

**PHYSICOCHEMICAL PARAMETERS AND FUNCTIONAL
PROPERTIES OF FLOURS FROM ADVANCED GENOTYPES AND
IMPROVED CASSAVA VARIETIES FOR INDUSTRIAL
APPLICATIONS**

MASTER OF SCIENCE (APPLIED CHEMISTRY) THESIS

LIFA CHIMPHEPO

**UNIVERSITY OF MALAWI
CHANCELLOR COLLEGE**

AUGUST, 2021

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MASTER OF SCIENCE (APPLIED CHEMISTRY) THESIS

BY

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B.Ed. (Science) -University of Malawi

Submitted to the Department of Chemistry, Faculty of Science in partial fulfillment of requirements for the degree of Master of Science (Applied Chemistry)

**UNIVERSITY OF MALAWI
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AUGUST, 2021

DECLARATION

I, the undersigned hereby declare that this thesis is my original work which has not been submitted to any other institution for similar purposes. Where other people's work has been used, acknowledgments have been made.

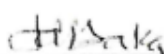
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CERTIFICATION OF APPROVAL

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DEDICATION

I dedicate this thesis to my late grandmother for her support in my early academic life. My late parents, Mr and Mrs Chimphepo, and my dear sister Tivis Kamanga, for their love and moral support.

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ABSTRACT

Cassava has potential for many industrial uses, which provide an opportunity for more rewarding markets. Therefore, significant research on improved varieties, targeting industrial applications, is required as a possible approach to spur improvements in the value chain. In addition, the promotion of cassava for production, targeting industrial applications, requires information on yield and stability performance of cassava genotypes and varieties in a diverse range of environments. To this end, this study evaluated the physicochemical parameters and functional properties of flours from ten (10) advanced genotypes and improved cassava varieties for industrial applications as well as the effect of genotype by environment (G X E) interaction on such physicochemical parameters and functional properties. The genotypes and varieties were collected from a multi-location (Uniform yield Trial) trial of the IITA breeding program in Malawi. Their flour samples were analysed for various physicochemical parameters and functional properties, and compared with currently marketed High Quality Cassava Flour (HQCF). The data were obtained using multiple analytical techniques and methodology such as oven-drying, sieving, colorimetry, titration, acid hydrolysis method, the Kjeldahl procedure, UV/VIS spectrophotometry, and centrifugation.

Results show that genotype effect on overall quality characteristics endearing to industrial applications was significant ($p < 0.05$), allowing identification of industry preferred genotypes and varieties. Starch and amylopectin content are the major determinants of variability in the cassava flours' functional properties, such as water binding and oil absorption capacities, solubility, and swelling power. Overall, genotypes I020452 and I010040, and the released variety Sagonja have a significantly ($p < 0.05$) high starch (ranged from 72.39 ± 5.23 g/100 g to 84.15 ± 2.81 g/100 g) and amylopectin content (ranged from 64.49 ± 5.48 g/100 g to 74.50 g/100 g), high bulk density (ranged from 0.65 ± 0.20 g/mL to 0.69 ± 0.03 g/mL), and all the analysed functional properties. These genotypes showed comparable/or superior functional properties to the marketed HQCF. The results also showed that environments (E) and genotypes (G) and their interaction (G x E) effects were highly significant ($P < 0.001$) in explaining the variance of the physicochemical parameters

and functional properties. Environment played a major role in influencing fresh root dry matter content (FR-DMC), bulk density and solubility. Genotype by environment (G X E) interaction played a major role in influencing starch and amylopectin content, swelling power, and water binding capacity. Additive main effect and multiplicative interaction (AMMI) analysis identified I010040, MM06/0045 and TMSL110080 genotypes and Mbundumali, Mpale and Sagonja varieties as the most stable with high yield performance. I010040 showed higher levels of starch related properties (starch and amylopectin content, bulk density, oil absorption capacity, solubility and swelling power) whereas Mbundumali yielded higher FR-DMC and also water binding capacity. Genotype TMSL110080 yielded highest FR-DMC. Therefore, the study recommends I010040, MM06/0045 and TMSL110080 genotypes and Mbundumali, Mpale and Sagonja varieties to be targeted for cultivation in wide range of environments for production of HQCF and starch for various industrial applications such as production of ethanol and bio fuels, starch and glucose syrup, and sweeteners in chemical industries; thickeners, stabilizers, and texture modifiers in food, bakery and confectionery industries; binders and adhesives in paper making and plywood industries; and fillers as well as stiffeners in textile and packaging industries.

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LIST OF ACRONYMS AND ABBREVIATIONS

ANOVA:	Analysis of Variance
CNP:	Cyanogenic potential
FAO:	Food Agricultural Organization
HCN:	Hydrogen cyanide
HQCF:	High quality cassava flour
IITA:	International Institute for Tropical Agriculture
WHO:	World Health Organization
PCA:	Principal Component Analysis
HCA:	Hierarchical Clustert Analysis
G X E:	Genotype – environment interaction
AMMI:	Additive main effects and multiplicative interactions
IPCA:	Interaction Principal Component Analysis
YSI:	Yield stability index
C:AVA:	Cassava: adding value for Africa
IFAD:	International Fund for Agricultural Development
WBC:	Water absorption capacity
OAC:	Oil absorption capacity

CHAPTER 1: INTRODUCTION

1.1 Background

Cassava (*Manihot esculenta* Crantz) is regarded as a food security crop with great potential for industrial applications. It is a staple food crop for millions of people in the world's tropical and subtropical regions and provides a livelihood for many farmers, processors, and traders (Abass et al., 2013; Nassar & Ortiz, 2006). Cassava is grown predominantly by small-scale farmers as a reserve famine crop. It does well in marginally fertile soils and tolerates unpredictable rainfall, and its cultivation does not require much labour and other farm inputs (Droppelmann et al., 2018). The roots of cassava are rich in carbohydrates. In contrast, the leaves provide a good source of dietary protein and minerals for low-income consumers in many parts of sub-Saharan Africa (Achidi & Ajayi, 2015; Montagnac, Davis, & Tanumihard, 2009).

Furthermore, in some parts of sub-Saharan Africa, cassava is the second-most important staple crop after maize, providing up to 15% of the energy intake (Kilic, Moylan, Ilukor, Mtengula, & Phangaphanga-Phiri, 2018). Therefore, the cultivation of cassava can contribute to improved food security and incomes for rural households (Droppelmann et al., 2018).

1.2 Problem statement and justification

There is a rising recognition of the cassava crop's role in improving rural livelihoods (Kambewa, 2010) through the inducement of rural industrial development (Abass et al., 2013). The Global Cassava Development Strategy and Implementation Plan envisioned improving livelihoods of the rural poor through commercialization of the cassava sector (FAO and IFAD., 2001; Haggblade et al., 2012). The strategy recognises that commercialisation may be catalysed by access to more rewarding markets than traditional household consumption (Abass et al., 2013). In this regard, cassava's almost unraveled utility in food and non-food industrial products offers a premium pathway to profitable markets for large-scale sales (Kambewa, 2010). Cassava flour and/or starch are used either directly or as a raw material in the processing of a wide range of products, including food, beer, textile, paper, adhesives, chemicals, glucose, detergents, ethanol, cosmetic powders, pharmaceuticals and insecticides (Chitedze, Monjerezi, Saka, & Steenkamp, 2012; Mweta, 2009; Nuwamanya, Baguma, Emmambux, Taylor, & Patrick, 2010).

Since more agro-enterprises and industries, including bakeries, timber and textile industries, are getting involved in the cassava value chain (Kambewa & Nyembe, 2008), then there is a need for cassava value chain to respond to industrial demand. As such, significant research, and extension on improved varieties, targeting industrial applications, is required as soon as possible to spur improvements in the value chain (Kambewa and Nyembe, 2008). The processing suitability of cassava roots for precise food and non-food applications is a function of their functional properties, which depend on the physicochemical parameters of the cassava roots hence high-yielding cassava genotypes

and varieties with suitable physicochemical parameters and functional properties will be identified for targeted industrial applications to fast-track the commercialization of the cassava crop for industrial utilization. Also, promotion of cassava crop for production requires information on stability and yield performance of cassava genotypes and varieties in a wide range of environments. A successful genotype or variety should produce high levels of physicochemical parameters and functional properties, required for industrial applications, consistently across different environments (Becker & Leon, 1988).

Malawi has diverse agro-ecological zones, in terms of edaphic and climatic factors. Therefore, genotype by environment ($G \times E$) interaction effect is inevitable (Benesi, Labuschagne, Herselman, Mahungu, & Saka, 2008), which complicates the recommendation of genotypes based on yield, physicochemical parameters and functional properties alone (Benesi, Labuschagne, Dixon, & Mahungu, 2004; Ebdon & Gauch, 2002; Kundy, Mkamilo, & Misangu, 2014; Nduwumuremyi, 2017). In this regard, this study reports the physicochemical parameters and functional properties of cassava flour produced from ten improved cassava genotypes and varieties as well as the effect of interaction of genotypes and varieties with environment on such physicochemical parameters and functional properties. In this context, the study evaluates the flours from improved cassava genotypes appropriate for the production of High-Quality Cassava Flour (HQCF) suitable for dietary and industrial applications, based on their physicochemical parameters, functional properties and $G \times E$ interactions. The study results contribute to the efforts of fast-tracking the selection of adaptable and preferred cassava genotypes for industrial applications.

1.3 General and specific objectives

This study was therefore undertaken to investigate the physicochemical parameters and functional properties of cassava flours for industrial applications.

The specific objectives were three-fold:

- (i) To determine physicochemical parameters and functional properties of flours from advanced genotypes and improved cassava varieties.
- (ii) To evaluate the relationship between physicochemical parameters and functional properties of flours from advanced genotypes and improved cassava varieties.
- (iii) To evaluate the effects of genotype by environment (G x E) interaction on functional properties of cassava flours from advanced genotypes and improved varieties.

1.4. Thesis outline

From this chapter, the thesis is outlined as follows: Chapter 2 of this thesis provides literature review of cassava root, flour, high quality cassava flour (HQCF) and starch while Chapter 3 deals with materials and methods used in this study. In Chapter 4, results of the study are presented and discussed. Conclusion and recommendations are provided in Chapter 5.

CHAPTER 2: LITERATURE REVIEW

2.1 Cassava (*Manihot Esculenta* Crantz)

Cassava is a staple food for at least 500 million people in the humid tropics and it is a source of low cost carbohydrates (Köcke, 2019; Kuipe et al., 2007; Ubwa et al., 2015). In Malawi, it is the second most important staple food after maize. Since cassava is very tolerant crop, it survives and grows on lands with low water supply, fertilizer and agrochemical inputs which allows for easier, cost effective cultivation and management (Droppelmann et al., 2018; James & Faleye, 2015; Ma'aruf and Abdul, 2020).

It is a significant food security and industrial crop, contributing as food, feed and industrial biomas in Africa, Asia, South America (Howeler, Lutaladio, & Thomas, 2013; Rogé et al., 2016; Sarma & Kunchai, 1991). On average, a typical mature cassava root is composed of 10-20 % peel, 0.5 -3 % cork layer, and 80-90 % edible portion which has 60-70 % moisture content, 30-35 % carbohydrates, 1-2 % protein, 0.3 % fats, 1-2 % fibre and 1 % ash content (Breuninger et al., 2009; Sivamani et al., 2018).

Cassava roots are an important source of starch (high starch yield about 30 % of fresh root or 80 % of root dry matter) with potential industrial uses, providing economic value to both farmers and industries (Atwijukire et al., 2019; Nuwamanya et al., 2010; Talip et al., 2018). Furthermore, cassava roots with high dry matter content (above 30 %) are desirable for both dietary and industrial applications in Malawi and worldwide (Teye et al., 2011).



Figure 2.1. Cassava plant (a) and roots (b)

2.2. Chemical properties of cassava

2.2.1. pH and total titratable acidity of cassava flour, HQCF and starch

The pH determines the quality of the cassava flour, HQCF and starch, and the recommended range for industrial use is pH of 4-7 (Eriksson, 2013). A study on the native starch stability and G X E interactions by Benesi et al. (2004) found pH of cassava starch ranging from 4.7 to 5.8. However, pH less than 4 for cassava is undesirable in confectionery industries because it indicates appreciable level of fermentation and starch breakdown (Apea-bah et al., 2007; Numfor & Walter, 1995). Total titratable acidity of cassava flour, HQCF and starch increases with decrease in pH during fermentation process (Adegunwa & Sanni, 2011; Moorthy & Mathew, 1998; Udensi & Ukozor, 2013) hence the more the acid produced the lower the value of pH (Ajifolokun, 2018). The production of organic acid during fermentation is responsible for a sour taste in a product (Inyang, 2016). In addition, total titratable acidity of cassava roots is influenced by genotype, fermentation and environment where the crop is grown (Onitilo et al., 2007).

2.2.2. Protein content of cassava

Protein is composed of amino acids and it is very low in cassava compared to other root crops ranging from 1 % to 4 % on dry basis (Balagopalan, 2002; Morgan & Choct, 2016; Samad et al., 2018; Shigaki, 2016; Tonukari, 2004). However, the lower the protein content, the higher the quality of starch which various industries set $\leq 1\%$ as its maximum protein content for industrial use (Benesi et al., 2004). On the other hand, proteins maybe lost due to fermentation processes which may occur during processing of cassava roots into flour, HQCF and cassava starch (Bayata, 2019; Montagnac et al., 2009; Sulistyo et al.,

2017). Therefore, the use of cassava in food industry and confectioneries should be supplemented by other protein sources such as wheat (Saranraj et al., 2019; Uchechukwu-Agua et al., 2015). Furthermore, protein influences functional properties of cassava such as swelling power and water absorption capacity (Tharise et al., 2014). However, it is not significantly different between cassava varieties hence it is not necessarily affected by the genotype (Chukwu & Abdullahi, 2015).

