \pm 0.48$	High GI
DT Max	$12.60^{ab} \pm 0.21$	$5.70^{de} \pm 0.10$	$13.54^{ab} \pm 0.22$	$5.24^{cd} \pm 0.10$	$14.75^{abc} \pm 2.74$	$80.45^{ef} \pm 0.05$	High GI
KH 500 43A	$12.06^{abc} \pm 0.57$	$6.19^{cde} \pm 0.32$	$12.95^{abc} \pm 0.61$	$5.69^{bcd} \pm 0.30$	$14.17^{abc} \pm 1.03$	$81.52^{cdef} \pm 0.32$	High GI
Longe 10H	$9.15^{de} \pm 0.78$	$8.56^{abc} \pm 0.73$	$9.83^{de} \pm 0.84$	$7.90^{ab} \pm 0.68$	$12.66^{abc} \pm 1.62$	$87.43^{a} \pm 1.09$	High GI
Longe 5	$8.97^{de} \pm 0.63$	$8.65^{ab} \pm 0.69$	$9.63^{de} \pm 0.68$	$7.98^{ab} \pm 0.64$	$14.09^{abc} \pm 0.01$	$79.38^{cdef} \pm 1.64$	High GI
Longe 5D	$11.65^{abcd} \pm 2.22$	$6.47^{bcde} \pm 1.50$	$12.51^{abcd} \pm 2.38$	$5.95^{bcd} \pm 1.39$	$15.19^{ab} \pm 0.03$	$81.38^{f} \pm 1.40$	High GI
MM3	$13.33^{a} \pm 0.25$	$5.28^{e} \pm 0.13$	$14.32^{a} \pm 0.27$	$4.85^{d} \pm 0.11$	$14.16^{abc} \pm 0.50$	$81.77^{cdef} \pm 1.32$	High GI
UH5051	$9.97^{bcde} \pm 1.58$	$7.78^{abcd} \pm 1.42$	$10.70^{bcde} \pm 1.70$	$7.17^{abc} \pm 1.33$	$11.56^{bc} \pm 0.56$	$83.35^{bcde} \pm 0.21$	High GI
UH5053	$9.10^{de} \pm 0.23$	$8.35^{abc} \pm 0.18$	$9.78^{de} \pm 0.24$	$7.71^{ab} \pm 0.17$	$10.65^{bc} \pm 0.37$	$84.65^{abcd} \pm 0.73$	High GI
UH5354	$9.10^{de} \pm 1.04$	$8.48^{abc} \pm 1.27$	$9.78^{de} \pm 1.12$	$7.82^{ab} \pm 1.18$	$12.12^{abc} \pm 0.24$	$84.80^{ab} \pm 0.91$	High GI
UH5355	$9.43^{cde} \pm 0.84$	$8.21^{abc} \pm 0.88$	$10.13^{cde} \pm 0.91$	$7.57^{ab} \pm 0.82$	$16.68^{a} \pm 1.21$	$86.27^{abc} \pm 0.09$	High GI
WE3106	$7.66^{e} \pm 0.66$	$10.15^{a} \pm 0.93$	$8.23^{e} \pm 0.70$	$9.38^{a} \pm 0.86$	$13.02^{abc} \pm 0.40$	$81.14^{def} \pm 0.27$	High GI
***p* Values**	***1.4e-11***	***2.29e-07***	***4.95e-08***	***2.3e-07***	***0.004***	***7.48e-06***	**–**

The values indicate the means of triplicates ± *SD*. Means with different letters in the same column differ significantly ($p \leq 0.05$). *Ratio of carbohydrate to estimated dietary fiber was calculated by considering that crude fiber analysis captures only the insoluble fraction, which on average comprises 92.60% of total dietary fiber (Lasek et al., 2020).

Table 3. Factors affecting starch digestibility of 13 maize grains sampled from Uganda (per 100 g dry matters).

