



Improving carbohydrate quality of polished maize flour using heat-moisture treatment

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DEDICATION

I dedicate this book to my family, namely, my wife **Racilia Senami E. Ganta** and my children **Shanelle Merveille Fenou** and **Ilyan Navid Tokpè**, for their daily support and prayers.

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LIST OF ABBREVIATIONS AND ACRONYMS

ANN	:	Annealing
BBD	:	Box–Behnken design
CCD	:	Central Composite Design
CHO	:	Carbohydrates
db	:	Dry Weight Basis
GI	:	Glycemic Index
GL	:	Glycemic Load
HCA	:	Hierarchical Cluster Analysis
HMT	:	Heat Moisture Treatment
MC	:	Moisture Content
NARO	:	National Agricultural Research Organization
NCDs	:	Non-Communicable Diseases
OAC	:	Oil Absorption Capacity
PCA	:	Principal Component Analysis
RDS	:	Rapidly Digestible Starch
RS	:	Resistant Starch
RSM	:	Response Surface Methodology
SDS	:	Slowly Digestible Starch
SOL	:	Solubility
SV	:	Swelling volume
T2DM	:	Type II Diabetes Mellitus
TAFSA	:	Train Agribusiness and Food Systems Scientists for African Agriculture
WAC	:	Water Absorption Capacity

THESIS ABSTRACT

Polished maize flour, consumed by more than 90% of Ugandan households, has fast starch digestibility and low resistant starch (low carbohydrate quality), which may make its high consumption a risk factor for non-communicable diseases. Among many methods used to modify starch and reduce its digestibility, heat moisture treatment (HMT) is a preferable and extremely attractive method due to its effectiveness for promoting high resistant starch development, flexibility in relation to heat sources, low cost and the non-generation of chemical residues. However, limited studies exist on application conditions of HMT to decrease starch digestibility of polished maize typically consumed in Uganda and other African countries. The overall objective of this study, therefore, was to improve the carbohydrate quality of polished maize flour using HMT. Out of 86 maize varieties promoted in Uganda, thirteen widely cultivated and consumed were selected for evaluation of their carbohydrate quality in terms of the carbohydrate-to-fiber ratio and glycemic index, and internal factors affecting their starch digestibility. Four varieties among the thirteen varieties, UH5354, DK777, Longe 10H, and DT Max, were further assessed for optimal HMT conditions using Box–Behnken design. The effect of optimal HMT conditions on the physicochemical and techno-functional properties of maize was assessed. Finally, the effect of polishing grade on the physicochemical, techno-functional properties, starch digestibility, and sensorial properties of HMT-treated maize grains was determined. The results revealed that the CHO-to-fiber ratios of the thirteen maize varieties ranged between 5.28 and 10.15, whereas their glycemic indices ranged from 79.38 to 87.43, indicating low carbohydrate quality. The internal parameters affecting starch digestibility varied significantly across the maize varieties ($p < 0.05$), and these subjected the varieties to be grouped into four distinct clusters ($p < 0.05$) based on hierarchical cluster analysis. The optimal HMT conditions to minimize starch digestibility for the four varieties selected from the identified clusters were as follows: UH5354 (23.17%, 80°C, 4.12 h), DK777 (20.83%, 95.43°C, 5.42 h), Longe 10H (34.51%, 90.46°C, 3.01 h), and DT Max (19.24%, 97.02°C, 5.88 h). These optimal conditions had no significant effect on the physicochemical composition of the maize varieties. However, HMT affected some techno-functional properties. Particularly, the swelling volume of DT Max (7.13 ml/g) and Longe 10H (8.23 ml/g) reduced by 0.45 and 0.60 ml/g, respectively while water absorption

capacity of Longe 10H (1.20 ml/g) and UH5354 (1.27 ml/g) increased by 0.08 and 0.10 ml/g, respectively. The pasting temperature increased from 82.4°C to 90.8°C for DK777, 86.0°C to 87.3°C for UH5354, 79.9°C to 82.3°C for Longe 10H and 89.2°C to 94.4°C for DT Max. These changes potentially may affect the maize dough and posho quality and thus may decrease flour acceptability. In contrast, polishing significantly improved the amylose content and color ($p < 0.05$) and the swelling volume of UH5354 and Longe 10H ($p < 0.05$). The solubility, water absorption capacity, and pasting temperature consistently decreased after polishing ($p < 0.05$), whereas the peak and holding viscosities increased ($p < 0.05$). Sensory quality improved substantially from unpolished to semi-polished forms, particularly for DK777, UH5354, and Longe 10H. Although HMT effectively reduces starch digestibility, it also negatively impacts the techno-functional properties of flour, which may affect flour acceptability. Conversely, polishing tends to improve these techno-functional properties of HMT-treated flour and thus enhances the sensory attributes of posho. It is therefore recommended that maize milling industries and processors in Uganda and other East African countries adopt the optimized heat-moisture treatment conditions together with an appropriate polishing grade to produce polished maize flour of improved carbohydrate quality and acceptable sensory quality. Policymakers should also integrate this technology into national food and nutrition strategies to help mitigate diet-related non-communicable diseases. Finally, further research should investigate the optimal conditions for both HMT and polishing to achieve lower starch digestibility as well as improved sensory qualities while minimizing nutrient losses.

Keywords: Carbohydrate quality, heat–moisture treatment, starch digestibility, techno-functional properties, polishing, sensory acceptability.

THESIS STRUCTURE

The thesis is structured into nine chapters. **Chapter one** contains the general introduction, consisting of background, problem statement, objectives, hypotheses, justification of the study and significance of the research. **Chapter two** presents the literature review, structured around each specific objective. It encompasses both the knowledge base and relevant literature, while systematically identifying the gaps that justify and support the present study. **Chapter three** presents the overview of the methodology used during the implementation of this study. It is organized according to each specific objective and shows the sample size, the treatment conducted, analysis done, and statistical analysis conducted. **Chapters four to seven** deal with the research conducted and are presented in paper-based format following the IMRaD structure (Introduction, Methodology, Results, Discussion and Conclusion). They are based on the study objectives defined in chapter one, studied in detail and thus constitute the core of the PhD work.

Chapter four investigated the carbohydrate (CHO) quality and the state of factors that affect starch digestibility of commonly consumed maize varieties in Uganda. Thirteen maize varieties available and most consumed in Uganda were considered out of the eighty-six varieties available with the National Agricultural Research Organization, Uganda. The CHO quality of the selected varieties in terms of the glycemic index and total CHO-to-fiber ratio was determined. In addition, the factors affecting the starch digestibility of these varieties were determined and grouped via principal component analysis (PCA) and hierarchical cluster analysis (dendrogram). Overall, the 13 varieties presented low CHO quality based on their high glycemic index status and were grouped into four significantly different clusters based on hierarchical cluster analysis results. From this chapter, a manuscript titled “Assessing starch digestibility and carbohydrate quality of Ugandan maize varieties: implications for diet-related non-communicable diseases risk” was produced and published in *Cogent Food & Agriculture* (Taylor and Francis).

Based on the low CHO quality ascertained in chapter four, **chapter five** optimized the heat moisture treatment (HMT) conditions to reduce starch digestibility (improve CHO quality) of the four maize varieties, each of which was selected from a distinct cluster identified in chapter four. The optimal HMT conditions (moisture content, temperature, heating time)

to maximize the slowly digestible and resistant starch contents of the maize varieties, were determined using the Box Behnken Design. From this chapter, a manuscript titled “Optimized heat–moisture treatment decreases the starch digestibility of polished maize flour from predominant varieties consumed in Uganda” was produced and published in *Applied Food Research* (Elsevier).

Chapter six investigated whether the optimal HMT conditions designed in chapter five would affect the physicochemical and techno-functional properties of flours. The findings showed that the physicochemical properties such as proximate composition, starch content and color characteristics were not significantly affected by HMT conditions applied. However, some techno-functional properties such as the swelling volume, water absorption capacity, pasting temperature and holding viscosity were affected and thus needed additional treatment to improve the acceptability. This was investigated in chapter seven. From this chapter, a manuscript titled “Effects of optimized heat–moisture treatment conditions on the physicochemical and techno-functional properties of flour from four maize varieties commonly consumed in East African countries” was produced and published in *Discover Food* (Springer Nature).

Chapter seven evaluated the impact of the polishing level (refining) on the physicochemical, functional, and sensory properties of HMT-treated maize flours and derived posho. This study demonstrated that polishing enhanced techno-functional and sensory properties but reduced nutritional composition. This illustrated the importance of the need to co-optimize HMT and polishing to achieve desirable starch digestibility, techno-functional quality, and sensory characteristics. This aspect is recommended for future research.

Chapter Eight provides a critical synthesis of the findings presented in Chapters Four through Seven. It highlights the interrelationships among the results, critically evaluates their strengths and limitations, and identifies areas for improvement as well as priorities for future research.

Finally, **chapter nine** presents the overall conclusion of the PhD research. The main findings of the study and contributions of the study are presented. The chapter revisits the objectives of the PhD study with the results achieved, draws conclusions, provides insights

into the contributions and limitations of the PhD study and provides relevant recommendations to advance knowledge and to inform practices and policy.

CHAPTER ONE

GENERAL INTRODUCTION

This chapter presents in subsection (1) Background of the doctoral research, (2) problem statement of the study followed by (3) objectives, (4) hypotheses, (5) justification of the study, and (6) the significance of the study.

1.1. Background

Dietary carbohydrates are a principal energy source, contributing more than 50% of total human energy intake. They are available in food like grains, legumes, fruits, and vegetables and play crucial roles in supporting human health, growth, and physical activity (Liu *et al.*, 2024). However, there has been debate on the amount and type of dietary carbohydrates required for health particularly because of their association with non-communicable diseases (NCDs) (Reynolds *et al.*, 2019). The 2017 Prospective Urban Rural Epidemiology study of individuals from 18 countries across five continents reported that consumption of low CHO quality foods with high glycemic index is associated with an increased risk of mortality (Seidelmann *et al.*, 2018). This is because such foods increase the prevalence of obesity and increase the blood glucose concentration, which are highly associated with NCDs (Bellmann *et al.*, 2018). Additionally, several studies have demonstrated a strong relationship between high carbohydrate consumption and cardiovascular disease (Mita *et al.*, 2025; Reynolds *et al.*, 2019) cancer development (Cai *et al.*, 2022; Clemente-Suárez *et al.*, 2022) and diabetes (Clemente-Suárez *et al.*, 2022; Suara *et al.*, 2021).

NCDs contribute up to 41 million deaths (74%) annually and account for more than three-quarters of all deaths globally especially in low- and middle-income countries (Müller *et al.*, 2024). Projections by the World Health Organization (WHO) indicate that by 2030, NCDs could lead to 52 million deaths, with diabetes, cardiovascular diseases, and cancers being major contributors (Azadnajafabad *et al.*, 2021; Wang and Wang, 2020). In 2019, Diabetes, especially type II diabetes mellitus (T2DM) alone was directly responsible for 1.5 million deaths worldwide, nearly half of which occurred prematurely before the age of 70 years (Makeri *et al.*, 2026). In sub-Saharan Africa, T2DM is particularly alarming, with the number of people living with the disease projected to increase from 24 million in 2021 to 55 million by 2045, a 129 % increase, and the highest among all regions potentially overtaking communicable diseases as the leading cause of mortality by 2030 (Hossain *et al.*, 2024; Müller *et al.*, 2024).

In Uganda, according to the International Diabetes Federation (IDF), the number of people living with the T2DM in 2024 is estimating at approximately to 369,100 adults (Makeri *et al.*, 2026). Alarmingly, 89 % of individuals with T2DM in Uganda are unaware of their

condition and are neither receiving medication nor engaged in routine T2DM care, leading to a high prevalence of undiagnosed and poorly managed disease. This prevalence has increased rapidly, and the number of people with T2DM is predicted to increase by 166.9% by 2035 (Chang *et al.*, 2019). However, although the Government of Uganda has made more efforts to control communicable diseases such as HIV/AIDS, little has been done about non-communicable diseases (Amanyire *et al.*, 2019). This highlights the urgent need to address carbohydrate quality in dietary recommendations to reduce the risk of NCDs and improve overall health outcomes.

Besides high carbohydrate consumption that enhances blood glucose concentration and obesity prevalence, factors such as a sedentary lifestyle (physical inactivity) and genetics influence the onset and development of T2DM (Azadnajafabad *et al.*, 2021; Cornil *et al.*, 2021; WHO 2020). Then, some strategies used are intense physical activity, anti-obesity drugs, oral hypoglycemic agents, glucose monitoring, and calorie restriction (CR) (Nyenwe *et al.*, 2011; Shakoor *et al.*, 2021). The global diabetes drug market size, including both oral antidiabetic drugs and insulin products, is expensive. It was valued at USD 48,753.1 million in 2018 and is projected to reach USD 78,261.7 million by the end of 2026 (Sokołowska *et al.*, 2022). In addition, most of these synthetic drugs have high secondary failure rates and undesirable side effects. Thus, calorie restriction appears the most potent non-pharmacological intervention to induce weight loss and then prevent T2DM (Most and Redman, 2020). It is a sustained reduction in energy intake from pre-intervention energy requirements while maintaining a sufficient nutrient supply to achieve weight stability. It involves the reduction of carbohydrates available for digestion in food and thereby the production of low glycemic index food.

Low glycemic index foods, due to their slow digestion and absorption, produce a more gradual rise in blood glucose and insulin levels increasingly associated with health benefits. Their consumption increases satiety, reduces appetite, delays hunger, and reduces excessive energy intake in adults and reducing the prevalence of obesity and high blood glucose level thereby T2DM (Chung *et al.*, 2008; Zafar *et al.*, 2019). Consumption of low glycemic index food appears to be a good alternative to control T2DM (Sokołowska *et al.*, 2022; Westman *et al.*, 2018) and they can be developed by the addition of ingredients such

as dietary fiber or phenolic compounds to control starch hydrolysis in the small intestine (Bello-Pérez *et al.*, 2021) or controlled starch modification through the development of resistant starch.

Based on their hydrolysis by enzymes, starch-based foods are classified as slowly digestible starch (SDS), rapidly digestible starch (RDS), or resistant starch (RS) (Bojarczuk *et al.*, 2022; Englyst *et al.*, 1992; Englyst *et al.*, 2018). RDS and SDS are the starch fractions that are hydrolyzed in the small intestine to dextrins by α -amylase within 20 to 120 min after ingestion respectively (Englyst *et al.*, 1992). RS is the portion that is not hydrolyzed after 120 min and then continues its passage from the small to the large intestine (Englyst *et al.*, 1992), where it is fermented by the intestinal microflora (Dupuis *et al.*, 2014). Based on its rapid digestion, RDS produces a high glycemic peak that elicits a more rapid insulin response than SDS which offers the advantage of a sustained glycemic index level over time, and RS is associated with a low glycemic peak (Giuberti and Gallo, 2018). From this point of view, frequent consumption of starch-based food with a high RDS may be a potential risk factor for high glycemic index and then insulin resistance, a precursor of T2DM (Bello-Pérez *et al.*, 2021). The development of carbohydrate food with a high proportion of SDS and RS becomes an alternative for preventing obesity and high blood glucose level and subsequently to alleviate T2DM (Halajzadeh *et al.*, 2020; Reshmi *et al.*, 2017).

Chemical, enzymatic and physical methods exist and have been explored to develop carbohydrate food with a high proportion of SDS and RS (Sandhu *et al.*, 2020). The chemical methods involve the use of different chemical reactions such as esterification, etherification, hydrolysis, oxidation, crosslinking, and bleaching have been employed to develop chemically modified starches globally. According to Haq *et al* (2019), the modified starches by the chemical method are expensive as compared to native starches due to the high cost of reagents used. In addition, Bojarczuk *et al* (2022) reported that chemical methods are associated with long production times, possible changes in product quality, low reaction rates, and environmental pollution. In addition, the chemical modifications of starch are highly complex reactions that might result in low or very high degree of substitution products or may result in promotion of side reactions if the process

conditions related to temperature, catalyst addition, pH, stirring, overhead sealing, and total solids in slurry are not properly controlled (Mazumder and Rahman, 2024). Whereas enzymatic starch modification has recently attracted interest for imparting functional properties with minimal environmental impact (Punia *et al.*, 2022), it can also be employed to generate novel molecules with broad applications in the food industry (Mazumder and Rahman, 2024). Otherwise, physically modified starch is considered natural as opposed to chemically modified starch. They are more accessible and do not produce any effluents containing salts or reagents (Amanyire *et al.*, 2019). With the increasing consumers demand for healthy and additive-free foods, physically modified starch is gaining interest because the functionality of starch can be improved without introducing any foreign substances (Deka and Sit, 2016). They can be easily applied in developing countries where industries are less developed. The physical modification method includes two hydrothermal methods: heat-moisture treatment (HMT) and annealing (ANN) which are mostly applied.

HMT method involves the treatment of starch granules at low moisture levels (<35% w/w) for a certain time (15 min–16 h) and at temperatures (80–140°C) above the transition temperature but below the gelatinization temperature (Li *et al.*, 2020; Wang *et al.*, 2021). ANN involves treatment in excess moisture content (>60% w/w) held at a temperature above the glass transition temperature but below the onset temperature of gelatinization for a set period (Chung *et al.*, 2009). HMT method is shown to improve starch resistant more than ANN in cereals grains (Chung *et al.*, 2009). In addition, during HMT application, the bioactive phenolic compounds well known to decrease starch digestibility are liberated in grains (Wang *et al.*, 2014). Nevertheless, HMT affects some functional properties of starch products such as the structural and functional characteristics of starch (Olayinka *et al.*, 2008), digestibility and the glycemic index (Kunyanee and Luangsakul, 2022; Sandhu *et al.*, 2020), and acceptability of the final product (Kim *et al.*, 2017; Mathobo *et al.*, 2021).

In Uganda, maize (*Zea mays*) is the most important cereal crop grown (Jun and Ross, 2018), a staple food for many Ugandans, for whom it provides over 40% of the calories consumed per capita in both rural and urban areas (Wacoo *et al.*, 2018). Maize flour is the most consumed form and is used for porridges, soups, and pastes by more than 92% of

households (Wacoo *et al.*, 2018). On the basis of nutritional classification, Camelo-Méndez *et al* (2017) reported that maize flour contains 33.0%, 55.9%, and 11.1% Rapidly Digestible Starch (RDS), Slowly Digestible Starch (SDS) and Resistant Starch (RS), respectively, whereas maize starch contains 74.76% RDS, 11.00% SDS and 14.24% RS, respectively (Zheng *et al.*, 2020). These findings indicate that the frequent consumption of maize-based food with this high RDS could be a potential risk factor for high-glycemic-response, a precursor of T2DM. Despite this evidence on maize and the importance of the carbohydrate quality of starch-containing food for diet design and health promotion, inadequate information exists on the CHO quality of Uganda's maize varieties whereas several varieties exist and are promoted across the country on the basis of their agronomical performance (Lee, 2020).

Meanwhile, the carbohydrate quality of these maize varieties remains unknown; in Uganda, aflatoxin contamination is a major concern in cereal crops and constitutes a significant food safety hazard. Aflatoxin in maize in Uganda ranges from 86 µg/kg to 3300 µg/kg, exceeding the maximum limit of aflatoxin B1 per kg of unprocessed maize (Bandé *et al.*, 2022). The aflatoxins of importance in Uganda maize are aflatoxin B1 (AFB1), aflatoxin B2 (AFB2), aflatoxin G1 (AFG1) and aflatoxin G2 (AFG2) (Lukwago *et al.*, 2019), with AFB1 being the most prevalent. Chronic exposure to aflatoxins has been associated with liver cancer in adults, growth retardation through blocking of nutrients in children and suppression of the immune system (Atukwase *et al.*, 2024). According to Lukwago *et al.* (2019), there is a high prevalence of cancer among the Ugandan population, and it is assumed that some of the food consumed could be the source of the carcinogens associated with cancer and other diseases that are caused by consumption of food contaminated with aflatoxin. According to Atukwase *et al* (2024), in Kampala, capital city of Uganda, aflatoxin exposure among mothers and children ranged between 0.01 and 1.0 µg/kg BW/day which is above the daily intake limit of 0.017 µg /kgBW/day recommended by the European Food Safety Agency. Around 20% of these women and children are exposed to 0.11 and 0.21 µg/kgBW/day, while more than 10% of children and 5% of women are exposed to high levels of aflatoxins (0.7–1.0 µg kgBW/day). Therefore, to address aflatoxin issues, in Uganda, maize grains are generally polished before being milled into maize flour for direct consumption (Van Campenhout and De, 2022).

Polishing significantly decreases aflatoxin contamination in maize grains through the removal of contaminated outer layers, thus improving their safety and making them suitable for food and feed (Naeem *et al.*, 2024; Sipos *et al.*, 2021). In addition to reducing aflatoxin contamination, polishing also improves sensorial quality of the maize flour, thus making polished maize flour the most preferred form of maize flour consumed in Uganda (Kiguli *et al.*, 2019). The polishing process removes most of the fiber and fat from the cereal grain, thereby improving the physical characteristics and storability (Ojediran *et al.*, 2020). Maize grains become soft, more digestible, light, have a shorter cooking time, and have better eating properties.

1.2. Statement of the problem

In Uganda, several maize varieties are promoted, and their flour constitutes the most starch-containing food consumed by approximately 92% of households. However, maize in Uganda has a high concentration of aflatoxins reaching 27.9 µg/kg (Atukwase *et al.*, 2024) exceeding the legal limit of 5 g of aflatoxin B1 per kg of unprocessed maize (Battilani *et al.*, 2013). Accordingly, the maize grain is generally polished before milling into flour (Van Campenhout and De, 2022). Polishing reduces aflatoxin contamination in maize grains (Hensawang *et al.*, 2020), removes the non-starch components of the grain, thus improving the physical characteristics especially the color and texture, storability and acceptability of the flour (Ojediran *et al.*, 2020). However, polishing maize increases starch digestibility by allowing easier access to digestive enzymes (Sivakamasundari *et al.*, 2022a). The high and frequent consumption of polished maize flour, which has a high glycemic index (Somaratne *et al.*, 2017), would raise the blood glucose level, a key pathway for the development of dietary associated NCD.

Heat moisture treatment (HMT) has been explored to modify cereal starch to reduce its glycemic index (Rafiq *et al.*, 2016; Sandhu *et al.*, 2020). Despite data on its ability to decrease starch digestibility in isolated maize starch (Asare *et al.*, 2021) or waxy maize starch (Chang *et al.*, 2020), limited information exist on its application and effect on maize flour or polished maize flour typically consumed in Uganda and other African countries. Since the effects of HMT vary with starch origin and the internal composition of isolated or waxy maize starch differs from that of polished maize flour, applying existing data to

develop high-resistant starch in polished maize flour using HMT is a challenge. Physicochemical, techno-functional and organoleptic characteristics are key factors that influence quality and acceptability of food (Korean *et al.*, 2024; Lin, 2025; Polania, 2023). However, it is largely unknown how HMT would affect these parameters for Ugandan maize varieties.

Given the limited literature on the application of HMT to maize grains, an important research question is “To what extent, does HMT improve carbohydrate quality mainly starch digestibility while maintaining desirable quality attributes across commonly consumed maize varieties in Uganda, and when considering their intrinsic characteristics affecting the starch digestibility?”. To address this overarching research question and bridge the identified knowledge gaps, the following questions were formulated:

- (i) What is the starch digestibility and state of internal factors affecting the starch digestibility of maize grain varieties available and consumed in Uganda?
- (ii) What are the optimal HMT conditions for decreasing starch digestibility of common maize varieties consumed in Uganda?
- (iii) What is the effect of HMT on the physicochemical and techno-functional properties of the maize flours from common varieties consumed in Uganda?
- (iv) What is the effect of polishing on the physicochemical, functional properties and sensory characteristics of HMT treated maize grains?

1.3.Objectives of the study

1.3.1. Main objective

The main objective of the study was to improve the resistant starch content of polished maize flour using heat moisture treatment.

1.3.2. Specific objectives

- (i) To characterize carbohydrate quality and internal factors affecting the starch digestibility across maize varieties
- (ii) To optimize the application conditions of HMT to improve the resistant starch content in polished maize grains

- (iii) To determine the effect of HMT on the physicochemical and techno-functional properties of maize grains
- (iv) To evaluate the effects of polishing on the physicochemical, techno-functional and sensory properties of HMT-treated maize grains.

1.4. Hypotheses

- (i) Maize varieties in Uganda differ in terms of their CHO quality and internal factors affecting starch digestibility
- (ii) HMT increases the resistant starch content of polished maize flour, which varies according to maize variety
- (iii) HMT affects the physicochemical, functional or organoleptic characteristics of polished maize flour
- (iv) Polishing decreases the physicochemical and techno-functional properties while increasing the sensory characteristics of HMT-treated maize grains.

1.5. Justification of the study

In Uganda, maize serves as the primary staple food for most households and is a vital source of sustenance across many regions. Before it is consumed as maize dough, commonly known locally as posho, the most popular form of maize, the grain undergoes polishing. This polishing process, however, diminishes the nutritional value and reduces the non-starch polysaccharides in the maize grain, thereby raising the glycemic index of the resulting flour. As a result, the widespread consumption of polished maize flour predisposes Ugandans to elevated glycemic conditions, which are a major risk factor for overweight, obesity, and non-communicable diseases, particularly Type II diabetes mellitus. The prevalence of Type II diabetes Mellitus in Uganda is 4.1% (Chiwanga *et al.*, 2016, IFD 2016) and is expected to increase by 166.9% by 2035 (Chang *et al.*, 2019), although the Government of Uganda has put in more efforts to control communicable diseases, little has been done about non-communicable diseases (Amanyire *et al.*, 2019). Based on the rising burden of non-communicable diseases (NCDs) in Uganda, there is an urgent need to establish effective preventive strategies. Despite its high glycemic index, polished maize flour remains the most consumed and preferred choice among Ugandans due to its favorable organoleptic qualities achieved by removing non-starch

polysaccharides. This preference makes it difficult to sensitize consumers to revert to unpolished maize. Therefore, it becomes essential to focus on improving the nutritional quality of polished maize flour by reducing its glycemic index, thereby contributing to the prevention of NCDs in Uganda.

1.6. Significance of the study

Diets with a low glycemic index are linked to better blood sugar control and reduced risk of NCDs, particularly diabetes, heart disease, and some cancers. Accordingly, the development of healthy starch-based foods with improved carbohydrate quality has gained global attention as a strategy to prevent and manage NCDs (Adegbaju *et al.*, 2025). Therefore, understanding the carbohydrate quality and intrinsic factors influencing starch digestibility in maize flour, combined with optimizing HMT to enhance these qualities, contributes to global efforts to reduce the burden of NCDs, particularly where maize is used as a staple food. Additionally, this study provides a scientific foundation for both food product development and public health interventions. The findings can be used by food scientists to develop high starch quality maize flour, while the public health professionals can draw on the findings to design dietary interventions that contribute to the prevention of NCDs. The findings also provide valuable evidence to guide nutrition-sensitive agricultural strategies, helping policymakers, plant breeders, and development practitioners prioritize maize varieties with better carbohydrate quality which support metabolic health, particularly in areas where maize is a key staple food. The study provides additional evidence that by optimizing HMT to reduce starch digestibility in maize flour, certain techno-functional properties of the grains may be compromised (Kaur and Singh, 2019). This evidence provides the rationale for future studies to carefully balance nutritional improvements with the preservation of key functional traits when designing HMT protocols.

CHAPTER TWO

LITERATURE REVIEW

This chapter presents the literature review and is organized in subsection as follow: (2.1) Conceptual explanation of key terms, (2.2) Carbohydrate quality and internal factors affecting starch digestibility (2.3) Improvement of carbohydrate quality of food, (2.4) Effect of HMT on physicochemical, techno-functional and sensory properties of derived product characteristics and (2.5) Effect of polishing on physiochemical, functional and organoleptic characteristics of starchy foods.

2.1. Conceptual explanation of key terms

To ensure clarity and consistency in interpretation, this subsection provides precise definitions of the key terms and concepts that underpin the present study.

- Carbohydrate quality

The concept of carbohydrate quality has been perceived as a means of describing the nutritional value of carbohydrate -rich foods based on how carbohydrates are digested, absorbed, metabolized in the body, as well as their associated metabolic outcomes , rather than simply focusing on the total quantity of carbohydrates contained in a given food (Seal and Courtin, 2021). Generally, when describing carbohydrate quality, it is imperative to consider how different types of carbohydrates affect the glycemic response, satiety, the rate of gastric emptying and inflammation and other associated metabolic health outcomes (Shapira, 2019). Thus, one key aspect of carbohydrate quality is linked to the magnitude and duration of postprandial blood glucose levels following the consumption of carbohydrate- containing foods (Mphasha, 2025). Since prolonged and sustained exposure to high blood glucose levels is a critical risk factor for metabolic diseases, especially the development of Type 2 Diabetes Mellitus (T2DM), carbohydrates such as refined grains and added sugars that are rapidly digested and give rise to high blood glucose concentrations are considered low carbohydrate quality foods compared with complex carbohydrates that are more slowly digested and result in lower blood glucose concentrations over time (Lamothe *et al.*, 2019).

- Glycemic Index and carbohydrate quality

Glycemic Index is a physiological classification of carbohydrate quality and a measure of how carbohydrate – containing food raises blood glucose after a given amount of food is eaten (C. Lee *et al.*, 2025). GI is defined as the incremental blood glucose area following the consumption of 50 g of available carbohydrates from the test food, expressed as a percentage of the corresponding area following an equivalent amount of carbohydrate from a reference product (typically glucose or white bread) (Arvidsson-lenner *et al.*, 2016) . In this study, GI is used as a measure of carbohydrate quality, with lower GI (lower than 56)

values indicating high carbohydrate quality of a given food, while higher GI (higher than 70) values indicate low carbohydrate quality.

- **Starch digestibility**

This refers to the rate at which starch molecules are being broken down into smaller molecules of starch in gastrointestinal tract in line with their rate of absorption in the blood system (Magallanes-Cruz *et al.*, 2017). The rate that starch is digested in the small intestine and released into the blood stream is a major determinant of the extent to which foods raise blood glucose and insulin levels after particular quantity of food is consumed (Kim *et al.*, 2024). This is of nutritional relevance because reducing glycaemic responses is generally considered to have beneficial metabolic outcomes (K. Englyst *et al.*, 2018).

- **Rapidly digestible starch (RDS)**

This is the starch fraction that causes a sudden increase in blood glucose levels after ingestion, resulting into a high glycemic response (Chung *et al.*, 2009). Carbohydrate- rich foods with a high proportion of this starch fraction are considered low carbohydrate quality, as they are rapidly hydrolyzed and absorbed in the upper gastrointestinal tract, eliciting a sharp postprandial increase in blood glucose and insulin concentration. These high glycemic responses have been associated with impaired glycemic control, thereby increasing the risk of diet- related metabolic disorders when rapidly digestible starch- rich foods are consumed frequently (Lovegrove *et al.*, 2017).

- **Slowly digestible starch (SDS)**

This starch fraction has been shown to digest slowly throughout the entire small intestine, generating slow and prolonged release of glucose into the blood stream, along with lower postprandial insulinemia (Wang *et al.*, 2023). Thus, SDS – rich foods are perceived as high carbohydrate quality owing to their potential to reduce postprandial hyperglycaemia and hyperinsulinemia, all of which are factors that exacerbate common chronic diseases related to diet, such as T2DM (Miao *et al.*, 2015).

- **Resistant Starch (RS)**

Resistant starch has been defined as the portion of starch that is not hydrolyzed by the enzymes in the small intestine and passes to the large intestine, with an accompanying low GI feature resulting in decreased postprandial glucose and insulin responses (Li *et al.*, 2023).

- **Relationship between carbohydrate quality, estimated glycemic index, starch digestibility and carbohydrate-to-fiber ratio**

Carbohydrate quality is less about how much carbohydrate a food contains and more about how that carbohydrate behaves once it's inside the body, and starch digestibility is what drives most of this behaviour. When a food is dominated by rapidly digestible starch (RDS), the starch is broken down and absorbed quickly in the upper gut. This produces steep rises in blood glucose and insulin, gives the food a high estimated glycemic index (eGI), and marks it as lower quality (Englyst *et al.*, 1999). Foods that carry more slowly digestible starch (SDS) and resistant starch (RS) do the opposite. They release glucose gradually or resist digestion altogether, which keeps postprandial glucose and insulin responses in check and brings the eGI down (Englyst *et al.*, 2007). Because SDS and RS behave in this favourable way, their combined share (SDS+RS) is often treated as a practical marker of carbohydrate quality. A higher SDS+RS proportion points to a lower glycemic index and a healthier metabolic profile (Englyst *et al.*, 2007). Fibre adds another dimension. It slows digestion and dampens the glycemic response, so a lower carbohydrate-to-fibre ratio, meaning more fibre relative to carbohydrate, usually goes hand in hand with a lower GI and a higher SDS+RS content (Mozaffarian *et al.*, 2013). Taken together, these four measures (eGI, the carbohydrate-to-fibre ratio, the starch digestibility fractions, and SDS+RS) make up a single connected framework. The core strategy for improving carbohydrate quality, and for lowering metabolic disease risk, is to shift starch composition away from RDS and towards SDS and RS while increasing the fibre content.

- **Improvement of carbohydrate quality**

Several researchers have identified the glycaemic index (GI) and the carbohydrate-to-fibre ratio as two key indicators of carbohydrate quality that are closely associated with

metabolic disease risk (Reynolds et al., 2019). Foods with a high GI and a high carbohydrate-to-fibre ratio are generally considered to be of low carbohydrate quality (Sievenpiper, 2020). Consequently, improving carbohydrate quality has been linked to strategies that lower the glycaemic index and/or increase dietary fibre content, thereby reducing the carbohydrate-to-fibre ratio (Schulz and Slavin, 2021a).

Given that the proportion of rapidly digestible starch (RDS) is strongly associated with the high glycaemic index of starch-rich foods (Eleazu and Kalu, 2017), increasing the proportions of slowly digestible starch (SDS) and resistant starch (RS) while reducing RDS has emerged as a promising strategy for lowering the glycaemic index and, consequently, enhancing carbohydrate quality, typical of starch modification techniques (Rostamabadi *et al.*, 2024).

- Polishing

This is the process of removing the outer layers from the maize kernels (mainly the bran and part of the germ) to obtain a smoother, finer and highly shelf -stable grits with majority of both germ and fiber removed depending on the polishing intensity (Gwirtz and Garcia-casal, 2014). In the context of the present study, and with reference to the Ugandan case, maize grains may undergo no polishing, single polishing, or double polishing, corresponding respectively to third-class (ordinary), second-class (semi-polished), and first-class (fully polished) maize flours.

- Techno-functional properties

Techno-functional properties of food refer to the physical and chemical characteristics of food constituents that determine their behavior during food processing, storage, and preparation, thereby influencing the texture, stability, appearance, sensory quality, and overall performance of the final food product (Angelis *et al.*, 2024). The carbohydrates, proteins, fats as well as other micromolecules interact with each other to form complexes which display different properties than the inherent properties of separate ingredients in the food system (Badia-olmos *et al.*, 2023). Thus, resulting in the modification of different food properties including water absorption capacity, swelling, solubility, viscosity, pasting properties, among others (Ramzan *et al.*, 2026).

2.2. Carbohydrate quality and internal factors affecting starch digestibility

2.2.1. Carbohydrate quality of food: definition and parameters for assessment

Carbohydrates are the major determinants of postprandial blood glucose and are directly related to the glycemic response (Afandi *et al.*, 2021). Their digestion results in the release of different sugars, such as glucose, in the blood, which affects the blood sugar level and induces the release of insulin. Accordingly, recent research has indicated that high CHO intake, especially from foods with high glycemic indices/loads, can cause spikes in insulin secretion, reduce energy expenditure, and thus increase the risk of obesity and non-communicable diseases (Hall, 2017; Liu *et al.*, 2024). This has led to the emergence of the concept of “Carbohydrate quality”.

The definition of carbohydrate quality is complex because of structural and functional variation among carbohydrate types (Schulz and Slavin, 2021a). The quality of carbohydrates has been defined based on how different CHO sources affect the glycemic response, satiety, and inflammation. Generally, whole grains, legumes, and non-starchy vegetables are considered high-quality sources, whereas refined/polished grains and added sugars are considered lower-quality sources because of their rapid glycemic impact and minimal nutritional benefit. Consequently, carbohydrate quality has been defined on the basis of the glycemic index (GI) or fiber content of the food and its postprandial insulin response (Schulz and Slavin, 2021a). Understanding the CHO quality of food provides useful information that can inform researchers, policymakers, the food industry, and consumers toward better food quality and overall healthier diets.

Carbohydrate quality is assessed through various approaches, with dietary fiber and free sugar playing central roles in defining its impact on health. Dietary fiber is resistant to digestion, and its non-absorption in the human small intestine reduces the risk of non-communicable diseases (Makki *et al.*, 2018). Therefore, one of the key methods of CHO quality assessment is the carbohydrate-to-fiber ratio, where a ratio below 10:1 (i.e., 1 g of fiber per 10 g of CHO) is indicative of high-quality carbohydrates (Liu *et al.*, 2020; Mello *et al.*, 2019). Diets adhering to this ratio support fiber intake targets such as 30 g of fiber from 300 g CHO in a 2000 kcal diet and help reduce NCD risk. Excessive free sugar intake has also been recognized as a key risk factor within the broader category of an unhealthy

diet (Benzian *et al.*, 2023). Consequently, refined metrics such as a 10:1:2 ratio, meaning at least 1 g of fiber per 10 g of carbohydrates and less than 2 g of free sugars, have been proposed (Liu *et al.*, 2020) for a healthy diet. These carbohydrate ratios were named the simple (10:1), modified (10:1:2) and dual (10:1 and 1:2) ratios (Blumfield *et al.*, 2021).

Another approach uses the glycemic index (GI) and glycemic load (GL), which assess how carbohydrate-containing foods affect blood glucose levels (Lal *et al.*, 2021). GI and GL were introduced to quantify the glycemic response to carbohydrates in different foods and by food serving, respectively. GI categorizes foods as high (>70), medium (56–69), or low (<55) on the basis of their postprandial blood glucose response. The consumption of high-GI foods causes a rapid and high postprandial response (hyperglycemia) and thus is not recommended (Lal *et al.*, 2021). GL is defined as the average GI of individual foods multiplied by the amount (%) of carbohydrate present in the individual food consumed. It incorporates both the GI and the amount of carbohydrate to estimate the overall impact on blood sugar and insulin resistance.

Maize is the major source of CHO in the diet of Ugandans and other East African Countries. It contributes over 40% of the dietary calories consumed per capita (Wacoo *et al.*, 2018) and several varieties are therefore promoted due to their agronomic performance. More recent estimates confirm that maize remains a leading source of dietary energy consumed per capita in Uganda and the wider East African region (Ekpa *et al.*, 2019; Galani *et al.*, 2022). Despite its significance in the diet, information on the variability of carbohydrate quality in these maize varieties remains unknown. Thus, it remains unclear whether or not maize consumption does not contribute to the increase of state of NCDs being experienced by Ugandan and other East Africa countries communities. This lacuna in knowledge provided the foundation for research conducted and reported in chapter four consistent with specific objective one of this thesis.

2.2.2. Carbohydrate quality and starch digestibility

Starch is the primary storage carbohydrate derived from plant sources. Throughout history, starch has played a crucial role in diets since ancient times, acting as a primary energy source for numerous cultures and bolstering agricultural economies worldwide (Puri *et al.*, 2025). Starch is a vital dietary component, enzymatically hydrolyzed by α -amylase into

glucose during digestion, thereby providing readily available energy for diverse cellular and metabolic functions. However, based on its hydrolysis, its digestibility influences the rate and extent of glucose release into the bloodstream, thereby impacting postprandial glycemia. Starch is a polysaccharide composed of amylose and amylopectin linked by glycosidic bonds. The physical structure and ratio of amylose to amylopectin significantly influence starch digestibility and postprandial glycemia (Lal *et al.*, 2021; Villas-Boas *et al.*, 2019). Amylopectin has a much larger surface area per molecule than does amylose, which makes it more susceptible to enzymatic degradation than amylose (Singh *et al.*, 2010). Therefore, foods with high amylose contents tend to have lower postprandial glucose and insulin levels.

Starch, on the basis of its glucose polymer content and structure, can be categorized into rapidly digestible starch (RDS), slowly digestible starch (SDS), and resistant starch (RS) (Chi *et al.*, 2021). RDS is hydrolyzed to dextrans by α -amylase within 20 minutes and rapidly elevates blood glucose levels, increasing the risk of obesity and type II diabetes. In contrast, SDS is hydrolyzed between 20 and 120 min after ingestion in the small intestine, resulting in gradual glucose release. RS completely escapes digestion in the small intestine, promoting satiety and beneficial gut fermentation (Dupuis *et al.*, 2014; Englyst *et al.*, 1992). Fermentation leads to the production of short-chain fatty acids (e.g. acetic, propionic and butyric acid) that play a protective role in the colon by reducing inflammation, a key factor responsible for many inflammations-associated diseases such as inflammatory bowel diseases, irritable bowel syndrome, type 2 diabetes, obesity, autoimmune disorders or in cancer patients (Markowiak-Kopéc and Slizewska, 2020). SDS and RS both support healthy glucose metabolism in individuals who are healthy or prediabetic, and they hold potential benefits for preventing non-communicable diseases.

RS is categorized into five types based on the response to enzymatic hydrolysis and process: RS1 (physically inaccessible starch in whole grains), RS2 (granules resistant due to conformation, e.g., in raw bananas), RS3 (retrograded starch formed upon cooling gelatinized starch), RS4 (chemically modified starch), and RS5 (amylose–lipid complexes) (Ashwar *et al.*, 2016; Fuentes-Zaragoza *et al.*, 2010; Kaimal *et al.*, 2021). RS3 is the most promoted resistant starch and is prepared by physical treatment (Ma *et al.*, 2020), during

which starch is first gelatinized and then retrograded. This process not only enhances RS formation but also contributes to the development of SDS, thereby improving the nutritional profile of starch-based foods. Looking at the effect of starch digestibility on glucose release in the bloodstream, the development of high-carbohydrate quality foods can be strategically linked to reducing starch digestibility through the enhancement of SDS and RS fractions. Consequently, increasing the proportion of slowly digestible and resistant starch in food formulations represents a promising approach to designing carbohydrate-rich products with improved nutritional and metabolic outcomes.

Despite the significance of starch digestibility for predicting better starch-based foods and the importance of RS3 in making flour healthier through technological processes, research on the starch digestibility of maize varieties in Uganda has been limited. Likewise, the application conditions for enhancing SDS and RS content in these maize varieties using physical starch modification remain underexplored. This gap formed the basis of specific objectives one and two, with Chapter Four addressing starch digestibility and Chapter Five examining heat-moisture treatment (HMT) to respond to this knowledge need.

2.2.3. Factors affecting starch digestibility (carbohydrate quality)

Starch digestibility is influenced by several intrinsic factors, including its molecular composition, granule size, and morphology. Starch granules are mainly composed of amylose and amylopectin, and normally starchy foods contain 20 to 30% amylose and 70 to 80% amylopectin. The amylose/amylopectin ratio affects both the gelatinization and retrogradation of starch. High amylose promotes starch retrogradation, forming a more organized and more rigid crystalline structure and thus high RS3 (Biduski *et al.*, 2018). For example, Yu *et al* (2009) reported that rice with a high amylose content retrogrades more quickly and easily compared to its counterpart and promotes the development of highly resistant starch.

Moreover, the size and morphology of starch granules play a significant role in determining their digestibility. Smaller granules tend to have a higher surface-to-volume ratio, facilitating greater enzyme–substrate interactions and thus increasing digestibility. For example, Singh *et al* (2010) and Kaur *et al* (2007) reported that native starches with a

greater proportion of small granules exhibited higher rates of enzymatic hydrolysis than starches with predominantly large or medium-sized granules.

Three key non-starchy components significantly influence starch digestibility: proteins, lipids, and phenolic compounds. The presence of proteins and lipids on the surface of starch granules plays a significant role in modulating enzymatic hydrolysis. These surface-bound substances can obstruct enzyme access by blocking adsorption sites, thereby delaying enzyme binding and reducing starch digestibility (Singh *et al.*, 2010). In particular, the formation of starch–protein or starch–lipid complexes involves a two-phase digestion process: rapid hydrolysis of amorphous regions, followed by slower degradation of the amylose portion (Annor *et al.*, 2017). This causes hydrolysis over time and requires additional production of enzymes. Phenolic compounds also inhibit digestive enzymes (α -amylase and amyloglucosidase) through chemical interactions, thereby reducing the breakdown of complex carbohydrates into glucose (Barrett *et al.*, 2018). Phenolic compounds interact with starch molecules to form complexes that are relatively resistant to digestion, thus reducing the glycemic impact of carbohydrate-containing foods (Ajayi *et al.*, 2021). Dietary fiber and crude fiber 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 contents often have 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).

As already indicated for the issue of carbohydrate quality, evidence remains inconclusive on the factors influencing starch digestibility in maize in Uganda and their impact on maize varieties, despite its significant contribution to dietary carbohydrate intake in Uganda and other East African countries. This knowledge gap was investigated as part of the specific objective one and reported in chapter four of this thesis.

2.3. Improvement of carbohydrate quality of food

2.3.1. Sources of starch consumed in Sub-Saharan Africa

Worldwide, cereals constitute approximately half of the energy requirements of humans and have been grown as staple foods for human consumption and feeds (Gani *et al.*, 2012). The major cereal grains consumed are wheat, rice, corn (maize), barley, oats, rye, millet,

and sorghum. In Sub-Saharan Africa, maize is the most widely cultivated crop and is a major staple consumed in many forms, including snacks and main dishes (Ekpa *et al.*, 2018). It is the staple food for 24 million households in East and Southern Africa and is planted annually over an area of 15.5 million hectares. Maize accounts for almost half of the calories consumed in Eastern and Southern Africa and one-fifth in West Africa (Tesfaye *et al.*, 2015). Dried maize grain is milled and consumed as a starchy base food in a wide variety of gruels, porridges, soups, and pastes (Badu-Apraku and Fakorede, 2017; Ekpa *et al.*, 2019).

2.3.2. Modification of maize starch to improve CHO quality

Chemical modification, enzymatic modification and physical modification are the most common methods applied to increase starch resistant in cereals.

Chemical modifications replace the hydroxyl groups (–OH) present in starch chains with other chemical substituents, such as carboxyl and acetyl groups (Bojarczuk *et al.*, 2022). Acid hydrolysis, oxidation, etherification, cross-linking, and grafting are examples of the approaches used to modify starch according to chemical methods (Haq *et al.*, 2019; Shaikh *et al.*, 2019). (Haq *et al.*, 2019; Shaikh *et al.*, 2019). Esterification is one of the most commonly used approaches to chemically modify starch and citric, lactic and acetic acids have been used for starch esterification (Mei *et al.*, 2015). The structure of starch macromolecules is changed, which significantly impacts the physicochemical properties of starch (Bojarczuk *et al.*, 2022). Xie and Liu (2004) reported that 78.8% resistant starch was obtained when citric acid (40% of starch dry weight) was reacted with normal corn starch at 140°C for 7 h. Shin *et al* (2007) showed that the SDS fraction of citric acid-treated rice starch increased to 54.1% after heat treatment. However, chemical modification methods are generally expensive because of the high cost of the reactants used (Haq *et al.*, 2019) and are associated with long production times, possible changes in product quality, low reaction rates, and environmental pollution (Bojarczuk *et al.*, 2022).