2.2.3. Mineral elements in cassava

Cassava is not particularly rich in all mineral nutrients, hence, diet based on cassava alone may not fulfil adequate mineral nutritional requirement in humans (Emurotu, Salehdeen, & Ayeni, 2012; Kalagbor, Ihesinachi, Dighi, James, 2015; Kanagarasu et al., 2014;). The contents of zinc, iron, calcium, magnesium, sodium, potassium and sulphur of cassava genotypes on dry basis ranged from 5.6 to 8.3 mg/kg, 8.41 to 11.73 mg/kg, 530 to 610 mg/kg, 713 to 1324 mg/kg, 46.9 to 63.7 mg/kg, 7930 to 9320 mg/kg, and 243 to 319 mg/kg, respectively (Ayetigbo et al., 2018; Edori, Ajuru, & Harcourt, 2015; Wilberforce, 2016). Furthermore, the total phosphorus content in cassava flour is as low as 70-120 mg/kg of the root while other mineral elements are usually in negligible amounts (Ayetigbo et al., 2018). The phosphate content in cassava starches is very low ranging from 23.5 nmol/mg to 25.3 nmol/mg, however, in other tuber crops especially potatoes, the phosphate content is relatively high, and influences a number of physicochemical and functional properties (Moorthy, 2004).

2.2.4. Cyanide in cassava

Cassava roots are generally categorised as bitter or sweet, depending upon their cyanide content in the tissue (Chinwendu, Ekaiko, Emmanuel, & Chukwu, 2015; Uchechukwu-Agua et al., 2015). Therefore, low-HCN or sweet cassava are those containing less than 50 mg HCN-equivalent/kg (fresh weight), the medium type (50-100 mg HCN-equivalent/kg) while the high-HCN or bitter cassava are those with more than 100 mg HCN-equivalent per kg in their roots (Breuninger et al., 2009; Richardson, 2013).

Cassava contains two cyanogenic glucosides namely; linamarin [2-(β -D-glucopyranosyloxy)isobutyronitrile] (A) and lotaustralin [methyl-linamarin or 2-(β -D-glucopyranosyloxy) methylbutyronitrile] (B) (Figure 2.2) which are synthesized from valine and isoleucine respectively.

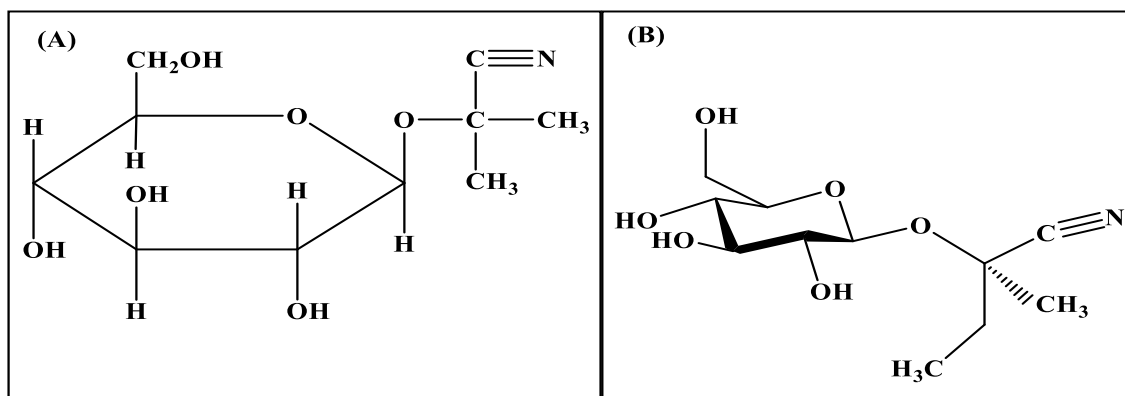


Figure 2.2: Molecular structure of linamarin (A) and lotaustralin (B), the major cyanogenic glucosides found in cassava.

Linamarin [2-(β -D-glucopyranosyloxy)isobutyronitrile] and lotaustralin [methyl-linamarin or 2-(β -D-glucopyranosyloxy) methylbutyronitrile] are both catalytically hydrolysed by linamarase (Figure 2.3) to release toxic hydrogen cyanide (HCN) when the

cassava root is crushed (Piero et al., 2015; Puonti-kaerlas, 1998). However, linamarin is more abundant than lotaustralin and accounts for over 90% of the total cyanogenic glycosides (Burns et al., 2012; Shigaki, 2016).

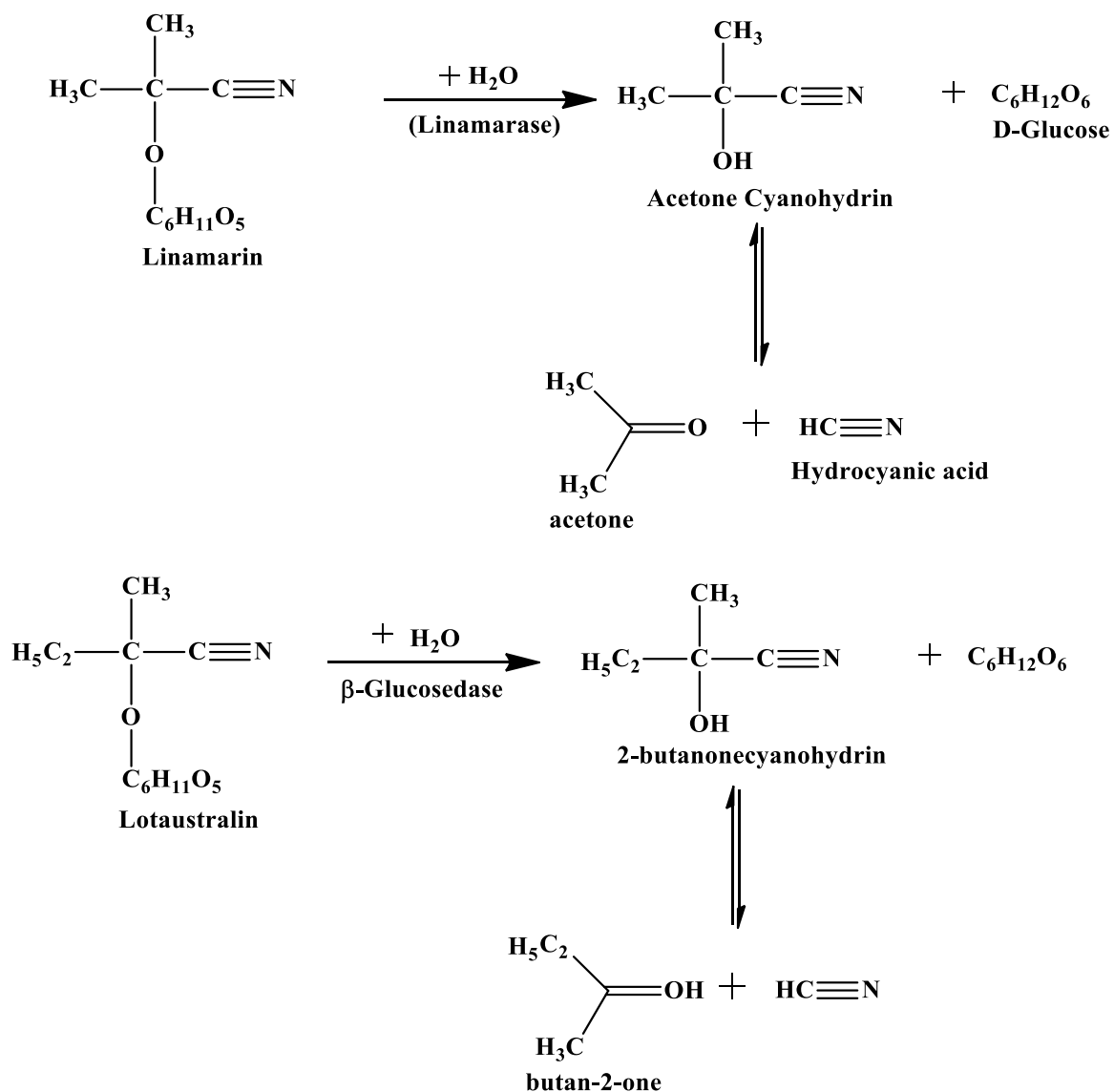


Figure 2.3: Enzymatic hydrolysis of linamarin and lotaustralin.

Since cyanide is very toxic (Aryee et al., 2006), Food and Agriculture Organization/World Health Organization (FAO/WHO) set 10 mg/kg cyanide as its safe level in cassava products (Ezeh, Okeke, & Ou, 2018; Iwe et al., 2017). Therefore, cassava genotypes with low cyanide are desirable for various applications in food industry (Sawyerr, Odipe, Olalekan, & Ogungbemi, 2018; Wheatley et al., 2003). Furthermore, high exposure to cyanide is lethal especially at a dose of 0.5-3.5 mg HCN/kg body weight (Bradbury, 1990).

It also causes nausea, vomiting, diarrhoea, dizziness, and weakness (Burns et al., 2012). However, about 100 mg of cyanide can be detoxified by the body per 24 hours by converting it to thiocyanate (Haque & Bradbury, 2002). On the other hand, Cyanide in cassava roots can also be reduced by processing techniques such as chipping, soaking, fermentation, cooking, steaming, frying, and roasting (Guédé, 2013). These methods permit enzyme linamarase to interact with the cyanogenic compounds to release HCN which either dissolves in the water or escape into the air (Piero et al., 2015) thereby reducing the cyanide content and improve palatability of cassava products (Wheatley et al., 2003). However, it is impossible to remove all cyanide from cassava roots through conventional processing hence genetic engineering is applied (Johnson, 2013). Furthermore, cyanide content in the cassava roots is influenced by genotypes, environmental factors and fertilizer application (Shittu et al., 2016). Shigaki (2016) observed that during drought, cyanide content increases in cassava roots as a physiological response to the stress hence elevating its levels. In addition, depletion of potassium content in the soil increases the cyanide content in the root (Tharise et al., 2014).

2.2.5. Total carbohydrates, starch, amylose and amylopectin contents of cassava

2.2.5.1. Total carbohydrates content of cassava

Carbohydrates is a main source of energy stored in the cassava root in the form of starch and ranges from 32 % to 35 % of the mass of the fresh roots and 80 % to 90 % of the mass of dried roots (Haritha & Jayadev, 2017; Samad et al., 2018). As such, carbohydrates influences the chemical and functional properties of cassava raw materials for various industrial uses because, as starch, it contains 80 % amylopectin and 20 % amylose (Bechoff et al., 2016; Adebowale, & Sanni, 2008). On the other hand, carbohydrates is higher in cassava than other roots i.e. potatoes, therefore, it has high nutritional value, desirable for industrial use than other roots (Rajapaksha et al., 2017).

2.2.5.2. Cassava starch

Cassava starch is a tasteless, odourless white polysaccharide of glucose which is made of two types of α -D-glucan chains (Figure 2.4) namely; amylose (amorphous) and amylopectin (crystalline). These chains of α -(1-4)-link D-glucose residues are interlinked with α -(1-6)-glucosidic linkages (glycosidic bonds: glucose linked to one another through the C1 oxygen), creating branches in the polymers (Ashogbon & Akintayo, 2014; Egharevba, 2019). Such polymers include amylose, which is linear with longer chains, and amylopectin, which is highly branched with short chains and significantly higher weight which define the size, structure and its particular industrial use (Chisenga et al., 2019; Tyler, Lindeboom, & Chang, 2004). Therefore, such starch polymers play a crucial compositional role and influence the functional properties such as solubility, swelling

power and water absorption which determine potential uses of cassava in food industries (Sangseethong et al., 2009) that are desirable in bulking, baking applications and consistency of products (Moorthy, 2002).

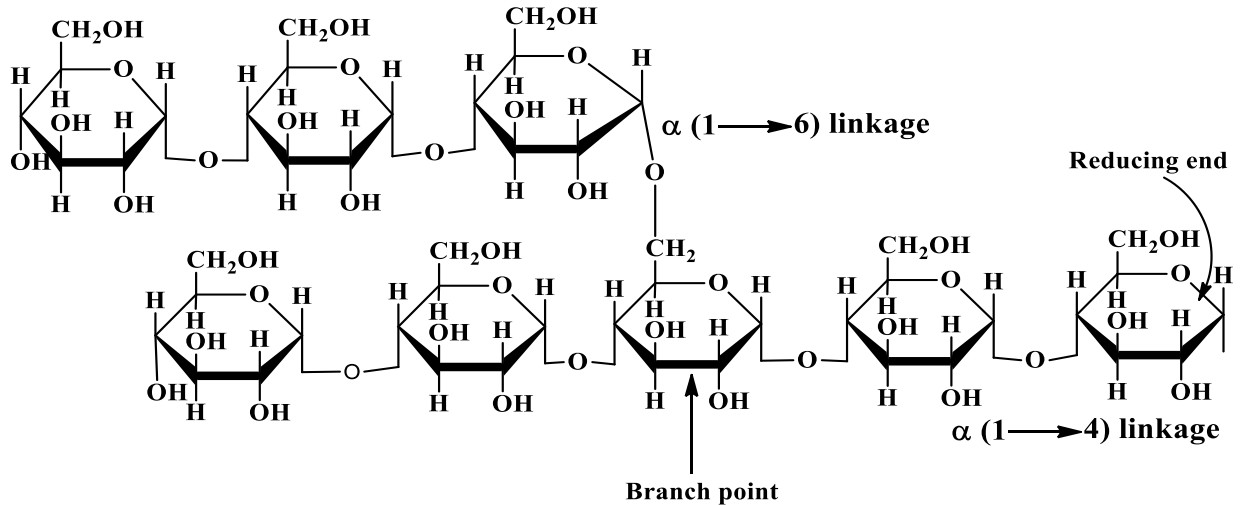


Figure 2.4: Starch structure.