Maize varieties	Dry matter (%)	Ash (g/100g dw)	Fat (g/100g dw)	Protein (g/100g dw)	Crude fiber (g/100g dw)	Estimated dietary fiber (g/100g dw)	Carbohydrates (g/100g dw)	Total starch (% of TS)	Amylose (% of TS)	Amylopectin (% of TS)	Phenolic compounds (GAE dw)
DK777	$88.17^{gh} \pm 0.13$	$1.24^{b} \pm 0.11$	$4.85^{a} \pm 0.27$	$8.74^{bc} \pm 0.42$	$13.39^{a} \pm 0.46$	$14.38^{a} \pm 0.05$	$71.79^{cd} \pm 0.70$	$63.01^{e} \pm 0.44$	$24.17^{bcd} \pm 0.23$	$75.83^{bcd} \pm 0.23$	$197.07^{bcdef} \pm 8.15$
DK9089	$87.20^{h} \pm 0.18$	$1.41^{ab} \pm 0.26$	$4.01^{abc} \pm 0.10$	$9.47^{ab} \pm 0.23$	$13.09^{a} \pm 0.90$	$14.06^{a} \pm 0.97$	$72.03^{cd} \pm 0.94$	$67.00^{d} \pm 2.46$	$22.51^{cde} \pm 0.32$	$77.49^{cde} \pm 0.32$	$189.81^{def} \pm 9.51$
DT Max	$90.50^{bc} \pm 0.17$	$1.39^{ab} \pm 0.32$	$4.08^{abc} \pm 0.28$	$10.14^{a} \pm 0.42$	$12.60^{ab} \pm 0.21$	$13.54^{ab} \pm 0.22$	$71.79^{cd} \pm 0.49$	$67.44^{d} \pm 1.24$	$26.32^{ab} \pm 0.94$	$73.68^{ab} \pm 0.94$	$216.45^{ab} \pm 7.00$
KH 500 43A	$91.05^{ab} \pm 0.13$	$1.30^{ab} \pm 0.14$	$4.10^{abc} \pm 0.89$	$8.08^{cd} \pm 0.42$	$12.06^{abc} \pm 0.57$	$12.95^{abc} \pm 0.61$	$74.46^{abc} \pm 0.48$	$75.69^{abc} \pm 0.28$	$27.59^{a} \pm 0.64$	$72.41^{a} \pm 0.64$	$180.25^{f} \pm 2.63$
Longe 10H	$89.31^{def} \pm 0.22$	$2.64^{a} \pm 1.00$	$3.09^{c} \pm 0.32$	$7.21^{e} \pm 0.23$	$9.15^{de} \pm 0.78$	$9.83^{de} \pm 0.84$	$77.90^{a} \pm 0.53$	$75.82^{abc} \pm 1.22$	$22.83^{cde} \pm 0.22$	$77.17^{cde} \pm 0.22$	$183.72^{ef} \pm 2.68$
Longe 5	$90.34^{bcd} \pm 0.12$	$1.86^{ab} \pm 0.30$	$3.58^{bc} \pm 0.32$	$8.36^{cd} \pm 0.25$	$8.97^{de} \pm 0.63$	$9.63^{de} \pm 0.68$	$77.23^{a} \pm 0.61$	$67.68^{d} \pm 1.20$	$22.54^{cde} \pm 0.44$	$77.46^{cde} \pm 0.44$	$187.74^{ef} \pm 4.59$
Longe 5D	$89.00^{efg} \pm 1.21$	$1.43^{ab} \pm 0.11$	$4.50^{ab} \pm 0.15$	$9.30^{ab} \pm 0.24$	$11.65^{abcd} \pm 2.22$	$12.51^{abcd} \pm 2.38$	$73.12^{bcd} \pm 2.40$	$68.12^{d} \pm 0.16$	$24.90^{bc} \pm 0.09$	$75.10^{bc} \pm 0.09$	$210.78^{abc} \pm 7.12$
MM3	$88.56^{fg} \pm 0.16$	$1.39^{ab} \pm 0.17$	$4.95^{a} \pm 0.11$	$9.95^{a} \pm 0.11$	$13.33^{a} \pm 0.25$	$14.32^{a} \pm 0.27$	$70.38^{d} \pm 0.46$	$73.97^{c} \pm 0.88$	$24.55^{bc} \pm 0.34$	$75.45^{bc} \pm 0.34$	$185.29^{ef} \pm 10.82$
UH5051	$88.33^{fg} \pm 0.19$	$1.54^{ab} \pm 0.24$	$3.08^{c} \pm 0.32$	$9.37^{ab} \pm 0.25$	$9.97^{bcde} \pm 1.58$	$10.70^{bcde} \pm 1.70$	$76.04^{ab} \pm 1.39$	$75.58^{bc} \pm 2.43$	$21.98^{de} \pm 1.56$	$78.02^{de} \pm 1.56$	$203.00^{abcde} \pm 2.71$
UH5053	$90.56^{bc} \pm 0.09$	$1.64^{ab} \pm 0.56$	$4.36^{ab} \pm 0.13$	$8.92^{bc} \pm 0.42$	$9.10^{de} \pm 0.23$	$9.78^{de} \pm 0.24$	$75.98^{ab} \pm 0.88$	$79.50^{a} \pm 0.12$	$20.47^{e} \pm 0.32$	$79.53^{e} \pm 0.32$	$193.38^{cdef} \pm 2.64$
UH5354	$91.95^{a} \pm 0.28$	$2.23^{ab} \pm 0.89$	$4.77^{a} \pm 0.53$	$7.60^{de} \pm 0.11$	$9.10^{de} \pm 1.04$	$9.78^{de} \pm 1.12$	$76.30^{ab} \pm 2.47$	$76.11^{abc} \pm 1.64$	$24.86^{bc} \pm 0.16$	$75.14^{bc} \pm 0.16$	$208.45^{abcd} \pm 10.41$
UH5355	$92.15^{a} \pm 0.14$	$1.34^{ab} \pm 0.15$	$3.56^{bc} \pm 0.27$	$8.76^{bc} \pm 0.09$	$9.43^{cde} \pm 0.84$	$10.13^{cde} \pm 0.91$	$76.90^{a} \pm 1.22$	$78.12^{ab} \pm 1.21$	$24.63^{bc} \pm 0.37$	$75.37^{bc} \pm 0.37$	$181.10^{f} \pm 6.88$
WE3106	$89.85^{cde} \pm 0.15$	$1.99^{ab} \pm 0.53$	$4.15^{abc} \pm 0.30$	$8.81^{bc} \pm 0.25$	$7.66^{e} \pm 0.66$	$8.23^{e} \pm 0.70$	$77.38^{a} \pm 0.51$	$74.51^{bc} \pm 0.08$	$23.70^{cd} \pm 2.05$	$76.30^{cd} \pm 2.05$	$222.64^{a} \pm 5.33$
***p* Value**	***1.26e-14***	***0.0258***	***3.24e-06***	***1.4e-11***	***4.95e-08***	***4.96e-08***	***2.58e-08***	***1.34e-14***	***5.06e-09***	***5.06e-09***	***5.15e-08***