Enzymatic modifications result in the hydrolysis of starch, resulting in its breakdown into lower-molecular-mass compounds. The role of the enzyme during this modification technique is to break down the α -1,4 bond between two glucose units. Endoamylases, exoamylases, debranching enzymes and transferases are the four groups of starch-

converting enzymes used (Van Der Maarel *et al.*, 2002). Starch debranching enzymes act on the α -1,6 linkages that form the branch points of branched polysaccharides. They are vital in industrial manipulation of starch because of their ability to effectively eliminate the limited dextrin that forms during amylopectin processing and consequently increase the utilization and conversion rate of starch raw material (Xia *et al.*, 2021). Compared with chemical compounds, they are free of toxic residues (Khan *et al.*, 2019). However, enzymes are highly efficient biocatalysts researched at the industrial scale (Bommarius and Paye, 2013; Chapman *et al.*, 2018). Enzymatic starch modification is costly, requires sensitive storage conditions for enzymes that are limited in the African context, and demands technical expertise for enzyme handling (Compart *et al.*, 2023). Moreover, the application of enzymatic starch methods is not feasible in rural areas where starchy foods are more commonly consumed as staple foods (He *et al.*, 2023). Furthermore, enzymatic processes can be time-consuming compared to chemical or physical methods, further reducing their practicality in settings where rapid, large-scale processing is needed (Compart *et al.*, 2023).

Physical methods have no harmful effects on the environment and are economically valuable. Compared with chemically modified starch, the physicochemical properties of starch and its potential applications improved significantly (Bojarczuk *et al.*, 2022; Xie *et al.*, 2018). Physically modified starch is considered natural as opposed to chemically modified starch. Physical methods are more accessible and often less expensive than chemical methods and produce no effluents (Amaraweera *et al.*, 2021). They are safe and innovative methods that improve the functional and technological properties of starch (Molavi *et al.*, 2018). They are generally divided into non-thermal (high-pressure processing, micronization, ultra-sonication, and pulse electric field) and thermal processes, including hydrothermal processes, which are most commonly used (heat–moisture treatment (HMT) and annealing) (Amaraweera *et al.*, 2021; Fonseca *et al.*, 2021).

Annealing and HMT are the most commonly used physical methods effective in altering starch properties, such as relative crystallinity, water absorption capacity, and pasting properties, maintaining the molecular integrity of starch (Fonseca *et al.*, 2021). During ANN starch granules in excess (>60% w/w) or at intermediate water content (40% w/w)

are held at a temperature above the glass transition temperature (T_g) but below the onset (T_o) temperature of gelatinization for a set period of time (Chung *et al.*, 2009). HMT is also a physical modification technique that involves treatment of starch granules at low moisture levels (<35% moisture w/w) for a certain time period (15 min–16 h) and at temperatures (84–120°C) above T_g but below the gelatinization temperature (Chung *et al.*, 2009). In addition, the use of HMT and annealing methods promotes thermal stability for starches, promising for the development of products that are exposed to high temperatures during production (Fonseca *et al.*, 2021). They are safe and innovative methods that improve the functional and technological properties of starch for industrial purposes (Molavi *et al.*, 2018).

Several studies have demonstrated the potential use of HMT and annealing in increasing SDS and RS in cereal pearl millet (Sandhu *et al.*, 2020), sorghum (Vu *et al.*, 2017; Singh *et al.*, 2011), and normal maize (Zou *et al.*, 2020; Chung *et al.*, 2009). HMT has shown more promising results than annealing for the promotion of RS and SDS in cereals (Dong *et al.*, 2021; Van Hung *et al.*, 2020). However, most of the applications of HMT applied to cereals focused on rice, extracted maize starch or waxy maize whereas studies remain limited in the case of normal maize (Asare *et al.*, 2021; Chang *et al.*, 2020; Liu *et al.*, 2014; Sui *et al.*, 2015). Waxy maize is a specialized maize whose endosperm starch is nearly all amylopectin, thus has low retrogradation tendency, and easy digestion in human gut whereas normal maize contains substantial amylose (Guo *et al.*, 2023). In addition, the normal maize is the whole kernel containing fiber and proteins whereas extracted maize starch is the refined, pure white carbohydrate separated from the kernel's endosperm (Li *et al.*, 2025). Because amylose strongly influences starch rearrangement, enzyme accessibility and digestion, and fiber and proteins affect digestibility, a starch modification developed in waxy maize and extracted maize starch will therefore not be effective in normal maize (Xu *et al.*, 2024). Above all, the influence of varietal differences has largely remained unexplored. This lacuna in knowledge provided the foundation for research conducted and reported in chapter five consistent with specific objective two of this thesis.

2.3.3. Methods applied to optimize HMT

HMT is a physical starch modification method that enhances starch functionality and decreases starch digestibility through the manipulation of three critical parameters: moisture content, treatment duration and temperature. Several studies have shown that HMT impacts starch digestibility according to variations in moisture content (Schafranski *et al.*, 2021; Sui *et al.*, 2015), treatment duration (Lin *et al.*, 2019), temperature (Yassaroh *et al.*, 2019) and the botanical origin of starch (Sun *et al.*, 2014). The optimization of these parameters is therefore essential for decreasing starch digestibility for a specific starch source.

HMT is strongly dependent on its botanical origin because starches from different sources differ in the intrinsic characteristics that govern their reorganization during treatment. Granule size and morphology, the amylose-to-amylopectin ratio, amylopectin chain-length distribution, the type of crystalline polymorph (A-, B- or C-type), and the amounts of associated lipids, proteins and phosphorus all vary with botanical source, and these differences determine how readily the starch gelatinises, retrogrades and forms enzyme-resistant structures during heat-moisture treatment (Schafranski *et al.*, 2021; Sun *et al.*, 2014). For example, cereal starches such as maize are predominantly A-type and comparatively low in phosphate, whereas tuber starches such as potato are B-type and phosphate-rich, so the same moisture, temperature and time combination produces different increases in slowly digestible and resistant starch across sources. Within a single species, differences in amylose, total starch and phenolic content among varieties further modulate this response, which is why optimum heat-moisture treatment conditions cannot simply be transferred from one starch source, or one variety, to another and must be established on a source- and variety-specific basis (Qi and Tester, 2016; Sun *et al.*, 2017). Researchers often apply response surface methodology (RSM) via designs such as central composite design (CCD) (Iuga and Mironeasa, 2021) and Box–Behnken design (BBD) for HMT optimization (Dai *et al.*, 2019; Deng *et al.*, 2022; Ye *et al.*, 2016) (Appendix 1). CCD and BBD had the advantage to predict responses based on few sets of experimental data in which all factors are varied within a chosen range (Jideani *et al.*, 2010). A CCD is defined as a five-level fractional factorial design that uses external points and one point at the center of the experimental region. The total number of needed design points (N) is determined by

the formula $N = 2^k + 2k + C_0$, where k is the number of factors and C_0 is the replication number of center points. Although CCD, a five-level factorial design, is ideal for modeling both linear and quadratic interactions and typically provides higher R^2 values and lower standard errors, it requires many experimental runs, making it relatively costly (Gündoğdu *et al.*, 2016). In contrast, BBD requires fewer data points ($N = 2k(k - 1) + C_0$), making it more cost-effective while still offering reliable prediction (Dutta, 2013; Ferreira *et al.*, 2007). Its structure avoids extreme experimental conditions, reducing the chance of unsatisfactory outcomes.

Ferreira *et al* (2007), Rakić *et al* (2014) and (Gündoğdu *et al.*, 2016) reported some advantages of BBD design. BBD is a three-level fractional factorial design developed by Box and Behnken that is applied to determine the nature of the response surface in an experimental region. This design has several advantages, such as having three levels that can be coded as -1 (low), 0 (middle) and +1 creating an independent quadratic design and providing an easier way to arrange and interpret the results. BBD is a class of rotatable or nearly rotatable second-order designs based on three-level incomplete factorial designs. The number of experiments (N) required for the development of BBD is defined as $N = 2k(k - 1) + C_0$, (where k is the number of factors and C_0 is the number of central points). Another advantage of BBD is that it reduces the number of additional experiments and eliminates the time-consuming and laborious laboratory studies required by the complete factorial design (Yetilmezsoy *et al.*, 2009).

Given its effectiveness, numerous studies have employed RSM specifically BBD for HMT condition optimization in cereals products (Dai *et al.*, 2019; Deng *et al.*, 2022; Gayary and Mahanta, 2020; Iuga and Mironeasa, 2021; Ye *et al.*, 2016). Despite this, a gap exists in its application to enhance resistant starch in maize flour typical of polished maize flour consumed in Uganda and other East African countries. This gap formed the basis for specific objective two and the research was carried out and reported in chapter five of this PhD thesis.

2.4. Effect of HMT on physicochemical, techno-functional and sensory properties of derived product characteristics

Several studies have investigated the impact of HMT and showed that HMT affects physicochemical, techno-functional, and sensory properties of starches, and highlighted that these changes vary according to the starch source even within the same crop with different varieties (Kaur and Singh, 2019). Therefore, studies applying HMT to starchy food should comprehensively assess its effects on these parameters to better guide their suitability and performance in food applications.

- ***Effect on derived product physicochemical composition***

In terms of physicochemical properties of starch food, heat-moisture treatment generally increases the apparent amylose content, which impacts starch digestibility and functionality (Dong *et al.*, 2021; Goel *et al.*, 2020; Marta *et al.*, 2022; Sun *et al.*, 2013). The increase in amylose results from the increase in amylose-amylose and amylose-amylopectin interactions, leading to more structured starch granules (Gunaratne *et al.*, 2010; Sun *et al.*, 2013). In addition, HMT influences starch color properties, a key quality indicator of food acceptability. The lightness (L^*) decreases, while the redness (a^*) and yellowness (b^*) increase, which is likely due to Maillard reactions between reducing sugars and amino groups during treatment (Fathi *et al.*, 2016; Kim *et al.*, 2017; Sindhu and Devi, 2019). These changes can affect product appearance and consumer acceptance and are therefore important to consider when HMT is applied in food product design (Chung *et al.*, 2012). However, HMT alters the physicochemical properties of starch without modifying its basic chemical composition, namely, its protein, fat, ash and total starch contents (Marta *et al.*, 2022).

- ***Effect on derived product functional properties***

In regard to the functional properties of a starchy food, several studies have reported that HMT decreases the swelling and solubility of cereals such as sorghum (Olayinka *et al.*, 2008), maize (Sui *et al.*, 2015) and rice (Hormdok and Noomhorm, 2007). These reductions are attributed to molecular rearrangements, increased crystallinity, and stronger interactions between the amylose and amylopectin chains (Goel *et al.*, 2020; Gunaratne *et*

al., 2010). These changes limit water penetration and starch granule expansion, leading to lower starch gelatinization and reduced enzyme accessibility, making starch less digestible. HMT significantly influences the water and oil absorption capacities (WAC and OAC) of starches, often enhancing their functional characteristics for food applications. A consistent finding across multiple studies is that WAC increases, whereas OAC decreases following HMT (Goel *et al.*, 2020; Liu *et al.*, 2016; Sindhu and Devi, 2019). This trend is attributed to structural modifications within the starch granules, particularly the disruption of hydrogen bonds between the amorphous and crystalline regions and the expansion of the amorphous domains, which improve hydrophilicity and reduce hydrophobic interactions. These alterations make HMT-treated starches more suitable for specific food processing purposes where enhanced hydration and reduced oil uptake are desirable.

- ***Effect on derived product pasting properties***

Pasting properties are supposed to provide information on intermolecular bonding between starch granules and the rigidity of swollen starch granules during the heating process (Sui *et al.*, 2015; Vandeputte *et al.*, 2003). Pasting temperature, peak viscosities, paste viscosity, final viscosities, breakdown and setback are the analysis of the main parameters. HMT has been demonstrated to affect the pasting properties of starchy food. In particular it has been shown to increase the pasting temperature, whereas the peak, breakdown, setback, and final viscosities decreased with respect to indica rice starch (Sun *et al.*, 2013), sorghum starch and flour (Sun *et al.*, 2014) and rice flour (Kim *et al.*, 2017). These changes affect starch functionality differently: (i) an increase in the pasting temperature suggests an increase in thermal stability and prolonged cooking time, which can influence formulation performance and energy costs (Awoyale *et al.*, 2022); (ii) a decrease in setback viscosity suggests a reduction in retrogradation and an increase in resistance to syneresis (Adebowale *et al.*, 2005; Sindhu and Devi, 2019); and (iii) a decrease in the final viscosity suggests a low capacity of starch to form viscous pastes (Asaam *et al.*, 2018). These imply that the modified starch or flour may gelatinize less readily when reconstituted with boiling water (Awoyale *et al.*, 2021). Finally, a decrease in peak viscosity suggests limited swelling and lower paste thickness, which may be advantageous for applications requiring softer textures and reduced gel strength (Awoyale *et al.*, 2021).

In summary, HMT affects starch pasting properties, reflecting changes in gelatinization behavior and granule integrity. These parameters should be carefully analyzed post-HMT to evaluate starch performance and functional applications in food systems. According to Balet *et al* (2019), the Rapid Visco Analyser (RVA) was developed by the Bread Research Institute of Australia in collaboration with CSIRO's Wheat Research Unit for pasting property analysis. It is a heating and cooling viscometer that measures the viscosity of a sample over time while stirring. The test runs through five stages: adding water to the sample, heating, holding at maximum temperature, cooling, and final holding stage (Figure 2.1).

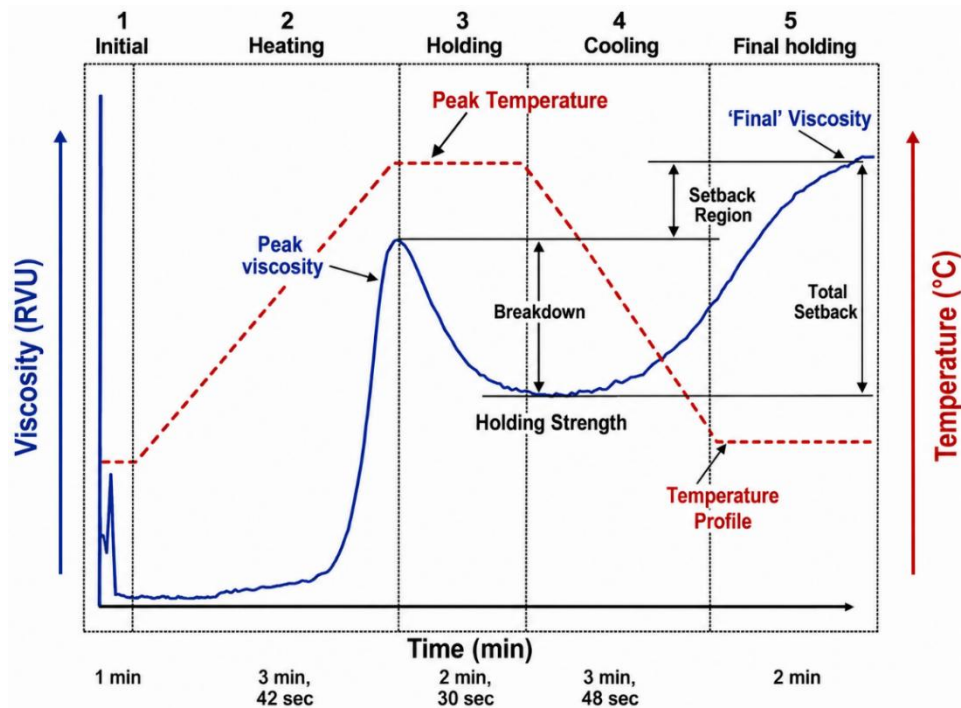


Figure 2. 1. A typical RVA pasting profile of a cereal (using the standard profile), indicating the main parameters measured during analysis (Balet *et al.*, 2019)

In the first stage, sample and solvent are combined in a canister placed into the RVA above ambient temperature, then mixed. This allows hydration, as water moves into the starch granules and binds to other components like protein. The heating stage simulates cooking and pasting appears as a sudden rise in viscosity, and the temperature at which this rise begins is recorded as the pasting temperature (PT) (Bemiller, 2011), indicating the minimum temperature needed to cook a flour. The highest viscosity reached during heating

is the peak viscosity (PV), occurring when swollen granules cause pasting (Balet *et al.*, 2019). PV reflects the starch's water-holding capacity and often correlates with other quality traits. During the holding stage, viscosity drops, and the curve flattens. Faster heating causes quicker granule swelling, letting more granules peak before breakdown (BD) begins. Breakdown viscosity is the difference between peak viscosity and holding strength (HS), the lowest point reached during holding (Lin *et al.*, 2023). The cooling stage that follows is known as setback (SB), where viscosity rises again as granules cool and retrogradation occurs. Setback viscosity is the difference between peak viscosity and final viscosity. The last stage holds temperature constant while viscosity continues rising until it plateaus, known as final viscosity (FV), or viscosity at the end of the pasting cycle (Vidaurre-Ruiz *et al.*, 2024).

- ***Effect on derived product thermal properties***

The thermal properties of starch are useful to understand the phenomenon of starch gelatinization and retrogradation, in particular the temperature range of gelatinization, and the structure of lipid amylose complexes. It is usually measured using the differential calorimeters method. Thermal transitions of starchy foods for gelatinization are generally characterized by their T_o (onset temperature), T_p (peak temperature), T_c (conclusion temperature), and DH (enthalpy of gelatinization). Several studies have shown that HMT generally enhances starch thermal stability and can reduce retrogradation tendencies (Adebowale *et al.*, 2005). For example, HMT increases the onset, conclusion, and gelatinization range while reducing the enthalpy of gelatinization in maize (Marta *et al.*, 2022), sorghum (Sun *et al.*, 2014), and rice flour (Kunyanee and Luangsakul, 2022). These increases in thermal transition points can be attributed to structural reorganization within the starch granule, particularly increased interactions between amylose chains and between amylose and lipids, which reduce chain mobility in the amorphous lamellae (Klein *et al.*, 2013). Overall, HMT alters starch granular architecture, enhancing thermal resistance while modifying gelatinization energy requirements.

- ***Effect on derived product sensory characteristics***

Although sensory quality is one of the most important indicators affecting consumer decision-making, HMT affects the sensory quality of modified starch-containing foods.

Kim *et al.* (2017) reported that although the color and flavor of HMT brown rice cakes improved with increasing HMT intensity, the hardness and overall acceptability scores decreased. Fathi *et al* (2016) reported that increasing HMT levels in millet flour reduced the taste, odor, and visual appeal of gluten-free cakes, particularly at 100% substitution and higher thermal treatments, which yielded denser, darker, lower-volume cakes. Despite these drawbacks, texture scores remained high, and general acceptance was neutral or better. Cahyana *et al* (2020) reported that substituting HMT-treated banana flour (25–75%) in biscuits led to reductions in all key sensory attributes, especially color and taste, underscoring the sensitivity of these properties to HMT treatment. The decline in sensory quality, particularly in color and flavor, has been attributed to Maillard reactions and thermal processes during HMT, leading to the formation of color pigments (Kim *et al.*, 2017).

The foregone literature has revealed that HMT affects food sensory properties differently depending on starchy food sources. However, a critical scan of literature on HMT revealed limited information on the effect on sensory quality characteristics of maize. Considering that sensory appeal is a key factor that influences acceptability of food, demonstrating the effect of HMT on sensory acceptability of maize becomes key if HMT technology is to be promoted to improve carbohydrate quality of maize. Physicochemical and techno-functional factors influence the sensory quality of food (Khan *et al.*, 2021; Saleh *et al.*, 2019). This implies that changes in sensory quality attributed to HMT are likely to be due to changes in physicochemical and techno-functional properties. Thus, understanding the effects of HMT on sensory properties of maize would require understanding the changes in physicochemical and techno-functional properties. This consolidated the basis for objective three and the research work conducted and reported in chapter six of this thesis.

2.5. Effect of polishing on physiochemical, functional and organoleptic characteristics of starchy foods

The polishing or refining is a common processing technique used in the food industry to improve the appearance and quality of starchy foods such as rice, wheat, and corn. It consists of removing the outer bran layer of cereals, resulting in grains with a smoother texture and more uniform appearance, which are preferred by consumers for their improved

palatability and visual appeal (Rao *et al.*, 2024; Sinkovič *et al.*, 2023). Polishing can have a significant impact on the physiochemical, functional, and organoleptic characteristics of starchy foods, which can ultimately affect consumer acceptance and overall product quality. It improves sensory qualities such as texture, appearance, and taste by making grains smoother, whiter, and less fibrous. This often enhances consumer acceptance and palatability, which are key organoleptic factors (Paiva *et al.*, 2016).

Polished cereals reduce the cooking time of cereal products and thus increase their acceptability. According to Paiva *et al.* (2016), the polishing of black and red rice decreased the cooking time by 37.0% and 48.4%, respectively. Similarly, Ojediran *et al.* (2020) reported that polishing reduced the cooking time of paddy rice from 21 minutes to 19 minutes, with further reductions to 15 minutes as the degree of polishing increased. The reduction in cooking time may be attributed to the physical modification of the grain caused by polishing, as the thick aleurone layer and pericarp in unpolished cereals can impede water penetration during cooking (Paiva *et al.*, 2016). Consequently, polished rice grains absorb water more readily during heating and hydration, thereby facilitating starch gelatinization and reducing the time required for cooking.

Polishing also improve the techno-functional properties of the cereals. Compared with unpolished flour, polished flour has greater swelling power. For example, in the Kullan Thondi rice variety, polished flour demonstrated a swelling power of 15.28 ± 2.07 , whereas the swelling power of unpolished flour was 12.27 ± 0.12 . This increase is attributed to structural changes during polishing, which reduce the fiber and protein contents, allowing starch granules to swell more readily during gelatinization (Wani, 2023; Yu *et al.*, 2024). Polishing reduces the water absorption capacity (WAC), as evidenced by the higher WAC of unpolished rice flour (4.0 ± 0.52) compared with that of polished flour (3.8 ± 0.87). The removal of bran and germ layers during polishing diminishes the flour's ability to bind water, as these components contain hydrophilic compounds such as fibers and proteins (Wani, 2023; Yu *et al.*, 2024) that are essential for water binding and subsequent effect on organoleptic characteristics and the derived products.

Although polishing significantly improves the techno-functional properties of maize grain, it can substantially reduce its nutritional and phytochemical value. Nutrients and bioactive

compounds are not uniformly distributed throughout the cereals (maize) kernel; rather, many are concentrated in the outer layers, including the pericarp, aleurone layer, and germ. Consequently, polishing removes these nutrient-rich fractions, resulting in a reduction in the concentrations of several nutrients and phytochemicals, while proportionally increasing the starch content of the remaining endosperm (Oghbaei and Prakash, 2016). The major compositional differences between whole maize grain and its polished form are therefore associated with the removal of dietary fibre and fibre-associated components such as phytic acid, tannins, polyphenols, as well as substantial amounts of minerals and some vitamins (Blandino *et al.*, 2017; Suri and Tanumihardjo, 2016). During polishing, the progressive removal of these outer fractions consequently results in a refined grain fraction that is relatively enriched in starch but depleted in essential nutrients and bioactive compounds and this decrease with the increase of polishing degree (Oghbaei and Prakash, 2016).

Whereas previous literature has demonstrated that HMT can influence the physicochemical, techno-functional, and sensory acceptability properties of cereals, literature showed that polishing can enhance these characteristics. This provided the basic for specific objective four and guided the research conducted and reported in chapter seven of this thesis.

CHAPTER THREE

METHODOLOGY

This chapter provides an overview of the methodology employed to address each specific objective, as well as the ethical approval and considerations associated with the study. It is organized according to the specific objectives and presents a summary of the samples used, the parameters analyzed, the methods applied, and the approaches used for data analysis.

3.1. Overview of methods applied

This study followed a multi-phase approach across four chapters (4 to 7) to assess the carbohydrate quality of thirteen maize varieties commonly consumed in Uganda, optimize HMT for four selected varieties, and evaluate the individual and combined effects of HMT and polishing on the physicochemical, techno-functional, and sensory attributes of maize flours (Table 3.1). Each chapter was built on the findings from the previous chapter.

The first objective, characterization of the CHO quality and state of factors affecting starch digestibility of popular maize varieties consumed in Uganda, necessitated a broad initial screening. Accordingly, thirteen maize varieties were characterized using a standardized analytical method. Starch fractions were quantified through simulated *in vitro* gastrointestinal digestion (Englyst *et al.*, 1992), while the factors potentially influencing starch digestion, such as fat, protein, ash, amylose, total starch, and phenolic compounds, were determined using standard methods (Table 3.1). One-way analysis of variance (ANOVA) was used to assess differences among varieties, while principal component analysis (PCA) and hierarchical cluster analysis (HCA) were applied to the combined dataset to characterize relationships among varieties and identify the compositional variables. The multivariate analyses transformed the initial descriptive characterization into a selection framework, providing an objective basis for selecting four varieties for further investigation. Thus, the reduction in the number of varieties from thirteen in Chapter 4 to four in Chapters 5–7, as presented in Table 3.1, represents a deliberate progression in the experimental design rather than a methodological limitation.

Response surface methodology specifically Box-Behnken design was employed in Chapter 5 for Heat Moisture Treatment optimization. The combined content of slowly digestible and resistant starch was used as the response variable to be maximized, and linear and quadratic models with or without interaction were evaluated based on model fit, predictive accuracy, and statistical significance before establishing the optimum conditions. Chapters 6 and 7 applied these validated conditions to assess additional changes in flour properties following the reduction in starch digestibility. Chapter 6 compared treated and untreated flour of the same variety using paired-sample t-tests, whereas Chapter 7 evaluated three polishing levels within a variety using one-way analysis of variance.

Table 3.1: Overview of the methods applied during the study data collection and analysis

Chapter	Number of Samples	Data collection method	Statistical analysis
Chapter 4: Carbohydrate quality and state of factors affecting starch digestibility of popular maize varieties consumed in Uganda	Thirteen (13) maize varieties most grown and consumed were considered	Starch digestibility: simulated in vitro gastro-intestinal digestion (Englyst <i>et al.</i> , 1992) and Megazyme procedure with some modifications (Neogen Cooperation, 2023)	One-way ANOVA
		Carbohydrate quality - Glycemic index: hydrolysis index of the starch and the linear model of Goñi <i>et al.</i> (1996) (Caballero-Rothar <i>et al.</i>, 2022). - Carbohydrate to fiber ratio: differential method for carbohydrate content - Weende method was used for crude fiber determination as described by Musembi <i>et al.</i> (2024)	One-way ANOVA
		Factors affecting starch digestibility - Fat content: Extraction using Soxhlet method (Afolayan <i>et al.</i>, 2022) - Protein : Nitrogen using micro-Kjeldahl AOAC (2015) (Afolayan <i>et al.</i>, 2022) - Ash content: gravimetric method of AOAC (2005) - Amylose content: iodine calorimetry method (Babatola <i>et al.</i>, 2021) - Total starch: sedimentation method of Goñi <i>et al.</i> (1996) - Phenolic compounds: colorimetric method applied by Kayodé <i>et al.</i> (2007)	- One-way ANOVA. - Principal component analysis (PCA) and hierarchical cluster analysis were performed to assess the relationships among the varieties.

Table 3.1: Overview of the methods applied during the study data collection and analysis (*Continuous*)

Chapter	Number of Samples	Data collection methods	Statistical analysis
Chapter 5: Optimization of heat–moisture treatment to decrease starch digestibility of polished maize flour from predominant varieties consumed in Uganda	Four (04) maize varieties selected based on the findings of chapter 4 were considered	• Response surface methodology (Box-Behnken design) was used to set the experimental design with moisture (20–30%), temperature (80–100°C), and heating (4–8 hours). • HMT was carried out following the laboratory protocol proposed by Sui <i>et al</i> (2015) • Maize grains treated using HMT were polished using a maize huller (Locally Fabricated, Gulu, Uganda) following the two common polishing steps typically used in Uganda	--
		Laboratory work • Composite SDS and RS (SDS+RS) were used in the optimization. • SDS and RS were determined according to the simulated in vitro gastro-intestinal digestion according to the method of Englyst <i>et al.</i> (1992) and using the Megazyme procedure (Neogen Cooperation, 2023)	• Shapiro and Levene tests were used to assess normality and homogeneous • Linear, interaction, and quadratic models were used, and appropriate models were identified using the goodness of fit, the predictive power of the model, and the global significance • A paired sample t test was used to compare the experimental results with the predictable value

Table 3.1: Overview of the methods applied during the study data collection and analysis (*Continuous*)

Chapter	Number of Samples	Data collection methods	Statistical analysis
Chapter 6 Effect of optimized heat–moisture treatment conditions on physicochemical and functional properties of maize flour	Four (04) maize varieties selected based on the findings of chapter 4 were considered	Design - Optimal HMT conditions for higher SDS and RS contents determined in chapter 5 for each variety was used - HMT was carried out under these conditions for each maize grain variety following the laboratory protocol proposed by Sui <i>et al</i> (2015)	--
		Laboratory work Physicochemical characteristics - Ash, fat content, protein, carbohydrate, amylose, amylopectin and total starch contents were determined according to the methods enumerated in chapter 4. - Color characteristics were measured using a Minolta CR-400 (Chávez-Murillo <i>et al.</i>, 2018). Functional properties - Swelling volume and solubility: sedimentation volume method Marta <i>et al.</i> (2022) - Water absorption capacity: differential thermogravimetric method (Cahyana <i>et al.</i>, 2019) - Oil absorption capacity: differential weight gain method (Ashwar <i>et al.</i>, 2016) - Bulk density: volumetric method (Adebiyi <i>et al.</i>, 2016) - Pasting properties: Rapid Visco Analyzer was used (Marta <i>et al.</i>, 2022, Nuwamanya <i>et al.</i>, 2023)	A paired sample t-test was employed to compare treated and untreated samples within the same variety.

Table 3.1: Overview of the methods applied during the study data collection and analysis (*End*)

Chapter	Number of Samples	Data collection methods	Statistical analysis
Chapter 7 Impact of polishing grade on physicochemical, functional, starch digestibility, and sensory properties of heat-moisture-treated flours	Four (04) maize varieties selected based on the findings of chapter 4 were considered	Design - Optimal HMT conditions and the HMT procedure described in chapter 5 were used - Polished: first, second and third classes were produced using a maize huller and following the common polishing steps typically applied in Uganda	-
		Laboratory work Physicochemical characteristics: ash, fat content, protein, carbohydrate, amylose, amylopectin and total starch contents were determined according to the methods enumerated in chapter 4 while color characteristics were determined according to the method described in chapter 6 Starch digestibility: simulated gastrointestinal in vitro digestion method of Englyst <i>et al.</i> (1992) applied by Cahyana <i>et al.</i> (2019) was used. Functional properties: swelling volume and solubility, water absorption capacity, oil absorption capacity, bulk density and pasting properties were determined according to the methods enumerated in chapter 6 Sensory analysis: derived posho from the treated flours and sensory parameters, including elasticity, hardness, color, flavor, and taste (Arnés <i>et al.</i>, 2022; Callejo, 2011) were determined using the five points hedonic scale method (Lawless and Heymann, 2010)	- Shapiro and Levene tests were used to assess the normality and homogeneity (equal variance) of the data - One-way analysis of variance (ANOVA) was used to compare the effect of polishing within the varieties

3.2. Ethical Clearance and consideration

The study was approved by the Gulu University Research Ethics Committee (Approval number: GUREC-2023-507) and the Uganda National Council for Science and Technology (Approval A338ES) (Appendix 2). Upon ethical clearance, consideration was made during the study to adhere to ethical principles. First, before maize sample collection, authorization was sought from the Chief Administrative Officer of each district where the samples were collected. The maize grain samples were collected following local and national guidelines for the use of plant products in research experiments, which complied with the requirements of Gulu University Research Ethics Committee and Uganda National Council for Science and Technology. As the samples were supposed to be used for a sensory acceptability test, they were kept in conditions that avoided any contamination during the transport and storage. The maize samples were stored in PICS (Purdue Improved Crop Storage) bags composed of two polypropylene bags and stored in the laboratory until use. Otherwise, during the sensory acceptability test, permission to conduct the study was sought before and only the participants willing to participate in the study were considered. Signed consent was obtained from the participants before their enrollment in the study (Appendix 3).

CHAPTER FOUR

Carbohydrate quality and state of factors affecting starch digestibility of popular maize varieties consumed in Uganda

This chapter is adapted from the published manuscript below:

Fassinou, F. T. K., Olum, S., & Ongeng, D. (2026). Assessing starch digestibility and carbohydrate quality of Ugandan maize varieties: implications for diet-related non-communicable diseases risk. *Cogent Food & Agriculture*, 12(1), 1–20.
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4.1. Abstract

Consumption of low-quality carbohydrate food resources is a risk factor for the development and progression of diet-associated non-communicable diseases. In Uganda, maize is a major dietary carbohydrate source consumed by 92% of the households. However, its carbohydrate quality is largely unknown and as such it remains obscure as to whether or not it is a dietary carbohydrate-source risk factor for diet-associated non-communicable diseases in the country. This study examined the carbohydrate quality (in terms of carbohydrate-to-fiber ratio and estimated glycemic index) and the contents of associated intrinsic physicochemical factors (carbohydrate, lipid, protein, amylose, amylopectin, total phenolic compounds) that influence starch digestibility for thirteen (13) maize varieties commonly consumed in Uganda. In general, the maize varieties examined had a carbohydrate-to-fiber ratio ranging from 5.33 to 10.32 and exhibited high glycemic indices in the range of 79.38 to 86.27, indicating low carbohydrate quality. The physicochemical factors that influence starch digestibility varied significantly across varieties ($p < 0.05$). Hierarchical cluster analysis grouped the varieties into four significantly different clusters based on the level of chemical factors that influence starch digestibility ($p < 0.05$). The cluster composed of varieties DT MAX, Longe 5D, and WE3106 had the best matrix combination of physicochemical characteristics associated with better starch resistant while the cluster composed of varieties UH5051, UH5053, and UH5354 had the reverse. This study has revealed that maize varieties consumed in Uganda have low carbohydrate quality which suggests that their consumption may be a risk factor for diet associated NCDs in the country and surrounding countries. A concerted effort is necessary to improve the carbohydrate quality of the maize varieties.

4.2. 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 carbohydrate storage (Niaz *et al.*, 2020). They are found in cereals, tubers, legumes, vegetables, fruits, seeds, leaves, and rhizomes (Bojarczuk *et al.*, 2022) and provide 4 kcal/g of dietary energy, accounting for 40–75% of total energy intake, depending upon the population (Lal *et al.*, 2021). However, throughout the past decades, the amount and type of dietary carbohydrates required for health have been debated (Reynolds *et al.*, 2019). This is because carbohydrates are increasingly being implicated in 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. Carbohydrate 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 preventing diseases. The consumption of a significant amount of low-quality carbohydrate 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). Conversely, Reynolds *et al.* (2019) reported that consuming high-quality carbohydrate foods can reduce the prevalence of non-communicable diseases by 15 to 30%.

Several indices have been proposed for assessing the carbohydrate quality of food. They include glycemic index (GI), glycemic load, and fiber content of whole grains (Hardy *et al.*, 2020). The GI is a measure of the ability of the available carbohydrates 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) that 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 relatively high 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 is believed to assist in the management of weight loss and diabetes and lower the risk of other non-communicable diseases. Therefore, assessing the carbohydrate quality of commonly consumed foods has become an important strategy for monitoring nutrition-related health risks for populations and assisting in identifying, guiding, and promoting healthier food choices.

Maize accounts for nearly half of the calories consumed in East Africa (Macauley, 2015). In Kenya, maize and its products constitute 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 more than 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 products (Caballero-Rothar *et al.*, 2022; Chauhan and Singh, 2020, 2021; Ge *et al.*, 2023). Consequently, frequent consumption of highly processed maize products raises public health concerns because of their potential impact on blood glucose levels and associated non-communicable disease risk. Despite this high consumption of maize, information on its carbohydrate quality is limited. Instead, literature is largely available on studies that focused on improving starch quality through several methods, mainly for isolated maize 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 scanty. In addition, varietal effects on carbohydrate quality analysis are rarely considered and yet several maize varieties are available. This shows the need for selection of varieties with good carbohydrate quality.

Maize is consumed in various countries in Africa; however, 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 on the basis of their yield, drought tolerance, resistance to major biotic stresses, responsiveness to inputs, and

good nitrogen use (Simtowe *et al.*, 2019). In addition, the country supplies large quantities of maize grains to all East African countries. Despite the high level of maize production and consumption in Uganda, to the extent that it exports excess amounts to neighboring East African countries and the importance of carbohydrate quality in starch food assessment, there are few 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 and aimed to understand the factors that affect starch digestibility to identify candidate maize varieties that could help prevent diet-related non-communicable disease risks.

4.3. Materials and methods

4.3.1. Materials

Up to 86 varieties of maize are reported to be 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 were considered for the present study (Appendix 4). 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 that the samples reflected the natural characteristics as commonly consumed by households. Each maize variety was collected from at least three farmers in the regions where they are most cultivated, following the guidance of the seed companies (the most sold maize grain varieties in their respective regions). The seed companies guided the selection of farmers based on their distribution records and knowledge of the varieties grown by their customers, thereby enhancing the traceability and authenticity of the grain varieties collected.

Maize samples were collected from farmer fields across three agroecological zones (AEZ) of Uganda, specifically the Central, Eastern, and the Northern, where farmers cultivated

under natural conditions and applied agronomic practices until harvest. The maize varieties DK777 and DK9089 both released by Monsanto were collected from the Central Region, while Longe 5D, UH5053, and UH5355, all released by National Agricultural Research Organisation (NARO) were collected from the Eastern Region. The varieties KH 500 43A released by East African Seed, Longe 5, Longe 10H, MM3, UH5051, UH5354 and WE3106 released by NARO and DT Max released by Nalweyo Seed Company (NASECO) were purchased from the Northern Region. Central AEZ experiences a tropical climate with average temperatures generally ranging from 20.8°C to 29.5°C, annual rainfall typically falls between 700 and 950 mm, and the soil pH is between 5.6 and 6.5 (Mwangu *et al.*, 2025; Nsubuga and Rautenbach, 2018). In Eastern AEZ, temperatures range from 20.0 to 28.8°C, with the hottest months occurring from December to February. Rainfall is bimodal, with a pattern varying between 800–1100 mm, and the soil pH ranges between 5.4 and 6.8, which is moderately acidic and suitable for a wide range of crops, including maize (Lugoi *et al.*, 2022; Nsubuga and Rautenbach, 2018). For Northern AEZ, temperatures range from 21.5 to 30.2°C, and rainfall is a unimodal regime, varying between 650 and 850 mm, with most rainfall occurring from April to November (Atube *et al.*, 2022). Soil pH ranges between 5.5 and 6.8. All the maize grain samples were sampled from the harvested stock from December 2022 to February 2023.

Following sample collection, foreign matter, dirt, and damaged grains were removed, and the grains were sorted and cleaned. For laboratory analyses, the maize grains were milled under laboratory conditions using a laboratory blender 8010EG Model HGBTWTG4 (Torrington, Connecticut 06,700, USA) and sieved through a 100 µm sieve such that the granulometry of the whole maize grain flour sold on the local market was similar to that of the whole maize grain flour. Passing the ground material through a 100-micrometre sieve produced a flour with a particle size below 100 micrometres, which approximates the fine granulometry of the polished maize flour predominantly milled and consumed by households in Uganda and other East African countries. This ensured that the analyzed samples were representative of the flour typically eaten. The flour was packed in paper bags and stored under laboratory conditions that reflected the ambient environment of the Gulu area, Uganda. Specifically ground materials were stored in a clean, dry, and well-ventilated room with temperatures below 25°C and a relative humidity under 65%

throughout the 10–15 weeks of storage to minimize moisture absorption, microbial proliferation, and nutrient deterioration prior to analysis.

Megazyme Digestible and Resistant Starch Assay Kit (K-DSTRS) was purchased from Megazyme (Megazyme, Wicklow, Ireland). The enzymes used in the experiments were purchased from Sigma–Aldrich Co. (St. Louis, MO, USA). All the chemicals were of molecular and analytical grade and were purchased from Merck KGaA (Darmstadt, Germany).

4.3.2. Determination of in-vitro starch digestibility

The starch digestibility procedure was conducted following simulated in vitro gastrointestinal digestion according to the method of Englyst *et al.* (1992) and using the Megazyme procedure with some modifications (Neogen Cooperation, 2023). In fact, rather than extending the starch digestibility assay to 240 minutes for the estimation of the resistant starch fraction as proposed by the Megazyme procedure, the assay was limited to 120 minutes as applied by Cheng *et al.* (2022) and Kraithong *et al.* (2023). The RS content was subsequently derived by subtracting the determined RDS and SDS fractions from the total starch content according to the formula applied by Cahyana *et al.* (2019).

Before analysis, two enzyme working solutions were prepared: a pancreatic α -amylase/amyloglucosidase (PAA/AMG) solution and an amyloglucosidase (AMG) solution. PAA (40 KU/g) and AMG (17 KU/g) solutions were prepared by adding 0.5 g of the PAA/AMG powder mixture to 25 mL of 50 mM sodium maleate buffer, pH 6.0. The PAA/AMG solution was stirred on a magnetic stirrer for 5 min and stored on ice until used. The AMG solution was prepared by adding 1 mL 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 used. The reagents were prepared immediately before use.

The maize flour sample (1 g) was placed into 50 mL polypropylene screw-cap tubes and mixed with 1.0 mL 95% v/v ethanol and 35 mL sodium maleate buffer (50 mM, pH 6.0). The mixture was submerged in a magnetic stirred Wagtech OLS Model 200 water bath (Grant Instruments, Germany) to equilibrate at 37°C for 5 min at 170 rpm. Then, 5 mL of the PAA/AMG enzyme mixture prepared as described above was added, and the mixture

was incubated in a magnetic stirred Wagtech OLS Model 200 water bath (Grant Instruments, 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, and 120 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 via a Centurion Scientific Benchtop Model 1. C2015 centrifuge (Centurion Scientific Ltd, United Kingdom). After centrifugation, a duplicate aliquot (0.1 mL) was removed, and 0.1 mL of AMG enzyme solution prepared as described above was added and mixed well. 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 D-glucose standards were prepared by mixing 100 µg of D-glucose with 0.1 mL of 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 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 a Jenway 6305 UV/Visible spectrophotometer (Jenway, United Kingdom). 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 4.1.

$$\text{RDS or SDS} = (\Delta A * F * EV) / (W * 0.0189) \quad (\text{Eq. 4.1})$$

Where, ΔA For RDS (ΔA^{RDS}) is the absorbance of the reaction read against the reaction blank after 20 min; SDS (ΔA^{SDS}) is the absorbance of the reaction read against the reaction blank after 120 min minus the ΔA^{RDS} ; F is the conversion from absorbance to µg (the absorbance obtained for 100 µg of D-glucose in the GOPOD reaction was 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 the content of RS was determined and the glycemic index was estimated, the total starch contents of the maize flour and white bread samples were determined. The white bread was used as it replicates the actual, real-world consumption of carbohydrates and provides a more accurate physiological simulation of how starches are broken down

compared to a simple sugar solution (Domínguez-soberanes *et al.* 2026; Torres *et al.* 2024). The total starch content of each of the 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 effective extraction of reducing sugars from the sample. Then, 2 mL of 2 M potassium hydroxide solution was added and stirred with a magnetic bar (5×15 mm) for 20 min. After this stage, 8 mL of 1 M sodium acetate solution (pH 3.8) was added, followed by 3 mL of thermostable α -amylase (0.5%). The mixture was incubated at 95°C for 10 min in magnetic stirred Wagtech OLS Model 200 water bath (Grant Instruments, 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 $4000 \times g$ for 10 min using Centurion Scientific Benchtop Model 1. C2015 centrifuge (Centurion Scientific Ltd, United Kingdom). The sample (0.1 mL) was collected in a 10 mL glass tube to determine the glucose content following the phenol–sulfuric acid method of Dubois *et al.* (1956) as applied by Dhillon *et al.* (2018) and read with a Jenway 6305 UV/Visible spectrophotometer (Jenway, United Kingdom) at 490 nm. The soluble starch solution was used as the standard solution for calibration curve generation. The percentage of total starch was calculated via multiplication by a factor of 0.9 after the glucose content was obtained (Wang *et al.*, 2022). The amount of resistant starch (RS) in the products was subsequently calculated according to the formula of Cahyana *et al.* (2019) as follows $RS (\%) = TS - (RDS + SDS)$ where TS represents the total starch in each maize flour.