On the other hand, cassava starch ranges from 17 % to 35 % on fresh weight basis and 80 % to 90 % on dry weight basis, however, it's affected by region, climate, soil and cultivation (Fakir, Jannat, Mostafa, & Seal, 2012; Salvador et al., 2014). Cassava roots contain maximum starch content at the end of rainy season (Salvador et al., 2014). The less mature cassava roots have less starch content and high moisture content whilst overly mature roots have lower recovery starch content and woody texture which make starch processing difficult (Rodrigues et al., 2018). A comparative study by Abera and Rakshit (2003) on cassava starch at Siam Modified starch Co. Ltd , Thailand found starch yield ranging from 24 % to 28 % on fresh weight basis. Furthermore, its amylose/amylopectin

ratio influences texture of the product, impact starch compactness, starch granule size; and gives cassava a functioning quality in making confectioneries (Uchechukwu-Agua et al., 2015).

There are variations in amylose, amylopectin and starch contents in cassava genotypes cultivated in different geographical locations (Rodrigues et al., 2018). Therefore, starch, amylose and amylopectin contents in cassava flour as well as starch yield from cassava fresh roots (after extraction) are influenced by genotype and environmental (G X E) factors (Rodrigues et al., 2018). The yield and quality of starch are significant factors to be considered when it comes to industrial uses of starch. However, the quantity and quality of cassava starch are influenced by genotype, environmental factors and farm practices (Santisopasri, Kurotjanawong, & Chotineeranat, 2001; Shittu et al., 2016), affecting both yield and the content of starch in the cassava roots, granule size distribution, swelling power, paste viscosity, pasting temperature, gelatinization temperature, amylose content and root cyanide (Piero et al., 2015). Mejía-Agüero et al. (2012) examined twenty-five cassava genotypes planted and harvested simultaneously in single plantation and found significant differences in starch content influenced by genotype and environmental factors.

In addition, starch quality is affected by root cyanide content (Oyewole et al., 2013). Therefore, adequate water supply throughout the growth period of cassava root and application of potassium fertilizer is beneficial to the quality of cassava flour, HQCF and starch for it reduces cyanide in the roots and stimulates dry matter and starch content (Vanovic et al., 2015). The industrial application and trading of cassava starch, like

commercial starch from other sources, is based on its quality (Shittu et al., 2016). Breuninger et al. (2009) stated that the minimum content of cassava starch is 85 % for industrial uses. In addition, Table 2.1 shows codex standards and grades (by Codex Alimentarius Commission, CAC) of cassava starch for various industrial uses.

Table 2.1: Codex standards of cassava starch (Shittu et al., 2016)

Qualifications	Grade		
	1	2	3
Moisture Content (% maximum)	13	14	14
Starch (% minimum by polarimetric method)	97.5	96	94
Ash (% maximum)	0.15	0.3	0.5
Acid in insoluble ash (% maximum)	0.05	0.10	0.15
Protein (% maximum)	0.3	0.3	0.3
Fiber (cm ³ in 50 g starch before drying)	0.2	0.5	1.0
pH	4.5 to 7	3.7 to 7	3.0 to 7
Residue on 150 μm sieve (% maximum)	1	3	5

2.2.5.3. Amylose and amylopectin of cassava

Amylose is a straight-chain polymer of α -D-glucose unit which is 20-30 % starch, similar to most other starches (Bashir & Aggarwal, 2019; Moorthy, 2004). Unlike corn (0-70 % amylose content) and rice (0-40 % amylose content), no significant variation of amylose content has been found in cassava starch (Van Der Maarel et al., 2002). Furthermore, cassava amylose has a higher molecular weight than other starches (Breuninger et al., 2009). Its chemical bonding is $\alpha - (1 \rightarrow 4)$ glucosidic linkage (Figure 2.5) which link several hundred or even thousands of glucose molecules to form amylose chain (840-22,000 units) of $\alpha - D -$ glucopyranosyl residues with molecular weight ranging from

136,000 to 3.5×10^6 g (Kanagarasu et al., 2014; Sweedman et al., 2013). Since the number of anhydroglucose units depends on the genotype of cassava root, there exist variations in amylose content among varieties (Eliasson, 2004). However, the age of the crop and environmental factors do not necessarily affect amylose content (Moorthy, 2004). Amylose is soluble in water and part of it exist as soluble amylose in the amorphous regions of the starch granules, however, it does not swell in hot water rather, just mingles with water and turn a substance into a thickener (Salvador et al., 2014). Moreover, its contents influence clarity of starch paste i.e. high amylose content result in more opaque starch paste (Mweta, 2009). In addition, the low amylose, low lipid and low protein contents, combined with high molecular weight of amylose, make cassava starch a unique and excellent native starch for various industrial uses (Breuninger et al., 2009).

Amylose content is used to categorized starches into waxy (0-15 %), normal/regular (20-35 %) and high-amylose (40 %) starches (Chisenga et al., 2019). Hence, low amylose increases crystallinity of starch, corresponding to a reduced amorphous band whilst high-amylose starch retrogrades relatively easily during industrial applications (Sangseethong et al., 2009). Furthermore, its short chains adds opacity to starch suspensions and food products, and since it is anhydrous, amylose forms excellent firms (very strong, colourless, odorless and tasteless) which are important characteristics for industrial applications (Alcázar-Alay & Meireles, 2015). Furthermore, high amylose content provide stability, shape and integrity to confectionery products such as candy pieces and therefore, cassava with high amylose content is the best raw material in confectionery industry (Onitilo et al., 2007).

On the other hand, Defloor et al. (1998) studied four cassava genotypes from International Institute for Tropical Agriculture (IITA) Ibadan, Nigeria which were harvested at 6,9,12 and 18 months after planting and found that amylose content ranged from 18 % to 24 % with no significant genotypic differences. Franco et al. (2002) also found average amylose content of 18 % for cassava roots of the “Vassourinha” variety. Nonetheless, Moorthy (2002) observed that total amylose content may range from 14 % to 24 %.

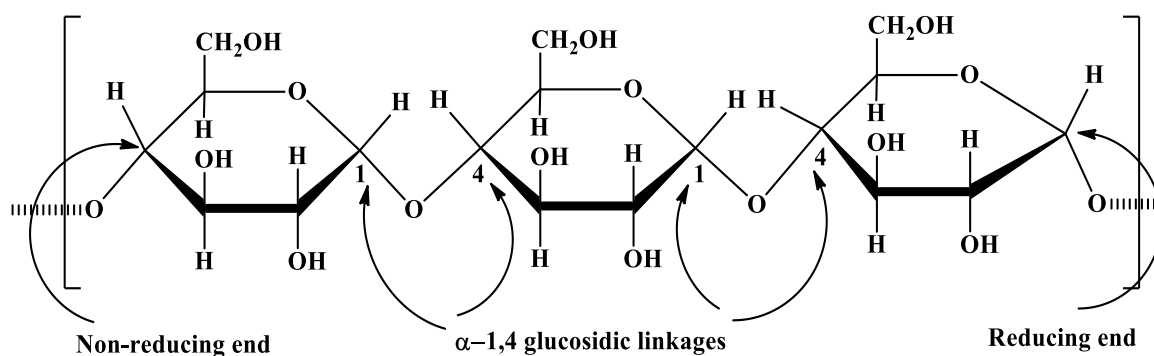


Figure 2.5: Amylose chains showing the α -1, 4 glycosidic linkages.

Amylopectin is a branched-chain polymer of α -D-glucose unit comprising 70-80 % of the starch content (Salvador et al., 2014). The cluster model shows amylopectin molecules as large flattened disks (Eliasson, 2004) with a chemical bond of α – (1 $\rightarrow$ 4) glycosidic linkage (short) and α – (1 $\rightarrow$ 6) glycosidic linkage at branch points (Figure 2.6). It's highly branched with 4 to 5 % of α – (1 $\rightarrow$ 6) glycosidic linkage, containing 2,000,000 glucose units and therefore, it's one of the largest molecule in nature (Egharevba, 2019).

Furthermore, its molecular structure is determined by degree of debranching, molecular weight, and chain length which is influenced by activity of starch branching enzymes of different polymorphic forms which influence swelling power of cassava flour, HQCF and starch thereby enlarging cassava's industrial applications (Ayetigbo et al., 2018).

Amylopectin stains reddish-brown with iodine and α & β amylase hydrolyze α – (1 $\rightarrow$ 4) glycosidic linkage but cannot hydrolyze α – (1 $\rightarrow$ 6) glycosidic linkage at its branch point (Kanagarasu et al., 2014). It is less soluble in water due to hydrogen bonding and crystallinity of its molecules, however, it is soluble in hot water with swelling (Alcázar-Alay & Meireles, 2015) which makes it form a starch gel or paste thereby making cassava flour, HQCF and starch desirable raw materials in food industry (Salvador et al., 2014).

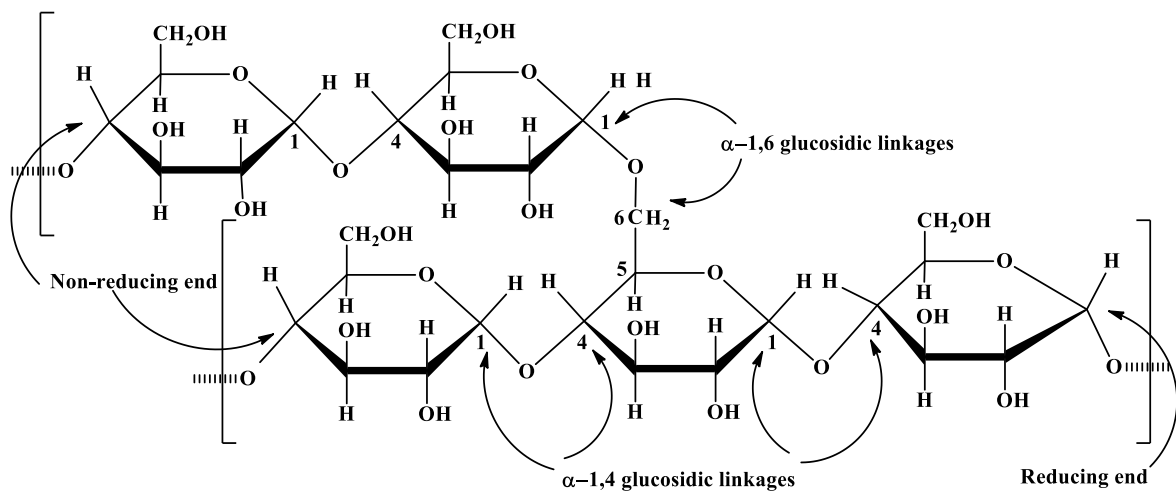


Figure 2.6: Amylopectin chains showing the α -1, 4 and α -1, 6 glycosidic linkages.

2.3. Physicochemical Properties of cassava

2.3.1. Moisture and dry matter content of cassava

Moisture content of cassava, one of the most important parameters, is used to select best cassava raw materials for confenctionery industries. Since cassava root has high moisture content (above 60 %) which makes it highly perishable with a short post harvest life of less than 72 h (Taylor et.al., 2011; Mehoudenou et al., 2016), is therefore, processed into industrial raw materials such as flour, high quality cassava flour (HQCF), starch, chips and pellets to extend the shelf life thereby promoting its industrial use (Chipeta & Bokosi, 2013). These processed raw materials are then packaged and stored properly to avoid moisture absorption from external sources, thereby ensuring quality, safety, and storage stability of such products prior to industrial utilization (Moorthy, 2004).

Furthermore, a safe moisture limit for starch storage and industrial uses is 13 % (Chukwu & Abdullahi, 2015). However, Moorthy (2002) observed that moisture content for dry cassava starch may range from 6 % to 16 % and usually affected by climatic factors. Benesi et al. (2004) working with native starch at Chitedze research station found moisture content in the range of 12-13 % which were within industrial range of 14-15 %. The dry matter content of cassava positively correlates with starch and ranges from 89.04 % to 96.41 % after starch extraction (Benesi et al., 2004). Cassava genotypes with high dry matter (above 90 %) are highly favoured for industrial use because they provide enough raw materials for product development in various industries (Bechoff et al., 2016). For example, cassava with appreciable dry matter content is desirable in ethanol production (Nuwamanya et al., 2009).

However, dry matter varies between genotypes and it is affected by environment where the crop is grown (Ayetigbo et al., 2018). Therefore, such genotype x environment (G X E) interaction is important for breeders and agronomists to choose a suitable and adaptable cassava genotypes for targeted industries (Benesi et al., 2004) hence there is need for stability analysis to deal with the effect of G X E interactions which will enable cassava breeders to select most stable genotypes and varieties with high yield performance for mass production, targeting specific industrial applications.