The values indicate the mean of triplicates ± *SD*. Means with different letters in the same column differ significantly ($p \leq 0.05$), GAE: gallic acid equivalent.

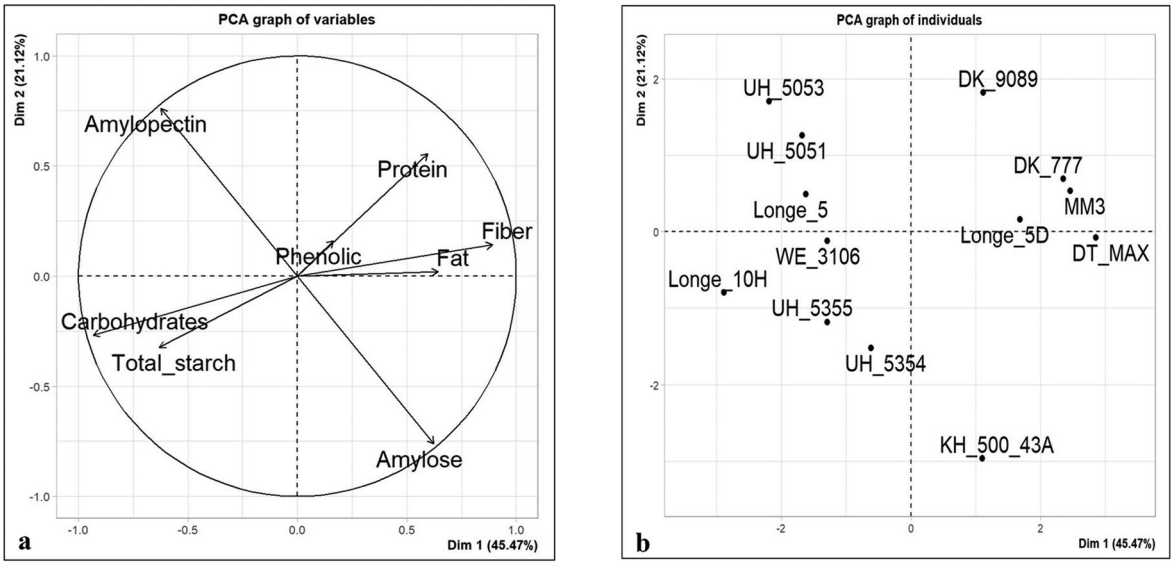

Figure 1. The principal component analysis (PCA) showing: (a) the correlation of the factors affecting the starch digestibility within PC1 and PC2, and (b) thirteen maize varieties distribution. PCA was performed on the correlation matrix of average values of factors affecting starch digestibility (Table 4).

components accounted for 66.59% of the overall variance. The distance between the locations of two maize varieties on the score plot (Figure 1(a)) is directly proportional to the degree of difference or similarity between these varieties. Figure 1(b), which represents the plot of the second principal component (PCA), provided valuable insights into the correlations between the measured parameters and the selected maize varieties.

Maize varieties UH5053, UH5051, and Longe 5 were located in the upper left of the score plot and are those having high content of amylopectin (Figure 1). The varieties Longe 10H, UH5355, and UH5354 were positioned in the lower left and have high carbohydrate and total starch contents. On the other hand, the phenolic compounds, protein, fiber, and fat contents were clustered together in the upper right and are correlated to the DK777, DK9089, MM3, DT Max, and Longe 5D varieties. KH 500 43 A was the only variety located in the lower right quadrant and had a high content of amylose.