4.3.3. Determination of carbohydrate quality

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

4.3.3.1. Determination of the estimated glycemic index

The estimated glycemic index was determined according to the hydrolysis index (HI) of the starch using the linear model of Goñi *et al.* (1996) as applied by Caballero-Rothar *et al.* (2022). The hydrolysis index (HI) of the starch was derived from the in vitro starch digestibility procedure described by Yaman *et al.* (2019). According to Costantini *et al.* (2024), in-vitro starch method mimicking in vivo human digestion are simple, reliable, accurate, fast, and cost-effective for predicting and estimating the glycemic response to food and can be largely used in the evaluation of the predicted GI of starchy products. During this 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 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 (Appendix 5). The AUC was determined via the trapezoid method for different time intervals (Pautong *et al.*, 2022). Owing to the linearity between the hydrolysis index (HI) and glycemic index (GI), the predicted GI (pGI) was estimated via equation 4.2 of Goñi *et al.* (1996).

$$\text{pGI} = 39.71 + (0.549 * \text{HI}) \quad (\text{Eq 4.2})$$

4.3.3.2. Determination of carbohydrate-to-fiber ratio

Before the carbohydrate–fiber ratio could be determined, the proximate composition of the maize samples was analyzed to determine the carbohydrate content via the difference method. The ash content was determined using the gravimetric method of AOAC (2005). The fat content was determined by the Soxhlet method using a solvent extraction system (Soxtec® Avanti 2050 Auto System, Foss Tecator AB, Höganäs, Sweden) with petroleum ether as the solvent according to the AOAC (2005). The micro-Kjeldahl AOAC (2015) method applied by Afolayan *et al.* (2022) was used for nitrogen determination via a Kjeldahl digester (VELP Scientifica, Model A00000176, Italy) and a Kjeldahl distiller (VELP Scientifica, Model F30200140, Italy). As most proteins contain 16% N, a conversion factor of 6.25 (100/16= 6.25) was used to convert the percentage of nitrogen to the percentage of crude protein. The carbohydrate (CHO dry weight) content was determined by a differential method according to equation 4.3 below.

$$\text{CHO (\%, db)} = 100\% - \text{Protein (\%, db)} - \text{Lipid (\%, db)} - \text{ash (\%, db)} \quad (\text{Eq. 4.3})$$

Following the determination of the carbohydrate content, the Weende method was used for crude fiber 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 of fresh sample (W_1) 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 the addition of 5 drops of octan-1-ol (antifoam). The samples were each boiled for 30 min at 100°C after being prepared up to the point of boiling using the fiber analyzer. After the sulfuric acid was removed, the samples were washed 3 times in hot deionized water (30 mL per wash). The procedure above was repeated, but 1.25% sulfuric acid was replaced with a 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 (W_2). This was followed by 3 h of ashing in a muffle furnace at 550°C and reweighing (W_3). Equation (4.4) was used to calculate the percentage of crude fiber.

$$\text{Crude fiber (\%)} = ((W_2 - W_3)/W_1) * 100 \quad (\text{Eq. 4.4})$$

However, crude fiber analysis using the Weende method involves the determination of only cellulosic compounds such as lignin, hemicellulose, and pectin and the insoluble fiber content (da-Silva and Walter, 2012). As maize grains have an average of 92.6% (91.75–93.45%) insoluble fiber, which is typically determined through the Weende method, and 7.4% soluble fiber (Lasek *et al.*, 2020; da Silva and de Lourdes, 2005), the dietary fiber represented by both soluble and insoluble fiber was estimated using equation 4.5 and the estimated dietary fiber was used for estimation of carbohydrate-to-estimate dietary-fiber ratio determination.

$$\text{Estimated dietary fiber (\%)} = \text{Crude fiber} * 1.074 \quad (\text{Eq. 4.5})$$

4.3.4. Determination of factors affecting starch digestibility

In this study, carbohydrates, lipids, proteins, fibers, total starch, amylose, amylopectin, and total phenolic compounds were analyzed as factors affecting starch digestibility (Cai *et al.*, 2015). The carbohydrate, lipid, protein, fiber and total starch contents were determined according to the procedures described in sections 4.3.2 and 4.3.3.2. Therefore, this sub section focuses exclusively on the determination of amylose, amylopectin, and phenolic compounds.

4.3.4.1. Determination of amylose and amylopectin contents

The amylose content of each maize variety was determined using the iodine calorimetry method applied by Babatola *et al.* (2021), which estimates the apparent amylose content (Zavareze *et al.*, 2011). Although this method measures apparent rather than absolute amylose content because some long external chains of amylopectin may also bind iodine, it remains one of the most widely used methods for starch analysis due to its simplicity, reproducibility, and suitability for comparative evaluation of starch samples (Wang *et al.*, 2024). The method is particularly valuable for screening and comparing crop varieties based on their starch characteristics and has been extensively applied in food quality evaluation, agricultural breeding, and nutritional studies (Park *et al.*, 2020; Y. Zou *et al.*, 2021). Since the objective of the present study was to compare the intrinsic starch characteristics of different maize varieties rather than determine their absolute amylose content, the iodine-binding method was appropriate and provided a consistent basis for evaluating varietal differences. Future studies aimed at absolute quantification of amylose or detailed starch structural characterization may complement these findings by employing more specific analytical techniques, such as enzymatic methods or iodine affinity analysis of defatted whole starch (Halim *et al.*, 2023)

Prior to amylose determination, the maize starch was initially extracted from the maize flour using the method previously applied by Nuwamanya *et al.* (2024). One 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 (to avoid underestimation of the amylose content (Neogen Corporation, 2024)), and mixed with 1 N of NaOH (9.2 mL). The mixed sample was heated in a magnetic stirred water bath (Wagtech OLS, Model 200, Germany) at 100°C for 10 min and allowed to cool. Approximately 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 brought to 10 mL with distilled water, allowed to stand for color development and read with a Jenway 6305 UV/Visible spectrophotometer (Jenway, United Kingdom) at 620 nm. Potato amylose was prepared in 1, 5, 10, 20 and 35% ethanol and NaOH (1 N) and used as a standard amylose solution. Standard curve construction procedure applied to the samples was also applied to the standard, and the absorbance was read at 620 nm using a Jenway 6305 UV/Visible spectrophotometer (Jenway, United Kingdom). The developed standard curve was used to calculate the amylose content in the maize flour samples. The amylopectin content was calculated by subtracting the amylose content from 100.

4.3.4.2. Determination of total phenolic compounds

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 5,000 × g for 10 min using Centurion Scientific Benchtop Model 1. C2015 centrifuge (Centurion Scientific Ltd, United Kingdom), after which the supernatant was removed and re-centrifuged. 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, United Kingdom). Blanks were freshly prepared, in which Folin–Ciocalteu's reagent was replaced with water to correct for interfering compounds. Because the Folin–Ciocalteu reagent measures total reducing capacity, non-phenolic reducing substances such as reducing sugars, ascorbic acid and some proteins can interfere with the assay (Dominguez-lópez *et al.*, 2024; Lawag *et al.*, 2023). This interference was minimised by

extracting phenolics in acidified methanol (1% v/v HCl–methanol), which reduces the co-extraction of interfering compounds while enhancing extraction efficiency (Petreska Stanoeva *et al.*, 2020). In addition, reagent blanks, in which the Folin–Ciocalteu reagent was replaced with water, were used to correct for background absorbance, and the results were expressed as gallic acid equivalents (GAE) to account for any residual non-phenolic reducing capacity (Singleton *et al.*, 1999).

Gallic acid was used as the reference standard, with results expressed as gallic acid equivalents (GAE) (Bastola *et al.*, 2017; Singleton *et al.*, 1999). Although the assay response varies with the structure of the phenolic compounds present, expressing results as GAE provides a standardized basis for comparing the total phenolic components across samples (Pérez *et al.*, 2023; Platzter *et al.*, 2021). This approach was therefore considered appropriate for estimating overall phenolic content rather than individual constituents, which could be characterized in future work using mixed phenolic standards (Bastola *et al.*, 2017) and or another standard for quantifying the specific phenols present in these samples (Sunanta *et al.*, 2023). Gallic acid was used as a standard and was calibrated at 0, 50, 100, 200, 300 and 500 µg/mL using 1% (v/v) HCl/methanol. A standard curve was generated, and the content of phenolic compounds in the maize flour was estimated and expressed as gallic acid equivalent (GAE) per 100 g dry weight of the sample.

4.4. Statistical analysis

All analyses were carried out in triplicate, except for the starch digestibility analysis, which was carried out in duplicate. Thus, the average values and standard deviations of the determined parameter (means $\pm$ SD) were calculated. One-way analysis of variance (ANOVA) was used to determine the significant differences in the estimated parameters among the various varieties and the differences between the clusters. Tukey's multiple comparison method was used as a post hoc test in the case of any significant difference. A significant difference level of 5% was used. Multivariate analyses, specifically principal component analysis (PCA) and hierarchical cluster analysis, were performed to assess the relationships among the varieties. PCA was computed to determine the established quality traits within varieties using the composition parameters and the FactoMineR package (Khan *et al.*, 2021). Maize varieties with similar characteristics were classified using the

plug-in heatmap with dendrogram, a major statistical method for finding homogeneous groups of cases on the basis of their 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 dendrogram validation. All the analyses were performed using R software version 4.2.2.

4.5. Results and discussion

4.5.1. In vitro starch digestibility

Starch digestibility was determined in terms of the proportions of rapidly digestible starch (RDS), slowly digestible starch (SDS), and resistant starch (RS) in the maize samples. RDS, SDS and RS varied significantly ($p < 0.05$) among the maize varieties (Table 4.1) and the difference in digestibility status among maize varieties could be related to differences in the factors affecting starch digestibility, which also varied significantly across varieties (Table 4.3). The RDS fraction ranged from 27.80% to 39.12% for the UH5355 and DT Max varieties, respectively, whereas the RS content ranged from 9.90% in the DK777 variety to 16.68% in the UH5355 variety. The combined content of slow digestible starch (SDS) and resistant starch (RS), referred to as SDS+RS, ranged from 61% to 72%. This level of RDS is within the proportion reported by Camelo-Méndez *et al.* (2017) for white maize flour (33%), which is lower than the proportion reported by Giuntini *et al.* (2022) for maize flour (49%) and Utrilla-Coello *et al.* (2011) for maize bar flour. The relatively low content of rapidly digestible starch (RDS) of these maize varieties may offer dietary benefits in preventing non-communicable diseases.

Overall, variety DT Max had the highest RDS and thus the lowest SDS+RS content, whereas variety UH5355 had the lowest RDS and the highest SDS+RS. The maize variety UH5355 has the highest RS (16.68%) and SDS+RS (72.19%). Therefore, if all other factors remain constant, the UH5355 maize variety may represent a suitable candidate for dietary interventions aimed at preventing non-communicable diseases. However, relying solely on starch digestibility fractions may not be sufficient for determining the quality of maize flours. Considering the overall carbohydrate quality of the maize varieties, as it looks beyond starch digestibility and considers aspects such as dietary fiber content and the

glycemic index, offers a more comprehensive assessment of the potential health benefits that can be derived from consuming a high-quality carbohydrate maize variety.

Table 4.1: Starch digestibility fraction of 13 maize varieties sampled from Uganda

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

All values are means ± Standard Deviation. Means with different letters in the same column differ significantly ($P \leq 0.05$). RDS: rapidly digestible starch; SDS: slowly digestible starch; RS: resistant starch.

4.5.2. Carbohydrate quality

Diets of low carbohydrate quality characterized by a high glycemic index, and low fiber content have been found to increase the prevalence of obesity, increase 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 a $\leq 10:1$ carbohydrate-to-dietary fiber ratio for the promotion of 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). Carbohydrate quality of the maize samples was estimated using two parameters: the carbohydrate-to-crude fiber ratio and the estimated glycemic index (Table 4.2). The carbohydrate-to-crude fiber ratios varied significantly ($p < 0.05$) among the different maize varieties. The carbohydrate-to-crude fiber ratio ranged from 5.28 to 10.15, and the lowest ratio was observed for the MM3 variety,

whereas the highest value was found for WE3106. Twelve of the thirteen examined maize varieties met the recommended carbohydrate-to-fiber ratio of 10:1. Only variety WE3106 slightly exceeded this ratio, with a value of 10.15. These findings suggest that many of the maize varieties that are commonly consumed in Uganda have acceptable carbohydrate quality based on the carbohydrate-to-crude fiber ratio. As fiber has the potential to reduce blood cholesterol levels, increase glucose tolerance, and decrease the glycemic response (Giuntini *et al.*, 2022), while keeping other factors constant, the consumption of these maize varieties can be promoted to control the development of metabolic diseases.

Furthermore, the study also applied more stringent carbohydrate-to-fiber ratios $\leq 8:1$ and $\leq 5:1$ (Mello *et al.*, 2019) to evaluate varieties with higher fiber content. Among the thirteen maize varieties investigated, seven of them (DK777, DK9089, DT Max, KH 500 43A, MM3, Longe 5D, and UH5051) fulfilled the recommended $< 8:1$ carbohydrate-to-fiber ratio, whereas none met the stricter $\leq 5:1$ ratio when the crude fiber content was used. Seven varieties, namely, DK777, DK9089, DT Max, KH 500 43A, Longe 5D, UH5051, and MM3, had ratios between five and eight. The ratios for Longe 5, Longe 10H, UH5354, UH5053, and UH5355 varieties were greater than 8 but less than 10. Therefore, it is advisable to prioritize consumption of the seven varieties over the six varieties that have a ratio higher than 8:1. The ratios for the varieties DK777, DK9089 and DT Max had almost similar values, that ranged between 5.37 and 5.70. The ratios for varieties Longe 10H, UH5053, UH5354 and UH5355 varied between 8.21 and 8.56 and were similar ($p>0.05$).

The current study assessed fiber content using the Weende method, which involves sequential acid and alkali digestion and quantifies only the indigestible dietary fiber fraction, which is referred primarily to as insoluble fiber (da-Silva and Walter, 2012). However, considering the role of soluble fiber in conferring various health benefits, including its ability to control glycemia and manage 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 and Agriculture Organization of the United Nations, 1992), the present study estimated total dietary fiber contents for these maize varieties. On the basis of these estimations, the carbohydrate to

dietary fiber ratios ranged from 4.85 to 9.38, with MM3 and DK777 exhibiting values below 5, whereas all other maize varieties except WE3106 had ratios between 5 and 8, and WE3106 having the highest ratios (9.38). This range falls below the 10:1 threshold recommended by the American Heart Association, suggesting nutritionally favorable 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 enzymatic digestion in the small intestine and reduces blood glucose and postprandial insulin levels following consumption (Giuntini *et al.*, 2022). Its typical low content in cereals, particularly in maize (Bader Ul Ain *et al.*, 2019; Food and Agriculture Organization of the United Nations, 1992; Lasek *et al.*, 2020), could explain the high glycemic index of the maize varieties examined and yet they have an acceptable carbohydrate quality according to the carbohydrate-to-fiber ratio. Maize varieties studied exhibited high glycemic index values ranging from 79.38% to 86.27% (≥ 70). The range of glycemic index values observed is consistent with previous findings reported by Caballero-Rothar *et al.* (2022), who showed values ranging from 75.6 to 81.0 for eight varieties of maize, and Nurjanah *et al.* (2020), who reported a value of 85.02 for maize isolated starch. However, the observed glycemic index values are lower than the value of 89.4 reported by Chauhan and Singh (2020) for maize grains, the value of 91.9 reported by Tovar *et al.* (2003) for maize tortillas. However, the observed values were higher than the in-vivo glycemic index level of 71.4 reported by RamyaBai *et al.* (2019) for maize flour. The discrepancy may be due to the choice of reference, white bread commonly used. This is because white bread is familiar, easy to prepare, and has a relatively high and consistent rate of starch hydrolysis and serves as an effective benchmark. However, its formulation can vary across regions and manufacturers, thereby influencing its digestibility and resulting glycemic response (Hettiaratchi *et al.*, 2012).

In summary, despite the acceptable quality of the maize varieties in terms of their carbohydrate-to-fiber ratio, they could contribute to an increase in blood glucose levels due to their high glycemic index. This discrepancy indicates that the carbohydrate-to-fiber ratio alone is insufficient to comprehensively assess carbohydrate quality. Although a

carbohydrate-to-fiber ratio below 10:1 reflects a favorable nutritional composition by indicating adequate dietary fiber, it does not capture the physiological response to carbohydrate consumption (Tan *et al.*, 2023). In contrast, the glycemic index directly measures the rate at which carbohydrates are digested, absorbed, and converted into blood glucose, thereby integrating the effects of starch digestibility, amylose-to-amylopectin ratio and starch structure (Ngo *et al.*, 2023). Despite their adequate fiber content, the high glycemic index of these maize varieties suggests that their starch is rapidly digested, leading to a rapid release of glucose that outpaces the moderating effect of dietary fiber. Therefore, for a starchy food with an acceptable carbohydrate-to-fiber ratio ($\leq 10:1$), but with a high glycemic index ($GI > 70$), the glycemic index should be given greater weight when evaluating carbohydrate quality. Despite the carbohydrate-to-fiber ratio remains a useful indicator of the nutritional composition of maize, while not accounting for the type of fiber, the glycemic index should be given greater weight when evaluating carbohydrate quality in relation to metabolic health because it more accurately reflects the physiological impact of carbohydrate consumption. Accordingly, the maize varieties evaluated in this study can be considered to have poor carbohydrate quality because of their high glycemic index.

Compared with other varieties, DK777, DK9089, and DT Max, that had the lowest carbohydrate-to-fiber ratios (5.37, 5.52, and 5.70, respectively), also exhibited low estimated glycemic indices (80.02, 81.37, and 80.45, respectively). These results indicate a strong correlation between high fiber content and a low glycemic index. On the other hand, variety WE3106, which had the highest carbohydrate-to-crude fiber ratio, did not have, by contrast, the highest estimated glycemic index value (81.14). The present study evaluated glycemic index of the maize flour through an in-vitro method. However, the results revealed the same trend as published results of in-vivo tests, although absolute values of in-vitro test tended to be greater than results of in-vivo tests (Afandi *et al.*, 2021). In addition, studies have reported that roasting maize and boiling maize flour, which are typically applied in African countries, can decrease the glycemic index. Chauhan and Singh (2021) reported that boiling and roasted maize can decrease the glycemic index of raw maize by 8.58% and 3.99%, respectively. Therefore, future studies could investigate the

effects of processing on the glycemic response or blood glucose level of maize varieties through in-vivo tests.

Table 4.2: Carbohydrate-to-fiber ratios and glycemic indices of 13 maize varieties sampled from Uganda and consumed in East Africa

Maize varieties	Ratio of Carbohydrate-to-Crude Fiber	Ratio of carbohydrate to estimated dietary fiber *	Estimated glycemic index (%)	ESI classification
DK777	5.37 ^{cd} ± 0.24	4.93 ^{cd} ± 0.22	80.02 ^{ef} ± 0.87	High GI
DK9089	5.52 ^{cd} ± 0.46	5.07 ^{cd} ± 0.43	81.37 ^{cdef} ± 0.48	High GI
DT Max	5.70 ^{cd} ± 0.10	5.24 ^{cd} ± 0.10	80.45 ^{ef} ± 0.05	High GI
KH 500 43A	6.19 ^{bcd} ± 0.32	5.69 ^{bcd} ± 0.30	81.52 ^{cdef} ± 0.32	High GI
Longe 10H	8.56 ^{ab} ± 0.73	7.90 ^{ab} ± 0.68	87.43 ^a ± 1.09	High GI
Longe 5	8.65 ^{ab} ± 0.69	7.98 ^{ab} ± 0.64	79.38 ^f ± 1.64	High GI
Longe 5D	6.47 ^{bcd} ± 1.50	5.95 ^{bcd} ± 1.39	81.38 ^{cdef} ± 1.40	High GI
MM3	5.28 ^d ± 0.13	4.85 ^d ± 0.11	81.77 ^{cdef} ± 1.32	High GI
UH5051	7.78 ^{abc} ± 1.42	7.17 ^{abc} ± 1.33	83.35 ^{bcd} ± 0.21	High GI
UH5053	8.35 ^{ab} ± 0.18	7.71 ^{ab} ± 0.17	84.65 ^{abcd} ± 0.73	High GI
UH5354	8.48 ^{ab} ± 1.27	7.82 ^{ab} ± 1.18	86.27 ^{ab} ± 0.09	High GI
UH5355	8.21 ^{ab} ± 0.88	7.57 ^{ab} ± 0.82	84.80 ^{abc} ± 0.91	High GI
WE3106	10.15 ^a ± 0.93	9.38 ^a ± 0.86	81.14 ^{def} ± 0.27	High GI

All values are means ± Standard Deviation. Means with different letters in the same column differ significantly ($P \leq 0.05$). *: The 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 the total dietary fiber (Lasek et al., 2020); ESI: Estimated glycemic Index.

4.5.3. State of factors that affect the carbohydrate quality of maize

Proximate composition, total starch, amylose and amylopectin, and phenolic compound contents were analyzed as factors affecting starch digestibility of maize (Table 4.3). Only the crude fat and ash contents were similar ($p > 0.05$) across all the varieties. Level of other parameters varied significantly ($p < 0.05$) with variety, and the trend of the variation differed depending on the parameter.

Proximate composition of maize varieties (g/100 g dw) varied from 3.09 to 4.95 for fat, 7.21 to 10.14 for protein, 7.66 to 13.39 for fiber, and 70.30 to 77.38 for carbohydrates. These values are within those reported for different maize varieties in Nigeria and Ethiopia

by Ndukwe *et al.* (2015) and Demeke (2018). The amylose content recorded for the maize varieties studied varied between 20.57% and 27.75%, which are in line with most maize varieties, which contain 25% amylose (fraction of total starch content). The phenolic compounds content varied from 180.25 to 222.64 mg GAE/100 g dry weight, a range higher than that reported by Lopez-martinez *et al.* (2009) for white maize (170 mg GAE/100 g dry weight) and lower than the range reported by Žilić *et al.* (2012) (522.71–57777.82 mg GAE/100 g dw) for colored Maize. Traditionally, resistant starch 5 (RS5) has been classified as a type of starch–lipid V-type complex. 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, and starch-other polysaccharides, are classified as RS5 (Gutiérrez and Tovar, 2021). The new information in other elements that constitute RS5 suggests that starch digestibility decreases with increasing parameters that starch interacts and can potentially promote the development of RS5. In this context of the current study, insight from the findings of literature and Gutiérrez and Tovar (2021) provides an indication that Ugandan maize varieties that have the highest protein and phenolic compound contents would have low starch digestibility compared to varieties with reverse scenarios.

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, offering beneficial effects against non-communicable diseases. According to the study conducted by Kwasnny *et al.* (2022), an increase in phenolic compounds has a statistically significant effect on the development of resistant starch in wheat starch gels. For example, Barros *et al.* (2012) reported that sorghum, which is rich in phenolics, namely, tannins, had a significantly lower starch digestibility than sorghum without tannins because of its tannin composition. Phenolic compounds extracted from various plants such as oregano, raspberry and rowanberry have been shown to inhibit porcine pancreatic α -amylase activity.

It is largely known that proteins reduce starch digestibility through the formation of complexes with amylose, which resists enzymatic degradation (Kim *et al.*, 2016). In particular zein, the principal storage protein found in maize, creates a coat with starch granules, enabling a core–shell structure that restricts water and enzyme access (Wang *et al.*, 2022). This reduces starch swelling and gelatinization during digestion and thus starch digestibility (Xu *et al.*, 2019). Based on these mechanisms, it becomes apparent that high levels of protein and phenolic compounds in Ugandan maize varieties such as DT Max decrease starch digestibility and lead to improvements in carbohydrate quality. Longe 10H variety has the lowest protein content and second lowest phenolic content. These properties potentially make the variety capable of releasing high amounts of soluble starch after hydrolysis.

In contrast, DT Max variety has the highest protein content, the second highest phenolic content, an intermediate fiber content and the second-highest amylose content. These properties combined have the potential to promote resistant starch formation during the cooking process of maize compared to other varieties. Combining DT Max's characteristics of low carbohydrate-to-fiber ratio and estimated glycemic index (Table 4.2) with others that decrease the release of starch during digestion, makes it a promising maize variety that should be promoted for consumption to prevent non-communicable diseases in Uganda. However, these analyses are based on isolated analyses of the parameters. The results from principal component analysis (PCA) and hierarchical cluster analysis (HCA), which are two main approaches widely used for classification in the field of food research (Yu, 2005), should be further considered to guide decisions on the varieties to promote.

Table 4.3: Factors affecting starch digestibility in 13 maize varieties sampled from Uganda (per 100 g dry weight)

Maize varieties	Dry matter (%)	Ash (g/100 g dw)	Fat (g/100 g dw)	Protein (g/100 g dw)	Fiber (g/100 g dw)	Estimated dietary fiber (g/100 g dw)	Carbohydrates (g/100 g dw)
DK777	88.17 ^{gh} ± 0.13	1.24 ^b ± 0.11	4.85 ^a ± 0.27	8.74 ^{bc} ± 0.42	13.39 ^a ± 0.46	14.38 ^a ± 0.05	71.79 ^{cd} ± 0.70
DK9089	87.20 ^h ± 0.18	1.41 ^{ab} ± 0.26	4.01 ^{abc} ± 0.10	9.47 ^{ab} ± 0.23	13.09 ^a ± 0.90	14.06 ^a ± 0.97	72.03 ^{cd} ± 0.94
DT Max	90.50 ^{bc} ± 0.17	1.39 ^{ab} ± 0.32	4.08 ^{abc} ± 0.28	10.14 ^a ± 0.42	12.60 ^{ab} ± 0.21	13.54 ^{ab} ± 0.22	71.79 ^{cd} ± 0.49
KH 500 43A	91.05 ^{ab} ± 0.13	1.30 ^{ab} ± 0.14	4.10 ^{abc} ± 0.89	8.08 ^{cd} ± 0.42	12.06 ^{abc} ± 0.57	12.95 ^{abc} ± 0.61	74.46 ^{abc} ± 0.48
Longe 10H	89.31 ^{def} ± 0.22	2.64 ^a ± 1.00	3.09 ^c ± 0.32	7.21 ^e ± 0.23	9.15 ^{de} ± 0.78	9.83 ^{de} ± 0.84	77.90 ^a ± 0.53
Longe 5	90.34 ^{bcd} ± 0.12	1.86 ^{ab} ± 0.30	3.58 ^{bc} ± 0.32	8.36 ^{cd} ± 0.25	8.97 ^{de} ± 0.63	9.63 ^{de} ± 0.68	77.23 ^a ± 0.61
Longe 5D	89.00 ^{efg} ± 1.21	1.43 ^{ab} ± 0.11	4.50 ^{ab} ± 0.15	9.30 ^{ab} ± 0.24	11.65 ^{abcd} ± 2.22	12.51 ^{abcd} ± 2.38	73.12 ^{bcd} ± 2.40
MM3	88.56 ^{fg} ± 0.16	1.39 ^{ab} ± 0.17	4.95 ^a ± 0.11	9.95 ^a ± 0.11	13.33 ^a ± 0.25	14.32 ^a ± 0.27	70.38 ^d ± 0.46
UH5051	88.33 ^{fg} ± 0.19	1.54 ^{ab} ± 0.24	3.08 ^c ± 0.32	9.37 ^{ab} ± 0.25	9.97 ^{bcde} ± 1.58	10.70 ^{bcde} ± 1.70	76.04 ^{ab} ± 1.39
UH5053	90.56 ^{bc} ± 0.09	1.64 ^{ab} ± 0.56	4.36 ^{ab} ± 0.13	8.92 ^{bc} ± 0.42	9.10 ^{de} ± 0.23	9.78 ^{de} ± 0.24	75.98 ^{ab} ± 0.88
UH5354	91.95 ^a ± 0.28	2.23 ^{ab} ± 0.89	4.77 ^a ± 0.53	7.60 ^{de} ± 0.11	9.10 ^{de} ± 1.04	9.78 ^{de} ± 1.12	76.30 ^{ab} ± 2.47
UH5355	92.15 ^a ± 0.14	1.34 ^{ab} ± 0.15	3.56 ^{bc} ± 0.27	8.76 ^{bc} ± 0.09	9.43 ^{cde} ± 0.84	10.13 ^{cde} ± 0.91	76.90 ^a ± 1.22
WE3106	89.85 ^{cde} ± 0.15	1.99 ^{ab} ± 0.53	4.15 ^{abc} ± 0.30	8.81 ^{bc} ± 0.25	7.66 ^e ± 0.66	8.23 ^e ± 0.70	77.38 ^a ± 0.51

All values are means ± Standard Deviation. Means with different superscript in the same column differ significantly ($P \leq 0.05$), GAE: gallic acid equivalent. dw: dry weight.

Table 4.3: Factors affecting starch digestibility in 13 maize varieties sampled from Uganda (per 100 g dry weight)

Maize varieties	Total starch (% of TS)	Amylose (% of TS)	Amylopectin (% of TS)	Phenolic compounds (GAE dw)
DK777	63.01 ^e ± 0.44	24.17 ^{bcd} ± 0.23	75.83 ^{bcd} ± 0.23	197.07 ^{bcdef} ± 8.15
DK9089	67.00 ^d ± 2.46	22.51 ^{cde} ± 0.32	77.49 ^{abc} ± 0.32	189.81 ^{def} ± 9.51
DT Max	67.44 ^d ± 1.24	26.32 ^{ab} ± 0.94	73.68 ^{de} ± 0.94	216.45 ^{ab} ± 7.00
KH 500 43A	75.69 ^{abc} ± 0.28	27.59 ^a ± 0.64	72.41 ^e ± 0.64	180.25 ^f ± 2.63
Longe 10H	75.82 ^{abc} ± 1.22	22.83 ^{cde} ± 0.22	77.17 ^{abc} ± 0.22	183.72 ^{ef} ± 2.68
Longe 5	67.68 ^d ± 1.20	22.54 ^{cde} ± 0.44	77.46 ^{abc} ± 0.44	187.74 ^{ef} ± 4.59
Longe 5D	68.12 ^d ± 0.16	24.90 ^{bc} ± 0.09	75.10 ^{cd} ± 0.09	210.78 ^{abc} ± 7.12
MM3	73.97 ^c ± 0.88b	24.55 ^{bc} ± 0.34	75.45 ^{cd} ± 0.34	185.29 ^{ef} ± 10.82
UH5051	75.58 ^{bc} ± 2.43	21.98 ^{de} ± 1.56	78.02 ^{ab} ± 1.56	203.00 ^{abcde} ± 2.71
UH5053	79.50 ^a ± 0.12	20.47 ^e ± 0.32	79.53 ^a ± 0.32	193.38 ^{cdef} ± 2.64
UH5354	76.11 ^{abc} ± 1.64	24.86 ^{bc} ± 0.16	75.14 ^{cd} ± 0.16	208.45 ^{abcd} ± 10.41
UH5355	78.12 ^{ab} ± 1.21	24.63 ^{bc} ± 0.37	75.37 ^{cd} ± 0.37	181.10 ^f ± 6.88
WE3106	74.51 ^{bc} ± 0.08	23.70 ^{cd} ± 2.05	76.30 ^{bc} ± 2.05	222.64 ^a ± 5.33

All values are means ± Standard Deviation. Means with different superscript in the same column differ significantly ($P \leq 0.05$), GAE: gallic acid equivalent. dw: dry weight.

4.5.4. Clustering of maize varieties based on quality parameters that affect starch resistant

Principal component analysis (PCA: Figure 4.1) was employed to classify the 13 maize varieties on the basis of the factors that impact their starch digestibility (Table 4.3). This procedure reduces the dimensionality of the data matrix while retaining most of the information of the original data and explains the relationships among objects and the correlation structure of the variables. The first and second principal components (PCs) explained 45.47% and 21.12% of the variance, respectively (Supplementary material 3). Thus, the cumulative contribution rate of the first two PCs accounts for 66.59% of the total variance, indicating that the first two plots explain more of the data than the other combinations do. The distance between the locations of two maize varieties in the score plot (Figure 4.1a) is directly proportional to the degree of difference or similarity between these varieties. Figure 4.1b, which represents the plot of the second principal component (PCA), provides valuable insights into the correlation 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 had high amylopectin contents (Figure 4.1a). The varieties Longe 10H, UH5355, and UH5354 are positioned in the lower left and had high carbohydrate and total starch contents. KH 500 43A was the only variety located in the lower right quadrant and had a high content of amylose. On the other hand, the maize varieties DK777, DK9089, MM3, DT Max, and Longe 5D were clustered together in the upper right and were strongly correlated with the phenolic compound, protein, fiber, and fat contents, which are all factors that contribute to lower starch digestibility and thus high carbohydrate quality (Ayua *et al.*, 2021; Brennan, 2024). Accordingly, when other factors remain constant, these varieties have the potential to elicit a lower postprandial glucose response than others do, thereby contributing more significantly to the prevention of diet related NCDs. In addition, variety KH 500 43A was strongly correlated with high amylose content, a key driver of starch retrogradation. Given its elevated amylose content, KH 500 43A 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.

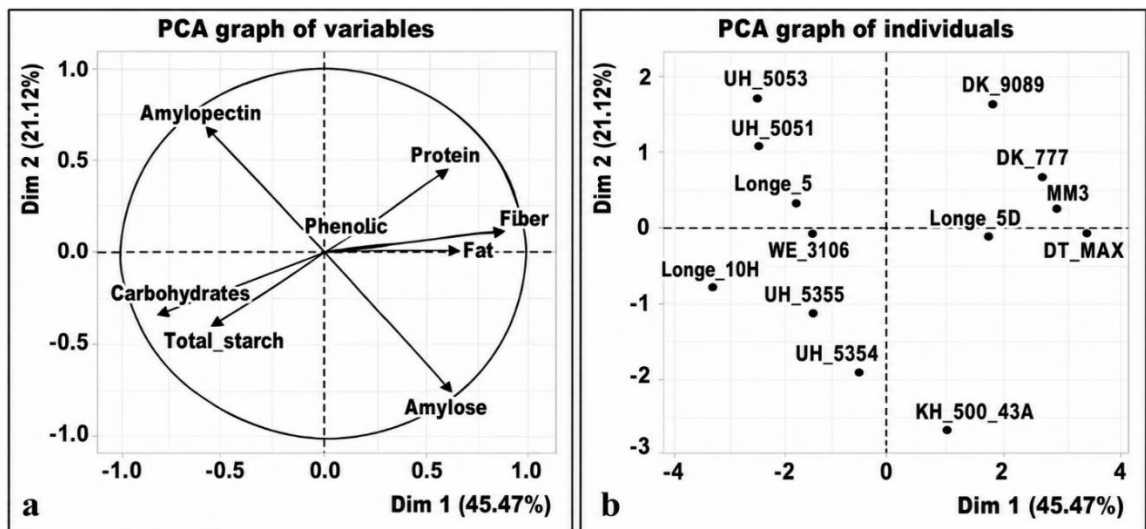


Figure 4. 1: Principal component analysis (PCA) showing (a) the correlation of the factors affecting starch digestibility within PC1 and PC2 and (b) the distribution of the thirteen maize varieties. PCA was performed on the correlation matrix of average values of factors affecting starch digestibility (Table 4.3).

Maize varieties with average similar characteristics based on factors that affect starch digestibility were grouped using the hierarchical cluster analysis (HCA) particularly the plug-in heatmap and a dendrogram (Figure 4.2). HCA identifies subgroups within datasets, distinguished by distinct differences in the measured variables, thereby enabling meaningful classification on the basis of shared traits or patterns (Dalmaijer *et al.*, 2022). The level of factors used in the clustering of varieties are provided in Table 4.3. As shown, in Figure 4.2, 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 heatmap. The darker the red color is, the greater the component, and the darker the blue color is, the lower the component in the respective maize variety. 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% dissimilarity (Appendix 6), the 13 maize varieties could be clustered into four statistically significant groups (1, 2, 3, and 4). The agglomeration coefficient was 0.85, which was very close to 1, indicating a good fitness of the dendrogram (Appendix 6). Otherwise, the cophenetic correlation coefficient

of the dendrogram generated from cluster analysis was 0.67, indicating a moderate representation of the data.

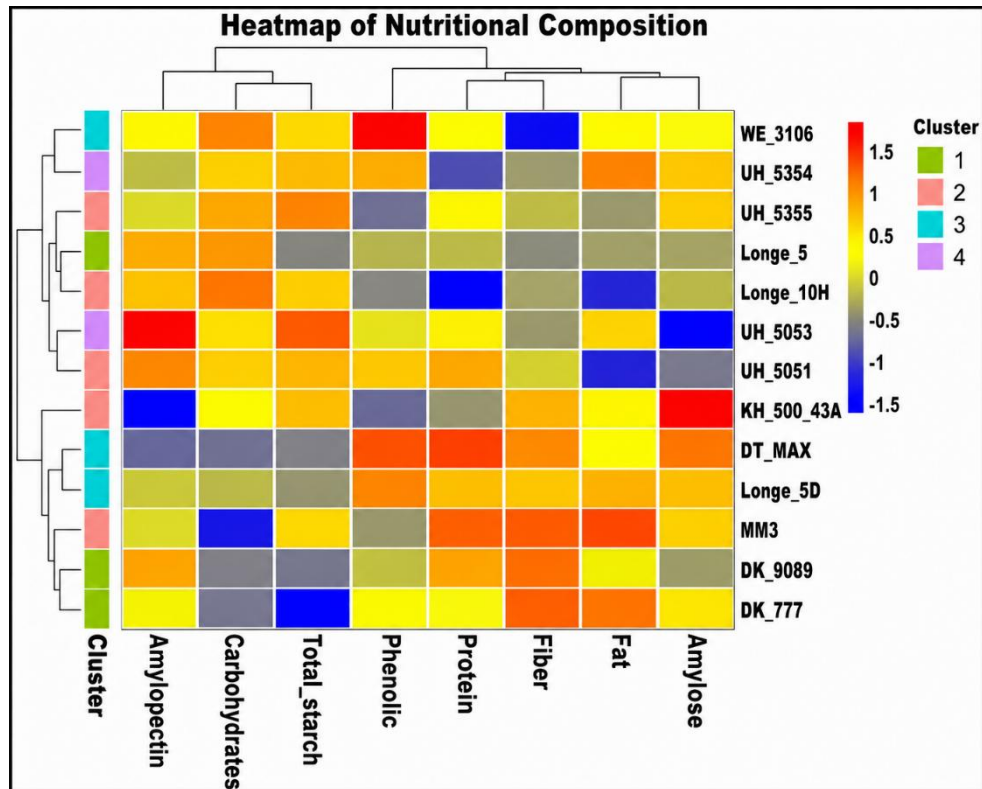


Figure 4. 2: Heatmap clustering showing maize variety clusters on the basis of factors that affect starch digestibility

Cluster 1 included the DK9089, DK777, and Longe 5 varieties and presented the lowest total starch content and the second lowest content of phenolic compounds. Four maize varieties were included in Cluster 2: MM3, KH 500 43A, UH5355, and Longe 10H (Figure 4.2). These varieties had the second highest levels of total starch and amylose content but the lowest levels of phenolic compounds. Cluster 3, which consists of the varieties DT MAX, Longe 5D, and WE3106, had the highest phenolic and amylose contents. Finally, cluster 4, comprising UH5051, UH5053, and UH5354, had the lowest amylose content and the highest total starch content.

Each cluster has its own characteristics, and the maize varieties within each cluster would probably develop a similar effect in terms of starch resistant development after processing and following digestion. The four clusters are similar in terms of their proximate

composition (fat, protein, fiber, carbohydrate) and amylose contents ($p>0.05$) but differ in their phenolic compounds and total starch content ($p<0.05$, Table 4.4). 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, with the highest (216.61) content of phenolic compounds, would promote higher resistant starch development during digestion than varieties in other clusters. On this basis, varieties in cluster 3 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 on the basis of HCA. These results show that HCA can uncover discrete subgroups that PCA might overlook; therefore, combining PCA and HCA improves the classification, which aligns with the findings of Mohamad Asri *et al.* (2020).

The average amylose contents of clusters 1, 2, 3, and 4 were 23.08, 24.90, 24.97, and 22.44%, respectively. Although statistically not significant, clusters 2 and 3 presented similar and higher amylose contents than did cluster 4, which presented the lowest amylose content (Table 4.4). Accordingly, varieties within Clusters 2 and 3, with similar high amylose contents, would likely experience similar and high levels of retrogradation when subjected to processing (e.g., hydrothermal methods, such as HMT or parboiling), whereas those in Cluster 4 would experience a lower level of retrogradation.

Looking forward to the link between phenolic compounds inactivating α amylase during digestion and the importance of amylose in increasing resistant starch during processing, cluster 3, which has the highest phenolic content and high amylose content, would probably develop more resistant starch than varieties in other clusters. Given the promising quality profiles of cluster 3 varieties, characterized by high contents of phenolic compounds and amylose, they need to be harnessed technologically for better outcomes for nutrition-associated NCDs. Therefore, 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 the other hand, since the proximate composition of cluster three is similar and there is no significant difference between the amylose contents

of the clusters, maize varieties in clusters 1 and 4, which have higher phenolic contents than those in cluster 2, would release less soluble starch than those in cluster 1 would and could also be adequate candidate maize varieties to be harnessed for nutritional intervention in diet-associated non-communicable disease.

Table 4.4: Average characteristics of the clusters of thirteen maize varieties selected from Uganda

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

All values are means ± Standard Deviation. Means with different letters in the same line differ significantly ($P \leq 0.05$).

4.6. Conclusion

This chapter 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-to-fiber ratio 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 internal factors leads to differences in starch digestibility among the maize varieties. On the basis of this variation, further analysis grouped the varieties into four significantly different groups. The cluster consisting of varieties DT MAX, Longe 5D, and WE3106 would develop more resistant starch, whereas varieties UH5051, UH5053, and UH5354 would develop the lowest starch resistant. On the basis of these results, varieties with compounds that promote higher starch

resistant are recommended for consumption to help in the control of NCD development. However, more research is needed to improve the carbohydrate quality of these varieties to limit their dietary contribution to the risk of NCD. Several methods are available to improve carbohydrate quality of starchy foods. These include chemical modification, enzymatic modification and physical modification. However, physical starch modification, mainly heat moisture treatment (HMT), has shown more promising results than the other methods and is most cost effective but has not yet been optimized for maize varieties produced and consumed in Uganda.

CHAPTER FIVE

Optimization of heat–moisture treatment (HMT) to decrease starch digestibility of polished maize flour from predominant varieties consumed in Uganda

This chapter is adapted from the published manuscript below:

Fassinou, F. T. K., Olum, S., Okidi, L., & Ongeng, D. (2025). Optimized heat-moisture treatment decreases the starch digestibility of polished maize flour from predominant varieties consumed in Uganda. *Applied Food Research*, 5(1), 101023. <https://doi.org/10.1016/j.afres.2025.101023>

5.1. Abstract

In Uganda, maize flour is the major carbohydrate food source for more than 90% of households. It is largely consumed in polished form characterized by high digestibility, rapidly releasing glucose into the bloodstream, making it unsuitable for metabolic health. This phenomenon calls for a reduction in maize flour starch digestibility by increasing the content of slowly digestible (SDS) and resistant starch (RS), which are typical functions of heat-moisture treatment (HMT). However, HMT efficacy depends on the botanical starch source and HMT conditions, necessitating its optimization. Therefore, this study investigated the optimal HMT conditions to produce polished maize flour with lower starch digestibility by increasing the SDS and RS contents of four maize varieties (UH5354, DK777, Longe 10H, and DT Max) selected as the most consumed in the study area in their respective clusters identified and reported in chapter four. Response surface methodology (Box-Behnken design) was used to set the experimental design with moisture (20–30%), temperature (80–100°C), and heating (4–8 hours) conditions, and the corresponding SDS and RS contents were analyzed via a megazyme procedure. Composite SDS and RS (SDS+RS) were used in the optimization. The quadratic function was the most appropriate model that fitted the data, as indicated by a nonsignificant lack of fit ($p>0.05$) and explained at least 80% of the variance for each variety considered. The optimum moisture (%), temperature (°C) and heating time (hours) were 23.17, 80.00, and 4.12 for UH5354; 20.83, 95.43, and 5.42 for DK777; 34.51, 90.46, and 3.01 for Longe 10H; and 19.24, 97.02, and 5.88 for the DT Max varieties. Relative to the control, optimal HMT conditions improved the contents of SDS+RS by 8–14%. This demonstrates the potential of optimal HMT conditions to reduce the high state of starch digestibility in polished maize flour making it more healthy.

5.2. Introduction

Starch is the main storage polysaccharide in plants such as cereal grains, roots, and tubers. It is quantitatively the most dominant carbohydrate source in the human diet and contributes greatly to the 4 kCal/g energy provided. For nutritional purposes and because of its hydrolysis by enzymes during digestion, it is classified as rapidly digestible starch (RDS), slowly digestible starch (SDS), or resistant starch (RS). RDS undergoes rapid digestion within the first 20 minutes in the small intestine, resulting in a high postprandial glycemic response (Englyst *et al.*, 1992), which increases the risk of non-communicable diseases. In contrast, SDS is digested slowly (20 and 120 minutes), whereas RS remains indigestible in the small intestine and is subsequently fermented by gut microorganisms, thereby reducing their contribution to the postprandial glycemic response (Liu *et al.*, 2020). Several studies have demonstrated that SDS and RS play roles in regulating blood glucose levels, body weight, and associated metabolic diseases (Miao *et al.*, 2015; Sun *et al.*, 2018; Wang *et al.*, 2019). This makes SDS and RS vital nutritional attributes of carbohydrate quality necessary to reduce the risk of diet associated non-communicable diseases.

In Uganda, maize grain is the staple food that provides carbohydrates to approximately 92% of households (Wacoo *et al.*, 2018). Therefore, several maize varieties, such as longe (5, 5D), UH (5354, 5355, 5051), DK (777 & 9089), Longe 10H, and DT Max, are promoted across the country and East African community on the basis of their agronomical performance (Lee, 2020). However, these varieties exhibit variable and relatively low levels of SDS and RS, ranging from 55.9--59.8% and 5.14--14.0%, respectively (Camelo-Méndez *et al.*, 2017; De-Alcântara *et al.*, 2020). The aforementioned statistics suggest that the maize varieties have high starch digestibility. This makes the glucose from their digestion rapidly absorbed into the bloodstream (high glycemic index) and thus unsuitable for metabolic health. In addition, the polishing of these maize grains before milling, which is a common practice in Uganda and surrounding countries to improve the organoleptic properties of maize flour, such as sweetness, digestibility, lightness, and shelf-life, and reduce the cooking time (Kiguli *et al.*, 2019) exacerbates this problem. This is largely because polishing removes bran, which is rich in SDS and RS, hence decreasing the SDS and RS contents of polished maize flour (Sivakamasundari *et al.*, 2022). Consequently, the

reduction in SDS and RS leads to increased starch digestibility and a high glycemic index, which are associated with health risks such as type II diabetes, obesity, and other non-communicable diseases (NCDs) that are increasing in the country according to recent National Demographic Health Surveys (UDHS, 2022). Thus, there is a need to improve the SDS and RS contents of polished maize flour most consumed in Uganda and surrounding countries for better nutritional outcomes.

Whereas several starch modification techniques exist and their advantages in increasing the SDS and RS of cereals have been demonstrated, physical starch modification, mainly heat moisture treatment (HMT), has shown more promising results than the other methods (Dong *et al.*, 2021; Sandhu *et al.*, 2020; Van Hung *et al.*, 2020). HMT has been shown in several studies to be effective in enhancing SDS and RS in starch-based foods such as cereals (Asare *et al.*, 2021; Marta *et al.*, 2022). The treated starches obtained from HMT are generally considered safe for use in food (Sandhu *et al.*, 2020). HMT is a natural, more accessible, low-cost physical starch modification method that does not produce any effluents, such as salts or reagents. It involves the treatment of starch granules at low moisture levels (<35% w/w) for a certain time (15 min–16 h) and at temperatures varying between 80–140°C, below the gelatinization temperature (Wang *et al.*, 2021). In addition, the increase in SDS and RS due to HMT has been shown to decrease the starch digestion rate (Cheng *et al.*, 2019; Gayary and Mahanta, 2020). However, the efficacy of HMT varies according to the botanical source of the starchy food and the conditions applied (Wang *et al.*, 2021); thus necessitating the optimization of HMT conditions for a specific starch source.

Response-surface methodology (RSM) is a useful technique for investigating several input variables that influence the performance measures or quality characteristics of the product or process under investigation (Giri and Prasad, 2007). RSM allows not only exploration of the optimal process but also further in-depth analysis of the extent to which different processes affect a given response factor typical of starch properties (Liu *et al.*, 2022). RSM is an effective and frequently used tool for optimization, and several studies have employed RSM, mainly the Box–Behnken design (BBD), to optimize HMT conditions, resulting in acceptable responses (Dai *et al.*, 2019; Deng *et al.*, 2022; Ye *et al.*, 2016). In particular,

BBD reduces the number of additional experiments, takes a shorter time, and eliminates the need for laborious laboratory studies (Yetilmezsoy *et al.*, 2009).