2.3.2. Ash content of cassava flour

The ash content indicates the mineral richness and non-volatile contents in cassava (Dewage et al., 2017). It represents all inorganic minerals in cassava flour or starch and it is lowest in cassava starch than cassava flour (Rajapaksha & Wickramasinghe, 2017). Furthermore, the recommended maximum content in cassava starch for industrial use is 0.5 % (Benesi et al., 2004). Moreover, Benesi et al. (2004) found that ash content ranges from 0.09 % to 0.25 %. Cassava processing significantly reduce ash content of the roots, with similar trend for the minerals (Omowonuola et al., 2017). Therefore, severe processing techniques such as those involving application of high temperature and chemicals and excessive fermentation, washing and milling treatments could significantly reduce ash content in cassava-dominated products (Omowonuola et al., 2017).

2.3.3. Colour of cassava flour, HQCF and starch

Colour is an important organoleptic quality attribute that determines the visual appearance and eye appeal of finished products in the confectionery industries (Shittu et al., 2016). As such, the reduction in whiteness of flour colour affects the quality and its industrial application (Oladunmoye et al, 2014). Therefore, the measurement of colour has gained much attention from food scientists and industry (Yeboah et al., 2010). Furthermore, it is affected by processing i.e. properly processed cassava starch is very white, however, when the roots are crushed without removing the rind, the resultant starch's colour is very dull and undesirable in food applications (Yeboah et al., 2010). The colour of cassava flour, HQCF and starch is determined by colour measurement systems which measure broad range of food products (Dziedzoave et al., 1999), but may also be assessed sensory-wise. The Hunter or the Commission Internationale de l'Eclairage (CIE)-Lab colour indices are often used. This system consists of lightness (L^*), greenness-redness (a^*) and blueness-yellowness (b^*) colour spaces. Furthermore, cassava flour or starch with $60 < L < 110$ (whitish) and $12 < b < 15$ (low yellowish tint) are desirable for industrial uses (Shittu et al., 2016), moreover, high L-value shows bright colour.

Delta E levels (ΔE) of cassava flour are the differences between the displayed color of cassava flour and the original colour of the standard. Lower ΔE levels indicate greater accuracy, while high ΔE levels indicate a significant mismatch. Therefore, ΔE levels of less than 3.5 for cassava flour, HQCF and starch are considered to be low and desirable in confectionery industries (Yeboah et al., 2010).

2.3.4. Bulk density of cassava flour, HQCF and starch

Bulk density is an important element in the ability of starchy foods to absorb water. It is divided into loose bulk density (LBD) and packed bulk density (PBD). The PBD of cassava starch ranges from 0.695 g/ mL to 0.793 g/mL (Ayetigbo et al., 2018; Chisenga et al., 2019) and there is a strong negative relationship between PBD and starch content in cassava flour, HQCF and starch. Therefore, starches of high PBD have a low void volume, high surface area to volume ratio, hence can be arranged tightly, making disintegration of intergranular integrity more difficult there by influencing the swelling power of cassava (Defloor et al., 1998). As such, there exist a significant negative correlation between PBD and water absorption of cassava flour, HQCF and starches which in turn influence their industrial use (Ayetigbo et al., 2018; Chisenga et al., 2019). On the other hand, Loose bulk density (LBD) of cassava flour is the ratio of mass of loosely packed fine cassava flour particles without tamping to the total volume they occupy. The LBD of cassava flour ranges from 0.51 g/mL to 0.54 g/mL (Ayetigbo et al., 2018).

2.4. Functional Properties of cassava

2.4.1. Swelling power and water solubility of cassava

The swelling power and solubility of cassava provide evidence of non-covalent bonding between starch molecules (Nwokocha et al., 2009). They are both dependent on amylose-amylopectin ratio, chain length and molecular weight distribution, degree/length of branching and conformation (Moorthy, 2004). However, swelling power of cassava is attributed to its amylopectin and is negatively correlated with amylose content (Abera & Rakshit, 2003). Furthermore, when starch is mixed with water and heated, temperature

increases and the granules swell until they are ruptured and the crystalline structure is destroyed, breaking the hydrogen bonds that hold the molecules together within the granules and this is called gelatinization (Alcázar-Alay & Meireles, 2015; Altuna et al., 2018). Gelatinization leads to irreversible changes in properties such as water uptake, solubility and viscosity development (Alcázar-Alay & Meireles, 2015; Altuna et al., 2018; Lorenz et al., 1995; Perez, 1996). The gelatinized starch may partially recrystallize through a process called retrogradation which changes properties of the products resulting in reduced quality, short shelf life, and poor stability thereby being undesirable in food industry (Altuna et al., 2018).

Pasting properties are measured by applying a heating-cooling cycle to a suspension of starch in water with constant stirring while viscosity is measured as the resistance to mixing which rise due to starch granule swelling caused by the weakening of the internal structure of the granules (Altuna et al., 2018). Swelling power impacts functionality of cassava starch as thickener in both confectionery and non-food industries whilst solubility is crucial when starch is used as degradable excipients in drug delivery systems in the pharmaceutical industries (Ayetigbo et al., 2018). However, swelling power is influenced by a strong bonded micellar network and amylopectin molecular structure which decreases with the increase in crystallite formation by the association between long amylopectin chains (Mweta et al., 2015). Swelling power increases with increase in the associative forces. The short glycosidic linkages at branch points prevents intermolecular associations of carbohydrate polymers which allow water molecules to penetrate easily into the intermolecular spaces which enhances solubility (Moorthy, 2002).

Swelling power is negatively correlated with amylose content, as well as presence of stronger intermolecular bonds (Onitilo et al., 2007). Therefore, it increases with high protein content and low amylose content because high protein content is associated with high water absorption and that amylose act as a dilutor and swelling inhibitor, therefore, the lower it is, the higher is the swelling power, essential for texture of food products (Chinma et al., 2011). It is also affected by temperature i.e. suspension of cassava genotypes at 84 °C, swells about two to three times its initial volume at 64 °C and this enables cassava flour, HQCF or starch to be well suited as thickeners in food industry (Ayetigbo et al., 2018). Cassava has medium swelling power compared to tuber crops, and therefore cassava has higher solubility than tuber crops due to such high swelling it undergoes during gelatinization (Moorthy, 2004).

Furthermore, swelling power and solubility shows interactions between starch chains within the amorphous and crystalline regions which influence application of cassava flour, HQCF or starch in food industry, textile processing and confectioneries (Trinh, 2019). They both vary between varieties i.e. cassava genotypes with lower dry matter content have lower swelling power hence the application of cassava flour, HQCF or starch should be done by taking into account of genotypic effects (Ayetigbo et al., 2018).

2.4.2 Water-binding and oil absorption capacities of cassava

Water-binding capacity and oil absorption capacity entails ability of components of cassava to bind water and oil at hydrophilic and hydrophobic sites, respectively (Ayetigbo et al., 2018). They are influenced by proteins and carbohydrates which have polar or charged side chains known as hydrophilic constituents (Chinma et al., 2011). Therefore, the higher the protein and carbohydrates contents in cassava flour or starch, the higher the water and oil absorption capacities, hence, an important trait in food industry for development of bakery products (Chinma et al., 2011).

Furthermore, oil absorption capacity is useful in structure interaction in food for flavour retention, palatability improvement and shelf life extension in bakery products (Sirivongpaisal, 2008). It ensures stable and uniform pastes and emulsions which are important properties for confectioneries and use in paints, textile sizing, soluble adhesives or gums and release of active ingredients of drugs in vivo (Rusike et al., 2010). Therefore, it makes cassava flour, HQCF or starch desirable in such industries (Nuwamanya et al., 2011). However, both water absorption and oil absorption capacities depend on genotypes and their high dry matter and starch contents (Tharise et al., 2014). Cassava starch has fairly high oil absorption capacity compared to other starch sources (Ayetigbo et al., 2018). Therefore, the ability of the dry matter, starch, and other components to bind with water or oil films makes cassava desirable for various industrial uses (Nuwamanya et al., 2011).

2.5. Processing of cassava for industrial applications

2.5.1. Processing cassava roots into flour

Cassava fresh roots undergo post-harvest physiological deterioration (PPD) which affect the overall sensory characteristics and acceptability because it reduces starch content resulting in poor functional properties and therefore, cassava roots should be processed with 24 h soon after harvest to produce low-cost raw materials such as cassava flour, HQCF and starch for various industrial uses (Balagopalan, 2002; Bechoff et al., 2016).

The process involves removal of peels, washing and after that, the roots are grated, chipped, or sliced and dried to produce cassava flour (Wheatley et al., 2003). These processes improve shelf life, stability and reduces the cynide content in cassava (by 99 %) to safe levels (less than 10 mg/kg cyanide, FAO/WHO) for various industrial applications (Chipeta & Bokosi, 2013). The dried product is milled (hammer or disc attrition mill) and screened through a 0.25 mm metal mesh to produce a finely milled product containing minimum fibre levels desirable for industrial uses (Graffham, 2000). Cassava flour for industrial uses should adhere to codex standards (Table 2.2) and be free from abnormal flavours, odors and live insects and filth (Ospina et al., 2017).

Table 2.2: Codex standards for cassava flour for industrial use (Shittu et al., 2016)

Parameter	Standard
Moisture (max)	13%
Fiber (max)	2%
Ash (max)	3%
Fine Flour	90% of CF passes through 0.6 mm sieve
Course flour	90% of CF passes through 1.2 mm sieve
Hydrocyanic acid (db, max)	10mg/kg
Sulfated Ash	0.5%
Starch	65-70%
Titrateable Acidity	1%

2.5.2. High quality Cassava Flour (HQCF) processing for industrial applications

The processing of cassava roots into high quality cassava flour (HQCF) involves peeling, washing, grating, pressing, disintegration, sifting, drying, milling, screening, packaging and storage (Weigand, 2018). ‘High quality’ refers to the way of drying and processing cassava without fermentation and contamination (Wheatley et al., 2003). HQCF can be dried by sun drying or artificial drying hence, in sun drying, the process requires equipment for grating or squeezing cassava roots to remove moisture followed by drying the pulp in the sun usually done by processor groups engaged in seasonal small-scale processing, as evident in Malawi whilst in artificial drying (or “flash” drying), more advanced technology is used, passing hot air through the cassava pulp to dry rapidly (Forsythe et al., 2016).

A flash dryer dries one to three metric tons of HQCF per day and are operated by large-scale factories or small and medium enterprises (SMEs), using their own cassava roots or roots purchased from farmers as raw material (Forsythe et al., 2016; Weigand, 2018). Therefore, HQCF produced is white or creamy, unfemented and gluten free (Chukwu & Abdullahi, 2015). It is used as partial replacement of wheat flour in baking, pasta and confectioneries (Kaitano & Martin, 2009). In Malawi, the availability of such HQCF for industrial uses has been facilitated by Cassava: adding value for Africa (C:AVA), a non governmental organisation based at Chancellor College, University of Malawi which is specialized at establishing value chains for HQCF (Weigand, 2018).

2.5.3. Cassava starch processing for industrial applications

Processing of cassava starch from cassava roots involves peeling, washing, grating, sieving, sedimentation, decantation and starch recovery (Taylor et al., 2011). Starch obtained is pure white in colour if the the process is carried out properly. The starch is extracted under running water and separated from the fibre and other root components by screening, and then solid starch is separated from the starch/water slurry by sedimentation or centrifugation (Olomo & Ajibola, 2003). The resultant starch is dried to 12-14% final moisture content through oven drying at 35-40 °C for 12 h and sun-drying at a small-scale, and flash-drying in large-scale plants (Chisenga et al., 2019). This is sweet starch because it is obtained after extraction and separated from other constituents whereas sour starch is obtained by fermentation following extraction (Taiwo, 2007).

Both the sweet and sour starch have various industrial applications i.e. sweet starch is used as an adhesive in the textile, paper, and battery industries, whereas sour starch is used in food industry (Taiwo, 2007). Since the lipid content in the starch is very little ($<0.1\%$), the starch and its derivatives have a non-cereal taste which is desirable in many food products (Moorthy, 2004). Cassava roots should be processed within 24 h of harvest to overcome post-harvest physiological deterioration (PPD) and maximize starch yield since any delays to process the root may lower starch yield and increase micro-flora content (Breuninger et al., 2009). Since cassava roots contain very low quantities of proteins, fats, etc, its starch is easily extractable (Moorthy, 2004).

2.6. Industrial uses of Cassava flour and starch

Cassava has various industrial applications in confectioneries, bakeries and breweries, timber, textile, packaging, pharmaceuticals, batteries, and paper making industries due to its high amount of starch ranging from 25 % to 35% (Chitedze et al., 2012; Yajima, 2010). It is used to make bread, cakes, pastries, beverages; and in production of plywood, beverages, textiles, bio-fuels and animal feed (Oduro-Yeboah et al., 2010; Pudjihastuti et al., 2018). Such applications are possible because cassava starch act as a modifier of texture, viscosity, adhesion, moisture retention, gel formation and films (Silva et al., 2011). Furthermore, industrial use of cassava is dependent on its chemical, physicochemical and functional properties such as water-binding capacity, swelling power and solubility which have a bearing on the quality of the carbohydrates, and influence the viscosity and gelling, moisture, viscosity, texture, consistency, mouth-feel, and shelf-life of the finished products (Chinma et al., 2011; Ojo et al., 2016).