3.5. Clusters of maize varieties based on the starch digestibility factors

The similarity of the maize samples was classified using the plug-in Heat Map with Dendrogram (Figure 2). As shown, the samples were clustered horizontally, and the intrinsic factors affecting starch digestibility were clustered vertically. The peak volume of each factor was marked by a different color in the heat map. The darker the red, the higher the component, and the darker the blue, the lower the component in the respective maize variety. The samples clustered into the same category showed a high degree of correlation and were marked by the same color. These results show that at 20% of the dissimilarity (Supplementary Figure S3), the 13 maize samples can be classified into four statistically significant clusters (1, 2, 3, and 4). The agglomeration coefficient was 0.85, very close to 1, showing the good fitness of the dendrogram (Supplementary Figure S4). Otherwise, the cophenetic correlation coefficient of the dendrogram generated from cluster analysis was 0.67, showing a moderate representation of the data.

These clusters were found to have similar fat, protein, fiber, carbohydrate and amylose content ($p > 0.05$), but differed in terms of phenolic compounds and total starch content ($p < 0.05$, Table 4). Cluster 1 included DK9089, DK777, and Longe 5 varieties have the lowest total starch content and the second lowest phenolic compounds. Four maize varieties were included in Cluster 2: MM3, KH 500 43 A, UH5355, and Longe 10H (Figure 2). These varieties have the second highest levels of total starch and amylose content, but the lowest levels of phenolic compounds (Table 4). Cluster 3, which consists of the varieties DT MAX, Longe 5D, and WE3106, has the highest phenolic and amylose content. Finally, cluster 4, comprising of UH5051, UH5053, and UH5354, has the lowest amylose content and the highest total starch content.

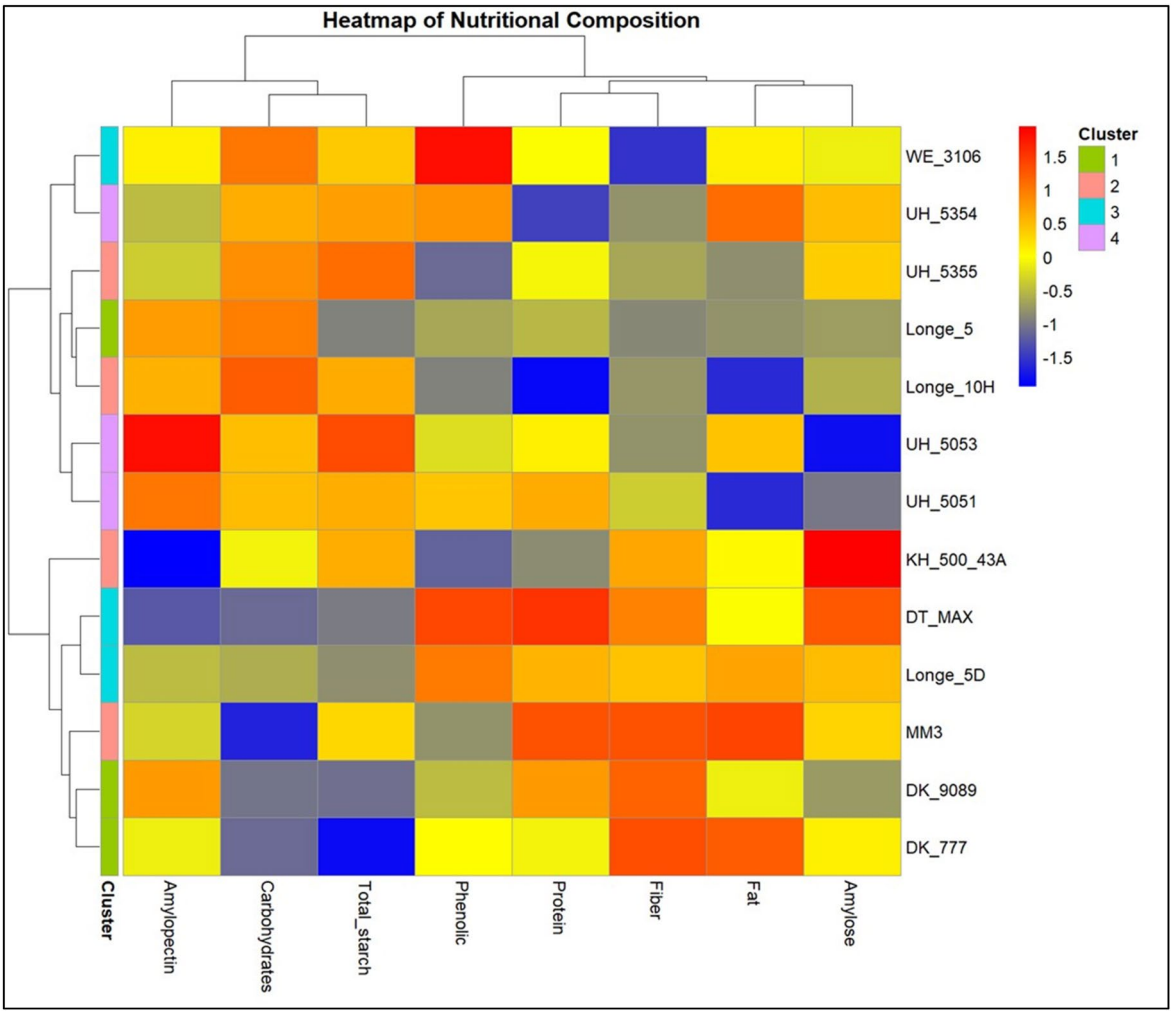

Figure 2. Heat map clustering showing maize varieties clusters based on the nutritional traits investigated as factors affecting the starch digestibility.