Although several studies have performed HMT on extracted maize starch (Asare *et al.*, 2021; Liu *et al.*, 2014) and waxy maize starch (Chang *et al.*, 2020; Sui *et al.*, 2015), limited information exists on experiments that have been carried out to reduce starch digestibility of polished maize flour typical of those consumed in Uganda and several African countries. Whereas the conditions of HMT application for starch modification of polished maize are unknown, they may be variety dependent (Piecyk and Domian, 2021). This could be due to differences in inherent characteristics (amylose, total starch, phenolic compounds, proximate composition) that affect the development of SDS and RS during HMT (Toutounji *et al.*, 2019; Yang *et al.*, 2023). Therefore, this study aimed to optimize HMT conditions to reduce the starch digestibility of polished maize flour, which is the predominant maize form consumed in Uganda, via RSM.

5.3. Materials and methods

5.3.1. Materials

In Uganda, while up to eighty-six maize varieties were recorded by the National Agricultural Research Organization (NARO) in 2022, only thirteen are commercialized and grown by farmers. Although these 13 varieties have different internal factors affecting starch digestibility (proximate composition, phenolic compounds, amylose, and total starch content), they can be grouped into four significantly different clusters on the basis of those internal factors (chapter four of the thesis). These internal factors affect the effectiveness of heat-moisture treatment in decreasing starch digestibility (Qi and Tester, 2016; Sui *et al.*, 2017). Therefore, the most consumed maize variety was selected from each of the four clusters (UH5354, DK777, Longe 10H, and DT Max) and subsequently used in the current study. Four varieties were selected to represent their respective clusters, as each belonged to one of the four statistically distinct groups identified based on intrinsic factors governing starch digestibility (Figure 4.2), and each also corresponds to the most widely consumed variety within its cluster. Because heat-moisture treatment is unique to starch origin, the optimum conditions derived for a cluster representative are expected to apply to the other varieties sharing that clusters characteristics. Seventy-five (75) kilograms of each variety

were purchased directly from farmers after the November–December 2023 harvest period. All foreign matter and dirt were removed; the grains were sorted and cleaned and damaged or broken grains were removed. The clean and sorted grains were placed in PICS (Purdue Improved Crop Storage) bags composed of two polypropylene bags and stored in the laboratory until use within 8–10 weeks.

5.3.2. Optimization of HMT conditions

5.3.2.1. Experimental design for heat moisture treatment optimization

RSM was employed to generate the experimental design used to optimize the HMT conditions, with a total of SDS and RS as response factors. The effects of the three HMT factors X_1 (moisture), X_2 (heating length), and X_3 (temperature) on response Y_1 (SDS + RS) were assessed.

According to Wang *et al.* (2016), the increase in moisture content during HMT leads to significant structural disorganization and molecular rearrangements in starch, which can convert RDS into SDS and RS. Although an increase in moisture content generally promotes the development of SDS and RS in cereals such as maize, subjecting maize starch to HMT with moisture contents above 30% tends to decrease the SDS and RS contents (Chen *et al.*, 2017a; Kumar *et al.*, 2023). On the other hand, according to de la Rosa-Millan *et al.* (2023), the application of gradual and higher temperatures between 80 and 100°C converts RDS into SDS and RS in maize grains due to optimal gelatinization and structural changes. However, when the temperature is above 100°C, maize starch granules swell significantly and lose their granular structure, resulting in SDS and RS (Chen *et al.*, 2017a). In contrast, with respect to the nutritional content of the maize grains, Ye *et al.* (2016) reported that SDS+RS increases when the maize is subjected to temperatures ranging from 80–100°C, moisture contents ranging from 20–30%, and heating durations ranging from 4–8 h. In contrast, SDS+RS decreases dramatically when the moisture content, time, and temperature are greater than 30%, 8 h, and 100°C, respectively (Ye *et al.*, 2016). Therefore, the ranges of intervals where SDS+RS increases, 20–30% moisture content, 4–8 h heating length, and 80–100°C temperature were considered in the present study for setting the experimental design.

A Box–Behnken design, an alternative to the central composite design, which is mostly applied to reduce the experimental cost and process (Gündoğdu *et al.*, 2016), was used. For three factors, Box–Behnken design, the design provides commonly a total of 15 runs, which comprise 12 experimental runs and 3 or 5 replications of the center points utilized to estimate pure experimental error and ensure model accuracy (Iwundu and Cosmos, 2022; Tan *et al.*, 2012). In the present study, the design scheme comprised 12 treatments with five replications of the central point to calculate the experimental error for each maize variety such as applied by Abul Kalam *et al* (2016) and Liu and Ho (2017). The experimental design was generated using R software version 4.2.2 with the rsm package. For the statistical calculations, the process variables were coded at three levels (-1, 0, and +1) representing lower, middle and higher values, and the coding was performed using equation 5.1.

$$x_i = (X_i - X_0)/\Delta X_i \quad i=1,2,3\dots k \quad (\text{Eq 5.1})$$

where x_i is the dimensionless value of an independent variable; X_i is the real value of an independent variable; X_0 is the real value of an independent variable at the center point; and ΔX_i is the step change in the real value of the variable i . The range of independent variables and their levels are presented in Table 5.1.

5.3.2.2.Heat Moisture Treatment Process

To minimize mechanical damage to starch granules, ensure controlled hydration kinetics, prevent excessive gelatinization, promote consistent structural reorganization during heat-moisture treatment (HMT), HMT process was applied to whole maize kernels prior to polishing (Gu *et al.*, 2024). Heat–moisture treatment (HMT) was performed according to the laboratory protocol applied by (Sui *et al.*, 2015). Prior to HMT application, the initial moisture content of each maize sample was determined gravimetrically using the hot air oven method following the standard AOAC method number 925.09 (Association of Official Analytical Chemists, 1990).

Table 5.1: Levels of factors used in the Box–Behnken design for the optimization of HMT conditions

Coded Process Variables			Actual variable non coded		
X ₁	X ₂	X ₃	Moisture content (%)	Heating length (Hours)	Temperature (°C)
0	-1	-1	25	4	80
-1	0	-1	20	6	80
-1	0	-1	30	6	80
0	-1	-1	25	8	80
-1	-1	0	20	4	90
-1	-1	0	30	4	90
0	0	0	25	6	90
0	0	0	25	6	90
0	0	0	25	6	90
0	0	0	25	6	90
0	0	0	25	6	90
0	0	0	25	6	90
-1	-1	0	20	8	90
-1	-1	0	30	8	90
0	-1	-1	25	4	100
-1	0	-1	20	6	100
-1	0	-1	30	6	100
0	-1	-1	25	8	100

X₁: Moisture content (%), X₂: Heating length (Hours) and X₃: Temperature (°C)

The moisture content of the maize grains was subsequently adjusted by immersing the grains in distilled water to obtain the desired moisture content using the proposed formula of Cordeiro *et al.* (2018) (Equation 5.2).

$$m_w = \left[\left(\frac{100 - M_i}{100 - M_f} \right) - 1 \right] * m \quad (\text{Eq 5.2})$$

where m_w (g) is the mass of water to be added, M_i (%) is the initial moisture of the sample, M_f (%) is the desired moisture, and m (g) is the maize grain mass used.

The wet maize grains were placed in polyethylene zipper bags, sealed, and equilibrated at 4°C for 24 hours in a Hisens refrigerator (Model RT488N4ASU, Guangdong, China) before heat treatment was applied. To avoid a decrease in moisture content during heat application, the samples were hermetically sealed in glass bottles to maintain the moisture content as described by Zou *et al.* (2023). The packed samples in glass bottles were heated using an electric hot-air drying oven DHG 9053A (Sui *et al.*, 2015) at the set temperatures

and durations according to the experimental design (Table 5.1), dried and polished as described in subsection 5.3.2.3.

5.3.2.3. Polishing and milling of maize samples

The dried heat–moisture–treated maize samples were polished and milled. Before polishing and milling, the maize grains were dried in an oven with forced air circulation at 50°C for 24 hours (Van Hung *et al.*, 2020). The maize grains treated using HMT were polished using a maize huller (Locally Fabricated, Gulu, Uganda) following the two common polishing steps typically used in Uganda. Briefly, whole maize grains were polished from the surface layer to the center. During polishing, the germ and bran layers were removed twice from the grain mechanically, as typically done in Uganda, resulting in what is locally known as "first class" maize flour. The first-class polished maize grains/grits were then ground in laboratory conditions using an 8010EG laboratory blender (Model HGBTWTG4, Torrington, Connecticut 06700 USA) and sieved through a 100 µm sieve. The flour was stored in paper bags at room temperature until laboratory analysis within 15–40 days.

5.3.2.4. Determination of starch digestibility in HMT-treated maize

The starch digestibility of polished maize flour obtained under section 5.3.2.3 was determined according to the gastro-intestinal simulated in vitro digestion method of Englyst *et al.* (1992) using the megazyme procedure (Neogen cooperation, 2023). Briefly, a pancreatic α amylase/amyloglucosidase (PAA/AMG) solution was prepared by adding 0.5 g of the PAA/AMG powder mixture to 25 mL of 50 mM sodium maleate buffer, pH 6.0. AMG solution was prepared by adding 1 mL of amyloglucosidase (3300 U/mL) to 30 mL of sodium acetate buffer (pH 4.5). The reagents were prepared immediately before use, stirred on a magnetic stirrer for 5 min, and stored on ice until use.

Specifically, 1 g of each sample of the polished maize flour was weighed, transferred into 50 mL polypropylene screw-cap tubes and mixed with 1.0 mL 95% ethanol (v/v) and 35 mL 50 mM sodium maleate buffer, pH 6.0. The mixture was submerged in magnetic stirred Wagtech OLS Model 200 water bath (Grant Instruments, Germany) to equilibrate at 37°C

for 5 min at 170 rpm. Specifically, 5 mL of PAA/AMG enzyme solution was added and incubated in a Wagatech OLS200 water bath at 37°C with agitation at 170 rpm for 180 minutes. Aliquots (1.0 ml) of the hydrolysate were taken at 0, 20, 120, 180, and 240 min and mixed with 20 ml of 80% ethanol to inactivate the enzyme. Exactly 2.0 mL of each solution was picked and centrifuged at 6500 rpm for 10 minutes using a Centurion Scientific Benchtop Model 1. C2015 centrifuge (Centurion Scientific Ltd, United Kingdom). After centrifugation, the aliquot (0.1 mL) was pipetted in duplicate and mixed with 0.1 mL of AMG solution, incubated at 50°C for 30 min, and mixed with 3.0 mL of freshly prepared D-Glucose Assay Kit Reagent (GOPOD). Reagent blank solutions were prepared by mixing 0.2 mL of sodium acetate buffer, pH 4.5, with 3.0 mL of GOPOD reagent, whereas D-glucose standards were prepared by mixing 100 µg of D-glucose with 0.1 mL of sodium acetate buffer, 100 mM, pH 4.5, and 3.0 mL of GOPOD reagent. The samples mixed with GOPOD, the reagent blank, and D-glucose standards prepared as described subsequently were incubated at 50°C for 20 min for color development before reading of the absorbance using a Jenway 6305 UV/Visible spectrophotometer (Jenway, United Kingdom) at 510 nm.

After 240 minutes of digestion, an aliquot of the sample suspension was mixed with 95% ethanol to precipitate the resistant starch fraction. The mixture was centrifuged at 4000 rpm for 10 minutes using a Centurion Scientific Benchtop Model 1. C2015 centrifuge (Centurion Scientific Ltd, United Kingdom), and the supernatant was decanted. The pellet was then suspended in 50% aqueous ethanol, vortexed, and centrifuged again at 4000 rpm for 10 minutes. This wash step was repeated twice to remove any residual digestible starch. This was then followed by the addition of 2 mL of 1.7 M NaOH to the washed pellet, and the mixture was incubated at 37°C for 20 minutes to solubilize the resistant starch. Specifically, 1.0 M sodium acetate buffer (pH 3.8) and AMG solution (3300 U/mL) were added. This mixture was incubated at 50°C for 30 minutes to hydrolyze the resistant starch to glucose, after which the GOPOD reagent was added. The absorbances of the sample and glucose standards at 510 nm against the reagent blank were measured. The contents of RDS (at 20 minutes), SDS (between 20 and 120 minutes), and RS (after 240 minutes) were calculated using the Megazyme formula (K-DSTRS), which was accessed from the Megazyme website (www.megazyme.com). All the experiments were conducted in

duplicate. The contents of SDS and RS were added to form a composite factor used during the optimization process.

5.3.2.5. Statistical models of heat moisture treatment factors

Statistical modeling was performed using R software version 4.2.2. According to Hsu *et al.* (2015), starch food exhibits distinct digestibility during processing, such as HMT, due to variations in their physicochemical properties (internal factors). As such, optimal processing conditions to reduce starch digestibility should be established on a variety-specific basis. The Shapiro test and Leven test were used to assess the normality and homogeneity of variance of the data, respectively. The results revealed that the data were normally distributed ($p > 0.05$) but violated the assumption of homogeneity of variance ($p < 0.05$). Based on these results, Welch's ANOVA test (not assuming equal variances) was used, as applied by Dervišević *et al.* (2024). These results showed that the starch digestibility fractions of polished maize flour in this study generally varied across the maize variety ($p < 0.05$, Appendix 7). Consequently, optimization was conducted for each variety in this study. The second-order polynomial regression (equation 5.3), which is generally able to describe the relationship between the responses and the independent variables, was used to check the fitness of the collected data.

$$Y = \beta_0 + \sum_{i=1}^3 \beta_i X_i + \sum_{i=1}^3 \beta_{ii} X_i^2 + \sum_{(i=1)}^2 \sum_{(j=i+1)}^3 \beta_{ij} X_i X_j \quad (\text{Eq 5.3})$$

where Y is the response, the SDS+RS yield (%); β_0 is the constant; β_i represents the linear coefficient; β_{ii} represents the quadratic coefficient; β_{ij} is the interaction coefficient; and X_i and X_j are the coding values of the independent variables or levels of each factor.

The linear, interaction, and quadratic relationship effects were expressed using the model. The appropriate model was identified after checking the goodness of fit (lack of fit), the predictive power of the model (R^2 value), and the global significance (model p value). The best model for fitting the data were selected when the lack of fit was not significant, higher, or equal to 0.05 (Fagerland and Hosmer, 2013) and the p value was significant (≤ 0.05). The acceptable model coefficient of defemination (R^2) was set at greater than 0.8, meaning that 80% of the data were explained by the model, the minimum R^2 value for good model fitting (Khodashenas and Jouki, 2020).

To describe the individual and interactive effects of the factors, three-dimensional (3D) response surface plots were developed. The selected models were used to plot 3D response surface contour graphs to study the interactive effects of independent variables on the response. The optimum application conditions for HMT yielding to the maximum (SDS+RS) development were determined via the selected predictive model. According to Oliveira *et al* (2019), constraining the predicted optimum strictly within the experimental design space may reduce its reliability. This limitation is particularly critical near the boundaries of the design, where the true optimum is most likely to occur. In such cases, the model's predictive capacity can be weakened, as the restrictions imposed prevent exploration of conditions that may better reflect the actual system behavior. Therefore, despite the experimental conditions being selected based on the literature, the predicted optimum was not constrained by the experimental design boundaries but must remain within the feasible range of HMT application conditions. The generated HMT solutions were validated.

5.3.2.6. Validation of the selected models

Separate validation tests were performed to determine the suitability of the model equations for the prediction of the optimum response variables. The optimized conditions were validated for the maximum SDS+RS contents (%) based on the predictive values obtained using RSM. The composite SDS+RS content was determined following the procedure described under subsection 5.3.2.4.

5.3.3. Determination of the optimal HMT conditions effect

The maize flour was prepared in two batches: control maize flour without HMT and maize flour subjected to HMT-optimized conditions for each variety. The HMT treatment was carried out according to a laboratory protocol applied by (Sui *et al.*, 2015) described in subsection 5.3.2.2, except that the optimal HMT conditions found for moisture content, temperature and heating length were applied. After HMT application, the maize grains were dried as described in section 5.3.2.3. The dried maize grains were polished and ground according to the methods described in subsection 5.3.2.3, and the starch digestibility fraction was analyzed. The starch digestibility was analyzed according to the procedure described in section 5.3.2.4.

5.3.4. Statistical analysis

The results were statistically tested using one-sample t test and paired t test at the significance level of $p = 0.05$. To determine the validity of the model selected, the experimental values were compared with the values predicted via a one-sample t test. The null hypothesis was that there was no significant difference between the actual and the predicted values (test value). The one-sample t-test was retained here as a recognised approach for validating response-surface predictions, in which the mean of the experimental replicates obtained under the optimum conditions is compared against the single value predicted by the model as the test value (Giri and Prasad, 2007). Statistically significant values indicated non-validity, whereas statistically insignificant values indicated validity (Giri and Prasad, 2007). The effect of optimal heat moisture treatment conditions was also assessed by comparing control maize flour without HMT to maize flour subjected to optimized HMT conditions using a paired t test. All the analyses were performed in duplicate and analyzed using R software version 4.2.2 and Design Expert 13 software.

5.4. Results and discussion

5.4.1. Model fitting and adequacy checking

The effects of three independent variables, moisture (X_1), heating duration (X_2), and temperature (X_3), on the development of the SDS and RS were determined by fitting second-order polynomial models to the experimental data obtained (Appendix 8). The quadratic model was selected because it provided the best fit for the experimental data for each variety of maize. This model generally had the highest values of lack of fit, R-squared (0.825-0.913), and adjusted R-squared (0.600-0.801), indicating that it fits the experimental data better compared to other models (Quinino *et al.*, 2012). This model explains and predicts the response variable better than other models. In addition, the quadratic regression is the only one with an R-squared value greater than 0.8, which is generally considered a good fit (Khodashenas and Jouki, 2020). The quadratic model was statistically significant at the 5% level for each variety, suggesting that the effects modeled by the quadratic function were meaningful and can be used to make predictions.

The lack of fit of the quadratic regression was 0.23, 0.61, 0.09, and 0.82 (>0.05) for the UH5354, DK777, Longe 10H, and DT Max varieties, respectively. This indicated a good fit for the models (Song *et al.*, 2015). The R-squared values were 0.89, 0.91, 0.86, and 0.82 for UH5354, DK777, Longe 10H, and DT Max, respectively, which revealed the high accuracy of the models (Polat *et al.*, 2020). This means that the quadratic regression could explain up to 89%, 91%, 86%, and 82% of the experimental data obtained for each variety. The regression coefficients of the linear, quadratic, and interaction terms for each response are presented in Table 5.2, and their significance was determined using analysis of variance (ANOVA). The model equations for the SDS and RS contents (SDS+RS) in terms of the coded factors for the quadratic model for each variety are presented in equations 5.4 to 5.7 obtained through the ANOVA test.

$$Y_1 = 50.37 + 1.46 X_1 - 0.86X_2 - 5.89X_3 + 4.15X_1X_2 + 10.53X_1X_3 + 6.20X_2X_3 + 0.91 X_1^2 + 4.15X_2^2 - 1.52X_3^2 \quad (\text{Eq 5.4})$$

$$Y_2 = 47.47 + 0.19 X_1 - 1.06 X_2 + 2.75X_3 + 1.79X_1X_2 - 3.26X_1X_3 - 3.85X_2X_3 - 0.10 X_1^2 - 7.32X_2^2 - 5.49X_3^2 \quad (\text{Eq 5.5})$$

$$Y_3 = 36.71 + 4.87 X_1 - 2.09X_2 - 0.86X_3 - 2.72 X_1X_2 + 1.54X_1X_3 + 6.56X_2X_3 - 1.75 X_1^2 + 5.74X_2^2 - 0.95X_3^2 \quad (\text{Eq 5.6})$$

$$Y_4 = 43.90 - 2.33X_1 - 0.81X_2 + 0.51X_3 - 1.95 X_1X_2 - 1.55X_1X_3 + 0.41X_2X_3 - 0.75 X_1^2 + 1.83X_2^2 + 3.65X_3^2 \quad (\text{Eq 5.7})$$

Where Y_1 , Y_2 , Y_3 , and Y_4 , are the composite SDS+RS for UH5354, DK777, Longe 10H, and DT Max, respectively, and X_1 , X_2 , and X_3 , are the coded moisture content, length of heating, and temperature, respectively.

The effects of independent variables (moisture content, heating length, and temperature) on the SDS and RS were strongly predicted with linear, interaction, and quadratic terms depending on the maize variety (Table 5.2). For the variety UH5354, the linear term for temperature (X_3) and its interactions with moisture content (X_1X_3) and heating length (X_2X_3) were statistically significant ($p < 0.05$). In contrast, for the variety DK777, the linear terms for moisture content and temperature, as well as the interactions between moisture and heating length and moisture and temperature, along with the quadratic term of moisture content (X_1), were significant ($p < 0.05$) (Table 5.2). These results show that the development of the SDS and RS were strongly modeled with linear and interaction terms for the UH5354 variety but with quadratic and interaction terms for the DK777 variety. For the Longe 10H variety, all three terms strongly model the development of SDS and RS,

whereas only the linear and quadratic terms strongly modeled the development of SDS and RS for the DT Max variety (Table 5.2). These results indicate that the suggested models appropriately describe the development of SDS and RS for each of the four maize varieties. The observed and predicted data in terms of coded factors for the quadratic model are presented in Appendix 9.

Beyond the coefficient of determination, the models were evaluated using the adjusted R-squared, the significance of individual model terms and residual diagnostics. The adjusted R-squared values, which penalize the inclusion of non-significant terms, were 0.762, 0.801, 0.687 and 0.600 for UH5354, DK777, Longe 10H and DT Max, respectively (Table 5.2), and remained reasonably close to the corresponding R-squared values, indicating that the quadratic models were not over-fitted. In addition, the analysis of variance in Table 5.2 shows that the interaction terms were significant for several varieties, notably the moisture-temperature interaction (X_1X_3) and the temperature-heating time interaction (X_2X_3) for UH5354, and the moisture-heating time interaction (X_1X_2) and X_2X_3 for DK777 and Longe 10H ($p < 0.05$), confirming that the effect of one factor on the development of SDS and RS depended on the level of the others. Residual analysis was also carried out to verify the adequacy of the models: the normal probability plots of the residuals showed the points falling approximately along a straight line, indicating that the residuals were normally distributed, while the plots of residuals against predicted values showed a random scatter with no systematic pattern, confirming constant variance and the absence of model misspecification (Appendix 10). The predicted R-squared values, however, were low and, for two varieties, negative: -0.096 for UH5354 and -0.742 for Longe 10H, against 0.442 for DK777 and 0.323 for DT Max (Table 5.2). Although the model has a negative predicted R^2 suggesting overfitting, meaning the model describes the observed data better than it predicts independent observations, it does not mean that the models are meaningless within the region explored (Myers *et al.*, 2016). Together with the non-significant lack-of-fit values, these diagnostics confirm that the quadratic models adequately describe the experimental data.

Table 5.2: Results of the quadratic polynomial models showing the effects of linear interaction, and quadratic terms on slow digestible (SDS) and resistant starch (RS) development across maize varieties

Model Statistics	UH5354			DK777			Longe 10H			DT Max		
	Quadratic			Quadratic			Quadratic			Quadratic		
	Coef	F value	P value	Coef	F value	P value	Coef	F value	P value	Coef	F value	P value
Model		6.690	0.010**		8.170	0.006***		4.910	0.024**		3.670	0.050**
X₁	1.460	0.981	0.355	0.187	7.970	0.026**	4.870	14.170	0.007***	-2.330	9.500	0.018**
X₂	-0.859	0.338	0.579	-1.060	0.037	0.854	2.090	2.620	0.150	-0.813	1.160	0.317
X₃	-5.900	15.940	0.005***	2.750	8.170	0.006***	-0.862	0.444	0.527	0.511	0.459	0.520
X₁X₂	4.150	3.940	0.087*	1.790	7.830	0.027**	-2.720	2.220	0.180	-1.960	3.350	0.110
X₁X₃	10.530	25.380	0.002***	-3.270	1.180	0.313	1.550	0.713	0.426	-1.560	2.130	0.188
X₂X₃	6.200	8.800	0.021**	-3.860	5.620	0.050**	6.560	12.840	0.009***	0.412	0.149	0.711
X₁²	0.915	0.202	0.667	-0.103	16.710	0.005***	-1.750	0.959	0.360	-0.754	0.525	0.492
X₂²	4.150	4.150	0.081*	-7.320	0.006	0.941	5.740	10.340	0.015**	1.830	3.080	0.123
X₃²	-1.520	0.555	0.481	-5.490	1.680	0.236	-0.955	0.287	0.609	3.650	12.330	0.010**
Lack of Fit		2.180	0.233		0.676	0.611		4.560	0.088		0.254	0.856
R²	0.896			0.913			0.863			0.825		
Adjusted R²	0.762			0.801			0.687			0.600		
Predicted R²	-0.096			0.442			-0.742			0.323		
Adeq Precision	10.634			8.796			8.033			6.912		

*, **, ***: denote statistical significance at the 10%, 5% and 1% levels, respectively, **X₁**: moisture content, **X₂**: heating length and **X₃**: **heating** temperature, UH5354, DK777, Longe 10H and DT Max are maize varieties used in the study.

5.4.2. Effect of independent variables and optimal HMT conditions for the maize varieties

5.4.2.1. Interactive effect of independent variables on the response

Prior to determining the optimal heat moisture conditions, the effects of the independent variables on the composite SDS+RS were analyzed. The quadratic regression enabled the prediction of the effects of the three independent variables on the development of the SDS and RS contents of flour for each maize variety. The relationships between the independent and dependent variables are depicted through two-dimensional contour plots (Figures 5.1-5.4: a₁, b₁, c₁) and three-dimensional response surface plots (Figures 5.1-5.4: a₂, b₂, c₂). In these plots, the dependent variable was obtained with two continuous independent variables (heating length and moisture), whereas the third independent variable was fixed (temperature), as shown in Fig. 5.1-a₁. The circular contour plot indicated that the interactions between the corresponding variables were negligible, whereas the elliptical contour plot indicated perfect interactions (Guo *et al.*, 2010).

As shown in Figures 5.1-5.4, the development of resistant starch fractions varied significantly when the moisture content and temperature of the HMT varied (Fig. 5.1-b₁). The elliptical contour plots revealed a significant interaction between the three independent variables. This interaction effect was more pronounced for the variations in moisture and temperature (Figure 5.1-b₂) but less pronounced for the heating variation (Figure 5.1-b₁). These findings indicate that the variation in moisture content and temperature influenced SDS and RS formation in these maize varieties. When maize starch is exposed to high moisture contents and temperatures during HMT, the starch granules absorb water, swell, and undergo partial gelatinization. This process disrupts the native crystalline structure of starch, allowing for molecular rearrangement. However, because HMT occurs under limited water availability (compared with full gelatinization in excess water), complete granule breakdown is avoided, resulting in the formation of SDS and RS (Liu *et al.*, 2016; Wang *et al.*, 2016). For instance, Ye *et al* (2016) and Zou *et al.* (2023) reported that increasing both moisture content and temperature increases the SDS and RS contents in maize starch, which aligns with the findings of the present study.

The increase in moisture content during HMT promotes molecular rearrangement, transforms the crystalline structure of starch, often from B-type to A-type, decreases the relative crystallinity, and increases the SDS and RS contents (Piecyk and Domian, 2021; Sun *et al.*, 2024; Xiao *et al.*, 2017). For instance, increasing moisture levels during HMT between 25–35% has been shown to lead to increases in the SDS and RS contents of maize starch (Wang *et al.*, 2016), waxy maize and native maize starch (Chen *et al.*, 2017) and native maize flour (Ye *et al.*, 2016). On the other hand, increasing the HMT temperature has been shown to increase the SDS and RS contents due to structural changes that facilitate starch retrogradation and crystallinity (Liu *et al.*, 2016; Wang *et al.*, 2016). Higher temperatures promote the conversion of amorphous starch into more ordered structures, which resist enzymatic hydrolysis (Chung *et al.*, 2009; Wang *et al.*, 2018). For example, Gu *et al.* (2024) reported an increase in the SDS and RS contents in high-amylose maize starch when the HMT temperature increased, which aligns with the findings of the present study. However, the length applied during HMT is not a critical factor influencing the formation of SDS and RS in most cases (Ye *et al.*, 2016). As such, the variation in moisture content and temperature influenced SDS and RS formation in these maize varieties regardless of the heating duration.

The maximum SDS and RS value of 46 was obtained after HMT application for the maize variety DK777 (Figure 5.2: a₁, b₁, c₁). Generally, the variation in the independent variable affects the development of SDS and RS. The elliptical contour plots revealed significant interaction between the independent variables, except for the temperature and heating length, where the plot was circular, implying that there was no perfect interaction between these two independent factors (Figure 5.2: c₁₋₂). For the maize variety Longe 10H, the variation in the independent variables significantly affected the development of SDS and RS. As shown in Figure 5.3: a₁₋₂, the maximum SDS and RS value reached 48 when the moisture content was high for a short duration and when the temperature was kept constant. The elliptical contour plots for this variety further revealed a significant interaction between the independent variables (Guo *et al.*, 2010). For the variety DT Max, the elliptical contour plots revealed a significant interaction between the independent variables expect for the temperature and heating length (Figure 5.4: c₁₋₂), as depicted by the circular contour plot.

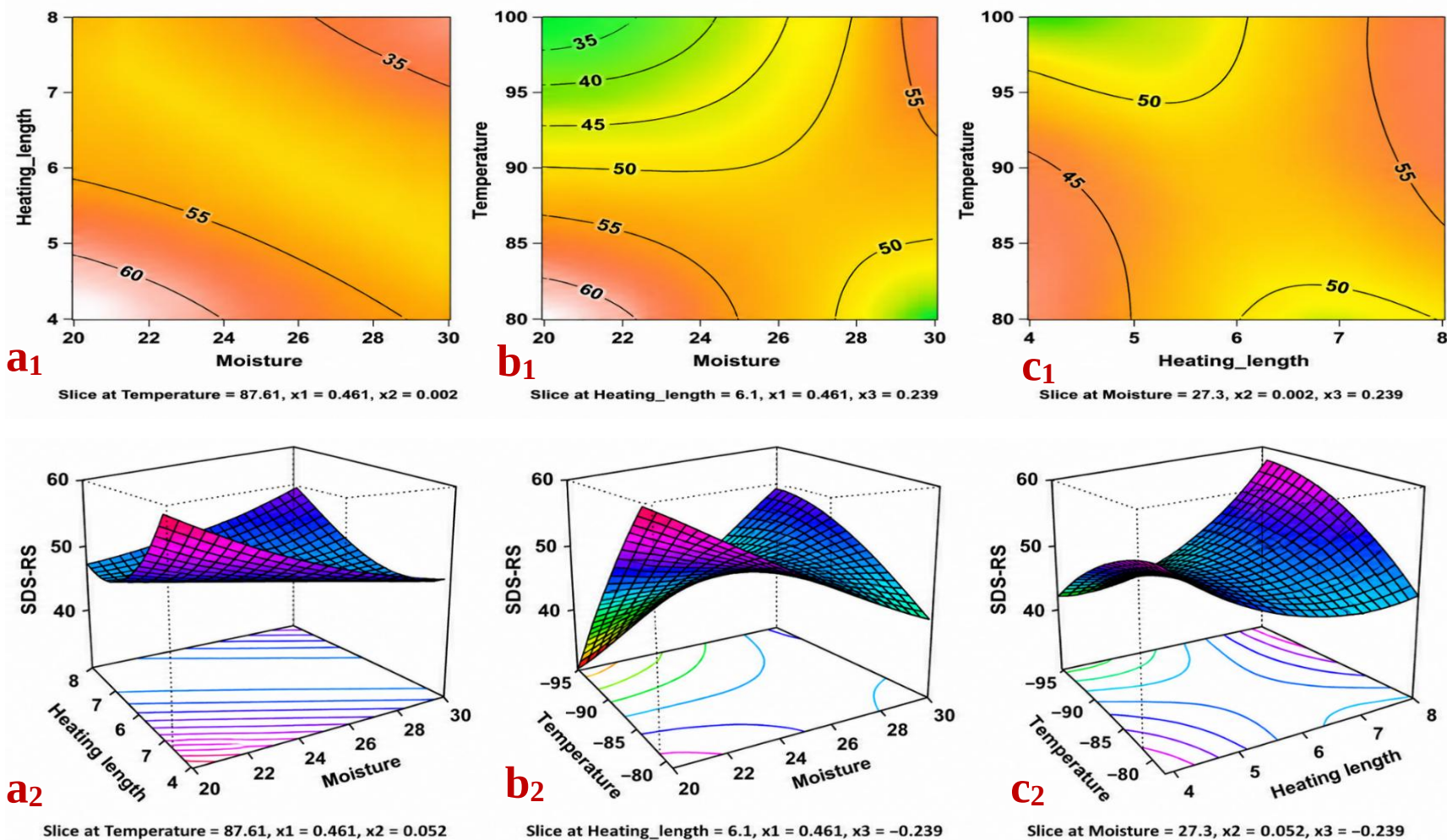


Figure 5.1: Response surface plots and contour plots showing the effects of moisture content, length of heating, and heating temperature on the development of composite slow digestible (SDS) and resistant starch (RS) contents of UH5354 maize variety

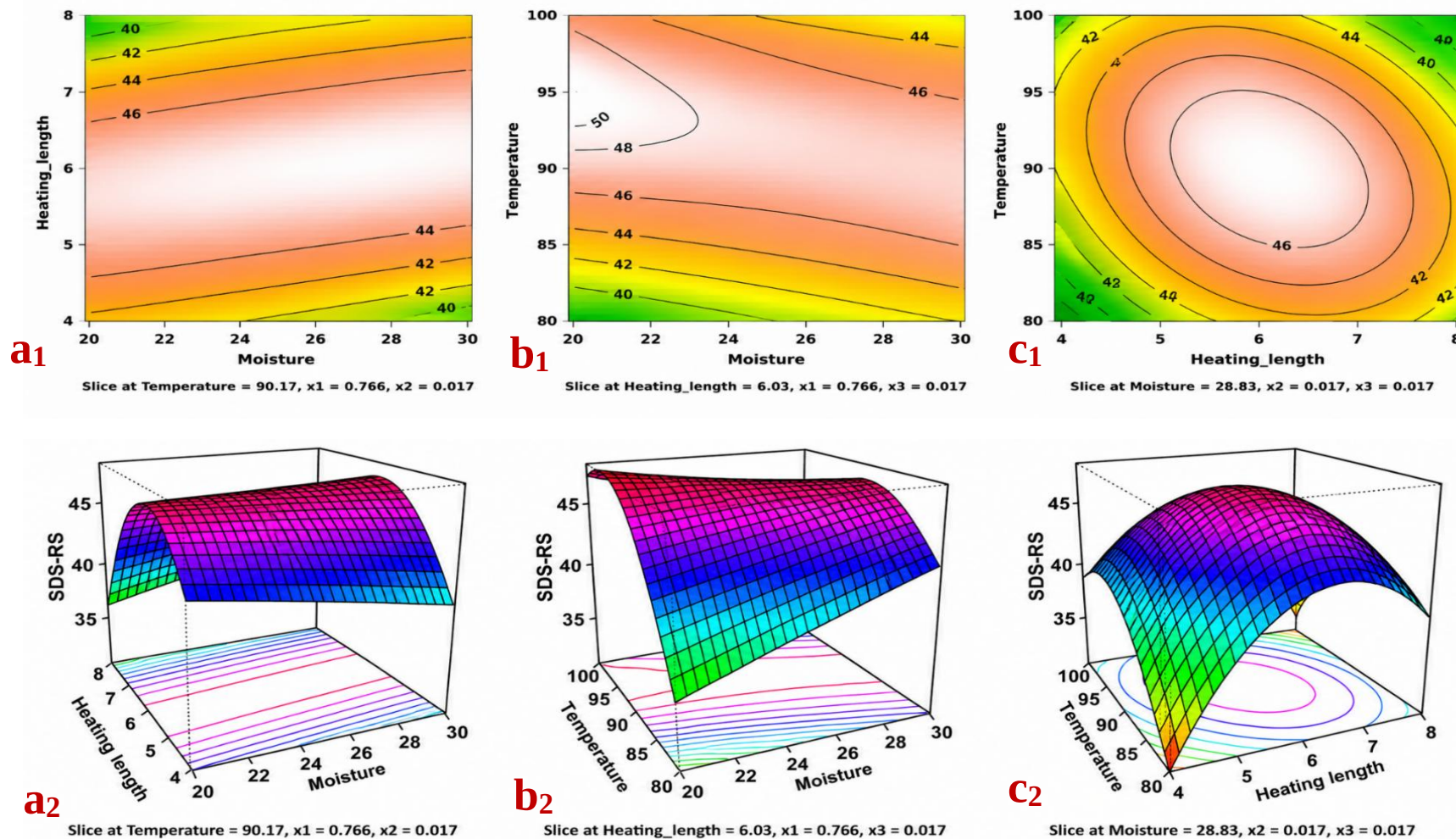


Figure 5.2: Response surface plots and contour plots showing the effects of moisture content, length of heating, and heating temperature on the development of composite slow digestible (SDS) and resistant starch (RS) contents of DK777 maize variety

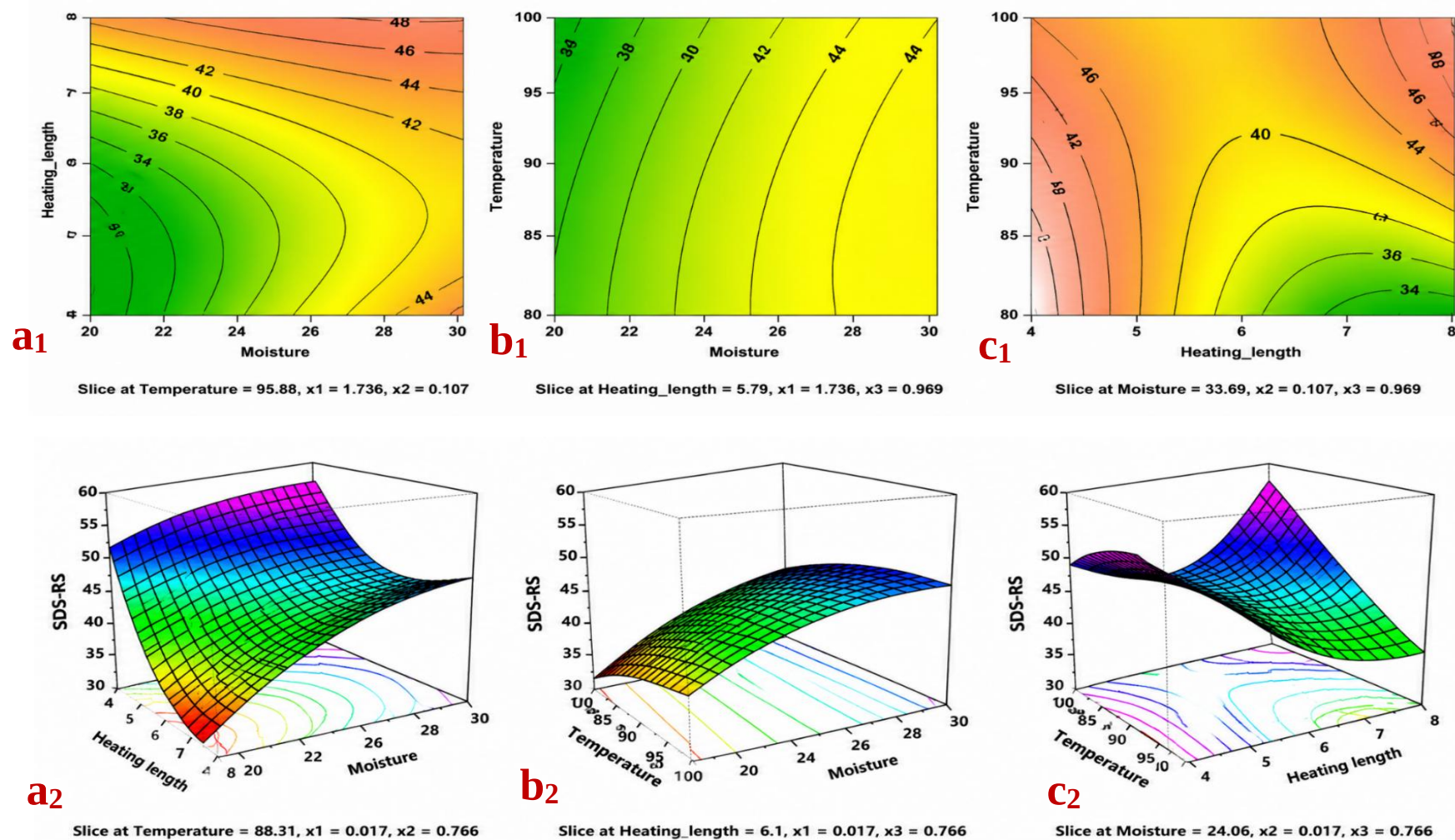


Figure 5.3: Response surface plots and contour plots showing the effects of moisture content, length of heating, and heating temperature on the development of composite slow digestible (SDS) and resistant starch (RS) contents of Longe 10H maize variety

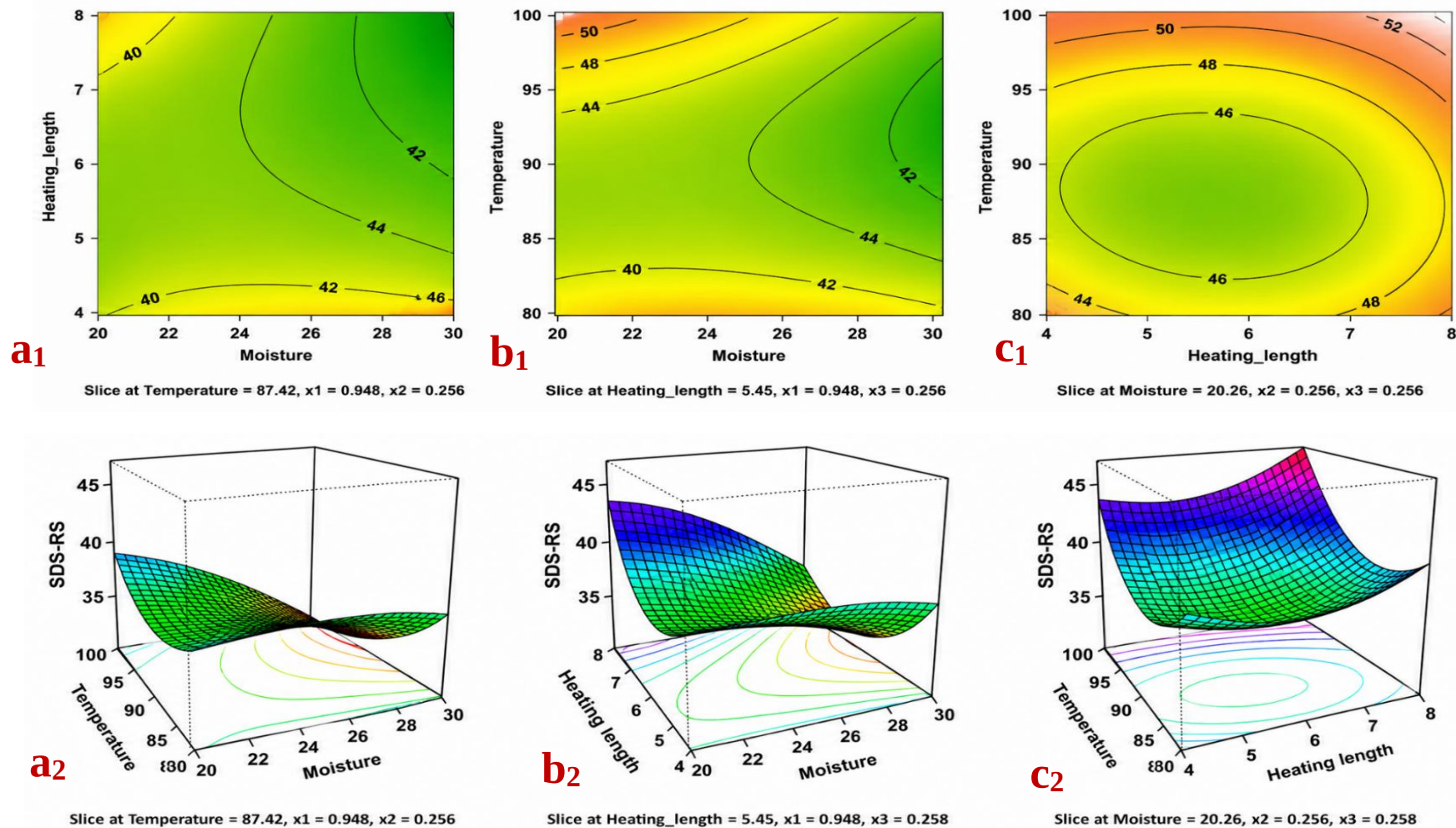


Figure 5.4: Response surface plots and contour plots showing the effects of moisture content, length of heating, and heating temperature on the development of composite slow digestible (SDS) and resistant starch (RS) contents of DT Max maize variety

5.4.2.2. Optimal HMT conditions

The heat–moisture treatment (HMT) parameters were optimized to maximize the development of slowly digestible starch (SDS) and resistant starch (RS) in the maize varieties. The optimal conditions varied according to variety, which is in agreement with the findings of Hsu *et al* (2015), which suggested that optimum conditions should be established per variety, albeit for non-polished maize. This implies that the polishing of maize does not affect the HMT conditions among varieties. The optimal combinations of moisture content, heating length and temperature were (23.17%, 4.12 hours, 80.00°C) for UH5354, (20.83%, 5.42 hours, 95.43°C) for DK777, (34.51%, 3.01 hours, 90.46°C) for Longe 10H and (19.24%, 5.88 hours, 97.02°C) for DT Max (Table 5.3). Although the experimental design was guided by literature-based parameters, the predicted optima namely the moisture contents of 19.24% for DT Max, 34.51% for Longe 10H, and a heating duration of 3.01 hours for Longe 10H, extended beyond the predefined experimental boundaries. Nevertheless, these values remained within the technically feasible operational range of HMT and are therefore considered acceptable in light of previous reports (Kadam *et al.*, 2023; Martínez-Castro *et al.*, 2024; Oliveira *et al.*, 2019). The occurrence of optima outside the design parameters suggests that the process may perform better than initially anticipated (Mohammed and Jarullah, 2023), indicating that the original design space may have been overly conservative. However, when optimal conditions lie beyond the tested range, it becomes essential to expand the experimental parameters and conduct confirmatory validation to establish the robustness and practical acceptability of these predictions (Kadam *et al.*, 2023; Martínez-Castro *et al.*, 2024), addressed accordingly in the subsequent section of this study.