Due to cassava's low amylose content and excellent starch paste, it is used in pie fillings and makes the products appear appealing (Moorthy, 2004). The Malawian industries that use cassava flour and starch as raw material include David Whitehead and Sons, Universal Industries, RAIPLY, Mapanga Furniture, Rab Processors, Bakemans, Export-Rab Processors, Export-Transglobe and export-Mohammed (Chipeta & Bokosi, 2013).

2.6.1 Application of cassava in food processing industry

In food industry, HQCF and cassava starch are used as a modifier of texture, viscosity, adhesion, moisture retention, gel formation (gelling agent) and films, stabilizer, bulking agent, colloidal and thickeners (Alcázar-Alay & Meireles, 2015; BeMiller & Roy, 2009). Furthermore, they are used as thickener (for soups, sauces, and baby food) due to their paste and physical properties of texture, stability and low flavour contribution; as filler due to its viscosity after gelatinization; as a binder (for sausages and other processed meat) and stabilizer due to its high water-binding capacity (Shigaki, 2016). Therefore, such uses require cassava flour, HQCF or starch to be of high quality i.e. finely milled (0.25 mm), white flour, low fibre, no odour or stains, and not fermented (Graffham, 2000). Furthermore, food industry require that cassava starch or flour should be of high clarity which depends on its associative bonds between starch molecules in the granules which are weaker compared to cereal starch hence better clarity (Moorthy, 2004). Therefore, when these hydrogen bonds are broken in the amorphous portions of starch (gelatinization at 52 °C), crystallinity is decreased since amylopectin (crystalline) gets altered resulting into solubilization, starch granule swelling which increase the randomness in the starch granule (entropy) and loss of birefringence (Bashir & Aggarwal, 2019). The starch granule

is finally ruptured and the amylose is released resulting in the formation of starch paste which increase the viscosity of the product (Bashir & Aggarwal, 2019). However, starches of higher amylose content are undesirable in production of noodles due to its retrogradation which results to more difficult rehydration (Breuninger et al., 2009).

The bakery industries use cassava flour, HQCF and starch, supplemented by wheat flour to achieve desirable products (Chilungo, 2013; Tonukari, 2004). For example, 10-20% of cassava flour or starch is used with wheat flour to make bread and cookies which do not significantly change in terms of taste and appearance (Iwe et al., 2017). However, the quality of bread made is affected by cassava variety used (Chandra, Singh & Kumari, 2015). Cassava flour is desirable for flavour retention, improvement in palatability and shelf life extension in bakery products due to its oil absorption capacity (Egharevba, 2019). Furthermore, cassava starch is desirable in confectionery, pastry and biscuits industry as molding powder for sweets, candy, gums, pastes as well as stabilizer for candy's shape and integrity due to its high amylose contents (Onitilo et al., 2007). It's also used to increase volume and crispness of biscuits due to its low fat content and longer storage life (Taiwo, 2007). Moreover, biscuits made from 40-50% substitution of cassava for wheat flour have better texture and colour than those with 100% wheat flour (Lin et al., 2009; Wheatley et al., 2003). In addition, pasta made from cassava starch is used to prepare noodles (Silva et al., 2011).

2.6.2 Application of cassava in Chemical industry

Chemical industry uses cassava in production of starch and glucose syrup; sweetener, ethanol and industrial alcohol; explosives and adhesives, glues, paints and cements; soaps, detergents, bleaches and insecticides, oil drilling materials, biodegradable plastics and polyesters; combustibles, ethanol and oils; cosmetics; and water treatment agents (Balagopalan, 2002). Glucose syrup is produced from cassava with high starch yield and contents which influence viscosity properties for its production (Nuwamanya et al., 2010). The process involves direct hydrolysis of starch to produce glucose syrups and maltose syrups by using α -amylase plus glucoamylase or β -amylase, respectively which have bland taste, clean flavour and high purity (Breuninger et al., 2009). However, brewery industries use cassava starch to make sweeteners due to its high starch contents (influenced by the genotype), and produced by acid-catalysed hydrolysis of starch to improve winey (BeMiller & Roy, 2009). It is also used to manufacture adhesives, dextrans, pastes and paints (Ayetigbo et al., 2018) and it's considered the main raw material in glue and adhesive industries. In the cosmetics industries, cassava starch is the best raw material for powder production, improving recovery and shelf life of the detergents. On the other hand, in the rubber and foam industries, starch is employed for getting better foaming and color (Tonukari, 2004).

Cassava is also used in production of pharmaceuticals, vit.C. vit.B12 and antibiotics in pharmaceutical industry (Henry et al., 1998). Its starch is used as an excipient, a type of bonding agent to active drugs, and filler for making pills and tablets thereby contributing to their solid content (Saranraj et al., 2019). It's also desirable due to its amylose which

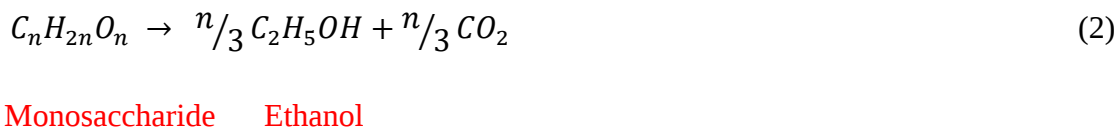
forms an inclusion complex with many ingredients such as flavouring ingredients (Silva et al., 2011). Furthermore, it acts as an encapsulant and increases the shelf life of pharmaceutical products (Silva et al., 2011). In addition, cassava is an effective ingredient for drugs used to treat prostate cancer (Shigaki, 2016).

Cassava starch is also used as an additive in cement to improve the setting time, and to improve the viscosity of drilling muds in oil wells due to its excellent viscosity properties (Alcázar-Alay & Meireles, 2015; Nuwamanya et al., 2010). It is also used to seal the walls of bore holes and prevent fluid loss (Tonukari, 2004).

2.6.3 Application of cassava in energy industry

Cassava is exploited by energy industry for bio-fuels production due to its high starch yield hence genotypes with large biomass are desirable to produce first generation bio-fuel (Pattiya, 2011). It is easily turned into ethanol by fermentation process due to its appreciable amounts of starch and total carbohydrates (Nuwamanya et al., 2009). Therefore, breeding cassava genotypes with high dry matter, total carbohydrates and starch would facilitate consistent production of bio-ethanol (Okudoh et al., 2014) hence there is need to identify most stable cassava genotypes and varieties with high yield performance through stability analysis. Biobutanol and bioethanol are produced by fermentation of sugars with microbes and used as substitute for gasoline (Ziska et al., 2009). However, ethanol fuel is similar to the alcohol in alcoholic beverages and can be used for biofuel as an alternative as well as oxygenates to gasoline (Kosugi et al., 2009; Shanavas et al., 2011).

The chemistry of bioethanol production is shown in equations (1 & 2) where starch is converted into fermentable sugar (glucose) via a two-stage process involving the hydrolysis of starch slurry. Chemical reaction between starch and water which breaks down the long chain of starch polymer into glucose from which ethanol is directly obtained by the anaerobic action of yeast (Sorapipatana & Yoosin, 2011; Zvinavashe et al., 2011).



Such alcohol can be used to operate alcohol-driven vehicles as well as production of different chemicals i.e. cellulose triacetate, vinyl acetate, PVC, styrene, and polystyrene (Balagopalan et al., 1998).

2.6.4. Application of cassava in paper making and plywood industry

High quality papers, cartons and different plywoods are produced in the paper making and plywood industries respectively using cassava HQCF or cassava starch of high quality i.e. finely milled (0.25 mm) cassava flour with low fibre, not fermented (Graffham, 2000). Cassava starch is used as flocculant and retention aid, bonding agent, surface size, binder for coatings and as adhesive in corrugated board, laminated grades and other products due to its high paste viscosity, stability and low swelling power (BeMiller & Roy, 2009).

It is also used as bin fibers, retain additives and increase strength due to its high amylose content (Graffham, 2000). On the other hand, low-amylose starches are used as adhesives due to their ability to form viscous pastes with a cohesive texture when heated and a low tendency of retrograde (Breuninger et al., 2009). In addition, cassava starch is an excellent remoistenable adhesive for use on postage stamps, envelop flaps, labels and gummed tape since it's odorless, tasteless and its dried film can reabsorb water rapidly (Breuninger et al., 2009).

2.6.5. Application of cassava in textile and packaging industry

Cassava is used in textile industries in production of fillers, stiffeners and leather goods due to its high quality (finely milled, 0.25 mm; white in colour, low fibre, no odour or stains, and not fermented), high paste viscosity and stability (Graffham, 2000). Its starch is used in textile sizing, printing purposes, maximizing brightness and shaping for fashion or ceremonial wears due to its amylose and amylopectin polymers which influence its functional properties which impact strong film formation, good film flexibility and stable viscosity (Silva et al., 2011). Moreover, starch films are also used during textile production as fiber coatings in this textile industry (Alcázar-Alay & Meireles, 2015). Cassava starch has also replaced plastics obtained from oil due to its ability to form films in food packaging applications which reduces loss, add value, extend shelf life, maintain quality and wholesomeness of a product (Uchechukwu-Agua et al., 2015).

2.6.6. Application of cassava in feed industry

Feed industry uses cassava in production of animal feed products which act as protein substitutes and carbohydrates sources (Henry et al., 1998). Usually, these are in form of chips and pellets which are desirable in feed industry due to their shape, uniformity and moisture content (Bechoff et al., 2016). Therefore, cassava genotypes with high dry matter content and less cyanide are the best in this industry (Taiwo, 2007).

2.7. Genotype and environment (G X E) interactions, stability and ranking of cassava genotypes and varieties

There are variations of fresh root dry matter and cassava flour quality parameters with genotype and environments (G X E). As such, there is need to understand the inter-character relationships among genotypes, to identify parameters that determine root yield and find out the influence of other parameters associated with the yield. Estimates of phenotypic correlation among characters are useful in planning and evaluating breeding programs (Aina et al., 2007). Plant breeders need to ascertain if improvement in one parameter will simultaneously result in changes in the other, and this could be achieved by estimating inter-character correlations among genotypes. The traits to be taken into account will vary depending on the end-use(s) of the genotypes and varieties. It is well-recognized, however, that in some cases desirable parameters may be negatively correlated (Barandica et al., 2016). Cassava breeders should therefore, aim at understanding the relationship between different variables with dry matter content at fresh root basis within the same trials (phenotypic correlations) and decide which genotypes to breed for higher yields.

However, the selection of stable and high yielding genotypes is difficult due to the complexity of the genotype responses across environments (Hagos & Abay, 2013). These differential genotypic responses when exposed to different environments are commonly known as genotype $\times$ environment (G $\times$ E) interaction. This kind of interaction leads to bias in the prediction of genetic advance and decreases gain from selection (Barandica et al., 2016). In plant breeding and varietal release programs, G $\times$ E interaction enables plant breeders to identify genotypes that are superior with better stability and adaptability (Aina et al., 2007) hence promoting them to be grown in wide range of environments for production of high quality cassava flour (HQCF) and starch for various industrial applications.

Additive main effect and multiplicative interaction (AMMI) analysis is the most reliable statistical method for determining stable cassava genotypes and varieties for specific adaptations (Hagos & Abay, 2013). AMMI biplot analysis enables a simple view of the specific interactions between genotypes and environments. The AMMI model in multi- environmental trial (MET) data analysis combines analysis of variance (ANOVA) and principal component analysis (PCA) into an integrated approach (Crossa et al., 1990; Gauch & Zobel, 1996). AMMI uses ANOVA to study the main effects of genotypes and environments and a PCA for the residual multiplicative interaction among genotypes and environments. It also helps in grouping environments with the best genotypes into mega-environments using the principal component axis scores and AMMI stability value (ASV) (Hagos & Abay, 2013).

The ASV is derived from the Interaction Principal Components Axes 1 and 2 (IPCA1 and IPCA2) scores of the AMMI model (Purchase et al., 2000). Stability parameter alone does not provide much information about the yield performance of a genotype and cannot be used as the only selection parameter since most stable genotypes would not necessarily be the best yield performer (Purchase et al., 2000; Gauch & Zobel, 1996). Therefore, yield stability index (YSI) which incorporate high yield performance with stability is used. The YSI is based on the sum of the ranking due to ASV scores and yield or performance ranking. Low YSI value indicates desirable genotypes with high mean yield or performance and stability (Hagos & Abay, 2013).

CHAPTER 3: MATERIALS AND METHODS

3.1. Study sites and design

Cassava samples were collected from an IITA uniform yield trial at three Agricultural Research Stations of Chitala, Chitedze and Mkondezi, and Njuli farm, in Malawi. The research sites are located in different agro-ecological zones representing heterogeneity in terms of soil type, elevation, and meteorological conditions (Figure 3.1). In Malawi, the agro-ecological zones are categorised, mainly according to elevation, as Lower Shire valley (altitude below 200 m.a.s.l), Lake shore, middle and upper Shire valley (>200 to 760 m.a.s.l), mid-elevation (>760 to 1300 m.a.s.l) and highlands (>1300 m.a.s.l) (Matchaya and Nhlengethwa, 2014). The spatial variation of climatic variables (temperature, humidity and rainfall) depends on elevation and thus, the agro-ecological zones represent spatial climatic zonation of the country (Ngongondo, Chong-yu, Lars, & Berhanu, 2011).