Table 4. Average characteristics of the clusters of thirteen maize varieties selected from Uganda.

Nutritional parameters (per 100g dw)	Cluster 1 DK9089 DK777 Longe 5	Cluster 2 KH 500 43A Longe 10H MM3 & UH5355	Cluster 3 DT mAX Longe_5D WE3106	Cluster 4 UH5051 UH5053 UH5354	*p* Values
Fat (g)	$4.15^{a} \pm 0.65$	$3.92^{a} \pm 0.80$	$4.24^{a} \pm 0.22$	$4.07^{a} \pm 0.88$	0.935
Protein (g)	$8.86^{a} \pm 0.56$	$8.50^{a} \pm 1.16$	$9.42^{a} \pm 0.67$	$8.63^{a} \pm 0.92$	0.885
Fiber (g/)	$11.81^{a} \pm 2.47$	$10.99^{a} \pm 2.04$	$10.64^{a} \pm 2.62$	$9.39^{a} \pm 0.50$	0.145
CHO (g)	$73.68^{a} \pm 3.08$	$74.91^{a} \pm 3.35$	$74.10^{a} \pm 2.92$	$76.10^{a} \pm 0.17$	0.364
Phenolic (mg Eq GA)	$191.54^{c} \pm 4.90$	$182.59^{d} \pm 2.33$	$216.62^{a} \pm 5.93$	$201.61^{b} \pm 7.63$	0.019
Amylose (% of TS)	$23.08^{a} \pm 0.95$	$24.90^{a} \pm 1.97$	$24.97^{a} \pm 1.31$	$22.44^{a} \pm 2.23$	0.679
Amylopectin (% of TS)	$76.92^{a} \pm 0.95$	$75.10^{a} \pm 1.97$	$75.03^{a} \pm 1.31$	$77.56^{a} \pm 2.23$	0.679
Total starch (%)	$65.89^{c} \pm 2.52$	$75.90^{a} \pm 1.70$	$70.03^{b} \pm 3.90$	$77.06^{a} \pm 2.13$	0.042

The values indicate the mean of triplicates ± SD. Means with different letters in the same line differ significantly ($p \leq 0.05$).

4. Discussion

4.1. Starch digestibility of maize varieties

Starch digestibility analysis revealed significant variation among maize varieties, and this difference in the starch digestibility percentage between maize varieties could be related to their difference in factors affecting starch digestibility, which also varied significantly across varieties (Table 3). The finding also shows that the RDS of the maize varieties ranges between 27.80% and 39.12% and thus SDS and RS between 61% to 72%. This level of RDS is within the proportion found by Camelo-Méndez et al. (2017)

for white maize flour (33%), lower than the proportion found by Giuntini et al. (2022) for maize flour (49%) and Utrilla-Coello et al. (2011) for the maize bars flour. The relatively low rapidly digestible starch (RDS) content in these maize flours may offer dietary benefits in preventing non-communicable diseases. The maize variety UH5354 has the highest RS (16.68%) and SDS+RS (72.19%). Therefore, this result suggests that if all other factors remain constant, the UH5354 maize variety may represent a suitable candidate for dietary interventions targeting the prevention of non-communicable diseases. However, relying solely on starch digestibility fractions may not sufficiently determine the quality of these maize flours; considering the overall carbohydrate quality of these flours, as it looks beyond starch digestibility and considers aspects like dietary fiber content, glycemic index, would provide a more comprehensive assessment and potential health impacts.

4.2. Carbohydrate quality of maize varieties

Diets with low carbohydrate quality with high glycemic index, and low fiber content, have been found to increase obesity prevalence, elevate blood glucose concentrations, and promote non-communicable diseases (Bellmann et al., 2018). In light of the association between carbohydrate quality and health, consumption of foods with a low glycemic index and high fiber content is recommended (Toh et al., 2020). Therefore, the American Heart Association has recommended ≤10:1 carbohydrate-to-dietary fiber ratio for overall well-being promotion (Liu et al., 2020). The 10:1 ratio captures the overall quality of carbohydrates, especially when the fiber component is derived from whole grains (Liu et al., 2020).