The variation in optimum HMT conditions among the four maize varieties could be due to variations in the internal factors that affect starch digestibility (phenolic compounds, total starch and amylose) (Chang *et al.*, 2020; Zurak *et al.*, 2023) which was revealed in chapter four (Subsection 2.6.3). The four maize varieties used in the current study were from an earlier assessment of thirteen (13) varieties of maize commonly consumed in Uganda and East African countries (See chapter four, subsection 4.3.1). Considering that the four varieties used were the predominant ones from the four distinct groups based on

factors that affect starch digestibility (phenolic compounds, total starch, and amylose) (Chang *et al.*, 2020; Zurak *et al.*, 2023), the optimized HMT conditions can be applied to other maize varieties with similar factors that affect starch digestibility.

Table 5.3: HMT optimal application conditions according to the characteristics of the maize varieties

Varieties	Varieties characteristics		Moisture (%)	Heating length (Hours)	Temperature (°C)
UH5354	Phenolic (mg Eq Galic Acid)	201.23 ± 6.68	23.17	4.12	80.00
	Amylose (%)	22.35 ± 2.13			
	Total starch (%)	76.79 ± 2.55			
DK777	Phenolic (mg Eq Galic Acid)	191.93 ± 7.20	20.83	5.42	95.43
	Amylose (%)	23.09 ± 0.83			
	Total starch (%)	66.05 ± 2.37			
Longe 10H	Phenolic (mg Eq Galic Acid)	183.64 ± 4.66	34.51	3.01	90.46
	Amylose (%)	24.94 ± 1.93			
	Total starch (%)	76.00 ± 1.61			
DT Max	Phenolic (mg Eq Galic Acid)	217.00 ± 8.08	19.24	5.88	97.02
	Amylose (%)	24.81 ± 1.33			
	Total starch (%)	70.14 ± 3.45			

The data for the varieties' characteristics were obtained from the results of chapter 4 (Table 4.4).

5.4.3. Validation of optimal HMT conditions

A separate validation experiment was conducted for the derived optimal conditions to verify the predictions and adequacy of the models. The heat–moisture treatment experiments were carried out under optimal conditions for each variety. The experimental values and the predicted values generated by the model are shown in Table 5.4. Notably, the experimental values were not significantly different from the predicted values for SDS and RS for all varieties investigated except for UH5354 ($p=0.022$). These findings suggest that the regression models are in line with SDS and RS development through HMT application. Thus, the models are adequately suited for assessing the behavior of HMT conditions for maize grains, except for the UH5354 variety. The variation in the predicted and experimental values for the variety UH5354 can be attributed to factors such as residual

errors of the model, which play a critical role in evaluating the model accuracy (Li *et al.*, 2021).

Additionally, variation could also be due to inherent composition, such as higher protein and lipid concentration of this maize variety, which could have promoted the development of starch–lipid or starch–protein complexes (Liu *et al.*, 2022). Starch–lipid complexes, particularly amylose–lipid complexes, can reduce the swelling of maize granules and increase their hydrophobicity (Wang *et al.*, 2025), which influences the development of SDS and RS (Sun *et al.*, 2021). In addition, starch–protein complexes change the structural and functional properties of starch, which is known to influence the development of SDS and RS (Liu *et al.*, 2022). As such, the lipid and protein composition of this variety could have affected the estimation of SDS and RS, hence the disparity in observed and predicted values reported in this study. In summary, the variability in SDS and RS between the predicted and experimental values may be attributed to residual errors in the regression model, as well as the potential interactions of starch components with proteins and lipids of this variety.

Table 5.4: Comparison of experimental and predicted values of starch digestibility

Maize varieties	Experimental Value	Predicted Value	Difference (p value)
	SDS+RS (%)	SDS+RS (%)	
UH5354	64.09	69.94	0.022*
DK777	54.08	49.34	0.067
Longe 10H	52.46	56.73	0.655
DT Max	54.64	48.90	0.053

* Denotes a statistically significant difference between the experimental and predicted values ($p \leq 0.05$).

5.4.4. Effect of optimal HMT conditions on starch digestibility

The effect of optimal conditions on the SDS and RS in maize varieties is presented in Table 5.5. Compared with that of the control/untreated sample, the amount of SDS and RS (SDS+RS) significantly increased ($p < 0.05$) after HMT application (Table 5.5). Under optimal conditions, SDS+RS increased from 8% to 14% across various varieties. Specifically, relative to the control, the increases were 9.35% for UH5354, 10.31% for

DK777, 7.57% for Longe 10H, and 14.39% for DT Max. These results demonstrate that the HMT process effectively reduced starch digestibility, primarily by increasing the SDS and RS contents of polished maize flour. According to the Wang *et al.*, (2023) and Karunarathna *et al* (2024), the consumption of cereal products rich in SDS and RS is associated with a slower rate of glucose appearance in the bloodstream, resulting in a lower postprandial glycemic response. Foods with high SDS contents have been reported to exhibit a lower glycemic index (GI) because they promote a sustained and balanced release of glucose over time (Gourineni *et al.*, 2017). Consequently, increasing the SDS and RS fractions through HMT is expected to reduce the glycemic index of maize flour by slowing starch digestion and glucose absorption, thereby attenuating the postprandial glycemic response. These changes enhance the carbohydrate quality of maize flour by promoting a more gradual glucose release and improved metabolic regulation. Therefore, the optimized HMT conditions represent a promising strategy for improving the nutritional quality of maize flour and may contribute to the dietary management and prevention of glycemia-related non-communicable diseases. Such an effect enhances the carbohydrate quality of maize flour by promoting slower glucose release and improved metabolic regulation, underscoring the nutritional relevance of HMT as a functional processing technique.

These optimal conditions therefore should be promoted to increase carbohydrate quality of polished maize flour and can be applied to similar maize varieties with comparable internal factors affecting starch digestibility. Although this study focused only on improving the SDS and RS of polished maize flour, their effects on physicochemical and functional characteristics have not been investigated, as reported by several studies in the literature. HMT affects the nutritional (proximate composition), color, functional (swelling, solubility), pasting, and thermal properties of starchy foods (Dong *et al.*, 2021; Olayinka *et al.*, 2008; Piecyk and Domian, 2021; Sandhu *et al.*, 2020), which may impact the acceptability of HMT-treated maize flour. Therefore, for better adoption of these HMT conditions, there is a need to assess the nutritional and functional properties, as well as the effects of processing conditions on the aforementioned parameters. These aspects are examined in chapter six of the thesis.

Table 5.5: Variations in the starch digestibility components of optimal HMT-treated maize varieties

Maize varieties	HMT	RDS (%)	SDS+RS (%)
UH5354	Untreated	45.26 ^a ± 1.46	54.74 ^b ± 1.46
	Treated	34.13 ^b ± 1.54	64.09 ^a ± 1.54
DK777	Untreated	55.72 ^a ± 4.26	44.28 ^b ± 4.26
	Treated	45.41 ^b ± 2.35	54.08 ^a ± 2.35
Longe 10H	Untreated	55.11 ^a ± 0.72	44.89 ^b ± 0.72
	Treated	47.54 ^b ± 1.48	52.46 ^a ± 1.48
DT Max	Untreated	59.75 ^a ± 2.18	40.25 ^b ± 2.18
	Treated	41.53 ^b ± 1.43	54.64 ^a ± 1.43

All values are means ± Standard Deviation. At the variety constant, means within columns with different superscripts are significantly different ($p < 0.05$).

5.5. Conclusion

RSM was successfully utilized to optimize the HMT conditions to reduce starch digestibility (yielding high contents of SDS and RS) for four maize varieties predominantly consumed in Uganda. The optimum moisture, temperature, and heating time varied according to the maize variety. The optimal conditions could be used as a strategy to mitigate non-communicable diseases associated with the consumption of high-starch digestibility polished maize flour. Decision makers should advocate the adoption of optimal HMT conditions to mitigate health risks associated with the high starch digestibility of polished maize flour. Given that maize flour production and distribution in Uganda and other East African countries are predominantly managed by industrial processors, promoting these optimal HMT conditions within the industry could improve the carbohydrate quality of polished maize flour available for commercialization and community consumption. Whereas the current study developed optimum HMT conditions that reduce starch digestibility among selected maize varieties, there is a need to consider the impact of HMT on the physicochemical, functional, pasting and thermal properties and acceptability of flour for widespread utilization among households. These aspects were examined in detail and reported in chapter six.

CHAPTER SIX

Effects of optimized heat–moisture treatment conditions on physicochemical and techno-functional properties of maize flour

This chapter is adapted from the published manuscript below:

Fassinou, F. T. K., Olum, S., & Ongeng, D. (2025). Effect of optimized heat-moisture treatment conditions on physicochemical and techno- functional properties of flour from four maize varieties commonly consumed in East African countries. *Discover Food*, 5(379), 1–19. <https://doi.org/10.1007/s44187-025-00694-y>

6.1. Abstract

Maize varieties commonly consumed in Uganda exhibit high starch digestibility, making them release high glucose in the bloodstream, a risk factor for non-communicable diseases. In a previous study, we optimized heat moisture treatment (HMT) conditions to reduce starch digestibility from four commonly consumed varieties (DK777, Longe 10H, UH5354, and DT Max). The current study investigated the effect of the optimized HMT conditions on physicochemical (proximate composition, total starch, amylose, and color) characteristics and techno-functional (swelling, solubility, water/oil absorption capacity, bulk density, and pasting) properties of flour from the four maize varieties. A paired sample t-test revealed that the optimized HMT conditions had no effect on the physicochemical properties of all maize varieties used in the study. However, it had various effects on techno-functional properties and depended on variety. Swelling volume decreased by 7.29% and 13.02% for Longe 10H and DT Max varieties, respectively. Water absorption capacity increased by 6.66% for Longe 10H and 7.87% for UH5354. Oil absorption capacities and bulk densities remained constant after HMT ($p>0.05$). Pasting temperature increased by 1.49%, 3.00%, 5.51%, and 10.35% for UH5354, Longe 10H, DT Max, and DK777 varieties, respectively, while holding viscosity decreased by 15.93% for DT Max and 97.78% for DK777. The observed changes could affect the maize posho quality (e.g., elasticity, cohesiveness, hardness, energy need, and cooking time), thus decreasing the acceptability of the flour. These results have demonstrated that while optimizing HMT solely for decreasing starch digestibility, the conditions may affect techno-functional properties, and thus sensory acceptability of the flours and derived products.

6.2. Introduction

Maize is a vital staple food in Sub-Saharan Africa (SSA), especially in Eastern Africa, where it is highly important for food security and nutrition (Falconnier *et al.*, 2023). Compared to wheat and rice, maize is a versatile multipurpose crop and an important human food in several SSA countries. It accounts for 40% of the cereal production in the region, with over 80% used as food (Ekpa *et al.*, 2019), with its intake ranging between 52 and 450 g/person/day. Uganda is one of the top ten maize-producing countries in SSA. In 2023, maize production was projected to reach 3.2 million metric tons (MTs), which corresponds to an 8% increase over 2022 production levels (Food and Agriculture Organization, 2024). Maize is consumed by more than 90% of Ugandan households, making it a key source of energy, especially in the northern region of Uganda, where maize starch is the primary energy source in the human diet.

Upon consumption and digestion, starch is classified as rapidly digestible starch (RDS), slowly digestible starch (SDS), and resistant starch (RS). RDS and SDS are digestible in the small intestine, leading to high and moderate increases in glucose levels in the blood, respectively, due to their glycemic response (Englyst *et al.*, 1992). In contrast, RS is fermented in the large intestine and does not impact blood glucose levels (Englyst *et al.*, 1992). Given that a high glycemic response increases the risk of non-communicable diseases, increasing attention has been given to reducing RDS in foods for potential health benefits (K. C. Cheng *et al.*, 2019). Unfortunately, maize has been shown to have high levels of RDS (De-Alcântara *et al.*, 2020; Zurak *et al.*, 2023). The high consumption of maize flour as it is in Uganda has therefore become a risk factor for the development of non-communicable diseases, which account for more than 30% of deaths in the country yearly (World Health Organization (WHO) African Region, 2023). Consequently, there is an urgent need to decrease the starch digestibility of commonly consumed maize varieties in Uganda to make them more suitable for health purposes.

In this context, heat moisture treatment (HMT), an environmentally friendly and cost-effective physical modification method (Kumar *et al.*, 2023) was optimized (chapter five of this thesis) to increase the SDS and RS contents of four maize varieties selected based on better state of factors that influence starch digestibility. The optimal HMT conditions

improved the composite SDS+RS content by 8-14%, offering promising health benefits. HMT was chosen for its ability to reduce starch digestibility without chemical additives, making it ideal for resource-limited settings while preserving the native starch structure of maize flour (Amaraweera *et al.*, 2021; Fonseca *et al.*, 2021). Whereas HMT decreases starch digestibility, several studies have demonstrated that HMT can significantly affect the physicochemical and techno-functional properties of cereal starch (Mathobo *et al.*, 2021; Ridwan Ariyantoro *et al.*, 2020). Research on sorghum (Olayinka *et al.*, 2008), maize (Sui *et al.*, 2015), and rice (Hormdok and Noomhorm, 2007) have shown that such modifications can positively or negatively influence the quality of flours, and thus, consumer acceptability (Dong *et al.*, 2021). However, these modifications are dependent on multiple factors, including the botanical source of the starch (Liu *et al.*, 2020), the variety of cereals (Fang *et al.*, 2023), and the HMT conditions applied (X. Tan *et al.*, 2017). For example, the swelling properties that enhance softness and texture significantly increase in waxy maize starch but not in normal maize starch under identical HMT conditions (Ma *et al.*, 2021). Likewise, under identical HMT conditions, the increase in amylose, a physicochemical property that contributes to the firmness, cohesiveness, and elasticity of dough and posho preferred in food applications, decreases in normal maize starch while increases in waxy maize starch (Ma *et al.*, 2021). The reduction in pasting properties, such as breakdown and setback viscosity, which results in stable, soft textures and lower gel strength of maize dough and posho favoured by consumers, varies among different maize varieties (Ma *et al.*, 2021).

Mathobo *et al.* (2021) and Maior *et al.* (2021) reported that HMT significantly changes the physicochemical and techno-functional properties of maize starch according to the conditions applied. A critical analysis of various literature sources revealed that the effect of HMT on the techno-functional and chemical properties of maize and other cereals depends on the variety and conditions applied (Fang *et al.*, 2023; Liu *et al.*, 2020; Ma *et al.*, 2021; Maior *et al.*, 2021; Mathobo *et al.*, 2021; Tan *et al.*, 2017), highlighting a gap in the direct applicability of findings across different maize varieties. Analyzing the potential changes caused by HMT in specific varieties is thus essential for proper utilization and anticipation of consumer acceptability of HMT-treated maize flour. In addition, HMT has been optimized to decrease starch digestibility in maize flour in Uganda (Fassinou *et al.*,

2025), but the effect of the optimal conditions on those techno-functional properties of maize is still scarce.

Accordingly, on the basis of the HMT conditions that decreased the starch digestibility of commonly consumed maize varieties in Uganda, the present study assessed the effects of optimized HMT conditions on the physicochemical and techno-functional properties of maize flour derived from four varieties commonly consumed in Eastern African countries, specifically in Uganda.

6.3. Materials and methods

6.3.1. Materials

Four maize varieties, DK777, Longe 10H, UH5354, and DT Max, were selected for this study. These varieties were selected because they are among the most locally available and commercialized maize varieties according to agro-input dealers in northern Uganda, Gulu district. They are generally grown by farmers in the study area and are the most consumed. The grains of each variety were purchased directly from the farmers after the harvest of November 2023. The maize grains were first cleaned by blowing to remove dust and other debris. They were then manually sorted to eliminate foreign materials and broken grains before being packaged for further use. They were then stored in PICS (Purdue Improved Crop Storage) bags composed of two polypropylene bags to avoid moisture gain. Storage was maintained under laboratory conditions for eight weeks prior to heat moisture treatment.

6.3.2. Methods

6.3.2.1. Sample preparation for HMT

Sample preparation involved two batches: one consisting of maize flour from HMT-treated maize grains and the other consisting of native flour from non-treated maize grains, which served as a control. For the maize grains treated with HMT, the optimal HMT conditions for higher SDS and RS contents were applied for each variety (See chapter 5, Table 5.3). The optimal HMT conditions (moisture content, heating time and temperature) for maximizing the SDS and RS contents of the four selected maize varieties were as follows: (23.17%, 4.12 hours, 80.00°C) for UH5354; (20.83%, 5.42 hours, 95.43°C) for DK777;

(34.51%, 3.01 hours, 90.46°C) for Longe 10H; and (19.24%, 5.88 hours, 97.02°C) for DT Max. HMT was carried out under these conditions for each maize grain variety following the laboratory protocol proposed by (Sui *et al.*, 2015) described in detail in section 5.2.2.2.

The moisture content of the maize grains previously cleaned and stored in the PICS bags was adjusted for HMT treatment. The initial moisture content of each maize grain was determined via the gravimetric method and a hot air oven according to the AOAC standard method 925.09 (AOAC, 1993). The moisture content of the maize grains was adjusted using distilled water, and the quantity to be added was determined according to the formula proposed by Cordeiro *et al.* (2018) (Eq 6.1).

$$m_w = [(100 - M_i/100 - M_f) - 1] * m \quad (\text{Eq. 6.1})$$

where m_w (g) is the mass of water to be added, M_i (%) is the initial moisture of the sample, M_f (%) is the desired moisture, and m (g) is the mass of the maize grain used.

One thousand grams of each maize grain variety (m) was then immersed in an appropriate quantity of distilled water determined via equation (6.1) and kept at room temperature for approximately 30 minutes, such as that applied for maize by Paliwal *et al.* (2023) for sorghum. The wet maize grains were sealed in a polypropylene zip bag and kept at 4°C in a Hisens refrigerator (Model RT488N4ASU, Guangdong, China) for 24 h to equilibrate the moisture content at 23.17, 20.83, 34.51, and 19.24% for UH5354, DK777, Longe 10H, and DT Max, respectively. They were then hermetically sealed in a glass bottle to avoid a decrease in moisture content during heat treatment and oven-heated using a hot-air oven (Sui *et al.*, 2015) at the corresponding temperatures and times mentioned above. After HMT application, the heat moisture-treated maize grains were dried in an oven with forced air circulation at 50°C for 24 h to decrease the moisture content up to 10–11% (Van Hung *et al.*, 2020) before being ground into flour. The HMT-treated maize grains and a similar quantity of native maize grains were ground into flour under laboratory conditions using the laboratory blender 8010EG (Model HGBTWG4, Torrington, Connecticut 06700 USA) and sieved through a 100 μ m British Standard Sieve. The resulting flour for each sample was stored in paper bags at room temperature until physicochemical, techno-functional, and pasting properties were analyzed. The polishing step was deliberately

omitted at this stage so that any change observed in the physicochemical and techno-functional properties could be attributed to heat-moisture treatment alone, without the confounding effect of polishing.

6.3.2.2. Determination of the physicochemical characteristics of flours from HMT-treated and native maize grains

Analyses were performed on flour from HMT-treated maize grains and native maize grains without treatment, which were used as controls. The proximate composition, amylose content, amylopectin content and color characteristics were the physicochemical characteristics considered.

6.3.2.2.1. Determination of proximate composition

The dry matter of the maize flour was determined using the gravimetric method following the standard AOAC number 925.09 (Association of Official Analytical Chemists, 1990). Ash content was determined in a Muffle furnace at 550°C until a grey ash result was obtained. The total nitrogen/protein content was determined according to the micro-Kjeldahl method as applied by Afolayan *et al.* (2022) and Jamil *et al.* (2020). Briefly, the samples were digested in concentrated sulfuric acid, and one Kjeldahl tablet was added as a catalyst using a Kjeldahl digester (VELP Scientifica, Model A00000176, Italy). The digested samples were distilled in 40% sodium hydroxide, and the liberated ammonia was absorbed in 4% boric acid containing methyl red as the indicator using the Kjeldahl distiller (VELP Scientifica, Model F30200140, Italy). Hydrochloric acid (0.1 N) was used for titration, and the nitrogen content was determined using equation 6.2.

$$\text{Nitrogen} = (\text{Na} * \text{Va} * 14 * \text{DF} * 100) / (\text{Wt} * 1000) \quad (\text{Eq. 6.2})$$

Here Va is the volume of acid used in the titration, Na is the normality of the acid used for the titration, and Wt is the mass of maize flour used.

As most proteins, such as those in maize flour, contain 16% N, a conversion factor of 6.25 (100/16= 6.25) was used to convert the percentage of nitrogen to the percentage of crude protein. The fat content was determined using the Soxhlet System according to standard AOAC method number 925.09 (Association of Official Analytical Chemists, 1990) as

applied by Afolayan *et al.* (2022) and Jamil *et al.* (2020). Crude fat extraction was performed using petroleum ether as a solvent and a Soxhlet system (Soxtec™ Avanti 2050 Auto System, Foss Tecator AB, Höganäs, Sweden). The percentage of crude fat content was calculated using equation 6.3:

$$\text{Crude fat (\%)} = [(W_2 - W_1)/\text{Weight of sample}] * 100 \quad (\text{Eq. 6.3})$$

Where: W2 = the weight of the extraction flask after the extraction process with fat. W1 = the weight of the empty extraction flask.

The Weende method was used for crude fiber content determination as described by Musembi *et al.* (2024). The crude fiber was extracted using a fiber analyzer (FIWE Raw Fiber Extractor, Velp Scientifica-Europe) by solubilizing non-cellulosic substances with 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 the addition of 5 drops of octan-1-ol (antifoam). The samples were boiled for 30 min at 100°C after being prepared until boiling. After the sulfuric acid was removed, the samples were washed 3 times in hot deionized water (30 mL per wash). The above procedure was repeated with a sodium hydroxide solution (1.25%). The samples were subsequently 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 (6.4) was used to calculate the percentage of crude fiber.

$$\text{Crude fiber (\%)} = \frac{\text{Oven-dried sample weight} - \text{Ashed sample weight}}{\text{Fresh sample weight}} * 100 \quad (\text{Eq. 6.4})$$

The total carbohydrate content was determined via a differential method according to equation (4.5) as described by Kandyliari *et al.* (2020):

$$\text{COH (\%, db)} = 100\% - \text{protein (\%, db)} - \text{lipid (\%, db)} - \text{ash (\%, db)} \quad (\text{Eq. 6.5})$$

6.3.2.2.2. Determination of the amylose and amylopectin contents

The amylose content of the maize flours was determined using the iodine calorimetry method applied by Babatola *et al.* (2021). A sample of maize flour (0.1 g) was mixed with 95% ethanol (1 mL) and 1 N NaOH (9.2 mL), heated in a Memmert Water Bath at 100°C for 10 min and allowed to cool. Approximately 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 brought to 10 mL with distilled water and allowed to stand for color development, and the color was read at 620 nm. Moreover, potato amylose was prepared between 1 and 35% in 95% ethanol (1 mL) and 9.2 mL of NaOH (1 N) and was used as a standard amylose solution. The curve was used to calculate the amylose content in the maize flour samples. The amylopectin content was calculated by subtracting the amylose content from the hundred (Huang *et al.*, 2024).

6.3.2.2.3. Determination of the total starch content

The total starch content of the maize samples was analyzed using the sedimentation method of Goñi *et al.* (1996), and the concentration of released glucose was read using the phenol–sulfuric acid method proposed by Dubois *et al.* (1956). Briefly, 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. Then, 2 mL of 2 M potassium hydroxide solution was added, and the mixture was stirred with a magnetic bar (5×15 mm) for 20 min in a water bath. Eight milliliters (8 mL) of 1 M sodium acetate solution (pH 3.8) were added, followed by the addition of 3 mL of thermostable α -amylase (0.5%). The mixture was incubated at 95°C for 10 minutes in a Memmert Water Bath. Amyloglucosidase (0.1 mL) was added to the mixture, which was subsequently incubated at 50°C for 30 minutes and cooled to room temperature. The volume of the solution was adjusted with deionized water to 50 mL and centrifuged at $4000 \times g$ for 10 min using Centurion Scientific Benchtop Model 1. C2015 centrifuge (Centurion Scientific Ltd, United Kingdom). The sample (0.1 mL) was collected in a 10 mL glass tube to determine the free sugar glucose (FSG) content following the phenol–sulfuric acid method (Dubois *et al.*, 1956). The tube containing the sample and phenol–sulfuric acid was incubated in a water bath at 30°C for 20 min for color development and cooled to room temperature. The absorbance of each sample was

measured at 490 nm using a Jenway 6505 spectrophotometer (Jenway, United Kingdom). A glucose solution ranging from 0–500 mg/mL in aqueous ethanol (80%, v/v) was used as the standard solution for curve generation. The percentage of total starch was calculated by multiplying glucose content determined by 0.9 (Englyst *et al.*, 1992).

6.3.2.2.4. Determination of the color characteristics

The color characteristics of the maize flour samples were measured using a Minolta CR-400 Chroma Meter (CR400 Model, Konica Minolta, Japan) according to the method applied by Chávez-Murillo *et al.* (2018). For each sample, 5 readings were taken from the flour samples and recorded. The color measurements included L* (lightness, 0 = black/100 = white), a* (+a* = redness/-a* = greenness), and b* (+b* = yellowness/-a*=blueness). The instrument was calibrated with a white plate (L=97.12, a=0.18, b=1.75) (Ahn *et al.*, 2013). The whiteness index of the maize sample was calculated using Equation (6.6) used by Marta *et al.* (2022).

$$\text{Whiteness index} = 100 - \sqrt{(100 - L^*)^2 + a^{*2} + b^{*2}} \quad (\text{Eq. 6.6})$$

6.3.2.3. Determination of techno-functional

The swelling volume (SV), solubility (SOL), water and oil absorption capacity (WAC and OAC), bulk density (BD), and pasting properties were the techno-functional properties determined.

6.3.2.3.1. Determination of the swelling volume and solubility

The swelling volume (SV) and solubility (SOL) were determined according to the sedimentation volume method applied by Marta *et al.* (2022) and Cahyana *et al.* (2019). SV was determined by adding 12.5 mL of distilled water to a centrifuge tube containing 0.35 g (dry basis) of maize flour. The samples were mixed using a vortex mixer for 30 s and then heated in magnetic stirred Wagtech OLS Model 200 water bath (Grant Instruments, Germany) at 92.5°C for 30 min with constant stirring. The mixture was cooled in iced water for 1 min before centrifugation at 2050 × g for 30 min using Centurion Scientific Benchtop Model 1. C2015 centrifuge (Centurion Scientific Ltd, United

Kingdom). The volume of the supernatant obtained was measured, and the sample was dried in a hot air oven. The swelling volume and solubility of the maize flours were calculated using equations 6.7 and 6.8 as applied by Marta *et al.* (2022).

$$\text{Swelling Volume (mL/g)} = \frac{\text{Total volume} - \text{Supernatant volume}}{\text{Weight of the sample (db)}} \quad (\text{Eq 6.7})$$

$$\text{Solubility (\%)} = \frac{\text{Weight of dried supernatant}}{\text{Weight of the sample (db)}} * 100 \quad (\text{Eq 6.8})$$

6.3.2.3.2. Determination of the water absorption capacity

Water absorption capacity (WAC) of the flours was determined according to the differential thermogravimetric method applied by Cahyana *et al.* (2019). One gram of maize flour in a centrifuge tube was suspended in 10 mL of distilled water. The mixture was left to stand at room temperature for 1 h, followed by centrifugation using Centurion Scientific Benchtop Model 1. C2015 centrifuge (Centurion Scientific Ltd, United Kingdom) at $2000 \times g$ for 30 min. The free water supernatant was removed from the wet maize flour and drained for 10 min, and the wet sample weight was measured. WAC was calculated as mL of water absorbed per gram of maize flour. The results obtained were expressed on a dry basis as grams of water bound per gram of flour sample (Cahyana *et al.*, 2019).

6.3.2.3.3. Determination of the oil absorption capacity

Oil absorption capacity (OAC) of the maize flours was determined using the differential weight gain method applied by Ashwar *et al.* (2016). Briefly, 2.5 g of maize flour samples on a dry weight basis (db) were each mixed with 20 mL mustard oil and then stirred for 30 min at 25°C using a Thermo Scientific MAXQ 4450 shaker (Model SHKE4450CC-1CE, United States of America). The slurry was then centrifuged at $3000 \times g$ for 10 min using a Centurion Scientific Benchtop centrifuge and the supernatant was decanted. The OAC was calculated as the difference between the initial weight of the sample and the weight of the sample after the oil was added. The OAC was expressed on a dry basis as grams of oil bound per gram of flour sample (Ashwar *et al.*, 2016).

6.3.2.3.4. Determination of the bulk density

Bulk density (BD) was determined according to the volumetric method applied by Adebisi *et al.* (2016). Fifty grams of each flour sample was weighed into a 100 mL graduated measuring cylinder. The bottom of the cylinder was gently tapped several times on a laboratory bench until there was no further decrease in the sample level, and the volume was recorded. The bulk density (g/cm³) was expressed as the weight of the samples per volume of the samples after tapping (Adebisi *et al.*, 2016).

6.3.2.3.5. Determination of the pasting properties

The pasting properties of the maize flours were determined using Rapid Visco Analyzer (RVA-4500, Perten Instruments, Australia) according to the method applied by Marta *et al.* (2022) and Nuwamanya *et al.* (2023). Approximately 3 g of flour sample was mixed with 25 g of distilled water to form a flour suspension. The suspension was poured into an RVA canister and subjected to a programmed heating and cooling cycle. The RVA test profile was held constant at 50°C, and the mixing speed was 960 rpm for 1 minute. After the sample was maintained at 50°C for 1 min, the mixing speed was decreased to 160 rpm, and the temperature was held at 50°C. The sample was then heated at a rate of 12°C/min to 95°C, held at 95°C for 2.5 min, cooled to 50°C for 2.5 min and then kept at 50°C for 2 min. The rotation speed of the stirrer was maintained at 160 rpm. The final viscosity was recorded after 13 minutes. The flour pasting temperature, peak viscosity and final viscosity were recorded. The breakdown viscosity and the setback viscosity were calculated using equations 6.9 and 6.10, respectively as applied by Vidaurre-Ruiz *et al.* (2024).

$$\text{Breakdown viscosity} = \text{Peak viscosity} - \text{Holding viscosity} \quad (\text{Eq 6.9})$$

$$\text{Setback viscosity} = \text{Final viscosity} - \text{Holding viscosity} \quad (\text{Eq 6.10})$$

6.3.3. Statistical analysis

In this study, for each maize variety, two independent HMT-treated samples were prepared and analyzed in the laboratory. All the samples were analyzed in duplicate and all the results are reported as the means $\pm$ standard deviations. As the primary aim of this study was to investigate the effect of heat–moisture treatment (HMT vs. non-HMT) on maize flour quality within each variety, each variety was subjected to its own optimized HMT

condition. Therefore, the HMT condition developed for one maize variety was not applied across all other varieties, and vice versa, resulting in a design that was not fully crossed (Schielzeth and Nakagawa, 2013). Consequently, a paired sample t test was employed to compare treated and untreated samples within the same variety, as it is statistically appropriate for analyzing two related conditions, such as pre- and post-treatment scenarios and compares the means between these two related groups (Langenberg *et al.*, 2023). A significance level of 5% was used. All these analyses were performed using R software version 4.4.2.

6.4. Results and Discussion

6.4.1. Effect of HMT on the physicochemical characteristics of maize flour

6.4.1.1. Effect of HMT on the proximate composition

Proximate composition of maize is essential for households, where maize-based diets are the main staple food such as in Uganda. Therefore, a decrease in its proximate content may affect diet quality and food security. The initial proximate composition of the native maize flours varied across the different varieties. The ash content varied from 1.05% in Longe 10H to 1.33% in UH5354, the protein content (g/100 g dry basis) ranged from 7.42 in Longe 10H to 8.79 in DT Max, fat content (g/100 g dry basis) ranged from 3.09 in UH5354 to 4.68 in Longe 10H, and crude fiber content (g/100 g dry basis) varied from 9.98 in DK777 to 13.43 in Longe 10H. These values are within the range of proximate compositions reported in the literature by Adeniyi and Ariwoola (2019) for 6 maize varieties in Nigeria, Sumbo and Victor (2014) in Nigeria for a common maize, by Mugabi *et al* (2026) for maize from Kampala while lower than values reported by Majamanda *et al.* (2022) for pigmented maize in Malawi and for maize from Eastern Uganda by Birungi *et al* (2023).

HMT generally did not significantly affect the proximate composition of the maize grains, regardless of the variety (Table 6.1, $p>0.05$). The protein content decreased slightly by approximately 10%, but not significantly, for DK777, UH5354 and DT Max but decreased by 5% for Longe 10H. Moreover, the crude fiber contents remained almost unchanged for UH5354 and Longe 10H but decreased slightly by 2 and 4% for DK777 and DT Max. The non-significant difference observed between the native and the HMT-treated maize flour

may be due to the HMT conditions applied. According to Ye *et al* (2016), HMT does not affect the nutritional content of maize flour when it is conducted with a moisture content ranging from 20--30%, a heating duration ranging from 4--8 h, and a temperature ranging from 80--100°C, which encompasses the optimal conditions applied. In addition, studies have shown that HMT can decrease starch digestibility but does not alter the proximate composition (Mathobo *et al.*, 2021). Therefore, these results indicated that while considering only the effect of the optimized HMT condition on proximate composition and starch digestibility, the HMT conditions applied could promote the beneficial reduction in starch digestibility in these maize varieties without affecting their proximate composition.

Similar findings were reported by Marta *et al.* (2022), Dong *et al.* (2021), and Goel *et al.* (2020) regarding the proximate composition of HMT-modified starch-containing foods. Dong *et al.* (2021) applied HMT at 30% at 110°C for 90 min to whole quinoa seeds and reported that HMT did not affect the protein and ash contents, whereas only the fat content decreased. Marta *et al.* (2022) applied HMT at 30% at 100°C for 16 h to maize starch and reported that HMT did not significantly affect the protein, fat, or ash contents of the maize starch, whereas only the moisture content decreased. The decrease in moisture may be attributed to the drying process always applied after HMT to reduce the moisture content for better storage. This could most likely induce a reduction in the moisture content compared with its original level before treatment. Due to this explanation, a slight and non-significant decrease in the moisture content was also observed in the present study. As maize grains with a low moisture content (below 15%) can be stored for a long time, the drying process applied to HMT-treated samples could then extend their storage time compared with that of the native flour (Sudheesh *et al.*, 2020). In summary, the proximate compositions of both the native and HMT-treated maize flours were largely consistent across the varieties, indicating that the applied optimal HMT conditions did not affect the proximate composition of the maize flours. Additionally, this treatment may potentially increase the storage stability of these flours through a decrease in moisture content.

Table 6.1: Proximate composition of flour from heat moisture-treated (HMT-treated) and native maize grains (per 100 g dry matter)

Maize varieties	HMT	Dry matter (%)	Ash (%)	Protein (g/100 g db.)	Fat (g/100 g db)	Crude fiber (g/100 g db)	Carbohydrate (g/100 g db)
DK777	Untreated	88.19 ^a ± 0.42	1.15 ^a ± 0.09	7.92 ^a ± 0.20	4.23 ^a ± 0.38	9.98 ^a ± 0.06	76.71 ^a ± 0.54
	Treated	89.62 ^a ± 1.07	1.09 ^a ± 0.01	7.13 ^a ± 0.20	3.91 ^a ± 0.05	9.77 ^a ± 0.20	78.09 ^a ± 0.04
UH5354	Untreated	88.38 ^a ± 0.09	1.33 ^a ± 0.02	8.42 ^a ± 0.26	3.09 ^a ± 0.40	10.17 ^a ± 0.17	76.97 ^a ± 0.47
	Treated	88.78 ^a ± 0.03	1.13 ^a ± 0.04	7.52 ^a ± 0.43	3.38 ^a ± 0.01	10.14 ^a ± 0.31	77.82 ^a ± 0.08
Longe	Untreated	88.94 ^a ± 0.05	1.05 ^a ± 0.04	7.42 ^a ± 0.23	3.37 ^a ± 1.59	13.43 ^a ± 0.17	74.91 ^a ± 1.98
10H	Treated	89.75 ^a ± 0.27	1.16 ^a ± 0.03	7.14 ^a ± 0.12	3.62 ^a ± 0.38	13.47 ^a ± 0.17	74.61 ^a ± 0.36
DT Max	Untreated	88.96 ^a ± 0.32	1.11 ^a ± 0.05	8.79 ^a ± 0.09	4.68 ^a ± 0.40	12.30 ^a ± 0.11	73.10 ^a ± 0.34
	Treated	89.88 ^a ± 0.37	1.01 ^a ± 0.05	8.08 ^a ± 0.10	4.45 ^a ± 0.80	11.87 ^a ± 0.01	74.57 ^a ± 0.65

All values are means ± Standard Deviation. With respect to variety, means within columns with different superscripts are significantly different ($p < 0.05$). HMT: heat moisture treatment.

6.4.1.2. Effect of HMT on the contents of starch and its macromolecules

The total starch, amylose and amylopectin contents and their ratios affect the gelatinization, viscosity, gel strength, and retrogradation behavior of maize flour, thereby affecting the processing performance and glycemic response of maize-based foods. The total starch and amylose contents of the native maize varied across the varieties. The total starch content varied between 65.40% in DT Max and 72.83% in UH5354, whereas the amylose content ranged between 21.97% in Longe 10H and 25.39% in DT Max. These values are similar to the total starch and amylose contents reported by Sreya Venadan *et al.* (2024) and Arora *et al.* (2024) for different maize varieties analyzed in Mexico and India. After HMT application, the total starch, amylose, and amylopectin contents remained constant (Table 6.2, $p>0.05$). Although the total starch content of native maize flour varied by variety (e.g., 0.34% for DK777 to 1.84% for Longe 10H), HMT did not affect significantly the total starch content of any variety ($p>0.05$). In addition, there was also a slight and non-significant difference in the amylose content.

Compared with that of native flour, the amylose content (in % of total starch) of the HMT-treated flour increased by 0.33% for DK777, 0.30% for UH5354, and 1.24% for Longe 10H, whereas it decreased by 0.09% for DT Max. Although several studies have reported a significant increase in amylose content after HMT, the lack of a significant difference observed in this study may be attributed to the formation of amylose–lipid and amylose–protein complexes, which could result in a decrease in measurable amylose content (Miyoshi, 2002). In fact, in solution, especially when the moisture content of cereals typical of HMT conditions is increased, the hydrophobic side of the lipid molecule interacts with the hydrophobic cavity of the amylose helix and thus forms a complex. This complex in binding form reduces the concentration of amylose molecules after analysis. Similarly, amylose can also interact with proteins through hydrogen bonding or hydrophobic interactions under heat treatment, which is typically applied during HMT. These complexes bind amylose to proteins and reduce the measurable amylose content (Yulianti and Liao, 2021). Sun *et al.* (2014) and Singh *et al.* (2009) reported similar results for heat-moisture-treated sorghum and water chestnut starch, respectively. Across other starch types, including sweet potato, potato, rice, and millet, the amylose content remained largely

unchanged after HMT. This is because the treatment primarily affects the physical and structural properties of starch rather than its chemical composition (Fang *et al.*, 2023). Nevertheless, the observed slight increase in amylose content may increase the firmness and elasticity of maize dough, thereby improving its suitability for consumption. Overall, the slight increase in amylose content may improve the quality of the flour and derived products.

Table 6.2: Total starch and starch macromolecule contents of flours from native and heat moisture-treated maize grains

Maize varieties	HMT	Total starch (% db)	Amylose (% db)	Amylopectin (% db)	Ratio Amylose/Amylopectin
DK777	Untreated	65.60 ^a ± 1.94	24.63 ^a ± 2.02	75.36 ^a ± 2.02	0.32 ^a ± 0.03
	Treated	65.94 ^a ± 1.82	24.93 ^a ± 0.31	75.07 ^a ± 0.31	0.33 ^a ± 0.01
UH5354	Untreated	72.83 ^a ± 0.06	25.10 ^a ± 0.59	74.89 ^a ± 0.59	0.33 ^a ± 0.01
	Treated	73.30 ^a ± 0.17	25.40 ^a ± 0.30	74.59 ^a ± 0.32	0.34 ^a ± 0.01
Longe	Untreated	71.07 ^a ± 0.29	21.97 ^a ± 1.73	78.03 ^a ± 1.73	0.28 ^a ± 0.02
10H	Treated	72.91 ^a ± 0.51	23.21 ^a ± 0.97	76.78 ^a ± 0.97	0.30 ^a ± 0.00
DT Max	Untreated	65.46 ^a ± 0.12	25.39 ^a ± 0.38	74.60 ^a ± 0.38	0.34 ^a ± 0.01
	Treated	65.75 ^a ± 0.52	25.30 ^a ± 0.01	74.70 ^a ± 0.01	0.34 ^a ± 0.00

All values are means of duplicate determinations ± S.D. With respect to variety, means within columns with different superscripts are significantly different ($p < 0.05$). HMT: heat moisture treatment

6.4.1.3. Effect of HMT on the color characteristics

Color is an important quality characteristic of any ingredient used in food products and determines its end use. HMT did not significantly affect the color characteristics of the maize flour (Table 6.3, $p > 0.05$). The lightness (L^*) decreased slightly for all varieties after HMT. The lightness decreased in percentage by 0.31, 1.77, 1.88 and 5.08 for Longe 10H, DK777, UH5354 and DT max, respectively. The a^* value, which indicates the red-green color axis, significantly decreased for the varieties DK777 (23.33%) and UH5354 (40.54%), but was not significant for Longe 10H (16.67%) and DT Max (2.78%) after HMT, suggesting a change toward a greener color in the treated maize flour. However, the b^* value, which indicates the yellow blue colour axis, slightly increased after HMT ranging

between 0.80% and 17.37%. These results are similar to those reported by Simić *et al.* (2024) for maize flours after being subjected to different HMT conditions. The observed changes in lightness (L^*), a^* , and b^* values following HMT are likely due to the thermal degradation of pigments, as well as heat-induced chemical interactions between starch and phenolic compounds. These processes can alter pigment stability, promote Maillard reactions, and affect light absorption and scattering, leading to shifts in flour color (Ma *et al.*, 2021; Simić *et al.*, 2024).

The whiteness degree, an important physical parameter that determines the color quality of maize flour, decreased by 2.69%, 1.72%, 0.65% and 5.95% for DK777, UH5354, Longe 10H and DT Max, respectively, but these differences were not significant (Table 6.3). The slight reduction in whiteness may be attributed to the initiation of Maillard reactions during the HMT process, where reducing sugars react with protein amino acids (Fang *et al.*, 2023). The interaction between protein and reducing sugars during heat processing produces a brown color characteristic of the Maillard reaction. Ridwan Ariyantoro *et al.* (2020) reported that physical starch modification using the heating process led to a non-enzymatic browning reaction between the reducing sugars and the protein. The slight change in color observed in the present study, mainly whiteness, is consistent with the effects observed in other starches and flours, such as sorghum flour (Ridwan Ariyantoro *et al.*, 2020) and potato starch (Subroto *et al.*, 2019).

The results of the present study contrast with those reported by Marta *et al.* (2022) for maize subjected to HMT, where HMT significantly affected the color characteristics of the flour. This difference is likely due to the variations in time and temperature applied during HMT. Marta *et al.* (2022) performed HMT at 30% moisture content for 16 hours at 100°C, which represents more severe conditions than the optimum conditions used in the present study. The long heating times applied by Marta *et al.* (2022) can lead to an increase in non-enzymatic browning reactions (caramelization), resulting in a darker color of the flour (Amin *et al.*, 2022). The HMT conditions used in the present study, i.e., short duration and low temperature, did not significantly affect the color parameters of the modified maize flour. Furthermore, in Uganda, maize grains are usually polished before consumption (Kiguli *et al.*, 2019), and polishing is known to increase the whiteness of maize flour

(Witoyo *et al.*, 2020). Therefore, the white color desired by Ugandan consumers can be achieved by polishing the treated maize grains, even if the HMT process results in a slight decrease in whiteness.

Table 6.3: Color characteristics of flour from native and heat moisture-treated maize grains

Maize varieties	HMT	L*	a*	b*	Whiteness Index
DK777	Untreated	79.69 ^a ± 0.74	0.60 ^a ± 0.02	11.66 ^a ± 0.07	76.57 ^a ± 0.68
	Treated	78.28 ^a ± 0.14	0.46 ^b ± 0.02	13.34 ^a ± 0.38	74.51 ^a ± 0.32
UH5354	Untreated	79.77 ^a ± 0.67	0.37 ^a ± 0.02	13.73 ^a ± 0.05	75.53 ^a ± 0.53
	Treated	78.27 ^a ± 0.69	0.22 ^b ± 0.03	13.84 ^a ± 0.04	74.23 ^a ± 0.61
Longe 10H	Untreated	82.97 ^a ± 1.06	0.18 ^a ± 0.04	11.74 ^a ± 0.21	79.31 ^a ± 0.75
	Treated	82.71 ^a ± 0.32	0.15 ^b ± 0.01	12.27 ^a ± 0.07	78.79 ^a ± 0.22
DT Max	Untreated	83.11 ^a ± 0.05	0.36 ^a ± 0.01	12.49 ^a ± 0.04	78.99 ^a ± 0.29
	Treated	78.89 ^a ± 0.93	0.35 ^a ± 0.01	14.66 ^a ± 0.11	74.29 ^a ± 0.70

All values are means ± Standard Deviation. For each variety, means within columns with different superscripts are significantly different ($p < 0.05$). L*: Lightness, a*: (+a* = redness/-a* = greenness), b*: (+b* = yellowness/-b* = blueness), HMT: heat moisture treatment

6.4.2. Effect of heat moisture treatment on techno-functional properties

Techno-functional properties such as swelling volume, solubility, water/oil absorption capacity, and bulk density are critical determinants of the processing characteristics and end-use applications of maize flour. Table 6.4 presents data on the swelling volume, solubility, water/oil absorption capacity and bulk density of the HMT-treated and untreated maize flours for the four selected maize varieties. The techno-functional properties of the initial maize flour varied across the varieties, with UH5354 having the lowest swelling volume of 6.85 mL/g and a solubility of 17.13%, whereas the Longe 10H had the highest, 8.23 mL/g and 18.13% swelling volume and solubility, respectively. The water absorption capacity ranged between 1.20 and 1.38 mL/g in Longe 10H and DT Max, respectively, whereas the oil absorption capacity varied between 0.86 and 0.94 mL/g for DK777 and UH5354, respectively and the bulk density ranged between 0.76 and 0.77 g/cm³. The

solubility volume and bulk density reported in this study are within the ranges reported by Ishara *et al.* (2018) for maize, with values of 18.64 ± 5.66 mL/g and 0.6 ± 0.2 g/cm³, respectively, while the swelling values are lower than the values for varieties used in the current study. Similarly, these values are within the range of values reported by Adedeji, *et al.* (2014).