Chitala and Mkondezi research stations are located in the lake shore, upper and middle Shire valley agro-ecological zone, whereas Chitedze and Njuli are located in the mid-elevation agro-ecological zone (Figure 3.1). All locations have a tropical wet and dry “savanna” climate (MetMalawi, 2006), characterised by a distinct rainy season between November and April, and hot and cool during the months of October to December and May to July, respectively (Ngongondo et al., 2011). In general, the lake shore, upper and middle Shire valley agro-ecological zone is characterized by higher mean monthly temperatures

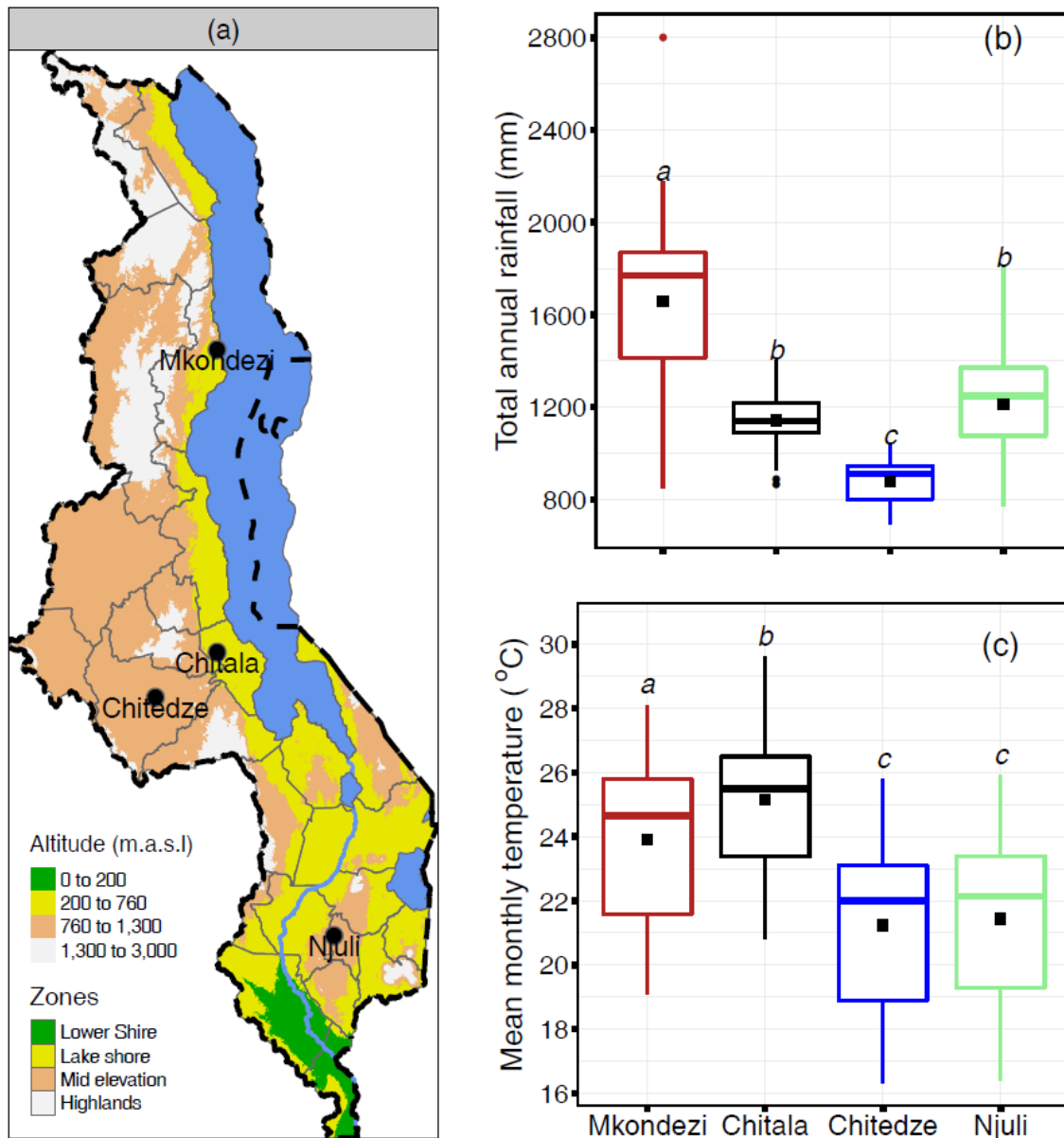


Figure 3.1: Location, temperature, and rainfall patterns of the research sites. (a) Map showing location of the research sites in Malawi's agro-ecological zones: Lower Shire valley (altitude below 200 m.a.s.l), Lake Shore, middle and upper Shire (>200 to 760 m.a.s.l), mid-elevation (>760 to 1300 m.a.s.l) and highlands (>1300 m.a.s.l). (b) Monthly average rainfall patterns and (c) monthly average temperatures at each research station (1995-2012).

than the mid-elevation zone. Monthly mean temperatures at Mkondezi, Chitala, Chitedze and Njuli fall in the range of 19 - 28 °C, 20.8-29.6 °C, 16-25.8 °C and 16-25.9 °C, respectively (Figure 3.1). In terms of rainfall, Mkondezi research station receives higher rainfall of over 1500 mm per year than the other stations (Figure 3.1). At Mkondezi, the dominant soils are strongly acidic sandy loam to sandy clay, with variable low to medium nutrient levels because of leaching. They are low in nitrogen, phosphorous and cation exchange capacity (CEC). Exchangeable potassium is marginally adequate (Table 3.1) where as calcium and magnesium are favourable only at selected sites (DAR, 2016). Chitala and Chitedze soils are slightly acidic sandy clay and sandy clay loam with low phosphorus (P) levels (Table 3.1). Chitedze soils have relatively higher levels of potassium (K).

Table 3.1: Edaphic description of trial sites

	Mkondezi	Chitedze	Chitala	Njuli
pH (H ₂ O)	4.0-5.6	5.2-5.8	5.6-6.1	5.73-6.41
Organic carbon(g/100 g)	2-5	2.39	1.07	2.88-3.47
Nitrogen (g/100 g)	0.04-0.2	0.16	0.04-0.07	0.25-0.29
Phosphorus (µg/g)	20-50	15.57-24.81	10.4-19.39	59.31-83.21
Potassium (cmol/kg)	0.08-0.14	0.31-0.40	0.23-0.51	0.18-0.26
Soil texture	Sandy clay to clay	Sandy clay loam	Sandy clay to sandy clay loam	Sandy clay loam
References	Benesi et al., 2007; DARS, 2016	Benesi et al., 2007; Bationo et al., 2012	Benesi et al., 2007; Bationo et al., 2012; Van Vlugt et al., 2017	

3.2. Study design, sample collection and preparation

The cassava samples comprised one local recommended variety (Mbundumali), three released varieties (Sagonja, Sauti, Mpale) and six new advanced genotypes (MM06/0045, TMSL110080, I010085, TMEB419, I010040, and I020452). They were planted on 22 December 2016 in a randomized complete block design with four replications and then harvested at 12 months later from only two middle rows. The roots were collected in four replicates and were processed within 24 h of harvesting. The cassava roots were washed to remove soil, and then woody ends of the roots were chopped off using a sharp stainless-steel knife. The roots were peeled, sliced into small pieces (approximately 20 mm thickness) and then oven-dried at 60 °C for 48 h (Kehinde, Udoro, Olasunkanmi, & Charles, 2014). The dried chips were ground into flour by using a laboratory mortar and pestle, and sieved through a 0.25 mm metal mesh to produce a consistent 0.25 mm particle size and then packaged in polythene bags and stored awaiting analysis.

3.2. Determination of physicochemical properties of cassava flours

3.2.1. Determination of moisture and dry matter contents of cassava flours

Moisture and dry matter contents of flours (cassava and HQCF) were determined using Eriksson's method (2013). The moisture content was determined in four replicates where 3 g of the flour sample was placed in pre-weighed dishes and dried in an oven at 105 °C for 4 h, and then samples were cooled in a desiccator for 30 min and weighed again. Then moisture content was determine using equation 3.

$$\text{Moisture content (\%)} = \frac{w_2 - w_3}{w_2 - w_1} \times 100 \quad (3)$$

where W_1 is the weight of the dish, W_2 is the weight of the dish + weight of the sample before drying, and W_3 is the weight of the dish + weight of the sample after drying.

Fresh root dry matter content was determined by weighing 10 g of fresh cassava sample in pre-weighed dishes and dried in an oven at 110 °C overnight and cooled in the following morning in a desiccator for 2 h and weighed again. Equation 3 was applied and dry matter-FWB was obtained by subtracting moisture content of fresh cassava from 100%.

3.2.2. Determination of ash contents of cassava flours

Ash content was determined using Eriksson's method (2013). The flour samples were prepared in four replicates and weighed (2 g) into pre-weighed, porcelain crucibles. The samples were transferred to muffle furnace (S302AU, England. S/N: 12/91/1994) and ashed at 550 °C for 8 h. The U crucibles were allowed to cool in desiccators and then weighed using Mettler Toledo (K.G. Goettingen, Germany, S/N 00365108). Then ash content was calculated as a percentage.

3.2.3. Determination of Bulk density of cassava flours

Bulk density was estimated following the method used by Iwe et al.(2017). Flour sample (10 g) was put into a 25 mL volumetric cylinder. The lower surface of the cylinder was tapped several times on the laboratory bench until there was no more diminution of the sample level. The sample weight was then determined, and bulk density was expressed as the weight/volume of the sample (g/mL).

3.2.4. Determination of colour of cassava flours

The colour of the cassava flour and HQCF was measured using a colorimeter, PCE instrument (PCE-CSMS, SN: 60158946). According to the Comission Internationale de l'Eclairage (CIE) colour space, data were expressed on the three colour coordinates that characterize colour points as L^* , a^* , and b^* . L^* is the “lightness” coordinate (0 = black to 100 = white), a^* is the “redness-greenness” coordinate ($+a^*$ = redness, $-a^*$ = greenness) and b^* is the “yellowness-blueness” coordinate ($+b^*$ = yellowness, $-b^*$ = blueness). A standard white background, supplied by the supplier, was used to standardize the instrument ($L^* = 97.63$, $a^* = -0.48$, $b^* = +2.12$). Values of chroma (C) and Whiteness Index (WI) were then calculated using equations 4 and 5 respectively (Zhu et al., 2016).

$$C = \sqrt{a^{*2} + b^{*2}} \quad (4)$$

$$WI = 100 - \sqrt{(100 - L^*)^2 + a^{*2} + b^{*2}} \quad (5)$$

Chroma indicates colour saturation, with higher values showing more colour purity. All measurements were performed at least in triplicate.

3.3. Determination of chemical properties of cassava flours

3.3.1. Determination of pH and total titratable acidity of cassava flours

pH and total titratable acidity (TTA) of cassava flour were determined using a method described by Eriksson (2013). Cassava flour sample (10 g) was weighed into a 250 mL beaker, to which distilled water (90 mL) was added and then mixed well. The mixture was left to stand for 1 h at room temperature, and then pH of the supernatant was measured in four replicates using a pH meter (Model: 8603, S/N: 1000329). TTA was then determined on the same supernatant of the sample mixture used for pH by titration using a 0.1 mol/L NaOH and 4 drops of phenolphthalein indicator. The volume of NaOH added was multiplied by 0.09 to obtain the g/100 g titratable acidity as lactic acid.

3.3.2. Determination of total cyanogens of cassava flours

Total cyanogens were determined by the acid hydrolysis method as described by Iwe et al. (2017). Extraction of cyanogens from cassava flour was done as follows: A 30 g of cassava flour sample was weighed in triplicate and blended with 160 mL of cold orthophosphoric acid, 0.1 mol/L H_3PO_4 and then homogenised at high speed for 15 s, at low speed for 60 s then at high speed for 60 s followed by 1 min rest. The sample was finally homogenised at highest speed for 60 s. This was done to avoid overheating of the blender and escape of HCN from the flour. The homogenate was transferred into a plastic beaker and covered tightly with para-film and stored in the refrigerator to settle for 30 min, and then the sample was removed from the refrigerator, and solution decanted into a sample bottle and kept in in deep freezer till analysis.

The Linamarin in cassava extracts was hydrolysed as follows: 1.0 mL of 4.0 mol/L H_2SO_4 solution (prepared by diluting 108.5 mL, 98% sulphuric acid with 50 mL of distilled water then made up to the mark of a 500 mL volumetric flask with constant shaking and cooling in ice water) was added to 1.0 mL of the sample extract. The acid hydrolysed samples were then vortex mixed and put in a water bath (in triplicates) at 100 °C for 120 min. The samples were removed from the water bath, cooled in ice for 10 min, and then to each sample, added 2.5 mL of 3.6 mol/L NaOH solution (prepared by dissolving 37.1134 g of Sodium hydroxide in 100 mL of distilled water and quantitatively transferring into a 250 mL volumetric flask containing a small volume of distilled water and cooled in the ice water bath. The solution was then diluted to the mark with distilled water) followed by addition of 0.5 mL of distilled water.