In the present study, it was observed that twelve out of the thirteen examined maize varieties met the recommended carbohydrate-to-fiber ratio of 10:1. Only the variety WE3106 slightly exceeded this ratio with a value of 10.15. These findings suggest that many of the maize varieties which are commonly consumed in Uganda have an acceptable carbohydrate quality based on the carbohydrate-to-crude fiber ratio. As fiber has the potential to reduce blood cholesterol levels, enhance glucose tolerance, and decrease the glycemic response (Giuntini et al., 2022), thus, keeping other factors constant, these maize varieties consumption can be promoted for controlling the development of metabolic diseases. Furthermore, the study also examined more stringent carbohydrate-to-fiber ratios ≤ 8:1 and ≤ 5:1 (Mello et al., 2019) to evaluate varieties with higher fiber levels. Among the thirteen maize varieties investigated, seven varieties (DK777, DK9089, DT Max, KH 500 43 A, MM3, Longe 5D, and UH5051) fulfilled the recommended < 8:1 carbohydrate-to-fiber ratio, while none met the stricter ≤ 5:1 ratio when based on their crude fiber content. Therefore, it is advisable to prioritize the consumption of these seven varieties over the six varieties that have a higher ratio than 8:1.

Otherwise, the current study assessed fiber content using the Weende method which approach, which involves sequential acid and alkali digestion and quantifies only the indigestible dietary fiber fraction, primarily referred to insoluble fiber (da-Silva & Walter, 2012). Considering the role of soluble fiber in conferring various health benefits, including its impact on glycemic control and managing obesity in individuals with type 2 diabetes (Giuntini et al., 2022), the absence of soluble fiber quantification represents a key limitation of the present study. Therefore, given that maize grains contain predominantly insoluble fiber, approximately 92.60% (Lasek et al., 2020) or 90% (Food & Agriculture Organization of the United Nations [FAO], 1992), the present study estimated total dietary fiber contents for these maize varieties. Based on these estimations, the carbohydrate-to-dietary fibre ratios range between 4.85 and 9.38, with MM3 and WE3106 having the lowest and highest ratios, respectively. This range falls below the 10:1 threshold recommended by the American Heart Association, suggesting nutritionally favourable carbohydrate quality of these maize varieties. Therefore, future studies could investigate the complete dietary fiber content of maize, including both soluble and insoluble fractions, to enable more accurate estimation of the carbohydrate-to-dietary fiber ratio.

Soluble fiber bypasses the enzymatic digestion in the small intestine and reduces blood glucose and postprandial insulin levels following consumption (Giuntini et al., 2022). Its typically low content in cereals, particularly in maize (Bader Ul Ain et al., 2019; FAO, 1992; Lasek et al., 2020) could explain the high glycemic index of these maize grains; meanwhile, they have an acceptable carbohydrate quality using the ratio carbohydrate-to-fiber. The maize varieties expressed high glycemic index values ranging from

79.38% to 86.27% (≥70). The range of glycemic index values observed in the present study is consistent with previous findings reported by Caballero-Rothar et al. (2022), who reported values ranging from 75.6 to 81.0 for eight varieties of maize, and Nurjanah et al. (2020), who found a value of 85.02 for maize starch. However, the glycemic index values obtained are lower than the value of 89.4 reported by Chauhan and Singh (2020) for maize grain, 91.9 reported by Tovar et al. (2003) for tortilla maize and higher than the in vivo glycemic index reported by RamyaBai et al. (2019) for ugali flour, 71.4. This discrepancy may come from the choice of reference, such as the type of white bread commonly used. White bread is used as a benchmark because it is a familiar and easy-to-prepare food that demonstrates a relatively high and consistent rate of starch hydrolysis. However, its formulation can vary by region and manufacturer, which can influence its digestibility and the resulting glycemic response (Hettiaratchi et al., 2012). In summary, it is becoming evident that despite the acceptable quality of these maize varieties in terms of their carbohydrate-fiber ratio, they could potentially contribute to an increase in blood glucose levels due to their high glycemic index.

The varieties DK777, DK9089, and DT Max, which expressed the lowest carbohydrate-fiber ratio, 5.37, 5.52, and 5.70, respectively, also had a low estimated glycemic index 80.02, 81.37, and 80.45, respectively, compared to others. These results indicate a strong correlation between high fiber content and low glycemic index. Nevertheless, the present study evaluates the glycemic index of the maize flour through the in vitro method, which had the same trend as in vivo tests, though in vitro tests tended to have absolute values (overestimation) greater than those of in vivo tests (Afandi et al., 2021). In addition, studies have reported that roasting of maize and boiling maize flour, typically applied in African countries, can decrease GI. Chauhan and Singh (2021) found that boiled maize and roasted maize can decrease the glycemic index by 8.58% and 3.99% of the raw maize, respectively. Therefore, future studies could investigate the processing effect on the maize variety's glycemic response or blood glucose level through an in vivo test.