Table 6.4: Techno-functional properties of flours from native and heat moisture-treated maize grains

Maize varieties	HMT	Swelling volume (ml/g)	Solubility (%)	Water absorption capacity (ml/g)	Oil absorption capacity (ml/g)	Bulk density (g/cm ³)
DK777	Untreated	7.79 ^a ± 0.06	17.96 ± 0.22 ^a	1.28 ^a ± 0.01 ^a	0.86 ^a ± 0.02 ^a	0.77 ^a ± 0.01
	Treated	7.25 ^a ± 0.06	18.11 ± 0.31 ^a	1.38 ^a ± 0.07 ^a	0.78 ^a ± 0.01 ^a	0.81 ^a ± 0.01
UH5354	Untreated	6.88 ^a ± 0.09	17.13 ^b ± 0.15	1.27 ^b ± 0.01	0.94 ^a ± 0.01	0.77 ^a ± 0.01
	Treated	6.63 ^a ± 0.02	18.19 ± 0.18	1.37 ^a ± 0.01	0.95 ^a ± 0.01	0.76 ^a ± 0.01
Longe	Untreated	8.23 ^a ± 0.09	18.13 ^b ± 0.41	1.20 ^b ± 0.01	0.87 ^a ± 0.02	0.76 ^a ± 0.01
10H	Treated	7.63 ^b ± 0.39	19.83 ^a ± 0.15	1.28 ^a ± 0.01	0.78 ^a ± 0.03	0.78 ^a ± 0.01
DT Max	Untreated	7.13 ^a ± 0.06	17.73 ^b ± 0.31	1.38 ^a ± 0.02	0.87 ^a ± 0.02 ^a	0.76 ^a ± 0.01
	Treated	6.68 ^b ± 0.12	18.94 ^a ± 0.90	1.49 ^a ± 0.08	0.82 ^a ± 0.01	0.75 ^a ± 0.01

All values are means ± Standard Deviation. For each variety, means with different superscripts within columns are significantly different ($p < 0.05$). HMT: Heat Moisture Treatment.

In addition, the swelling volume decreased after HMT, with a significant decrease observed for the maize varieties Longe 10H and DT Max (Table 6.4, $p < 0.05$), whereas the swelling volumes of DK777 and UH5354 did not significantly decrease (Table 6.4, $p > 0.05$). The swelling volume of the maize varieties Longe 10H and DT Max decreased by 7.26% and 6.31%, respectively (Table 6.4). In contrast, the solubility of the maize flour increased after HMT but not significantly (Table 6.4, $p > 0.05$). Similar findings have been reported in other studies, where the swelling volume and solubility of cereal grains such as sorghum (Olayinka *et al.*, 2008), maize (Sui *et al.*, 2015), and rice (Hormdok and Noomhorm, 2007) decreased and increased, respectively, after HMT. During HMT, the rearrangement of molecular chains results in ordered double helical amylopectin clusters that are more soluble in water, which in turn reduces starch swelling and increases its solubility. This reduction in the swelling volume of starch increases the strength of the gel and limits the accessibility of digestive enzymes to starch chains, thus resulting in a reduction in

starch digestibility. However, a decrease in swelling volume of the maize flour may affect firmness and cohesiveness, elasticity and thus may decrease the flour acceptability. On the other hand, a slight increase in flour solubility indicates a greater degree of starch degradation, which could compromise the structural integrity of the derived maize flour products such as dough.

WAC increased significantly for the UH5354 and Longe 10H varieties (Table 6.4, $p < 0.05$). These results align with the results of several previous studies, as HMT improved the native starch WAC (Olayinka *et al.*, 2008). While HMT increases the ability of the flour to bind water, it restricts its capacity to expand (decrease in swelling volume). These results could be explained by the fact that during the treatment, the crystalline and amorphous regions of starch undergo structural reorganization (Lee and Chung, 2022). The hydrogen bonds are thus strengthened, and the granules become denser and more compact. Those structural changes make granules more rigid and compact, limiting thus the free space available for swelling, so the granules resist expansion when hydrated, leading to a decrease in swelling volume (Chuwech *et al.*, 2023). At the same time, the treatment exposes more hydroxyl groups and amorphous sites, which enhances the hydrophilicity of the starch and allows it to absorb and retain more water molecules.

HMT consistently increases the WAC of flour, increasing its ability to hold more water during mixing and cooking. This phenomenon is observed across various grains, including maize (Miyazaki and Morita, 2005). The increase in water absorption of the flours means that the maize flour starch granules were damaged during HMT, causing an increase in hydroxyl–water molecule interactions and increasing the hydrophilicity of the flour granules (Rafiq *et al.*, 2016). In fact, during the HMT process, hydrogen bonds between amorphous and crystalline regions are disrupted, resulting in a slight expansion of the amorphous region, which increases hydroxyl-water molecule interactions and thus the increase in WAC. In addition, HMT promotes the formation of reduced crystalline regions, which increases the number of available binding sites in the amorphous region of the starch, thus increasing the hydrophilicity of the granules and the water binding capacity. According to Hussain *et al* (2021), the higher water absorption capacity of the flour results in softer derived product such as dough with reduced hardness of the final product. This is

due to the increased presence of weakly bound water, which makes the derived product (dough) less firm and more pliable. Therefore, the increase in the WAC of flour observed in this study could lead to changes in texture of the product derived from these flours, such as a decrease in the maize dough made from these flours, which in turn could affect the acceptability of the flour. As a result, water absorption capacity rises even though the physical swelling of the granules is reduced. This dual effect makes HMT starches more stable and functionally useful in food applications where controlled hydration and reduced stickiness are desirable.

The oil absorption capacities and bulk densities (volume) of the untreated and treated maize flour did not significantly differ (Table 6.4, $p>0.05$). The constant level of oil absorption capacity found in this study is similar to the results of Sindhu and Devi, (2019) for buckwheat starch. The constant oil absorption capacities after HMT indicate that the ability of flour to interact with oils remains stable, which is crucial for maintaining texture and mouthfeel in food products (Chen *et al.*, 2017; Yu *et al.*, 2023). Otherwise, the invariability of the bulk densities suggests that the flour packing and flow properties were unchanged, which is beneficial for processing and storage (Joshi *et al.*, 2015; Yu *et al.*, 2023).

6.4.3. Effect of heat moisture treatment on pasting properties

Pasting properties provide information on intermolecular bonding between starch granules and the rigidity of swollen starch granules during the heating process (Sui *et al.*, 2015). The pasting temperature, peak viscosity, paste viscosity, final viscosity, breakdown, and setback are the analyzed parameters in the present study (Table 6.5). The pasting properties of the native maize flour varieties generally varied across the maize varieties. Such variations are influenced by differences in starch granule rigidity, swelling capacity, and structural integrity among varieties and could be due to the differences in starch composition, particularly the ratio of amylose to amylopectin, as well as the presence of other constituents, such as proteins, lipids, and fibers. These components influence gelatinization behavior, viscosity development, and thermal stability during heating, which are reflected in parameters such as peak viscosity, breakdown, and setback values (Sandhu *et al.*, 2007; Sangeeta and Grewal, 2018).

The pasting temperature values obtained in this study ranged between 81.4 and 94.4°C, with the sample DT Max variety exhibiting the highest pasting temperature after HMT. These results aligned with the results reported by Kim *et al.* (2017) for rice and Sun *et al.* (2014) for sorghum. The pasting temperature increased in percentage by 1.49, 3.00, 5.51, and 10.35 for the UH5354, Longe 10H, DT Max, and DK777 varieties, respectively. This increase in pasting properties could be due to the crystalline structure of starch or the formation of starch-lipid complexes (Chen *et al.*, 2015; Tan *et al.*, 2017). These structural changes increase the resistance of starch granules to swelling and gelatinization, thereby increasing the temperature for gelatinization. The pasting temperature, which is the minimum temperature required for starch to gelatinize, indicates that compared with native maize flour, HMT-treated maize flour would require more energy and time during the cooking process (Awoyale *et al.*, 2021). This might be less desirable for consumers seeking convenience. In addition, an increase in pasting temperature can result in firmer textures and reduced stickiness of the final product derived from these flours (Sangeeta and Grewal, 2018). While this might be beneficial for certain applications, such as derived maize doughs requiring structural integrity, it could negatively impact products where softness in textures is preferred, such as derived maize flour porridges.

Although the pasting temperature increased, the peak viscosity of the modified maize flours slightly decreased, but not significantly (Table 6.5, $p > 0.05$), except for that of the DK777 variety (Table 6.5, $p < 0.05$). This slight decrease aligns with the results reported for other cereals (Kim *et al.*, 2017) and Sun *et al.* (2014) after HMT application. HMT enhances the interior interactions of starch, preventing the penetration of water into starch granules and thus causing a decrease in peak viscosity (Deng *et al.*, 2022). The peak viscosity expresses the ability of starches to swell freely before their physical breakdown and the strength of the pastes formed during gelatinization (Asaam *et al.*, 2018). Its low level in the treated maize flours indicates that they will not form a very thick paste; hence, they may be suitable for products requiring low gel strength and elasticity (Asaam *et al.*, 2018) but not suitable for maize posho derived from these flours, which is the typical form of consumption of these flours in Uganda, and requires high gel strength and elasticity.

HMT also affected the breakdown viscosity (BD) and holding viscosity (HV) of the maize flours (Table 6.5, $p < 0.05$). BD increased by 64 and 23 for the UH5354 and DT Max, respectively, but decreased by 64 and 8 for DK777 and Longe 10H, respectively. The breakdown viscosity of flour reflects the degree of disintegration of starch granules or their paste stability during heating (Kaur *et al.*, 2007). Its decreases suggest better stability of the granules or paste under stress conditions. These findings suggest that the pastes produced from these flours would be more stable and resistant to disintegration compared to the native pastes. This reduction in breakdown viscosity is linked to changes in the starch structure during HMT, including decreased swelling capacity of starch granules and increased interaction between starch chains and starch-protein, leading to a more stable starch granule structure that is less prone to breakdown during heating. Therefore, HMT derived flours, on account of their reduced BD, are more suitable for use in heat processing operations, as their starches are more stable during continuous heating and mechanical agitation (Kaur and Singh, 2019).

The maize flour HV (in centipoise) decreased after HMT for DK777, UH5354 and DT Max by 659.5, 110.5 and 70, respectively, whereas it increased by 57.5 for Longe 10H. The final and setback viscosities (FV and SB) decrease, respectively, after HMT, except for SB for the Longe 10H variety. The reduced SB of HMT starch may be due to reduced amylose leaching and additional amylose and amylopectin chain interaction. These results are in line with those of a previous study in cereals, in which the authors reported that, HMT generally increases the pasting temperature and decreases the peak viscosity, setback, breakdown, and final viscosity of starch in cereal flour (Kim *et al.*, 2017; Sun, Gong, *et al.*, 2014). Specifically, HMT decreases significantly and highly decreases DK777 pasting properties, namely, peak, holding, final and setback viscosities. The pronounced decrease in pasting viscosity compared with that of the other varieties is most likely due to its particular starch structure and composition of the former, which undergo stronger molecular rearrangements under HMT. Compared with other varieties, DK777 may possess a greater proportion of loosely bound amylopectin or lower crystalline stability, making its starch granules more susceptible to breakdown under heat and shear stress, thereby reducing viscosity parameters more markedly (Sandhu *et al.*, 2007; Sun *et al.*, 2014).

Nevertheless, while pasting properties provide valuable insights into the gelatinization behavior and viscosity profile of HMT-treated maize flour, they offer only a partial view of its techno-functional performance. Polishing, which is well-known for improving the techno-functional quality of cereals (Saleh *et al.*, 2019), is a prevalent practice in Uganda and neighbouring countries. Therefore, it is necessary to explore the potential effects of polishing on enhancing the techno-functional quality of these maize flours for better acceptability. This aspect is examined in detail and reported in chapter seven of this thesis

Table 6.5: Thermal properties of flours from heat-moisture-treated and untreated maize grains

Maize varieties	HMT	Pasting temperature (°C)	Pasting viscosity (cP)	Holding viscosity (cP)	Breakdown viscosity (cP)	Final viscosity (cP)	Setback viscosity (cP)
DK777	Untreated	81.4 ^b ± 0.1	1506.0 ^a ± 11.3	1334.0 ^a ± 14.1	172.0 ^a ± 2.8	4035.0 ^a ± 62.2	2701.0 ^a ± 48.1
	Treated	90.8 ^a ± 0.6	776.5 ^b ± 12.0	674.5 ^b ± 3.5	102.0 ^a ± 8.5	1523.0 ^b ± 11.3	848.5 ^b ± 7.8
UH5354	Untreated	86.0 ^b ± 0.7	1128.5 ^a ± 33.2	1086.5 ^a ± 26.1	42.0 ^a ± 7.1	2601.5 ^a ± 19.1	1515.0 ^a ± 7.07
	Treated	87.3 ^a ± 0.0	1081.5 ^a ± 91.6	976.0 ^a ± 105.0	106.0 ^a ± 14.1	2401.5 ^a ± 266.6	1426.0 ^a ± 161.0
Longe 10H	Untreated	79.9 ^b ± 0.0	1240.5 ^a ± 48.8	1144.0 ^a ± 33.9	96.5 ^a ± 14.8	2687.0 ^a ± 129.0	1543.0 ^a ± 94.7
	Treated	82.3 ^a ± 0.1	1290.0 ^a ± 67.9	1201.5 ^a ± 72.8	88.5 ^a ± 4.9	3678.5 ^a ± 19.1	2477.0 ^a ± 91.9
DT Max	Untreated	89.2 ^b ± 0.6	572.0 ^a ± 7.1	509.5 ^a ± 4.9	62.5 ^b ± 2.1	1393.5 ^a ± 0.7	884.0 ^a ± 4.2
	Treated	94.4 ^a ± 0.0	525.0 ^a ± 1.4	439.5 ^b ± 0.7	85.5 ^a ± 2.1	925.0 ^b ± 8.5	485.5 ^b ± 9.2

All values are means ± Standard Deviation. For each variety, means with different superscripts within columns are significantly different ($p < 0.05$), cP= centipoise.

6.5. Conclusion

The study investigated the effect of optimal heat moisture treatment (HMT) conditions on the physicochemical and techno-functional properties of flour from the maize varieties, DK777, Longe 10H, UH5354, and DT Max, which are widely consumed in Uganda. The optimized HMT conditions, primarily aimed at reducing starch digestibility, did not affect the physicochemical properties but rather some parameters of the techno-functional properties. Thus, this treatment maintains the nutritional integrity of maize flour, which is essential for communities in Uganda and other countries in East Africa, where maize varieties investigated constitute a staple food. Importantly, the starch and its macromolecules (amylose and amylopectin) and color characteristics remained constant after HMT, suggesting minimal compromise on fundamental flour properties. The swelling volume was reduced, which may lead to firmer, less cohesive dough, potentially compromising the elasticity required for traditional Ugandan maize posho. Additionally, the pasting temperature increases, and thus more energy and time are required for the cooking process, which is less desirable. The general decrease in peak, breakdown, and final viscosities suggests that HMT-treated flours produce more stable pastes with lower gel strength and elasticity, which is advantageous for soft-textured foods but unsuitable for posho that requires higher viscosity and elasticity. This study revealed that while HMT was optimized to decrease starch digestibility solely, it affected some techno-functional properties of the derived maize dough and posho and thereby the flour acceptability. This will enable scientists through further study to optimize simultaneously the HMT conditions that not only reduce starch digestibility but also preserve or improve the functional properties essential for the consumer acceptability of maize flour products. Therefore, future studies focused on optimizing HMT conditions to reduce starch digestibility should also consider alignment with the techno-functional properties of flour. In addition, future studies should empirically examine the extent to which the optimized HMT conditions affect the sensory acceptability of maize and the potential benefits of polishing in improving the techno-functional qualities of these HMT maize flours for greater acceptability.

CHAPTER SEVEN

Effect of polishing grade on physicochemical, functional, starch digestibility, and sensory properties of heat-moisture-treated maize grains

7.1. Abstract

Heat–moisture treatment (HMT) is a commonly used physical method for decreasing starch digestibility. However, HMT affects the functional properties and acceptability of modified starch, which could be improved through polishing. This study investigated the effects of polishing on the physicochemical, functional, and sensory characteristics of four widely consumed HMT-treated maize varieties in Uganda: the DK777, Longe 10H, UH5354, and DT Max. HMT was applied at moisture content (%), heating time (hours), and temperature (°C): 23.17, 4.12, and 80.00 for UH5354; 20.83, 5.42, and 95.43 for DK777; 34.51, 3.01, and 90.46 for Longe 10H; and 19.24, 5.88, and 97.02 for DT Max. HMT maize grains were polished once and twice using a locally fabricated maize huller to produce second- and first-class maize, respectively, while third-class maize remained unpolished. Polishing improved the physicochemical characteristics, mainly the amylose content and color characteristics ($p < 0.05$), which are important for improving product quality and acceptability. The protein content remained unchanged ($p > 0.05$) across varieties, and UH5354 (8.11 g/100 g dw) and DT Max (7.46 g/100 g dw) retained the highest contents after polishing. The fiber, fat, and ash contents decreased significantly, whereas the carbohydrate content increased significantly ($p < 0.05$). The swelling volume increased notably in UH5354 and Longe 10H ($p < 0.05$), whereas the solubility and water absorption capacities decreased. The pasting temperature consistently decreased after polishing ($p < 0.05$). The peak and holding viscosities increased with increasing polishing grade ($p < 0.05$). Sensorial quality notably improved from unpolished to semi-polished, especially in DK777, UH5354, and Longe 10H. The study revealed that HMT could be combined with polishing to reduce starch digestibility and enhance flour quality and acceptability.

7.2. Introduction

Maize is the main botanical source of starch in household diets and is used in the food industry (Maior *et al.*, 2021). Starch isolated from maize is used in many baked goods, such as cookies, cakes, icings and fillings, due to its functional properties, such as the ability to maintain moisture content, prevent the growth of crystals from sugars and increase juiciness (Yazid *et al.*, 2018). Maize starch exhibits a large range of structural and physicochemical properties, such as swelling, viscosity, and digestibility, that can be used to meet various end-uses, particularly in food applications (Wang *et al.*, 2022). It is an important ingredient in the production of many food products and has been widely used as a thickener, stabilizer, colloidal gelling agent, water retention agent and adhesive because of its very adaptive physicochemical characteristics (Omoregie Egharevba, 2020). In addition to being used for industrial purposes, maize starch is a major source of household dietary energy in many countries worldwide because of its accessibility and comparatively low cost.

Currently, 52% of starch is utilized by the food industry, while the remaining 48% is used in non-food sectors such as pharmaceuticals, paper, and various other applications. It is used in the food industry either as a food product or additive for thickening, preservation and quality enhancement in baked foods, confectioneries, pastas, soups and sauces, and mayonnaises. However, cereal starch is also responsible for undesirable metabolic disorders, such as high blood glucose levels, which are major risk factors of non-communicable diseases, such as type 2 diabetes. Starch-based foods are classified as rapidly digestible starch (RDS), slowly digestible starch (SDS), or resistant starch (RS) on the basis of their hydrolysis by enzymes (Bojarczuk *et al.*, 2022; Englyst *et al.*, 1992; Englyst *et al.*, 2018). RDS and SDS are hydrolyzed in the small intestine to dextrins by α -amylase within 20 to 120 min after ingestion, producing a high glycemic peak that elicits a more rapid insulin response than SDS, which offers the advantage of a sustained glycemic index level over time (Englyst *et al.*, 1992). From this point of view, frequent consumption of starch-based food with a high RDS becomes a risk factor for a high glycemic index and then insulin resistance, a precursor of T2DM (Bello-Pérez *et al.*, 2021).

Chemical, enzymatic, and physical starch modification techniques have been developed to improve the quality of starch. Physical methods have gained widespread acceptance for their low cost, safety, and effective characteristics and do not require chemical reagents to improve starch applicability (Fonseca *et al.*, 2021). Annealing and HMT are the most commonly used physical methods effectively used to modify starch properties (Fonseca *et al.*, 2021). Several studies have examined the influence of HMT on starch derived from different botanical sources, such as rice, maize, finger millet, African yam bean, pea, lentil, and navy bean (Fonseca *et al.*, 2021; Miyazaki and Morita, 2005; Schafranski *et al.*, 2021). HMT effectively reduces the digestion rate and GI value of starch through increasing SDS and RS while decreasing the RDS (Kumar *et al.*, 2023; Sui *et al.*, 2015). Although HMT can increase the SDS and RS contents, as well as improve certain functional qualities of derived products, it sometimes negatively impacts characteristics and acceptability of derived products (Marta *et al.*, 2022; Mathobo *et al.*, 2021).

In chapter six, it was observed that the optimal HMT conditions aimed at reducing starch digestibility affected some techno-functional properties (e.g. swelling volume, solubility, water absorption capacity, pasting temperature, breakdown viscosity) of the four maize varieties (UH5354, DK777, Longe 10H and DT Max) selected for the study. Polishing is a technique believed to improve the techno-functional properties of cereals (Paiva *et al.*, 2015, Sinkovič *et al.*, 2023). Polishing or refining of cereals consists of removing the outer bran layer, resulting in grains with a smoother texture and more uniform appearance, which are preferred by consumers for their improved palatability and visual appeal (Rao *et al.*, 2024; Sinkovič *et al.*, 2023). The polishing process is performed by cereal industries to improve their physical characteristics and sensory properties, as well as to increase their storage stability (Paiva *et al.*, 2016). It improves the softness and texture of cereal products and thus increases their acceptability. Although the effects of HMT on decreasing starch digestibility and improving the physicochemical and functional qualities of maize are well documented, there is limited information on how polishing can improve the physicochemical and functional properties of HMT maize grains. Therefore, the aim of the present study was to investigate the effect of polishing on the physicochemical and functional qualities of HMT-treated maize grains.

7.3. Materials and Methods

7.3.1. Materials

Maize varieties DK777, Longe 10H, UH5354, and DT Max were used. They were selected because of their widespread availability and commercialization by agro-input suppliers in Uganda. These varieties are commonly cultivated by farmers and are frequently consumed by households. Twenty kilograms of grain from each variety were obtained directly from farmers in November 2023. After collecting, the grains were manually sorted and cleaned to remove any foreign materials. They were then stored in PICS (Purdue Improved Crop Storage) bags, which consisted of two layers of polypropylene to prevent moisture absorption. The grains were kept under laboratory conditions for approximately eight weeks before they were subjected to HMT.

7.3.2. Methods

7.3.2.1. Heat moisture treatment sample preparation

HMT treatment was applied to the four maize grain varieties following the optimal conditions for reducing digestibility developed (See Table 5.3). The laboratory protocol proposed by Sui *et al* (2015) for HMT was used.

Table 7.1: HMT conditions applied for the selected maize varieties

Maize varieties	Moisture content (%)	Length of heating (Hours)	Temperature (°C)
UH5354	23.17	4.12	80.00
DK777	20.83	5.42	95.43
LONGE 10H	34.51	3.01	90.46
DT MAX	19.24	5.88	97.02

Prior to HMT application, the moisture content of each native maize sample was determined using the gravimetric method and a hot air oven according to the AOAC standard method 925.09 (Association of Official Analytical Chemists, 1990). The moisture content of the maize grains was adjusted using distilled water according to the proposed formula of Cordeiro *et al* (2018) described in chapter 6 subsection 6.3.2.1.

7.3.2.2. Polishing of heat-moisture-treated maize samples

After HMT application, to assess the effect of the polishing on the HMT treated maize grains, the maize grains were dried in an oven with forced air circulation at 50°C for 24 h to reach a moisture content of 10–11% (Van Hung *et al.*, 2020) and polished. Three batches of maize flour were produced (first, second, and third/ordinary maize flours) and used for laboratory analysis. To obtain the “third class” or “ordinary maize” flour, a portion of the HMT-dried maize grains was ground directly under laboratory conditions via an 8010EG laboratory blender (Model HGBTWTG4, Torrington, Connecticut 06700 USA) and sieved through a 100 µm sieve. This flour was used as the control for testing the effect of polishing.

The remaining HMT-dried maize grains were divided into two batches and used to obtain the “second class” and “first class” following the polishing step locally applied in Uganda. The HMT-dried maize grains were polished via a maize huller (Locally Fabricated, Gulu, Uganda). Briefly, whole maize grains were polished from the surface layer to the center, where the germ and bran layers were mechanically removed from the grain. One batch of HMT-dried maize grains was polished once to produce “second-class” maize grits, whereas the second batch was polished twice to obtain “first-class” maize grits. The polished maize grits obtained from each batch were then ground under laboratory conditions using an 8010EG blender (Model HGBTWTG4, Torrington, Connecticut 06700 USA) and sieved through a 100-µm sieve to obtain the second and first classes of HMT-polished maize flours. The samples were stored at room temperature until laboratory analysis within 15–40 days.

7.3.2.3. Analysis of proximate, color, starch digestibility and techno-functional properties

All the analyses were performed on first (polished twice), second (polished once) and third (not polished) classes of maize flour. The third class (non-polished) maize flour was used as a control for polishing. Proximate composition, amylose content, amylopectin content and color characteristics were analyzed as physicochemical characteristics. Techno-functional properties analyses included swelling volume, solubility, water and oil absorption capacities, bulk density and pasting properties. The analyses followed the

procedures already described in the preceding chapter six subsections 6.3.2.2 and 6.3.2.3 of the thesis. The starch digestibility, percentage of starch hydrolyzed in the maize grains was determined according to the simulated in-vitro gastrointestinal digestion method of Englyst *et al.* (1992) applied by Cahyana *et al.* (2019).

Prior to the analysis, two enzyme working solutions were prepared: pepsin enzyme solution A and pancreatic amylase, amyloglucosidase, and invertase enzyme solution B. The pepsin enzyme solution A was prepared by mixing 1 g of pepsin (250 U/mg solids, Sigma) with 200 mL of 0.05 M hydrochloric acid (HCl). For enzyme solution B, 3 grams of pancreatin-5.0 (PB0680, WO: B1, Biotech) was suspended in 20 mL of distilled water, magnetically stirred for 10 min and centrifuged for 10 min at $1500 \times g$. The cloudy supernatant was collected from each tube and then mixed with 4 mL of diluted amyloglucosidase (300 U/mL) (diluted into distilled water at a 1:7 ratio) and 6 mL of invertase (300 U of liquid). The reagents were prepared immediately before use.

The heat-treated maize sample flour (1 g) was placed into 50 mL polypropylene screw-cap tubes and mixed with 10 mL pepsin enzyme solution A. The mixture was left at 37°C for 30 min, 10 mL 0.25 M bicarbonate de sodium (NaHCO_3) was added, and the mixture was vortexed. The enzyme solution B (5 mL) prepared as applied above was added, and the sample was then incubated in a shaking water bath (37°C) with agitation (160 rpm) for 120 minutes. Successive aliquots (0.5 ml) of the hydrolysate were taken after 0, 20 and 120 min and mixed with 4 mL of 80% ethanol to inactive the enzyme. After centrifugation at $4000 \times g$ for 10 min, the hydrolyzed starch mixture was removed, and the total glucose concentration of the hydrolysate was determined via the phenol–sulfuric acid method (Van Hung *et al.*, 2020). An aliquot (50 μL) of the centrifuged sample was picked and mixed with 500 μL of phenol solution (6%) and 2500 μL of concentrated sulfuric acid (95.5%). The mixture was incubated at 30°C in a water bath for 20 minutes, and the absorbance was read at 490 nm using the UV/visible spectrophotometer. The percentages of rapidly digestible starch (RDS) and slowly digestible starch and resistant starch (SDS +RS) in the products were calculated using equations (7.1) and (7.2).

$$\mathbf{RDS\ (\%) = (G20 - FG) * 0.9 \quad (Eq.\ 7.1)}$$

$$\mathbf{(SDS + RS)(\%) = TS - RDS \quad (Eq.\ 7.2)}$$

With G20, the glucose was released after 20 min, FG was the free glucose at 0 min, and TS was the total starch weight of the sample used.

7.3.2.4. Sensory analysis

The heat-moisture-treated and polished maize flours obtained in section 7.3.2.1 and 7.3.2.2 were used for sensory analysis. Since posho, also called ugali in Swahili, a bread made out of maize flour and boiling water, is the most commonly consumed form of maize flour in Uganda, this derived product was considered for sensory analysis. Posho was cooked using conventional processing methods by a local restaurant, which serves maize posho daily. Sensory characteristics of the posho samples were analyzed using a five point hedonic scale, which is widely used as a method for quantitative measurement of the quality of food experienced by consumers (Lawless and Heymann, 2010).

The samples were presented to 62 non-trained panellists who were at least 18 years of age, had experience in the consumption of posho, and were interested in the sensory evaluation of posho. Before the analysis, a verbal explanation of the sensory acceptability objective was provided, and each panellist was guaranteed anonymity through consent agreements. The consent form was provided in both English and Acholi, the local language spoken in the study area. After consent to participate in the study, the panellist signed the informed written consent form. They were placed at least 2 meters apart to avoid interference and interaction among them.

The maize dough samples from each maize variety were served in random order in pre-coded white disposable plates using three-digit random numbers. During the test, the panellists received the samples and the sensory analysis questionnaire sheet. Five-point hedonic scales (from 1—dislike very much, 2—dislike moderately, 3—neither like nor dislike, 4—like moderately, 5—like very much) were used. Sensory parameters, including elasticity, hardness, color, flavor, and taste (Arnés *et al.*, 2022; Callejo, 2011), were evaluated. Mineral water at ambient temperature was supplied for the panellists to rinse their mouths between samples. At the end of the analysis, the participants were asked to identify the most preferred sample and the reasons for the choice of each sample.

7.3.3. Statistical analysis

In this study, for each maize variety, two HMT-treated and polished samples (independent treatment replicates) were prepared and subjected to laboratory analyses. All the treatments were analyzed in duplicate and reported as the means $\pm$ standard deviations. Shapiro and Levene tests were used to assess the normality and homogeneity (equal variance) of the data. Results showed that data were normally distributed and one-way analysis of variance (ANOVA) was used to compare the effect of polishing within the varieties. A significance level of 5% was used, and the Tukey multiple comparison test was applied as a post hoc pairwise test. The Kruskal–Wallis test was used for sensorial data analysis, as the normality assumption was violated. All the analyses were performed using R version 4.4.2.

7.4. Results and discussion

7.4.1. Effect of polishing on proximate composition

Polishing process significantly affected the proximate composition of the maize in the present study (Table 7.2). The ash, fat, and dietary fiber contents generally decreased after polishing. The decrease increased with polishing grade. The ash content decreased by approximately 50% from ordinary maize flour (Class 3) to first-class maize flour. The most significant reduction occurred while transitioning from ordinary to class 2 flour ($p < 0.05$), particularly in the DK777, UH5354, and DT Max varieties. However, no significant difference was observed between second-class and first-class flour for all varieties except for longe 10H variety. A similar trend was observed for the fat content of the maize varieties UH5354 and DT Max, whose fat content significantly decreased while transitioning from ordinary flour to class 2 ($p < 0.05$). The fat content decreased by approximately 61–78% from that of ordinary flour to that of first-class maize flour. The greatest decrease in fat content was observed for the variety DK777, where the fat content (g/100 g db) decreased from 3.91 in the ordinary flour to 2.49, around 37% decrease in second class while 0.84, around 78% decrease in the first class. However, no significant change was observed for the Longe 10H fat content after polishing ($p > 0.05$).

The fiber content decreased by approximately 15–47% after polishing. The greatest decrease of 47% in fiber content was observed for the DT Max variety (11.87 to 6.29 g/100 g dw), followed by the variety DK777, whose fiber content decreased by 42% (9.77 to 5.61 g/100 g dw) after polishing. In contrast, the total carbohydrate (g/100 g db) content generally increased by 6 to 12 when fully polished, especially for the UH5354 and DT Max varieties, respectively. Although the ash, fat, and fiber contents decreased and the carbohydrate content increased after polishing, no significant changes were observed in the protein content of the maize flour across all the varieties ($p > 0.05$), except for that of DK777, whose protein content decreased by 14.87% from ordinary to the first class ($p < 0.05$).

Overall, the results of the present study revealed that the proximate composition, especially the ash, fat, and dietary fiber contents of the flour, decreased with increasing polishing grade for all the maize varieties, whereas the carbohydrate content increased, and the

protein content remained largely unchanged. These results could be due to the components removed during the polishing process and the distribution of these elements within the maize grains. The polishing process involves the removal of bran and germ from the grain kernel, leaving behind the endosperm, which is primarily composed of starch. However, the bran of maize is rich in fiber, vitamins, and minerals, whereas the germ contains mainly fat (Paiva *et al.*, 2016). Their removal could cause a decrease in the aforementioned elements from the maize flour. In addition, these nutrients decrease with increasing polishing grade, which is in line with the findings of previous studies on rice (S. Puri *et al.*, 2014). For example, the dietary fiber content of DK777 decreased from 9.77 to 7.36 when it was semi-polished but reached 5.61 in the fully polished flour, a trend that is in line with Hansen *et al.* (2012) for polished rice. Similarly, the fat content in Longe 10H decreased from 3.62 to 2.80 and 1.38 g/100 g dw in the semi and fully polished flours, respectively. Nevertheless, the reduction observed in this study may have been greater if heat moisture treatment had not been applied to the maize grains, as reported by Ojediran *et al.* (2020) and Paiva *et al.* (2015), who noted that hydrothermal processing reduces the loss of proximate components during the polishing of rice. Future studies could investigate the effect of polishing on the proximate composition of heat- and moisture-treated versus untreated maize.

The protein content of the polished maize flour remained unchanged, and the carbohydrate content increased after the polishing process. Protein accumulates in the endosperm of maize grains, where it contributes to grain development (Rocha-Villarreal *et al.*, 2018). However, during the polishing process, the pericarp and the germ are affected, and the endosperm of maize grains containing a significant amount of protein remains intact (Wu *et al.*, 2022) and is preserved (Paiva *et al.*, 2016). This explains the invariant protein content after the polishing process. However, while most corn protein (75%) comes from the endosperm, Rocha-Villarreal *et al.* (2018) highlighted that the proteins with the best amino acid profile are concentrated in the germ. This means that the protein content was not generally affected but the amino acid content may be affected. Future studies could investigate the effects of polishing on the amino acid profile of maize flour.

Table 7.2: Composition of flour from heat-moisture-treated and polished maize grains (per 100 g dry matter)

Maize varieties	Maize class	Dry matter (%)	Ash (%)	Protein (g)	Fat (g)	Dietary fiber (g)	CHO (g)
DK777	3	89.62 ^a ±1.07	1.09 ^b ±0.01	7.13 ^b ±0.21	3.91 ^c ±0.05	9.77 ^c ±0.20	78.09 ^b ±0.04
	2	90.11 ^a ±0.06	0.53 ^a ±0.01	6.62 ^{ab} ±0.01	2.49 ^b ±0.38	7.36 ^b ±0.07	82.98 ^b ±0.32
	1	89.02 ^a ±0.01	0.57 ^a ±0.05	6.07 ^a ±0.09	0.84 ^a ±0.40	5.61 ^a ±0.08	86.89 ^a ±0.61
UH5354	3	88.78 ^b ±0.03	1.13 ^b ±0.04	7.52 ^a ±0.43	3.38 ^b ±0.01	10.14 ^c ±0.31	77.82 ^b ±0.08
	2	88.84 ^b ±0.09	0.52 ^a ±0.01	7.67 ^a ±0.56	1.40 ^a ±0.40	8.88 ^b ±0.09	81.50 ^a ±0.26
	1	89.38 ^a ±0.17	0.53 ^a ±0.01	8.11 ^a ±0.38	1.67 ^a ±0.01	6.98 ^a ±0.10	82.70 ^a ±1.51
Longe 10H	3	89.75 ^b ±0.27	1.16 ^b ±0.03	7.14 ^a ±0.12	3.62 ^a ±0.38	13.47 ^b ±0.17	74.61 ^b ±0.36
	2	88.59 ^a ±0.20	1.09 ^b ±0.01	6.91 ^a ±0.27	2.80 ^a ±0.79	13.36 ^b ±0.01	75.82 ^b ±0.50
	1	89.33 ^{ab} ±0.02	0.52 ^a ±0.03	7.01 ^a ±0.09	1.38 ^a ±0.39	11.05 ^a ±0.03	80.03 ^a ±0.22
DT Max	3	89.88 ^b ±0.37	1.01 ^b ±0.05	8.08 ^a ±0.10	4.45 ^b ±0.80	11.87 ^c ±0.01	74.57 ^c ±0.65
	2	88.89 ^a ±0.01	0.50 ^a ±0.01	8.02 ^a ±0.05	1.92 ^a ±0.38	8.62 ^b ±0.08	80.93 ^b ±0.53
	1	89.03 ^{ab} ±0.09	0.40 ^a ±0.01	7.46 ^a ±0.28	1.93 ^a ±0.39	6.29 ^a ±0.05	83.90 ^a ±0.06

All values are means ± Standard Deviation. For each variety, means with different superscripts within columns are significantly different ($p < 0.05$).

7.4.2. Effect of polishing on total starch and its constituents

Polishing process significantly increased the total starch and amylose contents in the maize flour (Table 7.3, $p < 0.05$). The total starch content increased significantly for the DT Max, Longe 10H, and UH5354 varieties, by approximately 4%, 5%, and 9.5%, respectively, from ordinary to first-class maize flour. The greatest variation in amylose content after polishing was observed in the DT Max variety (4.5%, from 25.31% to 29.83%), followed by UH5354 (3.6%, from 25.40% to 29.00%) (Table 7.3, $p < 0.05$). Longe 10H amylose content increased by 3% (from 22.81% to 25.21%), and that of DK777 increased by 1.2% (from 24.99% to 26.18%). A similar and opposite trend was observed for the amylopectin content after polishing. The amylopectin content decreased and varied across the varieties as the polishing progressed from ordinary class to first class, and the greatest decrease was observed in the DT Max variety (Table 7.3, $p < 0.05$).

The starch content generally increased for all varieties after polishing due to its location in the endosperm (90% of the total weight) (Zheng and Wang, 2015). Polishing removes the non-starch components and thus, the starch content per 100g weight of remaining grain increases significantly (Wang *et al.*, 1997). The results of the present study show that polishing significantly increases the amylose content of maize, and the trend varies according to the variety of maize, which aligns with results reported in the literature for Sri Lankan rice varieties (Darandakumbura *et al.*, 2015). In addition to the variation according to variety, the amylose content increased with the grade of polishing, which is also consistent with findings observed across different rice varieties (Handoko *et al.*, 2021; Tumanian *et al.*, 2021). During the polishing process, the removal of bran and germ results in a starch-rich endosperm composed of amylose and amylopectin. This enhances the measurable amylose fraction, which then contributes relatively to a high amylose percentage in the remaining grains (Reddy *et al.*, 2017; Xu *et al.*, 2021). On the other hand, an increase in the amylose content of these flours, particularly of DT Max and UH5354 when the samples were passed from unpolished flour to fully polished flour, indicates that when cooked, the derived product (posho) would become harder after cooling due to an increase in the retrogradation of the amylose molecules (Chettry *et al.*, 2024), which is

beneficial for managing blood sugar levels (Chungcharoen *et al.*, 2015; Gunaratne *et al.*, 2020).

Table 7.3: Content of total starch and its constituents' macromolecules in flour from heat-moisture-treated and polished maize grains

Maize varieties	Maize class	Total starch (% db)	Amylose (% db)	Amylopectin (% db)
DK777	3	64.25 ^a ± 0.00	24.99 ^b ± 0.36	75.01 ^b ± 0.36
	2	64.36 ^a ± 0.05	25.11 ^b ± 0.10	74.89 ^b ± 0.10
	1	64.80 ^a ± 0.81	26.18 ^a ± 0.06	73.82 ^a ± 0.06
UH5354	3	73.30 ^c ± 0.17	25.40 ^b ± 0.30	74.59 ^b ± 0.32
	2	75.01 ^b ± 0.28	27.04 ^{ab} ± 0.76	72.96 ^{ab} ± 0.76
	1	82.83 ^a ± 0.35	29.04 ^a ± 0.44	70.96 ^a ± 0.44
Longe 10H	3	72.91 ^b ± 0.51	22.81 ^b ± 0.09	77.19 ^b ± 0.09
	2	74.57 ^b ± 0.52	24.42 ^a ± 0.29	75.58 ^a ± 0.29
	1	77.97 ^a ± 1.12	25.21 ^a ± 0.16	74.79 ^a ± 0.16
DT Max	3	65.75 ^b ± 0.52	25.31 ^c ± 0.01	74.69 ^c ± 0.01
	2	68.67 ^{ab} ± 0.58	28.91 ^b ± 0.11	71.09 ^b ± 0.11
	1	69.09 ^a ± 0.11	29.83 ^a ± 0.03	70.17 ^a ± 0.03

All values are means ± Standard Deviation For each variety, means with different superscripts within columns are significantly different ($p < 0.05$). 1: First class maize flour, 2: Second class maize flour, 3: third or ordinary maize flour.

7.4.3. Effect of polishing on starch digestibility

The slowly digestible and resistant starch contents (SDS+RS) of heat-moisture-treated flour decreased with increasing polishing grade (Figure 7.1), whereas the rapidly digestible starch (RDS) content increased across all varieties. As maize grains undergo polishing, their outer layers, such as bran and germ, are progressively removed, leading to a relatively high concentration of pure starch. These changes decrease the physical barriers that typically delay enzyme access, particularly in fully polished flour, and thereby render starch less susceptible to enzymatic hydrolysis. This increased susceptibility to enzymatic hydrolysis enhances starch digestibility, leading to a more rapid postprandial glucose response. Over time, the frequent consumption of such high-glycemic foods may increase the risk of metabolic disorders, including type 2 diabetes and other non-communicable diseases (Kanbay *et al.*, 2025).

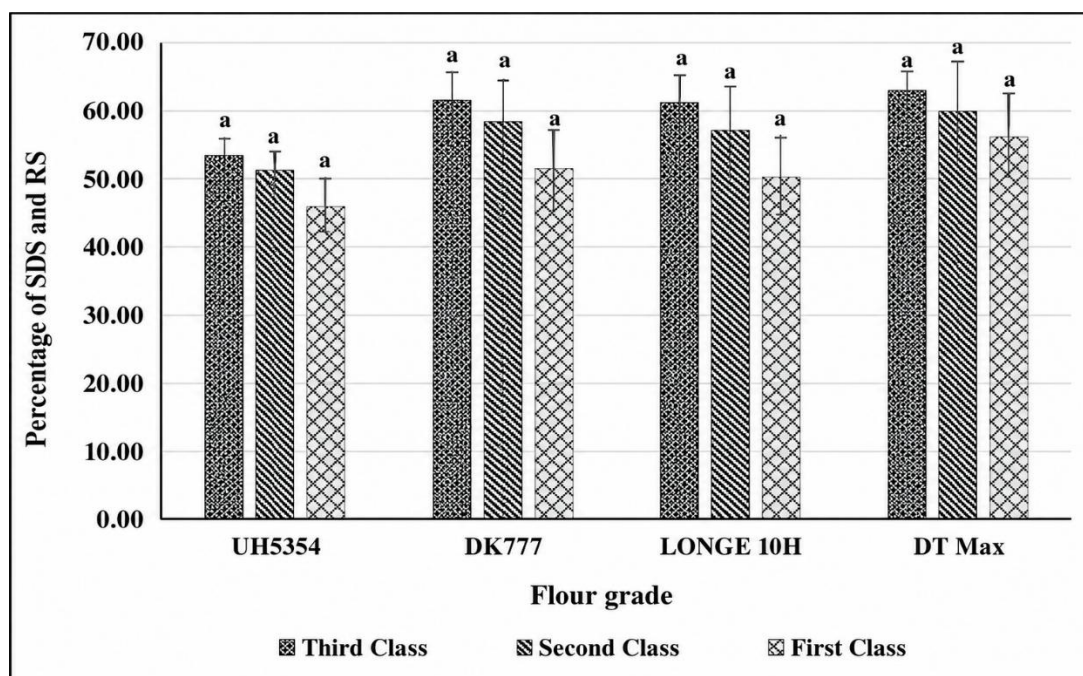


Figure 7.1: Content of slowly digestible and resistant starch fractions in flour from heat-moisture-treated maize grains according to polishing grade

Otherwise, the reduction in SDS+RS was most pronounced during the transition from semi-polished to fully polished states, highlighting the critical role of additional non-starch component removal in enhancing starch digestibility. Although the decreases were not significantly different, the SDS+RS decreased by 4-11% from the ordinary or third class to the second class but by 15-28% when fully polished ($p>0.05$). A large decrease in SDS+RS was generally observed when the HMT-treated maize grains passed from semi to fully polished, except in the DT Max variety. In particular, the SDS+RS decreased by 4.90 and 10.54 for UH5354, 8.27 and 16.71% for DK777, and 10.31 and 15.66% for Longe 10H when passing from ordinary to semi and semi and fully polished, respectively. Similar results have been shown for rice; as the degree of polishing increases, the total starch content in cooked rice increases, whereas the resistant starch content decreases. This leads to higher starch hydrolysis rates and a greater estimated glycemic index in polished grains compared to brown rice. The presence of the bran layer in less polished or brown rice significantly reduces starch digestibility, as it limits enzyme access to the starch within the grain (Tamura *et al.*, 2022; Yang *et al.*, 2019). These findings demonstrate that the transition from semi-polished to fully polished maize flour is critical, as the additional

removal of non-starch components further exposes starch granules, resulting in increased digestibility following HMT. Conversely, the increase was greater when passing from ordinary to semi-polished for DT Max (8.63%) compared to when passing from semi-polished to fully polished (8.47%).

Given that at least second-class (semi-polished) flour can reduce aflatoxin contamination due to partial bran removal (Kimani, 2004) while maintaining lower starch digestibility compared to fully polished flour, public health policies should prioritize and promote the consumption of second-class flour over fully polished flour. In addition, the combined influence of HMT and polishing must be carefully considered when formulating maize-based products aimed at delivering specific nutritional or functional profiles.

7.4.4. Effect of polishing on flour color characteristics

Color is one of the most significant aspects of quality that determines the acceptance or rejection of food. It is one of the first aspects of quality that the human senses perceive, and consumers use it to judge the quality of food products (Juvinal *et al.*, 2023). Polishing maize significantly improved the color characteristics of the maize flour. The color characteristics, lightness (L^*), green-red (a^*), and blue-yellow (b^*) varied according to the polishing grade. The lightness or brightness (clarity) generally increased after being fully polished by 4 and 6, especially for the maize varieties UH5354 and DT Max ($p < 0.05$, Table 7.4), whereas it did not significantly increase for varieties DK777 and Longe 10H ($p > 0.05$, Table 7.4). Considering that consumers and buyers perceive brightness as fresh and of high quality (Carbas *et al.*, 2016), increasing the brightness of UH5354 and DT Max flour would enhance their visual appeal and increase their acceptability and marketability.

The green–red color decreased when passed to the polished form for the varieties DK777 and DT Max, whereas it increased for UH5354 and Longe 10H. The blue–yellow color of all the maize varieties decreased significantly, indicating that polishing increased the flour's yellowish color, making them more attractive. The blue–yellow color significantly decreased for all the varieties when flour was polished, except for UH5354, where it decreased with increasing polishing grade. Overall, the whiteness index combined with the previous three-color parameters significantly increased for the UH5354 and DT Max

varieties ($p < 0.05$, Table 7.4) but did not significantly differ for the DK777 and Longe 10H varieties ($p > 0.05$, Table 7.4). The whiteness of the maize samples increased from 74.73 to 80.12 for UH5354 and from 74.29 to 80.62 for DT Max as the level of polishing progressed from ordinary to first-class flour. The whiteness increases for UH5354 and DT Max, suggest good quality of the flour from the two varieties, which could increase their acceptability and marketability (Oyesigye *et al.*, 2024).