Potassium cyanide (KCN) Calibration Curve was obtained at pH 6 [pH 6 was prepared by mixing 0.13 mol/L Na_2HPO_4 (34.8491 g of di-sodium hydrogen phosphate was dissolved by 100 mL of distilled water and quantitatively transferred into a 500 mL volumetric flask and diluted to the mark with distilled water) with 0.26 mol/L KH_2PO_4 (9.03 g of potassium di-hydrogen phosphate was dissolved in 100 mL of distilled water and quantitatively transferred into 500 mL volumetric flask and diluted to mark with distilled water and shaken thoroughly) while stirring until the pH 6 was obtained. This was done while the pH meter's probe was dipped into the KH_2PO_4 and stirring the mixture on a magnetic stirrer. The pH meter was calibrated before using it to prepare the buffer. The KCN working solution were prepared from 1.0 mL of potassium cyanide stock solution was diluted to the mark of a 100 mL volumetric flask with pH 6 buffer solution to give 2.5 µg/mL KCN).

Then KCN Calibration Curve was obtained as follows: To 0.0, 0.2, 0.4, 0.6, 0.8 and 1.0 mL of 2.5 µg/mL KCN working solutions, the volume of 3.4, 3.2, 3.0, 2.8, 2.6 and 2.4 mL of pH 6 buffer solution were added respectively. Then the samples were vortex mixed and while cooling in ice, 0.6 mL of 0.2 mol/L NaOH solution was added. NaOH solution (0.2 mol/L) used was prepared by dissolving 8.0064 g sodium hydroxide pellets in 50 mL distilled water and quantitatively transferred into a 1000 mL volumetric flask placed in an ice-water bath for cooling and then diluted to the mark with distilled water.

After addition of 0.6 mL of 0.2 mol/L NaOH, the samples were vortex mixed again and 0.2 mL of Chloramine T (prepared by dissolving 0.500 g of Chloramine-T hydrated with 10 mL of distilled water and solution quantitatively transferred into a 100 mL volumetric flask and diluted to the mark with distilled water) was added to each solution and vortex mixed while cooling in ice. The solutions were left to cool in ice before adding 0.8 mL of colouring reagent. After adding the colouring reagent, the solutions were left at room temperature for 15 min before analysis on a spectrophotometer at 600 nm. 0.1 mL of hydrolysed extract was diluted to 4.0 mL with 3.9 mL of pH 6 buffer solution and then vortex mixed. While in ice, 0.2 mL of Chloramine T was added to each sample, vortex mixed and left to cool in ice for 5 min, and then 0.8 mL of colouring reagent was added to the sample, vortex mixed and left at room temperature for 15 min. The samples were analysed for cyanogens on UV/VIS Spectrophotometer (S/N:20-1901-0351; Model: T90+; PG instruments Ltd) at 600 nm, the absorbance was recorded, and total cyanogens were calculated in equation 6.

$$\text{mg HCN} = \frac{10 \times A_s \left(160 + \frac{\text{moist \%} \times \text{wt}}{100} \right)}{A_{\text{ref}} \times \text{wt}} \quad (6)$$

Where A_s –sample absorption at 600 nm

10 –dilution factor

Moist % -moisture content of cassava flour

A_{ref} –absorbance for 1.00 μg HCN equivalent

Wt –the weight of cassava flour used for cyanogens extraction

160-extraction volume (160 mL of 0.1 mol/L orthophosphoric acid)

3.3.3. Determination of starch, amylose and amylopectin contents of cassava flours

Total starch (anthrone reagent) content and amylose (iodine reagent) content were determined by UV/VIS spectrophotometry (Oladayo & Joseph, 2016). For starch analysis, a 100 mg of the flour samples (cassava and HQCF) was weighed into 50 mL centrifuge tubes and homogenised with 30 mL of hot 80 % ethanol to remove sugars and then centrifuged for 10 min (Gallenkamp, England. CAT. No: CF 405. App. No: 8A 8840E) and residue retained. The residue was washed repeatedly with hot 80 % ethanol until the washings did not give the colour with anthrone reagent. The residue was dried well over a water bath. 5.0 mL of water and 6.5 mL of 52 % perchloric acid were added to the dried residue and then total starch was extracted at 0 °C for 20 min. After 20 min of total starch

extraction, the sample was centrifuged for 5 min and supernatant was saved. The supernatant was made up to 100 mL by distilled water. Then 0.1 mL of the supernatant was pipetted into a boiling tubes using micro pipette and made up to 1.0 mL with distilled water.

Thus, to determine total starch content, calibration curves were derived using D (+) Glucose Anhydrous (SAAR2676020EM, Merch, Wadeville, Gauteng, RSA) where stock solution was prepared by dissolving 100 mg of glucose in 100 mL of distilled water and then working standards of glucose were prepared (by diluting 10 mL of stock solution into 100 mL flask to its mark) as 0.2, 0.4, 0.6, 0.8 and 1 mL of working standard of D (+) Glucose Anhydrous (SAAR2676020EM, Merch, Wadeville, Gauteng, RSA) which were also made to the mark of 1 ml volume and “0” served as a blank. Then 4 mL of anthrone (400 mg dissolved in 200 mL of ice cold 98 % sulphuric acid) were added to the samples, as well as to the standard solutions of glucose and boiled (100 °C) for 8 min on water bath, after cooling, standards and samples were read on UV/VIS Spectrophotometer (S/N: 20-1901-0351; Model: T90+; PG instruments Ltd) at 630 nm. Glucose concentration in the sample was found using calibration curve and equation 7.

$$100 \text{ mL of the sample} = \frac{x \times 100 \text{ mg}}{0.1 \text{ mL}} \quad (7)$$

Where x = concentration

Then starch content was found by multiplying value of glucose content found by a factor of 0.9.

To determine amylose content, 100 mg of cassava flour sample was added to 1 mL of 99.9 % ethanol, and then 10 mL of 1 N NaOH (4 g of NaOH pellets was dissolved in 100 mL of distilled water) was added, and left overnight. Then the volume was increase to 100 mL using distilled water. A 2.5 mL of the extract was taken, and 20 mL of distilled water was added to it followed by 3 drops of phenolphthalein. Then 0.1 N Hydrochloric acid was added drop by drop until the pink colour just disappeared. Then 1.0 mL of iodine reagent (1.0 g of Iodine and 10 g of KI dissolved in distilled water and made up to the mark of 500 mL volumetric flask) was added and blue black colour developed and the volume was increased to 50 mL using distilled water.

Calibration curves were derived using pure amylose from potato (A0512; Sigma–Aldrich, St. Louis, MO, USA), prepared (100 mg amylose was dissolved in 10 mL of 1 N NaOH and the volume increased to 100 mL using distilled water) as 0.2, 0.4, 0.6, 0.8 and 1 mL, and the colour was developed as in the case of the sample. For a blank, 1.0 mL of iodine reagent was diluted to 50 mL with distilled water. Hence the colour developed for samples and amylose standards was read on UV/VIS Spectrophotometer (S/N: 20-1901-0351; Model: T90+; PG instruments Ltd) at 590 nm. Equation 8 was used to calculate the amount of amylose in cassava flours.

$$\begin{aligned} \text{Absorbance that corresponds to 2.5 mL of the test solution} &= x \text{ mg amylose 100 mL contains} \\ &= \frac{x}{2.5} \times 100 \text{ mg} = \% \text{ Amylose} \end{aligned} \quad (8)$$

The amount of amylopectin was obtained by subtracting the amylose content from the total starch content.

3.3.4. Determination of protein content of cassava flours

Crude protein content was determined as total nitrogen content per Kjeldahl procedure following the method used by Bankole et al. (2013). 1.0 g of flour samples were dried, powdered, weighed, and digested with H₂SO₄ and K₂SO₄/Se catalyst tablets in a Foss Tecator Auto Digester (block digestion). The resulting digest was steam distilled into boric acid using a Labconco Rapid Still II, and then the distillate was titrated with 0.2 mol/L HCl. Then nitrogen content and crude protein were determined as follows:

$$\% \text{ Total nitrogen} = \frac{\text{Titre value} \times \text{Normality}}{\text{Weight of flour sample}} \times 0.014 \times 100 \quad (9)$$

$$\% \text{ Crude protein} = \% \text{ Total nitrogen} \times \text{conversion factor} = 6.25 \quad (10)$$

3.3.5. Determination of carbohydrates content of cassava flours

Total carbohydrates were determined as glucose content, using anthrone reagent, according to the method described by Oladayo et al. (2016) with modifications. 100 mg of sample was weighed into a boiling tube and hydrolyzed using 5 mL of 2.5 mol/L NaOH in a boiling water bath (100 °C) for 3 h. After cooling to room temperature, it was neutralized with 3 g sodium carbonate powder until effervescence ceased and then it was made up to 100 mL with distilled water and centrifuged. The supernatant was collected, and 0.5- and 1-mL aliquots were taken for analysis. Standard solutions of D (+) Glucose Anhydrous (SAAR2676020EM, Merch, Wadeville, Gauteng, RSA) were prepared by taking 0, 0.2, 0.4, 0.8 and 1.0 mL of working standard of D (+) Glucose Anhydrous (SAAR2676020EM, Merch, Wadeville, Gauteng, RSA) and "0" served as a blank. Distilled water was added to both samples and standards to make up to 1.0 mL volume, and then 4 mL of anthrone

reagent was added. The samples were then heated for 8 min in a boiling water bath (100 °C) and cooled rapidly. The intensity of a green to dark green colour was determined using a UV/VIS Spectrophotometer (S/N: 20-1901-0351; Model: T90+; PG Instruments Ltd) at 630 nm.

3.4. Determination of functional properties of cassava flours

3.4.1. Determination of swelling power and water solubility of cassava flours

Swelling power and water solubility were determined using methods described by Kusumayanti et al. (2014). To determine swelling power, 0.1 g flour sample was mixed with 10 mL distilled water and heated at 90 °C for 1 h, with constant mixing. Then, the suspension was cooled rapidly, equilibrated at 25 °C and centrifuged for 30 min at 1600 rpm (Gallenkamp, England. CAT. No: CF 405. App. No: 8A 8840E), and then the sediments were weighed. For solubility, a 0.5 g flour sample was heated in 10 mL distilled water at 60 °C (in a water bath) for 30 min, without mixing. The sample was centrifuged at 1600 rpm for 10 min rpm (Gallenkamp, England. CAT. No: CF 405. App. No: 8A 8840E). The supernatant (5 mL) was separated, dried and weighed. The swelling power and water solubility of the flour were calculated using the equations 11 and 12 respectively.

$$\text{Swelling power (g/g)} = \frac{\text{Weight of the sediments}}{\text{Weight of initial flour}} \quad (11)$$

$$\text{Solubility (\%)} = \frac{\text{Dried supernatant weight}}{\text{Weight of initial flour}} \times 100 \quad (12)$$

3.4.2. Determination of water binding and oil absorption capacities of cassava flours

Water binding capacity and oil absorption capacity were determined according to methods described by Agyepong and Barimah (2018) and Iwe et al. (2017), respectively. For water binding capacity, 2.0 g of the flour sample was dissolved in 40 mL of water in a centrifuge tube. The suspension was agitated for 1 h at room temperature on a shaker and centrifuged for 10 min at 2200 rpm. The free water was decanted from the pellet, drained for 10 min, and pellet weighed. For oil absorption capacity, 1 g flour sample was mixed with 10 mL soybean oil (Sp. gravity: 0.9092) and allowed to stand at ambient temperature (30 ± 2 °C) for 30 min centrifuged for 30 min at 300 rpm. Water and oil absorption capacities were determined using equation 13.

$$\text{Water (Oil) Absorption Capacity (\%)} = \frac{\text{Weight of absorbed water (oil)}}{\text{Weight of initial flour}} \times 100 \quad (13)$$

3.5. Data Analysis

All statistical analyses were performed using R: A language and environment for statistical computing version 3.6.3 (R Core Team, 2020). The analysis of variance was performed on all measured characteristics of the genotypes by pooling data from all the trials. The correlation matrix for physicochemical and functional properties was produced using *rcorr* (Hmisc package), incorporating Spearman's correlation as a type. The correlation matrix was displayed using *corrplot* (corrplot package) and ordered by hierarchical clustering (Friendly, 2002; Murdoch & Chow, 1996), using "Ward.D2" method. In addition, principal component analysis (PCA) was performed using FactoMineR package (Husson, Josse, L  ,

& Mazet, 2009) to link correlations among chemical and functional parameters to the cassava varieties and genotypes. Phenotypic and genotypic correlations across all trials was estimated using multi-environment trial analysis with R for Windows (META R Ver 5.0) (Alvarado et al., 2020). Analysis of variance was performed on physicochemical and chemical parameters (dry matter on fresh root weight basis, and bulk density, starch and amylopectin content of flour) and functional properties of cassava flour (swelling power, water binding capacity, oil absorption capacity and solubility) of each of the individual trials. Thereafter, analysis of variance and interaction principal component analysis were calculated with additive main effect and multiplicative interaction (AMMI) model using Agricolae package (DeMendiburu, 2015) were performed on the pooled data of all parameters, with significant GxE interaction, from all the four sites. AMMI combines analysis of variance for genotype and environment main effects with principal components analysis of the G x E and is useful in identifying stable genotypes and location (Akinwale, Akinyele, Odiyi, & Dixon, 2011; Gauch, 1988; Zobel, 1990) and allocating all unstable genotypes to a most suitable location (Hugh & Gauch, 2013).

The bi-plots of IPCA1 vs mean response variable were produced using ggplot2 package for visual evaluation on the performance and stability of genotypes and varieties, and environments. Moreover, Shukla's stability variance (Shukla, 1972) and Kang's yield-stability statistics (Kang, 1993) were calculated to identify most stable genotypes in wide range of environments by simultaneous selection for stability and response variable. The selected genotypes were then ranked according to AMMI yield stability index (YSI) (Purchase, 1997; Sabaghnia, Sabaghpour, & Dehghani, 2008) to identify crops with better response and improved stability according AMMI.