4.3. State of factors affecting the carbohydrate quality of maize varieties

Proximate composition of the maize varieties in g/100g dw varied from 3.09 and 4.95 for fat, 7.21 and 10.14 for protein, 7.66 and 13.39 for fiber, and 70.30 and 77.38 for carbohydrate. These values are within those found by Ndukwe et al. (2015) and Demeke (2018) for different maize varieties in Nigeria and Ethiopia. The amylose content of the maize samples varied between 20.57 and 27.75% which is in line with most maize, which contains 25% amylose of the total starch. The phenolic compounds varied between 180.25 and 222.64 mg GAE/100g dry weight, a value higher than the value found by Lopez-Martinez et al. (2009) for white maize (170 mg GAE/100g dry weight) and lower than the range values found by Žilić et al. (2012) (522.71–577.82 mg GAE/100g dw). Traditionally, resistant starch 5 (RS5) has been classified as starch-lipid V-type complexes. However, recent research has identified other V-type complexes formed from the interaction of starch with various non-starch molecules such as starch-glycerol, starch-amino acids, starch-peptides, starch-proteins, starch-lipid-protein, starch-polyphenols, starch-other polysaccharides, among others, classified as RS5 (Gutiérrez & Tovar, 2021). This shows that the starch digestibility decreases with the increase of these parameters as they interact with amylose and promote the development of resistant starch type 5 (RS5). Therefore, the varieties with the highest protein, the phenolic compounds would probably express low starch digestibility compared to the adverse varieties.

Phenolic compounds can attenuate starch digestion mainly by inhibiting the activity of α-amylase. They reduce starch digestibility by binding with starch and forming insoluble complexes of amylose phenol that resist the action of α-amylase. Phenolic compounds delay starch digestion and reduce postprandial glycemia, with beneficial effects on the prevention of non-communicable diseases. According to the study conducted by Kwaśny et al. (2022), the increase in phenolic compounds had a statistically significant impact on the development of resistant starch in wheat starch gels. For example, Barros et al. (2012) found that the sorghum with rich phenolic namely tannin, has significantly decreased the starch digestibility compared to the non-tannin sorghum due to the tannin composition. Different extracted plant phenolic sources (clonal oregano, raspberry and rowanberry extracts) have been shown to inhibit porcine pancreatic α-amylase activity. Otherwise, protein content decreases starch digestibility through the formation of

complexes with amylose, which resists enzymatic degradation (Kim et al., 2016) and the presence of zein (Wang et al., 2022; Xu et al., 2019). Zein, the principal storage protein found in maize, create a coat with starch granules, enabling a core-shell structure that restricts to water and enzyme access (Wang et al., 2022). The barriers reduce starch swelling and gelatinization during digestion and thus the starch digestibility (Xu et al., 2019). It reveals that high levels of protein and phenolic compounds in these maize varieties would decrease their starch digestibility and lead to the improvement of their carbohydrate quality.

The variety DT Max recorded the highest protein and the second highest phenolic content, would have low-release glucose following consumption, compare to the variety Longe 10H, which has the lowest protein and second-lowest phenolic could release high soluble starch after hydrolysis. Additionally, DT Max variety has intermediate fiber content and the second-highest amylose content, an essential starch component that promotes resistant starch formation during thermal processing. Therefore, this particular variety could promote the development of resistant starch during the cooking process of maize than other varieties. DT Max having a lower carbohydrates-fiber ratio and estimated glycemic index (Table 2) and exhibits significant factors that decrease the release of starch during digestion, emerges as a promising maize variety that should be promoted for consumption to prevent non-communicable diseases in Uganda. However, these analyses are based on isolated analysis of the parameters. The results from the principal component analysis (PCA) and hierarchical cluster analysis (HCA), which are two main approaches widely used for the classification in the field of food research (Yu, 2005), should further be considered to better guide decisions on the varieties to promote.

4.4. Classification of maize varieties based on quality parameters that affect starch resistance

Principal Component Analysis (PCA) was conducted on the investigated characteristics to assess the grouping of maize varieties based on these parameters. This procedure reduces the dimensionality of the data matrix while retaining most of the information of the original data, as well as explaining the relationship of objects and the correlation structure of the variables. The cumulative contribution rate of the first two PCs accounts for 66.59% of the total variance, and each less than 15%. This indicates that the first two plots explain more of the data than other combinations. The maize varieties DK777, DK9089, MM3, DT Max, and Longe 5D were more correlated with the highest phenolic compounds, protein, fiber, and fat contents, all factors contributing to the decrease of starch digestibility, thus high carbohydrate quality (Ayua et al., 2021; Brennan, 2024). Accordingly, assuming other factors remain constant, these maize varieties would elicit a lower postprandial glucose response compared to others, thereby contributing more significantly to the prevention of diet-related NCDs. Otherwise, the maize variety KH 500 43 A exhibits a strong correlation with high amylose content, a key driver of starch retrogradation. Given its elevated amylose content, KH 500 43 A is expected to yield significantly higher RS3 levels when subjected to hydrothermal treatment (Leeman et al., 2006), making it a promising candidate for developing functional maize-based ingredients aimed at glycemic control and metabolic health.