Table 7.4: Color characteristics of maize flour from heat-moisture-treated and polished maize grains

Maize varieties	Maize class	L*	a*	b*	Whiteness Index
DK777	3	80.16 ^a ± 1.08	0.46 ^b ± 0.02	13.34 ^b ± 0.38	76.08 ^a ± 0.69
	2	81.39 ^a ± 0.52	-0.01 ^a ± 0.01	11.04 ^a ± 0.14	78.36 ^a ± 0.38
	1	82.75 ^a ± 2.32	0.06 ^a ± 0.07	10.88 ^a ± 0.14	79.59 ^a ± 1.89
UH5354	3	78.86 ^b ± 0.81	0.22 ^b ± 0.03	13.84 ^c ± 0.04	74.73 ^b ± 0.71
	2	80.95 ^{ab} ± 1.20	0.25 ^b ± 0.02	10.93 ^b ± 0.09	78.03 ^a ± 1.09
	1	82.96 ^a ± 0.13	0.35 ^a ± 0.03	10.24 ^a ± 0.01	80.12 ^a ± 0.12
Longe 10H	3	82.71 ^a ± 0.32	0.16 ^a ± 0.01	12.27 ^b ± 0.07	78.80 ^a ± 0.22
	2	82.58 ^a ± 0.07	0.14 ^a ± 0.09	10.63 ^a ± 0.39	79.59 ^a ± 0.26
	1	83.28 ^a ± 0.94	0.28 ^a ± 0.02	9.69 ^a ± 0.03	80.67 ^a ± 0.79
DT Max	3	78.89 ^b ± 0.93	0.39 ^b ± 0.01	14.66 ^b ± 0.11	74.29 ^b ± 0.70
	2	83.30 ^a ± 0.64	0.24 ^a ± 0.03	11.82 ^a ± 0.12	79.54 ^a ± 0.45
	1	84.45 ^a ± 0.21	0.16 ^a ± 0.01	11.57 ^a ± 0.07	80.62 ^a ± 0.22

All values are means ± Standard Deviation. For each variety, means with different superscripts within columns are significantly different ($p < 0.05$). 1: First class maize flour, 2: Second class maize flour, 3: third or ordinary maize flour.

7.4.5. Effect of polishing on functional properties

Functional properties of maize flour varied across maize varieties and polishing grades. In general, as the degree of polishing increased, the swelling volume (SV) increased across all varieties, yet the change was significant for only the Longe 10H variety when it passed from semi-polished flour to fully polished flour. The SV generally increased by 0.52 to 1.79 when moving from ordinary to first-class maize flour for all varieties. However, the increase in SV was significant only for the UH5354 and Longe 10H maize varieties and only when passing from semi-polished to fully polished maize flour ($p < 0.05$, Table 7.5). On the other hand, the solubility decreased with an increasing number of polishing steps

and Longe 10H had the greatest increase in SV, followed by DT Max and UH5354. By removing the non-starch components, such as bran, which normally act as physical barriers to water uptake, starch granules become more exposed and accessible, allowing them to absorb water more readily and swell to a greater extent (Démé *et al.*, 2015). The removal of non-starch components during polishing also promoted an increase in the amylose content, which highly increased the SV of maize varieties (Tumanian *et al.*, 2021). An increase in the SV of maize flour can lead to elastic and soft maize posho (Miyazaki and Morita, 2005), resulting in a better final texture (Onyeka and Okwume, 2023). These qualities would promote a smooth, more appealing mouthfeel, consistency and pliable dough and posho, which are easier to handle during food preparation and thus increase the preference and market value of the flours (Onyeka and Okwume, 2023).

However, when polishing increased the SV, solubility (SOL) and water absorption capacity (WAC) of the maize flours decreased. WAC decreased significantly with increasing polishing grade ($p>0.05$), except for that of DK777. Polishing significantly reduced the SOL in the UH5354 and Longe 10H flour and the WAC in all the maize varieties except DK777 ($p>0.05$). Polishing removes the bran of maize, which is rich in dietary fiber. This reduction in fiber content decreases both the flour's SOL and WAC (Karakannavar *et al.*, 2021). A decrease in dietary fiber reduces the hydrophilicity of flour, thereby lowering its ability to absorb and retain water. A decrease in SOL could hinder the development of a cohesive structure in the dough and posho (Oghbaei and Prakash, 2016). On the other hand, the reduction observed for WAC could lead to firmer and less elastic dough, which may compromise its sensory appeal and reduce consumer acceptability (van Rooyen *et al.*, 2023).

The findings of this study reveal that while polishing enhances the swelling properties of maize flour and thus the quality and acceptability of the flour, it also reduces the flour's solubility and WAC, which may adversely affect flour quality during processing. In Uganda and many East African countries, cassava flour is commonly blended with maize or millet flour to produce composite flours, a practice driven by nutritional, economic, and functional benefits (Kleih *et al.*, 2012). Therefore, future research should explore strategies to increase the functional properties of polished maize flour through the incorporation of

cassava flour, which is recognized for its exceptionally high functional quality. The oil absorption capacity remained constant for most varieties, except UH5354, whose value significantly decreased from 0.95 to 0.74 ml/g after polishing. Bulk density, on the other hand, consistently increased with increasing polishing grade for UH5354, Longe 10H, and DT Max.

Table 7.5: Functional properties of flour from heat-moisture-treated and polished maize grains

Maize varieties	Maize classes	Swelling volume (ml/g)	Solubility (%)	Water absorption capacity (ml/g)	Oil absorption capacity (ml/g)	Bulk density (g/cm ³)
DK777	3	7.26 ^a ± 0.06	18.11 ^a ± 0.32	1.38 ^a ± 0.06	0.78 ^a ± 0.01	0.81 ^a ± 0.01
	2	7.74 ^a ± 0.25	17.65 ^a ± 0.27	1.26 ^a ± 0.01	0.78 ^a ± 0.02	0.81 ^a ± 0.01
	1	7.78 ^a ± 0.37	17.46 ^a ± 0.60	1.22 ^a ± 0.01	0.75 ^a ± 0.01	0.84 ^a ± 0.01
UH5354	3	6.63 ^b ± 0.02	18.19 ^b ± 0.18	1.37 ^c ± 0.01	0.95 ^b ± 0.01	0.76 ^b ± 0.01
	2	7.13 ^b ± 0.17	17.57 ^a ± 0.06	1.26 ^b ± 0.05	0.81 ^a ± 0.04	0.81 ^a ± 0.01
	1	8.14 ^a ± 0.21	17.14 ^a ± 0.14	1.20 ^a ± 0.03 ^a	0.74 ^a ± 0.03	0.84 ^a ± 0.01
Longe 10H	3	7.63 ^b ± 0.06	19.83 ^c ± 0.15	1.28 ^b ± 0.01	0.78 ^a ± 0.03	0.78 ^b ± 0.01
	2	8.30 ^b ± 0.05	18.29 ^b ± 0.10	1.21 ^a ± 0.01	0.77 ^a ± 0.01	0.83 ^b ± 0.01
	1	9.42 ^a ± 0.41	17.28 ^a ± 0.15	1.22 ^a ± 0.01	0.76 ^a ± 0.02	0.86 ^a ± 0.00
DT Max	3	6.68 ^a ± 0.13	18.94 ^a ± 0.90	1.49 ^a ± 0.08	0.83 ^a ± 0.01	0.75 ^a ± 0.01
	2	7.28 ^a ± 0.10	16.46 ^a ± 0.65	1.29 ^{ab} ± 0.03	0.79 ^a ± 0.05	0.84 ^a ± 0.01
	1	7.40 ^a ± 0.31	16.38 ^a ± 0.17	1.24 ^a ± 0.01	0.74 ^a ± 0.04	0.83 ^a ± 0.01

All values are means ± Standard Deviation. For each variety, means with different superscripts within columns are significantly different ($p < 0.05$). 1: First class maize flour, 2: Second class maize flour, 3: third or ordinary maize flour.

7.4.6. Effect of polishing on pasting properties

Polishing generally affected the pasting properties of the maize flour regardless of variety (Table 7.6). Pasting temperature (PT) consistently decreased with polishing grade, and the most significant reduction was observed when the samples were passed from semi-polished to fully polished maize flour ($p < 0.05$, Table 7.6), except for Longe 10H variety ($p > 0.05$). The most significant decrease in PT was observed in DK777 (8.1°C), followed by UH5354 (4.5°C), when fully polished. The decrease in PT implies that first-class flour would require less energy for gelatinization than second-class and ordinary flour. This would allow a reduction in cooking time and energy (Balet *et al.*, 2019), which is generally advantageous for both household and industrial processing (Balet *et al.*, 2019), and thus would increase

the acceptability of the flours (Mtelisi Dube *et al.*, 2020). Whereas PT decreased, both the peak viscosity (PV) and holding viscosity (HV) increased with increasing grade of polishing, with significant changes observed when the samples were passed from unpolished flour to polished flour ($p < 0.05$). PV represents the highest viscosity achieved by maize flour paste during heating, and HV represents the lowest viscosity during the high-temperature holding phase. An increase in PV enhances the dough viscosity and gel strength. This would lead to a softer, more elastic, and cohesive texture after cooking, an essential quality required for smooth, pliable consistency (Chhabra *et al.*, 2018).

In contrast, the breakdown viscosity (BD) mostly decreased with increasing polishing grade, with the most significant decreases observed for DK777, DT Max, and UH5354. The FV and SB increased with the increase of polishing grade and this could increase the structural integrity and elasticity of derived maize dough after cooling (Guan *et al.*, 2021). The findings of this study show that whereas polishing improves the overall functionality of maize dough, final viscosity and setback viscosity increased. This scenario is likely to affect the formation of resistant starch, which is crucial for non-communicable diseases. Thus, the promotion of HMT-treated and polished flour derived from the Longe 10H maize variety, where no significant change in final viscosity was observed, could be a better alternative variety for good functional quality with good resistant starch content.

Table 7.6: Pasting properties of flour from heat-moisture-treated and polished maize grains

Maize varieties	Maize class	PT (°C)	PV (cP)	HV (cP)	BD (cP)	FV (cP)	SB (cP)
DK 777	3	90.8 ^b ± 0.6	776.5 ^b ± 12.0	674.5 ^b ± 3.5	102.0 ^a ± 8.49	1523.0 ^b ± 11.3	848.5 ^b ± 7.8
	2	88.4 ^b ± 1.7	1041.5 ^{ab} ± 130.8	907.0 ^b ± 141.4	134.5 ^a ± 10.6	2025.0 ^b ± 246.0	1118.0 ^{ab} ± 104.6
	1	82.7 ^a ± 0.6	1793.5 ^a ± 304.8	1734.0 ^a ± 256.0	59.5 ^a ± 48.8	3806.5 ^a ± 673.9	2073.0 ^a ± 417.9
UH5354	3	87.3 ^b ± 0.0	1081.5 ^b ± 91.6	975.5 ^c ± 105.4	106.0 ^b ± 14.1	2401.5 ^b ± 266.6	1426.0 ^b ± 161.0
	2	86.8 ^b ± 0.5	1695.5 ^a ± 6.4	1453.5 ^b ± 37.5	242.0 ^a ± 31.1	3452.0 ^a ± 22.6	1998.5 ^a ± 14.8
	1	82.8 ^a ± 0.6	1842.0 ^a ± 5.7	1747.5 ^a ± 20.5	94.5 ^b ± 26.2	4114.5 ^a ± 88.4	2367.0 ^a ± 67.9
Longe 10H	3	82.3 ^a ± 0.1	1290.0 ^b ± 67.9	1201.5 ^b ± 72.8	88.5 ^a ± 4.9	3678.5 ^a ± 19.1	2477.0 ^a ± 91.9
	2	81.5 ^a ± 1.2	1798.5 ^a ± 67.2	1567.0 ^a ± 18.4	231.5 ^a ± 48.8	3568.0 ^a ± 121.6	2001.0 ^a ± 140.0
	1	78.3 ^a ± 1.2	1884.0 ^a ± 106.1	1757.5 ^a ± 36.1	126.5 ^a ± 70.0	4000.5 ^a ± 234.0	2243.0 ^a ± 270.0
DT Max	3	94.4 ^b ± 0.0	525.0 ^c ± 1.4	439.5 ^c ± 0.7	85.5 ^a ± 2.1	925.0 ^b ± 8.5	485.5 ^b ± 9.2
	2	93.2 ^b ± 0.6	702.0 ^b ± 43.8	620.0 ^b ± 37.5	81.5 ^a ± 6.36	1262.0 ^{ab} ± 76.4	641.5 ^b ± 38.9
	1	89.0 ^a ± 0.2	1262.0 ^a ± 16.3	1187.0 ^a ± 7.1	74.5 ^a ± 9.2	2268.5 ^a ± 521.0	1082.0 ^a ± 528.0

All values are means ± Standard Deviation. For each variety, means with different superscripts within columns are significantly different ($p < 0.05$). 1: First class maize flour, 2: Second class maize flour, 3: third or ordinary maize flour. **PT** = pasting temperature. **PV** = peak viscosity. **HV** = holding viscosity. **BD** = breakdown viscosity. **FV** = final viscosity. **SB** = setback viscosity

7.4.7. Effect of polishing on sensory characteristics

The sensory characteristics of posho prepared from heat-moisture-treated (HMT) and polished maize flour varied depending on the grade of polishing. In general, posho made from semi and fully polished maize flour were more preferred than that made from unpolished flour.

Polishing by increasing the functional quality of the flour, mainly the swelling volume, significantly improved UH5354 and DT Max consumer rating for the posho elasticity and texture. The elasticity and hardness of posho were significantly influenced by the grade of polishing and were between "Neither like nor dislike" (score of 3) and "Like moderately" (score of 4) for all varieties. UH5354 posho elasticity was the most preferred, specifically when polished. Its elasticity preference increased from 2.95 when unpolished to 3.41 when semi-polished and 3.49 when fully polished ($p < 0.05$). A similar trend was observed for DT Max posho in terms of elasticity when passing from unpolished (3.23) to semi-polished (3.48) and fully polished (3.77) ($p < 0.05$). The texture (hard) of the polished samples was greater than that of the unpolished samples and increased with increasing polishing grade for all the varieties ($p < 0.05$), except for the DT Max variety ($p > 0.05$).

In addition, color preference generally increased for all the maize varieties with increasing polishing grade ($p < 0.05$). The removal of the outer bran layer from the maize grain results in grains with a smoother texture, more uniform appearance, improved color, and greater preference by consumers (Kwon *et al.*, 2025). Polishing improved the color of the maize flours, thus increasing the appeal, brightness and uniformity of their color (Ojediran *et al.*, 2020). The greatest increase in color preference was observed when passing from the unpolished posho to the semi-polished posho. The color preference increased by 1.21 and 0.23 for DK777, 0.83 and 0.21 for UH5354, 0.99 and 0.00 for Longe 10H, and 0.77 and 0.49 for DT Max when passing from unpolished to semi and fully polished maize flour posho, respectively. A similar trend was observed for the posho flavor and taste, which generally increased from the unpolished form to the polished form in the DK777 and UH5354 varieties ($p < 0.05$). Polishing improved preference for flavor by reducing the branny taste and chewiness of the maize flour, making the final product more acceptable and palatable to consumers (Shobana *et al.*, 2011).

The overall acceptability of maize dough was lower for unpolished flour compared to semi-polished flour, particularly for the DK777, UH5354, and Longe 10H varieties. However, acceptability increased gradually for the DT Max according to the polishing grade. The overall acceptability of the flour dough increased by 0.91 and 0.39 for DK777, 0.71 and 0.20 for Longe 10H, and 0.45 and 0.57 for DT Max when passing from the unpolished to the semi-polished and fully polished posho, respectively. For UH5354, the increase was 0.94 for the semi-polished samples, whereas it remained constant when fully polished. Overall, this increase in acceptability with increasing polishing grade enables the posho made from first-class flour (fully polished) to be the most preferred flour for all varieties, followed by the posho made from second-class flour (semi-polished) (Figure 7.2).

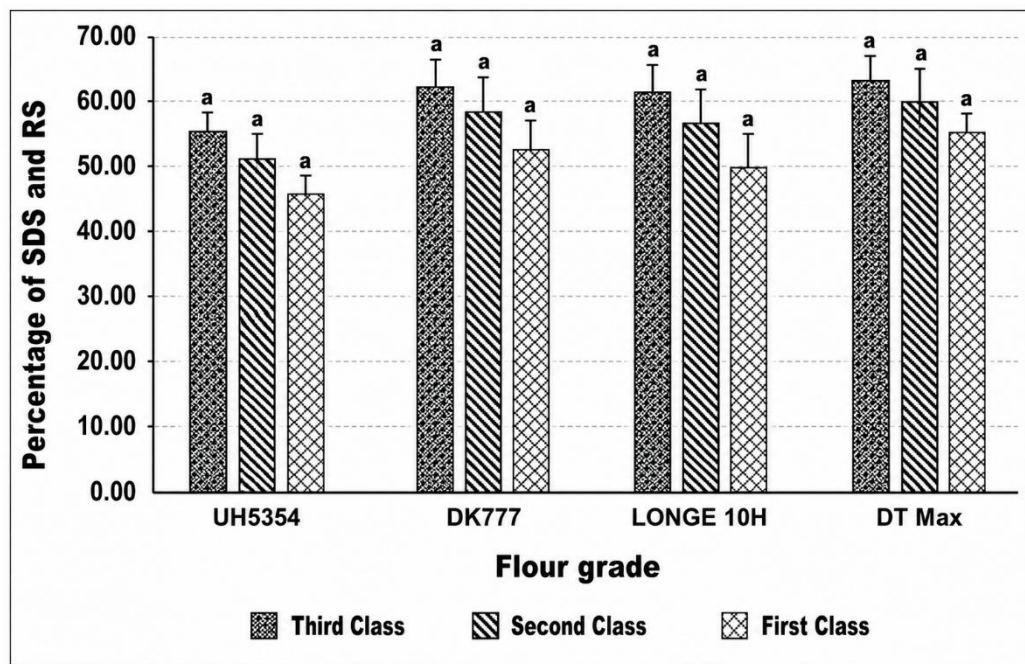


Figure 7.2: Panellist preference of maize dough/posho from heat-moisture treatment-polished maize grains

1. Most preferred 2. Moderately preferred and 3. Less preferred

The findings highlight the positive impact of polishing on sensory attributes and consumer preference. Polishing enables the consumer's desire for white cereal flour to be reached, promoting softness, digestibility, lightness, low cooking time, and better eating properties (Kyritsi *et al.*, 2011). However, it appears that polishing decreases the nutritional quality of these maize flours; thus, careful optimization of the polishing process can help balance sensory improvements with minimal nutrient loss.

Table 7.7: Consumers rating for the sensory characteristics of maize dough/posho from heat-moisture-treated and polished maize grains

Varieties	Maize class	Elasticity	Hardness	Color	Flavor	Taste	Overall Acceptability
DK777	3	3.24 ^a ± 1.25	3.33 ^{ab} ± 1.26	2.24 ^c ± 1.21	2.67 ^b ± 1.28	2.27 ^b ± 1.45	2.59 ^b ± 1.32
	2	3.35 ^a ± 0.97	3.25 ^b ± 1.22	3.66 ^b ± 1.04	3.45 ^a ± 1.02	3.47 ^a ± 1.10	3.50 ^a ± 0.98
	1	3.68 ^a ± 1.02	3.79 ^a ± 1.04	4.39 ^a ± 0.84	3.68 ^a ± 1.05	3.74 ^a ± 1.12	3.89 ^a ± 0.96
UH5354	3	2.95 ^b ± 1.20	3.12 ^b ± 1.18	2.66 ^b ± 1.14	2.89 ^b ± 1.21	2.57 ^b ± 1.20	2.95 ^b ± 1.13
	2	3.41 ^{ab} ± 1.07	3.73 ^a ± 1.00	4.19 ^a ± 0.80	3.49 ^a ± 0.98	3.70 ^a ± 0.98	3.89 ^a ± 0.86
	1	3.49 ^a ± 1.14	3.61 ^a ± 1.23	4.11 ^a ± 1.00	3.70 ^a ± 1.02	3.87 ^a ± 1.22	3.90 ^a ± 0.96
LONGE 10H	3	3.23 ^a ± 1.26	3.23 ^b ± 1.21	2.53 ^c ± 1.16	3.15 ^a ± 1.20	2.95 ^b ± 1.36	3.03 ^b ± 1.24
	2	3.48 ^a ± 0.95	3.58 ^{ab} ± 0.90	3.74 ^b ± 0.81	3.52 ^a ± 0.95	3.61 ^a ± 0.84	3.74 ^a ± 0.87
	1	3.69 ^a ± 1.17	3.92 ^a ± 0.98	4.24 ^a ± 0.94	3.50 ^a ± 1.13	3.7 ^a ± 1.08	3.94 ^a ± 1.10
DT Max	3	3.23 ^b ± 1.19	3.45 ^a ± 1.25	2.55 ^c ± 1.25	3.10 ^b ± 1.35	3.05 ^b ± 1.35	3.08 ^c ± 1.18
	2	3.48 ^{ab} ± 0.99	3.52 ^a ± 1.02	3.40 ^b ± 1.00	3.32 ^b ± 0.95	3.52 ^b ± 1.07	3.53 ^b ± 0.84
	1	3.77 ^a ± 1.00	3.85 ^a ± 1.02	4.56 ^a ± 0.86	3.81 ^a ± 0.99	4.05 ^a ± 1.08	4.10 ^a ± 1.04

All values are the means ± standard deviations of the rating provided by the 62 panelists members. For each variety, means with different superscripts within columns are significantly different ($p < 0.05$). 1: First class maize flour, 2: Second class maize flour, 3: third or ordinary maize flour. 1-Dislike very much, 2-Dislike moderately, 3- Neither like nor dislike, 4- Like moderately and 5- Like very much.

7.5. Conclusion

This study successfully investigated the effect of polishing on the physicochemical and functional properties of heat-moisture-treated (HMT) flour from four predominant Ugandan maize varieties. These findings indicated that polishing HMT-treated maize enhanced the physicochemical properties, particularly the amylose content and color characteristics. The protein content remained stable, and the protein contents of the maize varieties UH5354 and DT Max were the highest after polishing. The total mineral, fat, and fiber contents, which interact with starch and decrease its digestibility, decreased with the increase in polishing grade. This could explain the increase in starch digestibility of the flour compared with that of the ordinary flour. Nevertheless, polishing improved the functional properties of the maize flour, particularly the swelling volume for Longe 10H, DT Max, and UH5354, which could increase the elasticity and soft texture of the derived product (maize posho). In terms of pasting properties, polishing generally improved the pasting profile of the flour. This phenomenon lowers the energy needed for starch gelatinization through a decrease in the pasting temperature and enhances the gelatinization efficiency through an increase in the peak and holding viscosities. However, polishing decreases the breakdown, final, and setback viscosities of flour, which may contribute to a decrease in structural integrity and increase starch digestibility, which is critical for non-communicable diseases. Whereas the polishing of HMT-treated maize grains may increase their starch digestibility, it generally improves the sensory attributes and acceptability of their derived posho. Future research could investigate the optimal conditions for heat-moisture treatment and polishing to increase the nutritional quality and consumer acceptability of maize flour.

CHAPTER EIGHT

General Analysis and Discussion

This chapter is dedicated to a systematic analysis and discussion of chapters 4-7. It is organized into subsections: (8.1) Carbohydrate quality and state of factors affecting starch digestibility of maize varieties, (8.2) Optimization of HMT to decrease starch digestibility of polished maize flour, (8.3) Effects of optimized heat–moisture treatment conditions on physicochemical and techno-functional properties of maize and (8.4) Effect of polishing on physicochemical, functional and sensory properties of heat-moisture-treated maize grains.

8.1. Carbohydrate quality and state of factors affecting starch digestibility of maize varieties

Maize serves primarily as an energy source because of its high carbohydrate content, particularly starch ($C_6H_{10}O_5$)_n. However, beyond the quantity of carbohydrates for energy, the quality of starch in maize, plays a significant role in health outcomes, notably in the development of diet associated non-communicable diseases. This distinction between the quantity and the quality of dietary carbohydrate underpins the concept of carbohydrate quality (Hall, 2017; Liu *et al.*, 2024) investigated in this study.

In Chapter Four, the carbohydrate quality of thirteen maize varieties commonly consumed in Uganda was assed using the carbohydrate-to-fiber ratio and the estimated glycemic index, together with the intrinsic physicochemical constituents (carbohydrates, lipids, proteins, amylose, amylopectin, and total phenolic compounds) that influence starch digestibility. The varieties exhibited carbohydrate-to-crude fiber ratios of 5.28 to 10.15 and consistently high glycemic indices of 79.38 to 87.43 (Table 4.2). On the basis of the WHO recommended CHO to fiber threshold ratio (10:1) all varieties except WE3106 demonstrated acceptable carbohydrate quality, yet their glycemic indices exceeded 70, which classifies them as sources of low carbohydrate quality. This discrepancy can be explained by the fact that maize has high levels of insoluble fiber, which does not improve the glycemic control as it does not bypasses enzymatic digestion in the small intestine and thus increase the gastric emptying rate and/or glucose absorption through the intestinal mucosa (Giuntini *et al.*, 2022; Schulz and Slavin, 2021). Therefore, when the carbohydrate-to-fiber ratio is applied, particular attention should be directed to the type of fiber present in the food.

Although a carbohydrate-to-fiber ratio below 10:1 indicates an adequate fiber content, it does not capture the rate at which carbohydrates are digested and absorbed into the blood, integrating starch digestibility, the amylose-to-amylopectin ratio and starch structure measured through glycemic index (Ngo *et al.*, 2023). Therefore, for a starchy food with an acceptable ratio ($\leq 10:1$), as recommended by the WHO, but a high glycemic index ($GI > 70$), the glycemic index should be given greater weight when carbohydrate quality is evaluated, as it is the more comprehensive and reliable indicator. Because maize, like other

cereals, has a high proportion of insoluble fiber, approximately 90% to 92.60% (Food and Agriculture Organization of the United Nations, 1992, Lasek *et al.*, 2020), the varieties evaluated here can be considered of poor carbohydrate quality on account of their high glycemic index, which suggests that their frequent consumption may contribute to the risk of diet-related non-communicable diseases. Carbohydrate quality was, however, assessed using *in vitro* indicators, and the glycemic index was determined for maize flour in isolation, whereas the glycemic response in real diets is influenced by food processing and by interactions with the other foods consumed within a meal (Schulz and Slavin, 2021a). Additionally, the nutritional composition of the selected maize varieties may vary with environmental (agro-ecological) conditions. Further studies should therefore evaluate the *in vivo* glycemic response of these varieties in their commonly consumed forms and in combination with typical accompanying foods, as well as assess the same varieties grown under different agro-ecological conditions.

Given the poor carbohydrate quality, and because the daily and high consumption of these varieties may contribute to the risk of diet-related non-communicable diseases, there is a need to improve the CHO quality of these maize. Starch is the predominant carbohydrate component of maize, and the hydrolysed fraction, particularly rapidly digestible starch (RDS), substantially increases the glycemic index (Gujral *et al.*, 2019). Increasing the proportions of SDS and RS while reducing RDS is therefore a promising approach to lowering the glycemic index and improving carbohydrate quality. Starch modification techniques such as heat-moisture treatment have been widely demonstrated to reduce starch digestibility in cereals such as pearl millet (Sandhu *et al.*, 2020), sorghum (Vu *et al.*, 2017; Singh *et al.*, 2011), and normal maize (Zou *et al.*, 2020; Chung *et al.*, 2009). However, the effectiveness of such methods varies according to intrinsic factors influencing starch digestibility, and the conditions applied (Wang *et al.*, 2021); thus necessitating the optimization of HMT conditions for a specific starch source. Although the thirteen varieties exhibited distinct intrinsic characteristics (Table 4.3), they could be statistically classified into four significantly different clusters (Figure 4.2). Since varieties within a cluster share similar intrinsic factors affecting starch digestibility, HMT can be optimized for each cluster rather than for each individual variety.

8.2. Optimization of HMT to decrease starch digestibility of polished maize flour

Heat-moisture treatment (HMT) is one of the most widely used physical starch modification techniques. It induces structural rearrangements within the crystalline and amorphous regions of starch granules, reducing enzyme accessibility and thus decreasing RDS while increasing SDS and RS (Amaraweera *et al.*, 2021). As a low-cost, environmentally friendly and scalable process that preserves granule integrity, it is particularly suitable for resource-limited settings such as Uganda (Fonseca *et al.*, 2021). It involves treating starch at a low moisture content (<35%, w/w) for 15 min to 16 h at temperatures below the gelatinization point (80–140°C) (Wang *et al.*, 2021). Because its effect depends on the botanical source of the starch (Wang *et al.*, 2021), HMT must be optimized for each source, and response surface methodology (RSM) has accordingly been widely used for cereal-based products (Dai *et al.*, 2019; Deng *et al.*, 2022; Gayary and Mahanta, 2020; Iuga and Mironeasa, 2021; Ye *et al.*, 2016).

In Chapter Five, RSM, specifically a Box–Behnken design (BBD), was used to optimize the HMT conditions required to reduce the starch digestibility of polished maize flour from four predominant maize varieties consumed in Uganda. The optimal combinations of moisture content, heating length and temperature were (23.17%, 4.12 hours, 80.00°C) for UH5354, (20.83%, 5.42 hours, 95.43°C) for DK777, (34.51%, 3.01 hours, 90.46°C) for Longe 10H and (19.24%, 5.88 hours, 97.02°C) for DT Max (Table 5.3). These variety-specific optima reflect the intrinsic composition of each variety. Higher-amylose varieties such as Longe 10H (24.94%) and DT Max (24.81%) favour the formation of double helices and of amylose-amylose and amylose-lipid associations that retrograde into enzyme-resistant structures and therefore require different moisture and temperature combinations to maximize SDS and RS than lower-amylose varieties such as UH5354 (22.35%). In addition, protein forms starch-protein complexes and a network around the granules that restricts water mobility and swelling, so protein-rich varieties such as UH5354 respond differently to treatment. Phenolic compounds, which ranged from 183.64 mg GAE in Longe 10H to 217.00 mg GAE in DT Max and are known to bind to starch chains and inhibit amylolytic enzymes, further lowering digestibility and shifting the optimum

conditions. These could explain the variety-specific optimal conditions observed in this study.

Although the experimental moisture range was constrained between 20% and 30% (Table 5.1), the optimum generated by the quadratic model fell outside this boundary for the moisture content of Longe 10H and DT Max and for the temperature for UH5354, indicating that the design boundaries set for these varieties were too restrictive, which can lead to suboptimal results (Geremia *et al.*, 2026). Nevertheless, these values are still within the general conditions under which HMT is applied, and comparable moisture contents (20% to 35%) and durations (5 to 6 hours) have been used for cereals to improve SDS and RS contents (Ariyantoro *et al.*, 2022; Khunae *et al.*, 2007; Li *et al.*, 2011; Liu *et al.*, 2016). A significant difference between the predicted (69.94%) and observed (64.09%) SDS+RS values was found only for UH5354 (Table 5.4). Further studies should therefore expand the design boundaries to capture improved performance without appreciably affecting the nutritional content.

Despite these deviations, the optimized heat-moisture treatment increased the SDS and RS contents of the polished flour of all the maize varieties investigated. According to Olamiti and Ramashia (2025), processing methods such as polishing or refining reshape starch structure by disrupting the granules, reducing crystallinity and altering amylose-amylopectin interactions, which increases the surface area available for enzyme attack and therefore raises the glycemic index. Even so, SDS+RS increased by 9.35% for UH5354, 10.31% for DK777, 7.57% for Longe 10H and 14.39% for DT Max after HMT and polishing, higher than the 5% reported by Ye *et al* (2016) for normal maize starch and unpolished. These results confirm the effectiveness of HMT in reducing the starch digestibility of polished maize flour and suggest that the structural rearrangements induced within the granules are retained even after polishing. Since maize is commonly polished before being milled into flour, applying HMT to whole kernels prior to polishing remains effective, which demonstrates the practical applicability of the technique within conventional milling operations, where kernels can be heat-moisture treated, dried, polished and milled without compromising the beneficial modification of starch digestibility.

Several studies have nevertheless shown that HMT affects the proximate composition, color, functional (swelling, solubility), pasting and thermal properties of starchy foods (Dong *et al.*, 2021; Olayinka *et al.*, 2008; Piecyk and Domian, 2021; Sandhu *et al.*, 2020). Evaluating these effects, and the influence of subsequent processing on them, is therefore necessary to facilitate the adoption of the optimized HMT conditions; these aspects have been addressed in Chapters Six and Seven of this thesis.

8.3. Effects of optimized heat–moisture treatment conditions on physicochemical and techno-functional properties of maize

Previous studies report that HMT affects physicochemical and techno-functional properties of cereals (Kaur and Singh, 2019). However, the present findings show that the optimal conditions generally did not significantly affect the proximate composition of the maize grains, regardless of the maize variety (Table 6.1, $p > 0.05$). As maize is the principal staple food and is consumed by more than 92% of households in Uganda (Wacoo *et al.*, 2018), preserving its nutritional quality while reducing glucose release during digestion is essential. These findings support further development of maize-based products with a lower glycemic index, offering a practical tool to support management of diet-related non-communicable diseases while maintaining food security and cultural food preferences (Bello-Pérez *et al.*, 2021). This dual outcome, unchanged proximate composition alongside reduced starch digestibility, strengthens the case for using HMT as a low-cost, chemical-free, physical modification technique suitable for industrial or small-scale processing in low-resource settings like Uganda, where chemical modification may be less accessible or affordable (Fonseca *et al.*, 2021).

HMT did not significantly affect the total starch content as well as the contents of amylose and amylopectin fractions of the maize varieties examined (Table 6.2, $p > 0.05$). This implies that the reduction in starch digestibility following the application of HMT was not due to changes in starch fractions because amylose is more resistant than amylopectin (Liu *et al.*, 2024; Patel *et al.*, 2017). The optimized HMT conditions nevertheless significantly affected certain techno-functional properties of maize flour, particularly swelling volume, solubility, water absorption capacity and pasting temperature (Tables 6.4 and 6.5). The significant decrease in swelling volume is consistent with the literature, in which HMT

enhances interactions between starch chains, such as hydrogen bonding within the amorphous regions and the formation of amylose-lipid complexes, thereby restricting granule swelling (Kaur and Singh, 2019); a similar decrease was reported for normal and waxy maize starch and was largely determined by the moisture content during treatment (Sui *et al.*, 2015). The variation of SV across the varieties, for example 7.26% and 6.31% for Longe 10H and DT Max respectively (Table 6.4), may relate to differences in native amylose content (Table 6.2) and to granule architecture and the proportion of crystalline to amorphous regions (Gu *et al.*, 2024), which merits further study. The non-significant increase in solubility, despite the reduced swelling, may reflect the partial leaching of amylose from destabilized amorphous regions, as also noted in HMT-treated corn by Marta *et al* (2022) and Qi *et al* (2024).

The pasting temperature increased after HMT, ranging between 81.4 and 94.4°C, with DT Max exhibiting the highest value. This rise mirrors previous studies in which the pasting temperature increased while granule swelling was suppressed, owing to the formation of thermally stable ordered structures within the granule (Sui *et al.*, 2015), and it explains why greater thermal energy was required to initiate pasting, particularly in DK777 and DT Max. The relatively stable peak viscosity across most varieties, despite the elevated pasting temperatures, aligns with observations that HMT can raise the gelatinization temperature while affecting peak viscosity only modestly, depending on the degree of granule disruption and on the starch-lipid or starch-protein interactions induced (Gu *et al.*, 2024). The significant decline in peak viscosity specific to DK777 suggests more extensive granule modification, consistent with its markedly higher increase in pasting temperature.

Overall, the optimized HMT conditions designed to decrease starch digestibility preserved the proximate composition of the flour, which makes HMT a promising low-cost, chemical-free modification for improving the carbohydrate quality of Uganda's maize flour. Nevertheless, the optimal HMT condition significantly altered techno-functional properties such as swelling, solubility and pasting temperature, which emphasizes the need to improve these characteristics in order to ensure consumer acceptability, which is typically achieved through polishing or refining.

8.4. Effect of polishing on physicochemical, functional and sensory properties of heat-moisture-treated maize grains

Whereas heat-moisture treatment (HMT) alone negatively affected several functional properties of maize grains, polishing significantly improved their techno-functional characteristics, with the improvement increasing with the grade of polishing. Specifically, polishing significantly increased the swelling capacity of HMT-treated grains while significantly decreasing their solubility and water absorption capacity (Table 7.5). The increased swelling capacity indicates that the starch granules retain a strong ability to absorb water and expand upon heating, which translates into a firmer, more cohesive, and elastic paste (Joyner *et al.*, 2021). Because starch swelling is considered beneficial in food processing because the increased viscosity makes it a thickening agent in food products (Jia *et al.*, 2023), this is directly relevant to posho, Uganda's dominant maize-based product, which may be derived from these flours, since an increase of swelling capacity generally correlates with the desirable firm, non-sticky, and moldable texture that consumers associate with good-quality posho. In addition, the decrease in solubility suggests that fewer soluble solids leach out of the granule during cooking, which can be advantageous for maintaining a stable, non-cloudy paste structure and reducing stickiness in the final product (Kazem *et al.*, 2026; Rozendo *et al.*, 2026). However, while the increase of swelling and decrease of solubility have an advantageous impact, the decrease in water absorption capacity (WAC) implies lower water-binding capacity which reduces the ability of flour or starch to retain moisture, which lowers the product's yield (Kazem *et al.*, 2026).

Overall, these functional shifts suggest that HMT-polished maize flour is better for products requiring high swelling and paste firmness such as posho, ugali, or similar stiff porridges but may be functionally less suitable for products requiring high solubility or water-binding, such as instant porridges. These results may explain the improvement in sensory acceptability of derived posho prepared from semi- and fully polished maize flour, particularly regarding elasticity, texture, and color which increase with the polishing grade. The increased swelling volume and paste viscosity associated with polishing translate into a firmer, more cohesive, and visually more appealing posho texture, which is generally preferred by consumers over the less cohesive product obtained from unpolished flour

(Buenafe *et al.*, 2021). The parallel improvement in color preference likely reflects the removal of the darker-pigmented bran and germ fractions, yielding a lighter, more visually uniform product consistent with consumer expectations for refined maize flour (Shobana *et al.*, 2011) consumed in Uganda. In addition, the pasting temperature of the polished maize flour decreases significantly with the increase of polishing grade. In unpolished flour, residual bran, fiber, and protein form a matrix around starch granules that hinders water penetration and hydration, thereby requiring more thermal energy to initiate swelling resulting in higher pasting temperatures and reduced swelling volume (Yang *et al.*, 2022). Polishing removes this restrictive matrix, leaving purified starch granules that gelatinize at lower temperatures and swell more extensively, explaining the concurrent decline in pasting temperature and increase in swelling volume.

Polishing improved the techno-functional properties of the HMT-treated maize flour and thus impacted positively on the consumer sensory appeal of the posho derived from the flour. However, polishing significantly reduced the ash, fat, and fiber contents of maize flour, with losses increasing with polishing grade. Ash content dropped by ~50% from ordinary (Class 3) to first-class flour, with the sharpest decline between Class 3 and Class 2 (Table 7.2, $p < 0.05$), especially in DK777, UH5354, and DT Max. Fat content also decreased notably in UH5354 and DT Max during this transition ($p < 0.05$). Fiber losses ranged from 15–47%, with DT Max showing the greatest reduction around 47% and DK777 following with around 42%. In contrast, carbohydrate content increased while protein remained largely unchanged ($p > 0.05$), except in DK777 where it fell by around 15%. The observed decrease in ash, fat, and fiber contents with increasing polishing grade is consistent with the well-established finding that, in the maize kernel, the pericarp (bran) and germ are the main storage sites for these components, while the endosperm is mainly rich in starch (Álvarez-Iglesias *et al.*, 2021; Rocha-Villarreal *et al.*, 2018). As polishing abrades and removes these outer layers, the aforementioned nutrients are physically removed along with them (Wu *et al.*, 2022). The relatively stable protein content observed in the present study across most varieties suggests that maize protein, unlike fiber, fat, and ash, is more distributed between the germ and the protein matrix of the endosperm, making it comparatively less sensitive to the degree of polishing (Rocha-Villarreal *et al.*, 2018).

In addition to the non-significant change observed for protein, polishing significantly increased the total starch and apparent amylose contents (Table 7.3) and these results can be explained by a concentration effect. As polishing removes fiber, fat, and ash, which dilute starch on a dry-weight basis, the proportion of starch and apparent amylose in the flour increases, despite no change in the absolute amount of starch present in the grain. This compositional purification, in turn, explains the marked shift observed in starch digestibility fractions, where the slowly digestible and resistant starch (SDS+RS) content decreased while the rapidly digestible starch (RDS) fraction increased with polishing grade. This finding aligns with the broader principle that non-starch polysaccharides and fiber components physically encapsulate starch granules, restricting enzyme accessibility and thereby slowing the rate of starch hydrolysis; their removal exposes the starch more directly to amylolytic attack, accelerating digestion. This mechanism has been demonstrated in studies showing that soluble dietary fibers reduce rapidly digestible starch and increase resistant starch content by limiting enzyme access and thus lower postprandial glycaemic response (Giuntini *et al.*, 2022; Torres *et al.*, 2024; Zhang *et al.*, 2022).

As reported in Chapter Seven, it was demonstrated that polishing improves the techno-functional properties of the HMT-treated maize grain but decreases the proximate composition of the flour. While the improved sensory and functional qualities of polished flour make it the preferred choice among consumers, its consumption on a population scale may be contributing to a double burden: a reduction in the intake of dietary fiber and micronutrients naturally present in the bran and germ, alongside an increased glycemic burden arising from the elevated proportion of rapidly digestible starch. Given that maize is consumed by most Ugandan households, these findings highlight the need for processing strategies that balance nutritional quality with desirable techno-functional and sensory properties. Approaches such as combining HMT with moderate or semi-polishing may help preserve consumer-preferred characteristics while improving carbohydrate quality and reducing the glycemic impact of maize-based foods. These strategies merit further investigation as practical approaches for producing healthier maize products without compromising their technological performance or consumer acceptability.

CHAPTER NINE

Overall conclusions and recommendations

The overall objective of this study was to improve the carbohydrate quality of flour from commonly consumed maize varieties, using heat–moisture treatment (HMT) method. The research specifically addressed four key objectives: (1) evaluated the carbohydrate quality of selected maize varieties and identified those that are suitable for technology application for HMT; (2) designed the optimal HMT conditions for improving the carbohydrate quality of maize by reducing starch digestibility; (3) demonstrated how HMT affects physicochemical and functional properties of maize flour; and (4) assessed the impact of polishing on the physicochemical, functional, digestibility and sensory characteristics of HMT-treated maize flour. The following summary and conclusions are derived from the findings reported in chapters four to seven, highlighting the contributions to the body of knowledge.

9.1. Summary of the study findings

In the first phase of the study, the carbohydrate quality of thirteen widely cultivated maize varieties in Uganda was assessed using the carbohydrate-to-fiber ratio and estimated glycemic index, which ranged from 5.33-10.32 and 79.38-86.27, respectively. These results generally reflect the low carbohydrate quality of these maize varieties, making their high consumption a factor that may contribute to non-communicable diseases, although this requires confirmation through in vivo studies. In addition, the intrinsic chemical components affecting starch digestibility, such as amylose, amylopectin, lipids, proteins, and the total phenolic content of the selected maize, were analyzed, and these parameters generally varied according to the variety. Through principal component analysis (PCA) and hierarchical cluster analysis (dendrogram), the thirteen selected maize varieties were categorized into four statistically distinct clusters based on their intrinsic chemical components. Among these clusters, DT Max, Longe 5D, and WE3106 displayed more favorable profiles associated with lower starch digestibility, suggesting their potential suitability for increasing dietary resistant starch content, which is important for addressing metabolic disease due to diet.

To increase the carbohydrate quality of the thirteen widely cultivated maize varieties in Uganda, the second phase focused on optimizing heat moisture treatment (HMT) conditions for four selected varieties: UH5354, DK777, Longe 10H, and DT Max, each representing a distinct cluster identified in the previous analysis. Response surface methodology, particularly a Box–Behnken design, was utilized to investigate different combinations of moisture content, temperature, and heating time. BBD was used to determine the optimal conditions for maximizing the amount of slowly digestible starch (SDS) and resistant starch (RS) in polished maize flour. The optimal conditions differed across varieties, and the optimum moisture (%), temperature (°C) and heating time (hours) were 23.17, 80.00, and 4.12 for UH5354; 20.83, 95.43, and 5.42 for DK777; 34.51, 90.46, and 3.01 for Longe 10H; and 19.24, 97.02, and 5.88 for the DT Max varieties. These conditions increased the SDS+RS content of the flour by 8% to 14% compared with that of the untreated controls. These findings demonstrated that HMT could be effectively utilized to reduce starch digestibility in polished maize flour.

The third phase examined the implications of the optimized HMT conditions on the flour physicochemical and functional characteristics of the four selected maize varieties: DK777, Longe 10H, UH5354, and DT Max. The physicochemical composition, such as total starch, amylose, and protein contents, remained stable after optimal HMT conditions were applied. However, functional properties such as swelling volume, water absorption, and pasting behaviors were significantly impacted, although the extent of these effects varied among the varieties. After heat-moisture treatment (HMT), the swelling volume decreased, whereas the water absorption capacity increased. The pasting temperature also increased, whereas the holding viscosity decreased. These modifications in functional and pasting properties may impact essential quality attributes of maize flour dough, such as elasticity, cohesiveness, hardness, energy requirements, and cooking time, potentially lowering the flour's overall acceptability. These findings demonstrate that when HMT conditions are optimized to reduce starch digestibility, considerations should be made to reduce the negative effects on functional properties to improve acceptability by consumers.

To address the negative effects of HMT on functional properties, which could decrease the acceptability of flour by consumers, the fourth phase investigated whether polishing could complement HMT to improve flour quality. HMT-treated maize grains were obtained via the optimal HMT conditions obtained in the second phase. The three polishing levels typically applied in Uganda, namely, unpolished (ordinary), semi-polished (second class), and fully polished (first class), were applied to the HMT-treated maize grains using a locally fabricated huller. The findings revealed that polishing improved flour's physical and functional qualities, especially by increasing color, swelling, and viscosity properties, while reducing its solubility, water absorption, and pasting temperature. These changes contribute to better flour quality (e.g., elasticity, appearance, cooking time). These variations in functional and physical properties contribute to enhanced sensory qualities, especially in fully polished grains. These findings demonstrated that polishing can effectively enhance the functional and sensory properties of flour from heat moisture-treated maize grains.