Meanwhile, the findings from hierarchical cluster analysis show that the thirteen maize varieties can be grouped into four significantly different clusters. HCA identifies subgroups within datasets, distinguished by distinct differences in the measured variables, thereby enabling meaningful classification based on shared traits or patterns (Dalmaijer et al., 2022). Therefore, each subgroup has its characteristics and maize varieties within each group would probably develop a similar effect in terms of starch resistance development after processing and following the digestion. The four clusters are similar concerning their proximate composition, while different according to their phenolic compounds and total starch content (Table 4). Phenolic compounds are widely known to reduce starch digestibility by binding with starch and forming insoluble complexes with amylose that resist the action of α-amylase. Thus, cluster 3, composed of DT MAX, WE3106 and Longe 5D (Table 4), with the highest (216.61) phenolic compounds, would promote higher resistant starch development during digestion than varieties in other clusters. On this basis, varieties in cluster D could be chosen when the other factors are kept constant. The results of this study indicate that while PCA identified DK777, DK9089, MM3, DT Max, and Longe 5D as the most promising varieties with low starch digestibility, only DT Max and Longe 5D were clustered together within the same subgroup based on HCA. These results show that HCA can uncover discrete subgroups

that PCA might overlook, and therefore, combining PCA and HCA improves the classification, which aligns with findings of Mohamad Asri et al. (2020).

Otherwise, the average amylose content of 1, 2, 3, and 4 was 23.08, 24.90, 24.97, and 22.44%, respectively. Although statistically not significant, clusters 2 and 3 had almost similar and the highest amylose contents, compared to cluster 4, which had the lowest (Table 4). Accordingly, maize varieties within clusters 2 and 3, with similar high amylose content, would likely develop a similar and high level of retrogradation when subjected to processing (e.g. hydrothermal, such as heat moisture treatment or parboiling), while cluster C would have a lower level of retrogradation.

Looking forward to the link with phenolic compounds in inactivating the α amylase during digestion and the importance of amylose in increasing resistant starch during processing, cluster 3, having the highest phenolic and high amylose content, would probably develop more resistant starch than the other clusters. Given these promising nutritional profiles of cluster 3 varieties, characterised by a high content of phenolic compounds and amylose, plant breeders are encouraged to prioritise these varieties to develop cultivars that improve resistant starch formation and inhibit α-amylase activity. Policymakers should integrate these genotypes into national agricultural strategies aimed at combating non-communicable diseases, including targeted funding programs for their cultivation and distribution in high-risk regions. Alongside this, nutrition educators and public health advocates are encouraged to raise awareness of the health benefits associated with resistant starch and phenolic compounds. The maize varieties in this cluster could be promoted to encourage healthy dietary choices and support the prevention of non-communicable diseases across Uganda and neighboring countries. On another hand, as the proximal composition of the cluster is similar and no significant difference between the amylose content of the clusters, maize varieties in clusters 1 and 4 with higher phenolic content than cluster 2 would release less soluble starch than cluster A and could also be adequate candidate maize varieties to prevent diet-associated non-communicable diseases.

5. Conclusion

The present study evaluated the carbohydrate quality and state of factors affecting the starch digestibility of 13 maize varieties selected from Uganda and commonly consumed in East Africa. Despite having the recommended carbohydrate fiber ratios of less than 10:1, all the varieties presented a high glycemic index. The factors affecting the starch digestibility of the maize varied significantly across the varieties, except for the fat and ash contents. These results therefore show that the variation in the internal factors leads to differences in starch digestibility among the maize varieties. Based on this variation, further analysis grouped the maize varieties into four significantly different groups. The cluster consisting of varieties DT MAX, Longe 5D, and WE3106 would develop more resistant starch, while the maize varieties UH5051, UH5053, and UH5354 would develop the lowest starch resistance. On the basis of these results, varieties with compounds that promote higher starch resistance are recommended for consumption to regulate NCD development. However, further research is required to enhance the carbohydrate quality of these varieties to contribute to better nutritional outcomes, such as reducing NCD risks.

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Ethical approval

The study did not involve any human or animal testing. Despite this, ethical clearance was obtained from Gulu University Research Ethics Committee (Approval number: GUREC-2023-507) and the Uganda National Council for Science and Technology (Approval A338ES). Additional authorization to conduct sample collection in the community was obtained verbally from the Chief Administrative Officers of the study location.

Author contributions

CRediT: **Finagnon Toyi Kevin Fassinou**: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Validation, Visualization, Writing – original draft. **Solomon Olum**: Conceptualization, Methodology, Supervision, Validation, Writing – review & editing. **Duncan Ongeng**: Conceptualization, Funding acquisition, Methodology, Supervision, Validation, Writing – review & editing.

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Data availability statement

The datasets used in this study are available upon a reasonable request from the correspondent author (Finagnon Toyi Kevin Fassinou: ffinagnon93@gmail.com).

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