9.2. Conclusion of the study

The study revealed that the thirteen maize varieties commonly cultivated in Uganda showed low carbohydrate quality, have high glycemic index and carbohydrate-to-fiber ratios, that could make their high consumption, a potential risk factor for the development of food-based non-communicable diseases. Otherwise, these varieties varied in terms of their intrinsic components affecting starch digestibility but could be statistically grouped into four distinct clusters using hierarchical cluster analysis based on these characteristics. These results suggest that the application of starch modification method to improve starch digestibility would vary by variety but can be strategically developed by cluster. The cluster composed of DT Max, Longe 5D, and WE3106 seems to have the high potential content of intrinsic factors and thus may develop more resistant starch when subjected to starch modification compared to other clusters. The optimized heat moisture treatment (HMT) conditions enhanced the slowly digestible starch (SDS) and resistant starch (RS) content by 8–14% in the polished flour of four maize varieties, DK777, DT Max, Longe 10H, and UH5354, each selected from the previously four distinct clusters. These optimal HMT conditions did not affect the physicochemical characteristics of the maize flour, however, they negatively affected certain functional properties of the flour, which could reduce consumer acceptability. On the contrary, while HMT alone affected some functional properties, the application of polishing after HMT improved the flour's physical, functional, and sensory qualities, especially in fully polished (first-class) maize flour.

9.3. Recommendation and perspectives for future studies

The study shows that the thirteen maize varieties cultivated in Ugandan have low carbohydrate quality and diverse intrinsic factors affecting starch digestibility, with DT Max, Longe 5D, and WE3106 showing high potential for resistant starch improvement through starch modification. In addition, the findings show that the optimized heat moisture treatment (HMT) conditions improved the slowly digestible starch (SDS) and resistant starch (RS) content by 8–14%. As maize flour is generally consumed by households in Uganda, and the study revealed that HMT could be an effective method of decreasing starch digestibility in polished maize flour, policymakers, particularly from the Ministries of Agriculture and Health, should after in vivo test confirmation:

- (i) Promote the cultivation and dissemination of maize varieties with high potential for resistant starch development, particularly DT Max, Longe 5D, and WE3106, as part of national strategies to improve the carbohydrate quality of maize and contribute to the prevention of diet related NCDs
- (ii) Promote and facilitate the adoption of the optimized HMT conditions by maize flour processors and milling industries in Uganda and other East African countries through technical training, technology transfer, and supportive regulatory frameworks
- (iii) Incorporate the optimized HMT technology into national food and nutrition policies and maize processing guidelines as a strategic intervention to improve the carbohydrate quality of maize flour.

However, the reported 8–14% decrease is still quite small, particularly considering the high consumption of polished maize flour in Uganda. Additionally, the findings showed that while optimized HMT application alone negatively affects some functional properties of maize flour, which could affect the acceptability of the flours, adversely, application of polishing after HMT improved the flour's physical, functional, and sensory characteristics, especially when fully polished (first-class maize flour). Therefore, further studies should

- (i) Investigate the fortification of HMT-treated maize flour with banana flour, cassava flour, or other resistant starch-rich ingredients to further increase resistant starch content and improve the carbohydrate quality of maize flour
- (ii) Evaluate the in vivo glycemic response of maize flour produced under the optimized HMT conditions and different polishing levels to generate evidence for dietary recommendations and public health interventions
- (iii) Investigate the combined optimization of HMT conditions and polishing levels to develop polished maize flour with lower starch digestibility while maintaining desirable functional, sensory, and nutritional properties.

9.4. Contribution of the study

The present study investigated the carbohydrate quality of thirteen maize varieties available and commonly consumed in Uganda and other East African countries. It makes several

novel contributions to the understanding of maize carbohydrate quality and starch digestibility. First, it provides the most comprehensive characterization to date of carbohydrate quality across thirteen maize varieties commonly consumed in Uganda and East Africa, addressing a gap in the literature that previously focused on single varieties. Second, it identifies the intrinsic physicochemical factors influencing starch digestibility, thereby offering evidence-based guidance for crop selection and processing strategies aimed at improving public health outcomes. Third, the study also established, for the first time, optimal heat–moisture treatment conditions for reducing starch digestibility in polished maize flour. The implementation of these conditions offers a novel strategy for enhancing the nutritional profile of maize-based foods and evidence that HMT can effectively be used to design lower glycemic response diets and support dietary interventions aimed at managing non-communicable diseases. Finally, this study shows that while HMT effectively lowers digestibility, its impact on functional properties requires careful attention to balance nutritional quality with processing performance and consumer acceptability. This study provides an evidence-based foundation for future research to design optimal HMT conditions that reduce starch digestibility while optimizing the functional properties and acceptability of maize flour.

9.5. Limitations of the study

The present study established optimal heat moisture treatment (HMT) conditions for four maize varieties, each selected from clusters sharing similar intrinsic characteristics. Although these conditions are expected to reduce starch digestibility optimally across maize varieties within the same cluster, they were not individually validated on the other varieties in each cluster. Therefore, the study cannot conclusively determine which specific variety would achieve the highest increase in slowly digestible starch (SDS) and resistant starch (RS) content. In addition, the present study analyzed the intrinsic factors affecting starch digestibility and designed optimal HMT conditions for the thirteen maize varieties widely consumed in Uganda, although up to eighty-six varieties are reported to be available. Accordingly, this study could not determine optimal conditions for the remaining varieties. Future research should therefore investigate the intrinsic factors of these varieties and identify the most suitable HMT conditions based on their specific characteristics.

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APPENDICES

Appendix 1: Methods applied for HMT application conditions optimization (some case studies)

Objectives	Starchy foods	Optimization design used	Results	References
Optimize HMT application conditions for relative crystallinity (RC) of raw waxy maize starch.	Raw waxy maize starch.	Response surface methodology (RSM), mainly Box-Behnken design (BBD)	Temperature, treatment time, and moisture content were 99.2°C, 1.8 h, and 18.2%, respectively. The maximum RC of heat-moisture treated starch could reach 42.44% and more holes, depressions, and cracks appeared on the surface of HMTS	Dai <i>et al</i> (2019)
Optimize HMT reaction conditions for improving nutritional properties of treated maize starch	Maize starch	Response surface methodology (RSM), mainly Box-Behnken design (BBD)	The optimum reaction condition decreased RDS from 87.10% to 82.21% when NMS was subjected to HMT at 23.6% moisture content and heated at 114.8 °C for 9.04 h.	Ye <i>et al</i> (2016)
Optimize HMT application condition for rice starch isolated from broken rice	Rice starch	RSM based on face-centered central composite design (FCCD)	Temperature of 117°C and time of 35 min were the optimal conditions for OPT that gave final viscosity of 4718.42cP, setback viscosity of 2312.27cP, gel hardness of 73.12 g, swelling power of 16.21 g/g and solubility of 11.45%.	Gayary and Mahanta (2020)
Investigated the optimal condition of HMT on potato starch and study on its physicochemical properties	Potato starch	Response surface methodology (RSM), mainly Box-Behnken design (BBD)	23.56% of MC exposed at 90°C during 1.5 h were optimal for higher setback viscosity, gel strength and hardness.	Deng <i>et al</i> (2022)
sfEstablish the optimum HMT conditions for preparing sweet potato flour with increased SDS content	Sweet potato flour	Response surface methodology (RSM), mainly Box-Behnken design (BBD)	The optimum conditions for preparing slowly digestible sweet potato flour were MC of 22%, temperature of 103°C, and treatment time of 5.8 h. The highest SDS content in heat-moisture treated sweet potato flour was 57.6%.	Ahn <i>et al</i> (2013)

Appendix 1: Methods applied for HMT application condition optimization (some case studies) (continuous & end)

Objectives	Starchy foods	Optimization design used	Results	References
Optimize operating conditions of HMT to maximize RS and SDS fractions	Morado banana starch	Response surface methodology is mainly a central composite design (CCD)	The optimized conditions were 32.2% for moisture content and 6.5 h of heating time to achieve the highest slowly digestible starch (SDS) and resistant starch (RS) content	Hoyos-Leyva <i>et al</i> (2015)
Optimize the conditions for the preparation of sweet potato starch containing a high proportion of SDS by HMT.	Isolated sweet potato starch	Response surface methodology (RSM), mainly Box–Behnken design (BBD)	Maximum SDS content (50.9%) was obtained after 8.6 h at 113°C with a 22.8% moisture level	Na <i>et al</i> (2019)
To enhance the RS content of high amylose rice starch with HMT	Rice starch	Response surface methodology (RSM), mainly Box–Behnken design (BBD)	Temperatures of 100°C, MC of 24.2%, and a time of 11.5 h for achieving the highest RS content were established. The RS content increased from 32.1% for native starch to 46.4% in HMT starch with the optimized conditions.	Lee and Chung (2022)
Investigate the effect of HMT on the functional and rheological properties of cassava starch	Cassava starch	Response surface methodology (RSM), mainly Box–Behnken design (BBD)	Optimal processing conditions for cassava starch were achieved with a moisture content of 30%, drying temperature of 90 °C and time of 2 h. These variables are important to consider for processing cassava starch with improved consumer acceptability and potential large-scale production of baked foods, gel formation and custard production	(Sobowale <i>et al.</i> , 2022)
HMT optimization to increase the formation of SDS in waxy potato starch, and the structural and physiological properties of this starch	Waxy potato starch	Response surface methodology (RSM), mainly Box–Behnken design (BBD)	A maximum SDS content (41.8%) consistent with the expected value (40.1%) was obtained after 5 h 20 min at 120°C with a 25.7% moisture level	Lee and Chung (2022)

Appendix 2: Study approval received from the Gulu University Research Ethics Committee (GUREC) and Uganda National Council for Science and Technology (UNCST)

GULU		UNIVERSITY
P.O. Box 166, Gulu (U) Website: www.gu.ac.ug Email: gurec@gu.ac.ug		Tel: +256 471 432 096 Fax: +256 471 432 913 Mob: +256 772 305 621 +256 776 812 147
RESEARCH ETHICS COMMITTEE		
19/06/2023		
To: Finagnon Fassinou		
Faculty of Agriculture and Environment (FAE)/Gulu University +256762291319		
Type: Initial Review		
Re: GUREC-2023-507: IMPROVING THE CARBOHYDRATE QUALITY OF POLISHED MAIZE FLOUR USING HEAT-MOISTURE TREATMENT		
I am pleased to inform you that at the 97th convened meeting on 23/03/2023 , the Gulu University REC meeting voted to approve the above referenced application. Approval of the research is for the period of 19/06/2023 to 19/06/2024 .		
As Principal Investigator of the research, you are responsible for fulfilling the following requirements of approval:		
1. All co-investigators must be kept informed of the status of the research. 2. Changes, amendments, and addenda to the protocol or the consent form must be submitted to the REC for re-review and approval prior to the activation of the changes. 3. Reports of unanticipated problems involving risks to participants or any new information which could change the risk benefit: ratio must be submitted to the REC. 4. Only approved consent forms are to be used in the enrollment of participants. All consent forms signed by participants and/or witnesses should be retained on file. The REC may conduct audits of all study records, and consent documentation may be part of such audits. 5. Continuing review application must be submitted to the REC eight weeks prior to the expiration date of 19/06/2024 in order to continue the study beyond the approved period. Failure to submit a continuing review application in a timely fashion may result in suspension or termination of the study. 6. The REC application number assigned to the research should be cited in any correspondence with the REC of record. 7. You are required to register the research protocol with the Uganda National Council for Science and Technology (UNCST) for final clearance to undertake the study in Uganda.		
The following is the list of all documents approved in this application by Gulu University REC:		
GULU UNIVERSITY RESEARCH ETHICS COMMITTEE APPROVED UNTIL ★ 19 JUN 2024 ★ FACULTY OF MEDICINE P. O. Box 166, Gulu		

No.	Document Title	Language	Version Number	Version Date
1	Protocol	English	3.0	2023-06-16
2	Informed Consent forms	English & Acholi	3.0	2023-06-16
3	Informed Consent forms	English & Acholi	3.0	2023-06-16
4	COVID-19 risk management plan for the study	English	2.0	2023-05-05
5	Data collection tools	English	2.0	2023-05-03

Yours Sincerely




Dr. Gerald OBAI
For: Gulu University REC



Uganda National Council for Science and Technology

(Established by Act of Parliament of the Republic of Uganda)

Our Ref: A338ES

4 October 2023

Finagnon Fassinou
Faculty of Agriculture and Environment (FSA), Gulu
University (GU)
Gulu

Re: Research Approval: IMPROVING THE CARBOHYDRATE QUALITY OF POLISHED MAIZE FLOUR USING HEAT-MOISTURE TREATMENT

I am pleased to inform you that on **04/10/2023**, the Uganda National Council for Science and Technology (UNCST) approved the above referenced research project. The Approval of the research project is for the period of **04/10/2023** to **04/10/2024**.

Your research registration number with the UNCST is **A338ES**. Please, cite this number in all your future correspondences with UNCST in respect of the above research project. As the Principal Investigator of the research project, you are responsible for fulfilling the following requirements of approval:

1. Keeping all co-investigators informed of the status of the research.
2. Submitting all changes, amendments, and addenda to the research protocol or the consent form (where applicable) to the designated Research Ethics Committee (REC) or Lead Agency for re-review and approval **prior** to the activation of the changes. UNCST must be notified of the approved changes within five working days.
3. For clinical trials, all serious adverse events must be reported promptly to the designated local REC for review with copies to the National Drug Authority and a notification to the UNCST.
4. Unanticipated problems involving risks to research participants or other must be reported promptly to the UNCST. New information that becomes available which could change the risk/benefit ratio must be submitted promptly for UNCST notification after review by the REC.
5. Only approved study procedures are to be implemented. The UNCST may conduct impromptu audits of all study records.
6. An annual progress report and approval letter of continuation from the REC must be submitted electronically to UNCST. Failure to do so may result in termination of the research project.

Please note that this approval includes all study related tools submitted as part of the application as shown below:

No.	Document Title	Language	Version Number	Version Date
1	Informed Consent forms	English & Acholi	3.0	16 June 2023
2	Data collection tools	English	2.0	03 May 2023
3	COVID-19 & EBOLA risk management plan for the study	English	1.0	07 February 2023
4	Project Proposal	English	3.0	
5	Approval Letter	English		
6	Administrative Clearance	English		

Yours sincerely,



Hellen Opolot

For: Executive Secretary

UGANDA NATIONAL COUNCIL FOR SCIENCE AND TECHNOLOGY

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WEBSITE: <http://www.uncst.go.ug>

Appendix 3: Evaluation of the effects of heat–moisture treatment on the sensory acceptability of polished maize flour (consent letters and data collection sheets)

VERSION 3.0

Sub-topic: Evaluation of heat-moisture treatment effect on polished maize flour sensory acceptability and in vivo glycemic index

Informed consent for participation in the sensory acceptability test

Introduction

Hello, my name is Finagnon Toyi Kevin FASSINO, a Ph.D. student in Agriculture and Applied Bioscience at the Faculty of Agricultural and Environment (FAE), Gulu University. I am (we are) conducting a study in Gulu about the **improving of carbohydrate quality of polished maize flour using heat-moisture treatment**. I would very much appreciate your participation in this study.

Purpose

The study seeks to develop polished maize flour with a low glycemic index using hydrothermal processing mainly the heat moisture treatment. The findings of this study are expected to contribute at addressing the issue of overweight, obesity, insulin resistance, start point of non-communicable diseases.

Procedures

The test will take 30 minutes at most to be completed. It is individual and I need some of your personal information. For the test, I am going to serve you some improved maize doughs based on their glycemic index quality. You will eat, smell, and touch these doughs and will give us your appreciation about each of them using the five hedonic scales proposed in the test sheet.

Who will participate in the study?

You have been selected as participate in the purpose of this sensory evaluation study because you are a consumer of polish maize dough.

Risks/discomforts

There is no risk of discomfort that will arise from your participation in this study. The main issue is you time spend during the sensory analysis.



Benefits

Therefore, we are seeking your consent to participate in the study whose findings might be beneficial to your community and those who consumed the maize dough. Your involvement in this phase of the study will allow you to know the process of development of polished maize flour with low glycemic index. This will help you to prevent overweight and obesity and consequently the non-communicable diseases among your community. There is no financial or other personal benefits from participating in this study.

Confidentiality

All the information collected in this study will remain anonymous and treated with confidentiality, and will only be used for academic research purposes. The principal investigator will only access confidential information.

Alternatives

You do not have to participate in this study if you are not interested. You will not lose any benefit in case of no participation.

Cost

There will be no any additional cost incurred as a result of participating in this study.

Questions

At this time, please feel free to ask me about anything you would like to understand about the study. You can contact the principal investigator, Finagnon Toyi Kevin FASSINO on telephone number 0762291319 or via email on ffinagnon93@gmail.com

Statement of voluntariness

Participation in the research study is voluntary and you may join on your own free will. You have a right to withdraw from the study at any time without penalty.

If you have any issues pertaining to your rights and participation in the study, please contact the Chairperson, Gulu University Research Ethics Committee, Dr. Gerald Obai Tel: No., 0772305621; email: lekobai@yahoo.com/lekobai@gmail.com; or the Uganda National Council for Science and Technology, on plot 6 Kinera Road, Ntinda, Kampala on Tel 0414705500.



★ Page 2 sur 5

Statement of consent

..... has described to me what is going to be done, the risks, the benefits involved and my rights as a participant in this study. I understand that my decision to participate in this study will not affect me in any way. In the use of this information, my identity will be concealed. I am aware that I may withdraw at anytime. I understand that by signing this form, I do not waive any of my legal rights but merely indicate that I have been informed about the research study in which I am voluntarily agreeing to participate. A copy of this form will be provided to me.

Name Signature/thumbprint of participant.....Date

Name.....Signature of witness.....Date.....

Name.....Signature of interviewer.....Date.....



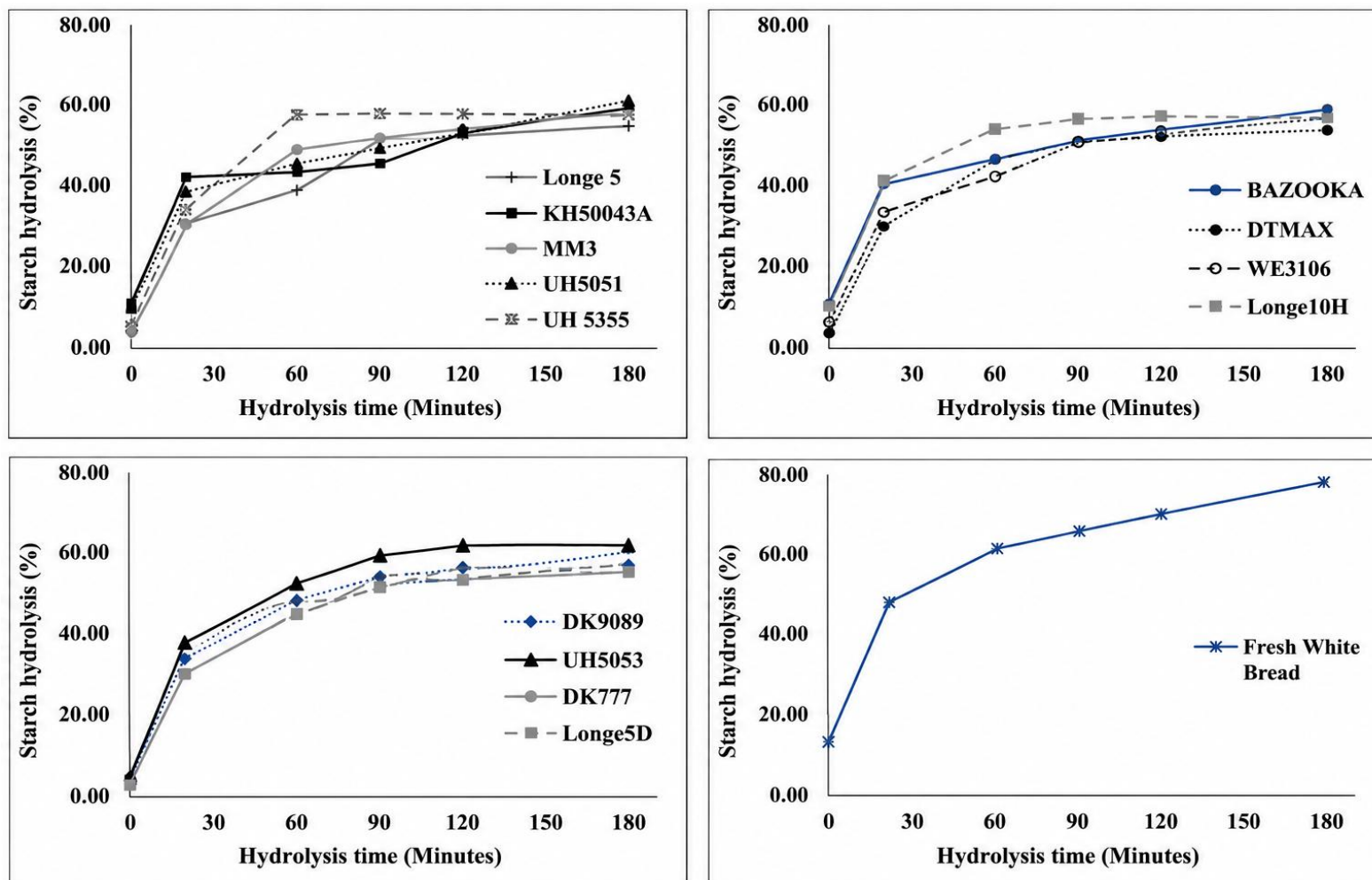
Appendix 4: Details of the 13 maize varieties studied

Variety name/code	Year of release	Owners	Special attributes
DK 777	NA	Monsanto	Resistant to MSV, NLB, GLS
DK 9089	NA	Monsanto	Resistant to MSV, NLB, GLS
KH 500 43A	2012	EASEED	Mid-altitude hybrid
Longe 5	2000	NARO	Resistant to MSV, stress-tolerant, flint grain type
Longe 5 D	2012	NARO	Resistant to MSV, good standability, flint grain type
Longe 10H	2009	NARO	Resistant to MSV, NLB, GLS, drought tolerant
MM3	2010	NARO	High yield stability, Drought tolerant
UH5051	2012	NARO	Drought tolerant, intermediate maturing, resistant to aflatoxin
UH5053	2012	NARO	High yield, good husk cover
UH5354	2013	NARO	Has snow white grains
UH5355	2013	NARO	Drought tolerant, intermediate maturing
DT Max	2012	NASECO	Tolerant to GLS
WE3106	2016	NARO	Early maturity (suitable for areas with short rains)

EASEED: EAST AFRICAN SEED COMPANY LIMITED, East African community, **NARO**=National Agricultural Research Organization, Uganda, **NASECO:** Nalweyo Seed Company, Uganda, Monsanto Company, Eastern and Southern Africa, **MSV**=Maize streak virus, **NLB**=Northern Leaf blight, **GLS**=Gray Leaf Spot

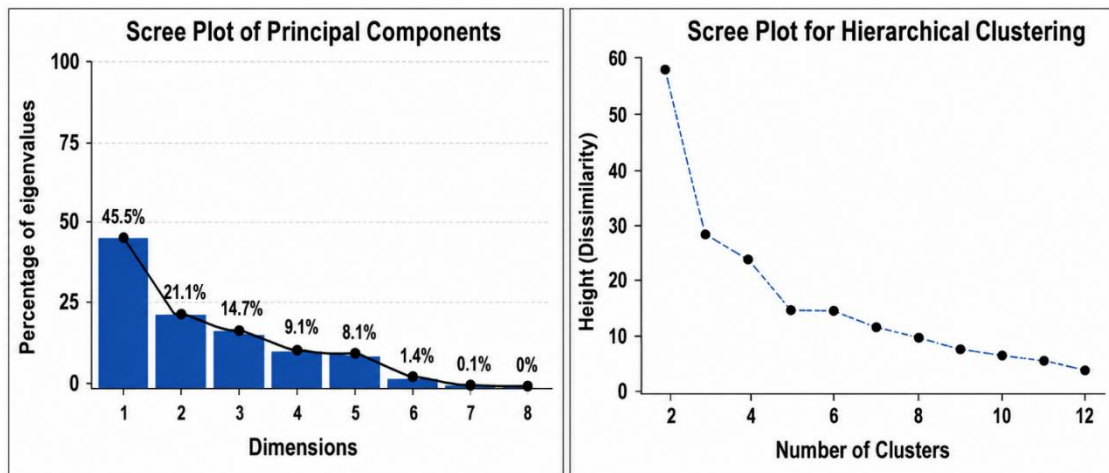
Source: National Crops Resources Research Institute (NaCRRI), (January 2023)

Appendix 5: Starch digestograms of the different maize varieties and fresh white bread (reference food)



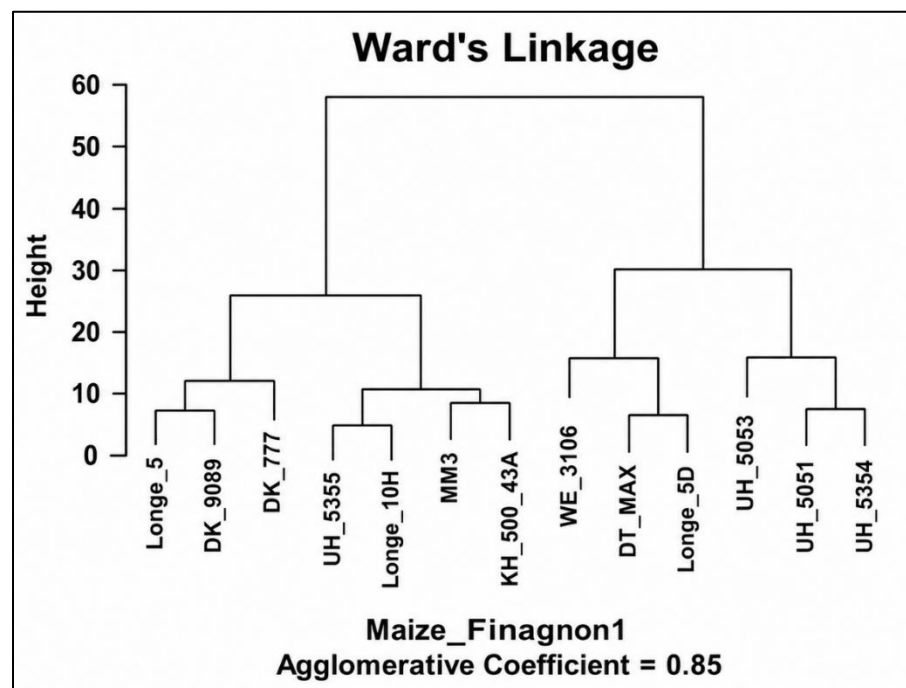
Supplementary Figure 1. Starch digestograms expressing the released starch according to time for the different maize varieties and fresh white bread

Appendix 6: Scree plot of principal component and hierarchical clustering analysis scree plot and dendrogram



Supplementary Figure S2: Scree plot of the principal component analysis

Supplementary Figure 3: Scree plot of the hierarchical clustering analysis



Supplementary Figure 4: Hierarchical cluster analysis showing the grouping of the maize varieties according to their dissimilarity

Appendix 7: Starch fraction released under different HMT conditions and the effects on maize varieties

X ₁	X ₂	X ₃	Rapid Digestible Starch (RDS)					Composite Slow Digestible Starch and Resistant Starch (SDS+RS)				
			UH	DK	LONGE	DT	P value	UH	DK	LONGE	DT	P value
			5354	777	10H	MAX		5354	777	10H	MAX	
25 (0)	4(-1)	80(-1)	35.60	69.89	55.16	50.72	0.006***	64.40	30.11	44.84	49.28	0.006***
20(-1)	6(0)	80(-1)	33.04	66.42	70.59	52.28	0.016**	66.96	33.58	29.41	47.72	0.016**
30(1)	6(0)	80(-1)	56.13	57.63	60.70	53.56	0.321	43.87	42.37	39.30	46.44	0.321
25 (0)	8(1)	80(-1)	46.10	63.99	59.12	53.12	0.099*	53.90	36.01	40.88	46.88	0.099*
20(-1)	4(-1)	90(0)	41.69	56.19	64.89	53.69	0.016**	58.31	43.81	35.11	46.31	0.016**
30(1)	4(-1)	90(0)	42.11	61.28	52.94	54.69	0.083*	57.89	38.72	47.06	45.31	0.083*
25(0)	6(0)	90(0)	49.43	57.38	64.49	55.59	0.026**	50.57	42.62	35.51	44.41	0.026**
25(0)	6(0)	90(0)	48.38	52.52	66.18	58.62	0.102	51.62	47.48	33.82	41.38	0.102
25(0)	6(0)	90(0)	53.30	50.07	60.00	51.90	0.149	46.70	49.93	40.00	48.10	0.149
25(0)	6(0)	90(0)	52.32	52.52	62.51	57.78	0.020**	47.68	47.48	37.49	42.22	0.020**
25(0)	6(0)	90(0)	44.72	50.12	63.29	55.97	0.002***	55.28	49.88	36.71	44.03	0.002***
20(-1)	8(1)	90(0)	55.33	62.19	60.22	51.45	0.077*	44.67	37.81	39.78	48.55	0.077*
30(1)	8(1)	90(0)	39.15	60.13	59.17	60.27	0.077*	60.85	39.87	40.83	39.73	0.077*
25(0)	4(-1)	100(1)	60.30	58.97	71.02	48.94	0.026**	39.70	41.03	28.98	51.06	0.026**
20(-1)	6(0)	100(1)	65.39	52.08	74.38	49.72	0.008***	34.61	47.92	25.62	50.28	0.008***
30(1)	6(0)	100(1)	46.37	56.35	58.31	57.23	0.046**	53.63	43.65	41.69	42.77	0.046**
25(0)	8(1)	100(1)	46.00	68.50	48.75	49.70	0.012**	54.00	31.50	51.25	50.30	0.012**

*, **, and *** denote statistical significance at the 10%, 5% and 1% levels, respectively.

X₁: moisture, X₂: heating length, X₃: Heating temperature

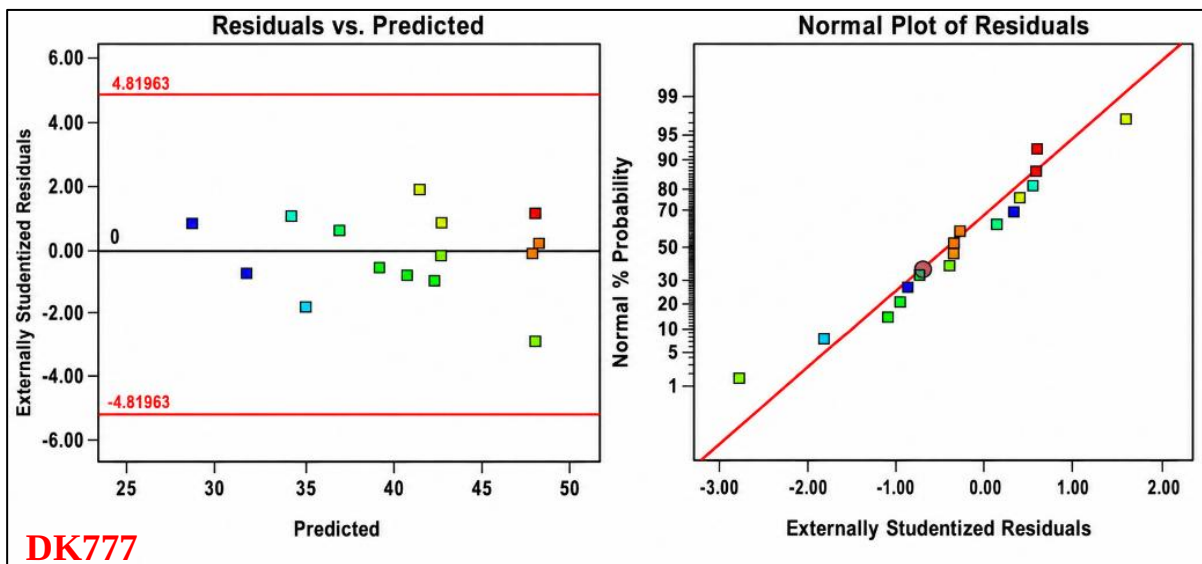
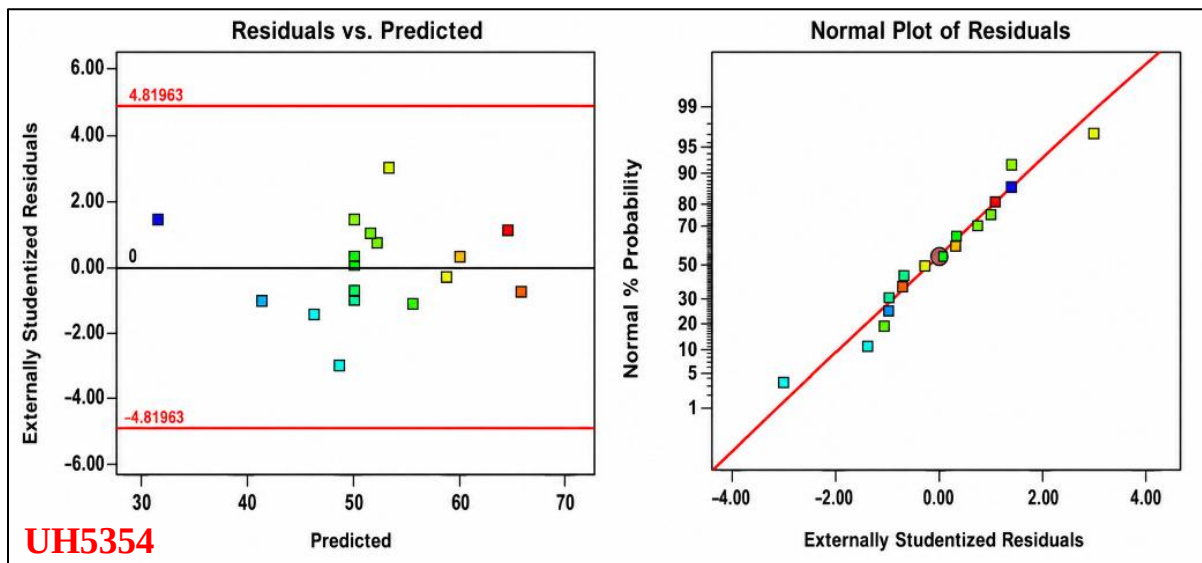
Appendix 8: ANOVA table showing the comparison of regression models for composite slow digestible and resistant starch according to maize variety

Models	Lack of fit (p)	R²	Adjusted R²	Model (p value)	Decision
Bazooka					
Linear	0.031	0.257	0.085	0.262	
Linear with interaction	0.220	0.824	0.718	0.003	
Quadratic without interaction	0.019	0.328	-0.074	0.582	
Quadratic	0.233	0.896	0.762	0.010	Selected
DK777					
Linear	0.045	0.114	-0.090	0.651	
Linear with interaction	0.037	0.309	-0.117	0.643	
Quadratic without interaction	0.197	0.725	0.561	0.019	
Quadratic	0.611	0.913	0.801	0.006	Selected
LONGE 10H					
Linear	0.024	0.337	0.184	0.137	
Linear with interaction	0.041	0.645	0.432	0.059	
Quadratic without interaction	0.026	0.555	0.288	0.147	
Quadratic	0.089	0.863	0.688	0.024	Selected
DT MAX					
Linear	0.312	0.278	0.111	0.223	
Linear with interaction	0.266	0.418	0.069	0.381	
Quadratic without interaction	0.634	0.685	0.495	0.036	
Quadratic	0.825	0.825	0.600	0.050	Selected

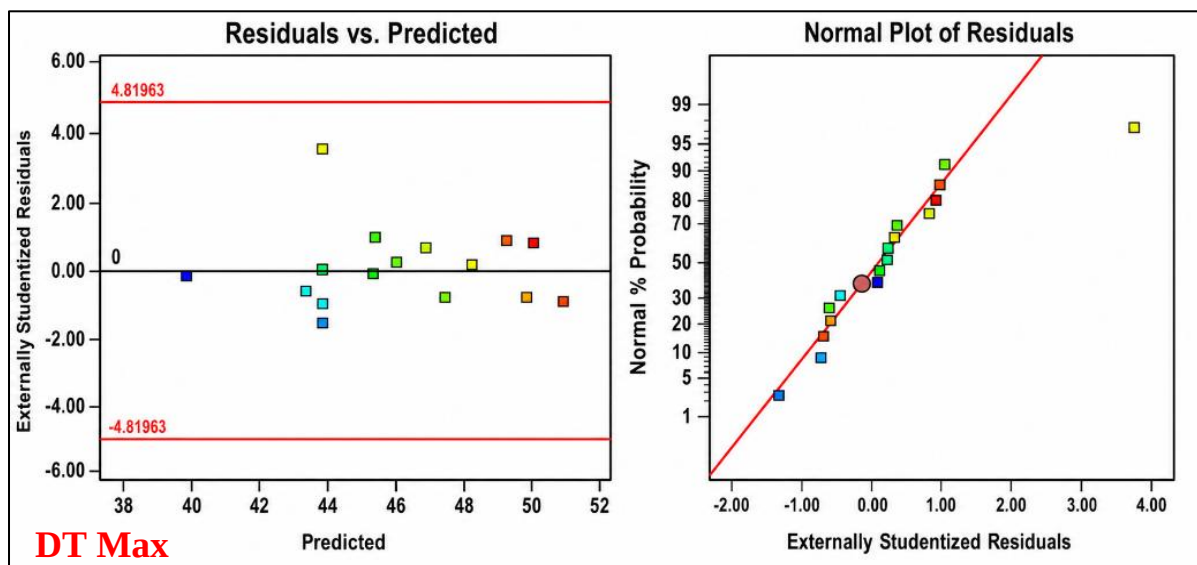
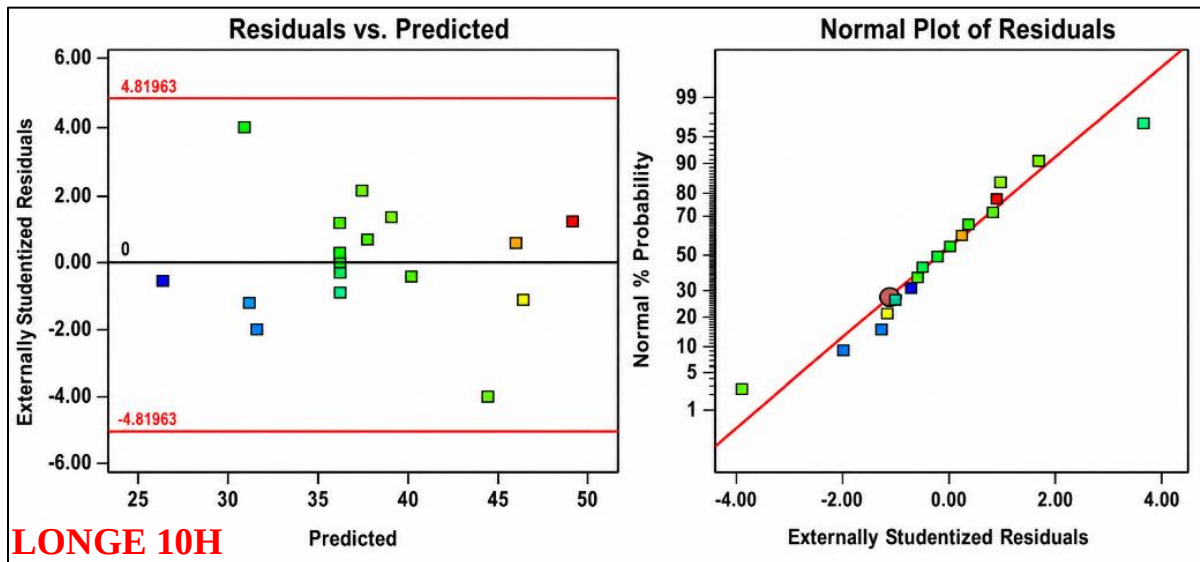
Appendix 9: Observed and predicted data in terms of coded factors for the quadratic model

HMT parameters			BSDS_RS		DSDS_RS		LSDS_RS		MSDS_RS	
Moisture	Heating length	Temperature	Observed	Predicted	Observed	Predicted	Observed	Predicted	Observed	Predicted
0	-1	-1	64.40	65.96	30.11	29.11	44.84	46.81	49.28	50.09
-1	0	-1	66.96	64.73	33.58	35.68	29.41	31.54	47.72	47.06
1	0	-1	43.87	46.60	42.37	42.58	39.30	38.19	46.44	45.52
0	1	-1	53.90	51.84	36.01	34.71	40.88	37.89	46.88	47.65
-1	-1	0	58.31	58.98	43.81	42.71	35.11	31.01	46.31	46.16
1	-1	0	57.89	53.60	38.72	39.51	47.06	46.20	45.31	45.42
0	0	0	50.57	50.37	42.62	47.48	35.51	36.71	43.90	43.90
0	0	0	51.62	50.37	47.48	47.48	33.82	36.71	41.38	43.90
0	0	0	46.70	50.37	49.93	47.48	40.00	36.71	48.10	48.44
0	0	0	47.68	50.37	47.48	47.48	37.49	36.71	42.22	39.88
0	0	0	55.28	50.37	49.88	47.48	36.71	36.71	43.90	50.29
-1	1	0	44.67	48.96	37.81	37.02	39.78	40.64	48.55	51.20
1	1	0	60.85	60.18	39.87	40.97	40.83	44.93	39.73	43.43
0	-1	1	39.70	41.76	41.03	42.33	28.98	31.98	51.06	49.49
-1	0	1	34.61	31.88	47.92	47.71	25.62	26.73	50.28	43.90
1	0	1	53.63	55.86	43.65	41.56	41.69	39.56	42.77	43.90
0	1	1	54.00	52.44	31	32.50	50.00	49.28	51.25	43.90

Appendix 10: Diagnostic graphs showing the diagnostic plots for the quadratic model fitting



Supplementary Figure 5: Residual diagnostic plots for the fitted response surface model. (Left) Residuals versus predicted values; (right) normal probability plot of the externally studentized residuals



Supplementary Figure 5: Residual diagnostic plots for the fitted response surface model. (Left) Residuals versus predicted values; (right) normal probability plot of the externally studentized residuals (End)

Appendix 11: Some pictures taken during the data collection



Photo 1: Maize Grains Sealed in Purdue Improved Crop Storage (PICS) bags composed of two polypropylenes for storage in laboratory condition



Photo 2: Maize Grains Sealed in Polyethylene Zipper Bags ready for Moisture Content Adjustment



Photo 3: Measurement and addition of water to maize grains for the adjustment of their moisture



Photo 4: Maize grains Sealed in Polyethylene Zipper Bags after adjusting moisture content, keep in Fridge at 4°C for 24 hours for moisture equilibration



Photo 5: Maize grains packing in glass bottles for the heat application

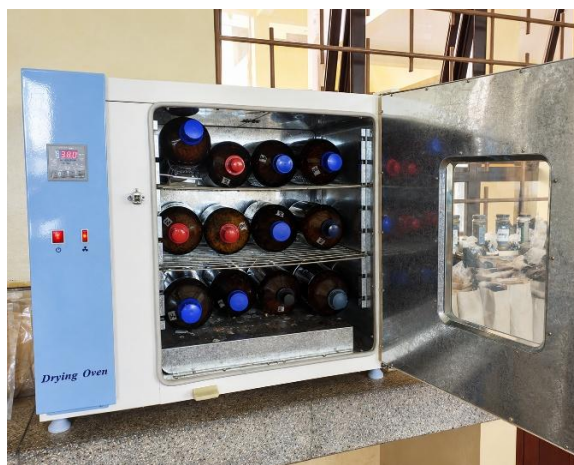


Photo 6: Maize grains packed in glass bottles and heated using an electric hot-air drying oven (Heat application of HMT)



Photo 7: Maize grain drying after application of HMT to reduce the moisture content to 10-11%



Photo 8: Polishing of HMT-treated maize grains using the traditional maize huller



Photo 9: Assessment of oil absorption capacity of heat moisture treated maize flour



Photo 10: Digestion of maize flour in magnetic stirrer water bath at 37°C for 5 min at 170 rpm



Photo 11: Centrifugation of digesta for glucose release quantification



Photo 12: Spectrophotometric Reading of Color Intensity Used to Calculate Released Glucose (Corrected vs. Blank)



Appendix 12: Plagiarism check certificate

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Department: FOOD SCIENCE AND POSTHARVEST TECHNOLOGY

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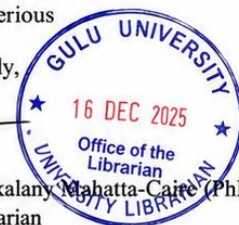
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AUTHOR BIOGRAPHY



Finagnon Toyi Kevin FASSINO was born on 3 June 1993 in Porto-Novo, the capital of the Republic of Benin. He holds a Bachelor's degree in Sciences and Techniques of Preservation and Processing of Agricultural Products from the National University of Agriculture (UNA), Benin (2014), and a Master's degree in Food Science and Nutrition from the University of Abomey-Calavi (FSA/UAC), Benin. (2018). In 2025 he also completed a Postgraduate diploma in Monitoring and Evaluation at Gulu University, Uganda.

Following his Master's degree, he was awarded a grant from the Regional Universities Forum for Capacity Building in Agriculture (RUFORUM) in 2019, under which he disseminated his research by training local communities and nutrition institutions to use locally available resources to develop nutrient-dense foods, aimed at addressing malnutrition among women of reproductive age and children under five. Between 2020 and 2022, he worked as a project assistant researcher and manager on several projects in Benin. Within the edible-insects project, he updated the national inventory of insects consumed in the country. He also coached students in agribusiness and entrepreneurship development and contributed to curriculum revision at the National University of Agriculture through the AgriEngage project, among other activities.

Since June 2022, he has been enrolled in a PhD programme in Agricultural and Applied Biosciences in the Faculty of Agriculture and Environment at Gulu University, Uganda, supported by a mobility scholarship from the Train Agribusiness and Food Systems Scientists for African Agriculture (TAFSA) project. During his doctoral studies, he has also served as a part-time lecturer and teaching assistant in the Department of Food Science and Post-Harvest Technology, contributing to courses on food security, food chemistry, and postharvest handling, and has supervised undergraduate research on food security among host and refugee communities in Northern Uganda. Over the course of his PhD, he has published four peer-reviewed articles arising directly from his doctoral research and co-authored several others, bringing his record to more than a dozen publications in peer-reviewed journals.

Kevin FASSINO is a researcher specializing in food formulation, food fortification, and food safety and quality management. Through his doctoral research, Kevin focused on improving the carbohydrate quality of predominant maize varieties using heat-moisture treatment, with the aim of contributing to the prevention of non-communicable diseases. Building on this work and his previous master's research, his research contributes to the achievement of the Sustainable Development Goals, particularly SDG 1 (No Poverty), SDG 2 (Zero Hunger), and SDG 3 (Good Health and Well-being), by promoting food security, improving nutritional quality, and supporting healthier and more sustainable livelihoods.