CHAPTER 4: RESULTS AND DISCUSSION

4.1. Proximate composition and physicochemical parameters of cassava flours

In this study, the proximate analysis involved determining the major components of cassava flour such as moisture, ash, crude protein, and total carbohydrates content. Proximate compositions were compared among the improved varieties and advanced genotypes (Figures 4.1 to 4.3). Dry matter content is a basis of accepting raw materials in the industry, and dry matter content above 30 g/100 g are preferred. Dry matter contents in cassava roots varied among the genotypes ($p < 0.05$), and the genotype average ranged from 27.58 g/100 g (Sauti) to 38 g/100 g (Mbundumali). Mbundumali did not differ significantly ($p > 0.05$) from the advanced genotypes of TMEB419 and TMSL110080 (Figure 4.1). Genotypes and varieties with high fresh root dry matter content are preferred in energy industries to provide enough raw material for production of bio-fuel. Therefore, genotypes TMEB419 and TMSL110080 and Mbundumali variety can be used to produce ethanol and bio-fuel in energy industries; and production of chips and pellets in feed industries.

The average bulk density of the flour samples from the genotypes ranged from 0.68 g/mL to 0.75 g/mL, with little significant variation ($P < 0.05$) among the genotypes. All the genotypes (except for Mpale variety) had significantly ($P < 0.05$) superior bulk density values to HQCF (Figure 4.1). Bulk density indicates the closeness of the cassava flours'

packaging and determines industrial handling and processing requirements (Iwe et al., 2017). All genotypes flour samples had average moisture content (ranged from 7.00 to 10.20 g/100 g) below the guideline value of 13 g/100 g (CODEX STAN 176-1989). Moisture content is an essential parameter in cassava flour storage as levels greater than 13 g/100 g may cause caking and/or may encourage microbial growth leading to spoilage (Hasmadi et al., 2020; Iwe et al., 2017). Therefore, the low moisture content would be suitable for long flour storage and hence amenable to industrial processing. Sagonja, sauti and mbundumali varieties, and MM06/0045, I010085, TMEB419, TMSL110080, I010040 and I020452 genotypes had superior bulk density values to HQCF and also had good moisture levels within codex standards hence have potential for better shelf life and therefore, are recommended for confenctionery industries.

The overall ash content of the samples was generally low. The mean ash content ranged from 0.11 g/100 g to 1.83 g/100 g (dry weight), and was not significantly different ($P > 0.05$) from HQCF (average, 0.41 g/100 g). All genotypes flour samples had ash content below the guideline value of 3 g/100 g (CODEX STAN 176-1989). Only flour from Sagonja variety had significantly ($p < 0.05$) lower ash content than HQCF Figure 4.1). Ash content reflects the amount of mineral matter in flour and non-volatile content of cassava (Montagnac et al., 2009) and it is routinely used as a measurement of the quality of flours in the food industry (Harris & Marshall, 2019). As such, flours from all genotypes and varieties in this study were of industrial quality with desirable mineral amounts and therefore, best suited for food industries.

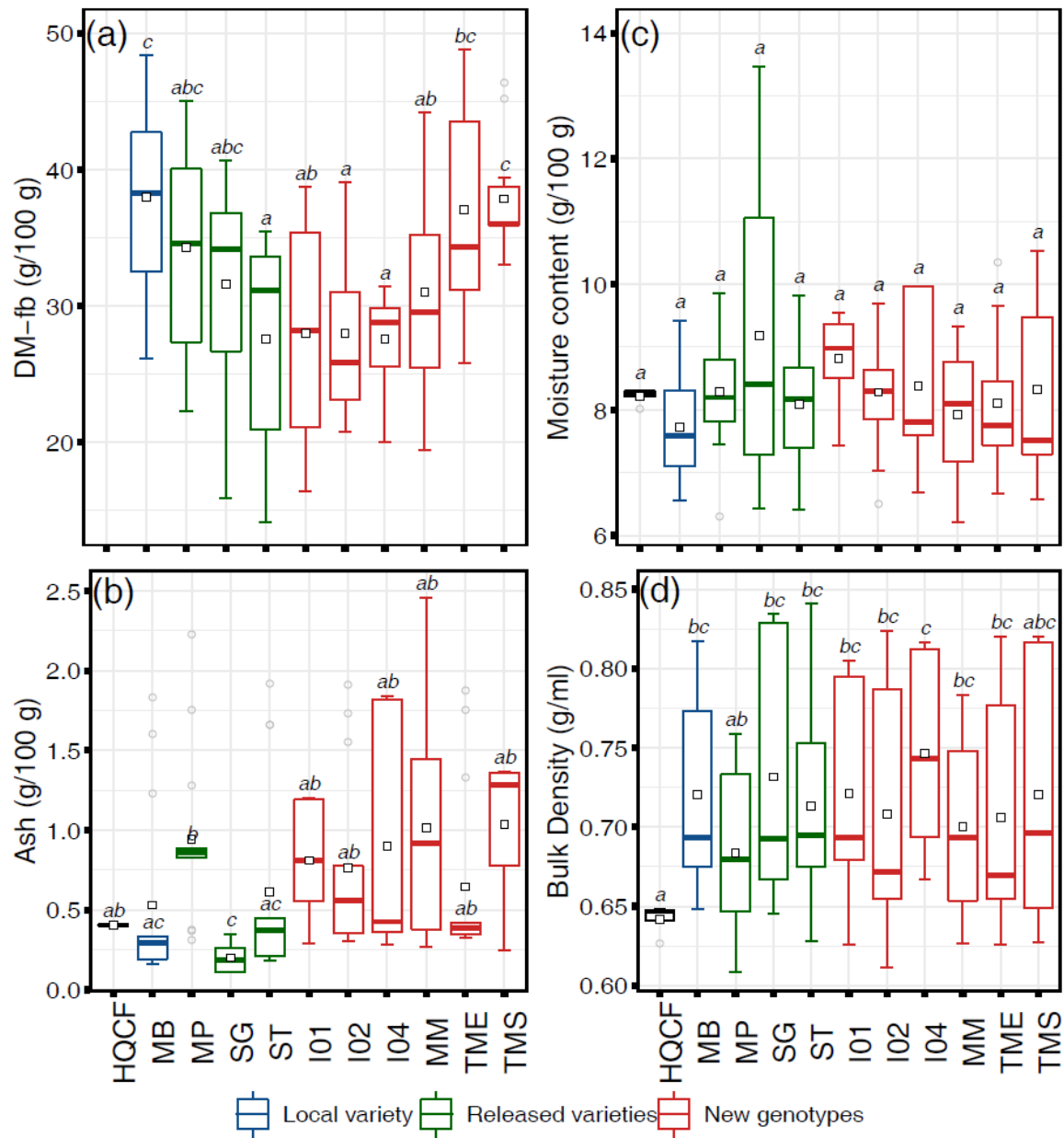


Figure 4.1: Distribution levels of (a) dry matter (fresh basis), (b) ash content, (c) moisture content and (d) bulk density, and for the studied cassava varieties, genotypes and HQCF. Apart from dry matter, all parameters are expressed on dry weight basis. Sample types with the same letter have insignificant differences ($p = 0.05$). The small square in the boxplots indicates an average for each sample type. The dots, line-ends and upper and lower ends of the box represent outliers, spread, and first and third quartiles, respectively. Genotype and variety codes: SG = Sagonja; ST = Sauti; MB = Mbundumali; MM = MM06/0045; I01 = I010085; TME = TMEB419; MP = Mpale; TMS = TMSL110080; I04 = I010040; I02 = I020452

Colour characteristics are an important quality attribute of flour for consumer acceptability and food industries. All flours had L^* (ranging from 82.84 to 84.75) values comparable to HQCF (mean: 84.60). However, the flour samples from most genotypes showed higher a^* and b^* values than HQCF (Table 4.1). Generally, all genotypes had lower ($p < 0.05$) WI values than HQCF. The genotype average WI values were in the range of 77.90–80.17, with some significant variations ($p < 0.05$) among the genotypes (Table 4.1), which is a lower range than other studies (Omolola, Kapila, Anyasi, Jideani, & Mchau, 2017).

WI values are affected by temperature and period of drying, with higher temperatures and longer drying periods giving higher b^* and a^* values, thus lower WI values (Omolola et al., 2017). The genotypes flour samples also had significantly higher ($p < 0.05$) chroma (higher colour saturation) values than HQCF (except for I020452 and TMEB419). The flours with high WI improve visual appearance and eye appeal of the finished products (Anyasi, Jideani, & Mchau, 2015). Genotypes MM06/0045, I010085, TMEB419, TMSL110080 and I010040 and varieties of sagonja, sauti and mbundumali had high WI and therefore, are recommended for food and confectionery industries.

Table 4.1: Colour parameters, whiteness index (WI) and chroma (C) of cassava flour from the ten genotypes under study

Genotype	Colour parameters			WI	Chroma, C
	<i>L*</i>	<i>a*</i>	<i>b*</i>		
Sagonja	83.67 ± 1.42 ^{ab}	4.82 ± 1.29 ^{cd}	13.14 ± 1.56 ^{df}	78.45 ± 1.96 ^{ab}	14.04 ± 1.64 ^c
Sauti	82.84 ± 2.72 ^a	4.66 ± 2.46 ^{abcd}	12.56 ± 3.24 ^{abcdef}	78.02 ± 3.73 ^{abc}	13.45 ± 3.88 ^{abc}
Mbundumali	83.19 ± 1.72 ^a	3.95 ± 1.47 ^{abc}	11.36 ± 1.99 ^{abce}	79.15 ± 1.06 ^{abcd}	12.06 ± 2.32 ^{abc}
MM06/0045	83.93 ± 2.27 ^{ab}	3.56 ± 1.63 ^{ab}	11.00 ± 2.22 ^{cef}	80.01 ± 2.10 ^{abcd}	11.60 ± 2.58 ^{ab}
I010085	84.05 ± 2.03 ^{ab}	5.29 ± 1.25 ^d	13.08 ± 1.77 ^{abdf}	78.69 ± 2.86 ^{abcd}	14.12 ± 2.11 ^{cd}
TMEB419	83.71 ± 1.79 ^{ab}	3.81 ± 1.60 ^{abcd}	10.91 ± 2.19 ^{eg}	79.83 ± 1.34 ^{acd}	11.59 ± 2.51 ^{ade}
Mpale	83.97 ± 2.01 ^{ab}	3.64 ± 1.29 ^a	11.11 ± 1.52 ^{ace}	80.00 ± 1.13 ^{cd}	11.72 ± 1.81 ^a
TMSL110080	84.75 ± 1.07 ^b	4.37 ± 0.75 ^{acd}	11.89 ± 0.79 ^{ace}	80.17 ± 1.39 ^d	12.68 ± 0.93 ^{abc}
I010040	83.29 ± 2.54 ^{ab}	4.85 ± 0.99 ^{cd}	12.67 ± 1.60 ^{bdef}	78.47 ± 3.06 ^{abcd}	13.57 ± 1.81 ^{cd}
I020452	82.89 ± 4.58 ^a	4.74 ± 2.29 ^{bcd}	12.57 ± 2.85 ^{fg}	77.90 ± 4.21 ^b	13.50 ± 3.40 ^{bce}
HQCF	84.60 ± 0.01 ^{ab}	0.05 ± 0.02 ^e	8.20 ± 0.02 ^g	82.55 ± 0.01 ^e	8.20 ± 0.02 ^e

Note: All values are means of triplicate analysis (from all study trials). Means ± standard deviation. Within the same column, the values with different letters are significantly different (p < 0.05).

The results presented in Figure 4.2 show that the pH values ranged from 4.24 to 7.72, with MM06/0045 showing significantly ($p < 0.05$) higher pH than HQCF and other genotypes (apart from Mbundumali and Sagonja varieties). On the other hand, I010040 and Mpale gave low pH values comparable to HQCF. Therefore, the results show that the pH values of the processed flour in this study were within the acceptable pH range of 5-7 (Sanni et al., 2005). Cassava flour with lower pH (< 4) is characteristically sour (from organic acids) due to appreciable levels of fermentation and hence not desirable for use in bakery products (Eriksson, 2013). The production of organic acid during fermentation is responsible for a sour taste in a product (Inyang, 2016). Thus, pH is an important criterion because low level of pH (acidity) will limit the substitution level of flour when used in preparing composite flour for baking. Sagonja, sauti, mpale and mbundumali varieties, and MM06/0045, I010085, TMEB419, TMSL110080, I010040 and I020452 genotypes had pH within acceptable pH range of 5-7 hence desirable for bakery products in bakery industries.

Correspondingly, the genotypes flour had higher ($p < 0.05$) total titratable acidity (TTA) than the HQCF (Figure 4.2). However, the TTA values range (0.36-0.53 g/100 g) is consistent with the results obtained by Eriksson (2013). The results of all tested genotypes were within codex standards of total acidity for typical cassava flour products (< 1 g/100 g), expressed as percent lactic acid (CODEX STAN 151-1989). Also, pH and total (titratable) acidity are two interrelated parameters used to depict acidity. Organic acids in foods influence flavor, microbial stability (and keeping quality). Titratable acidity provides better insight into the impact of acidity on flavour, whereas pH is better suited to predicting the potential for microorganism growth in the food sample (Harris & Marshall, 2019).

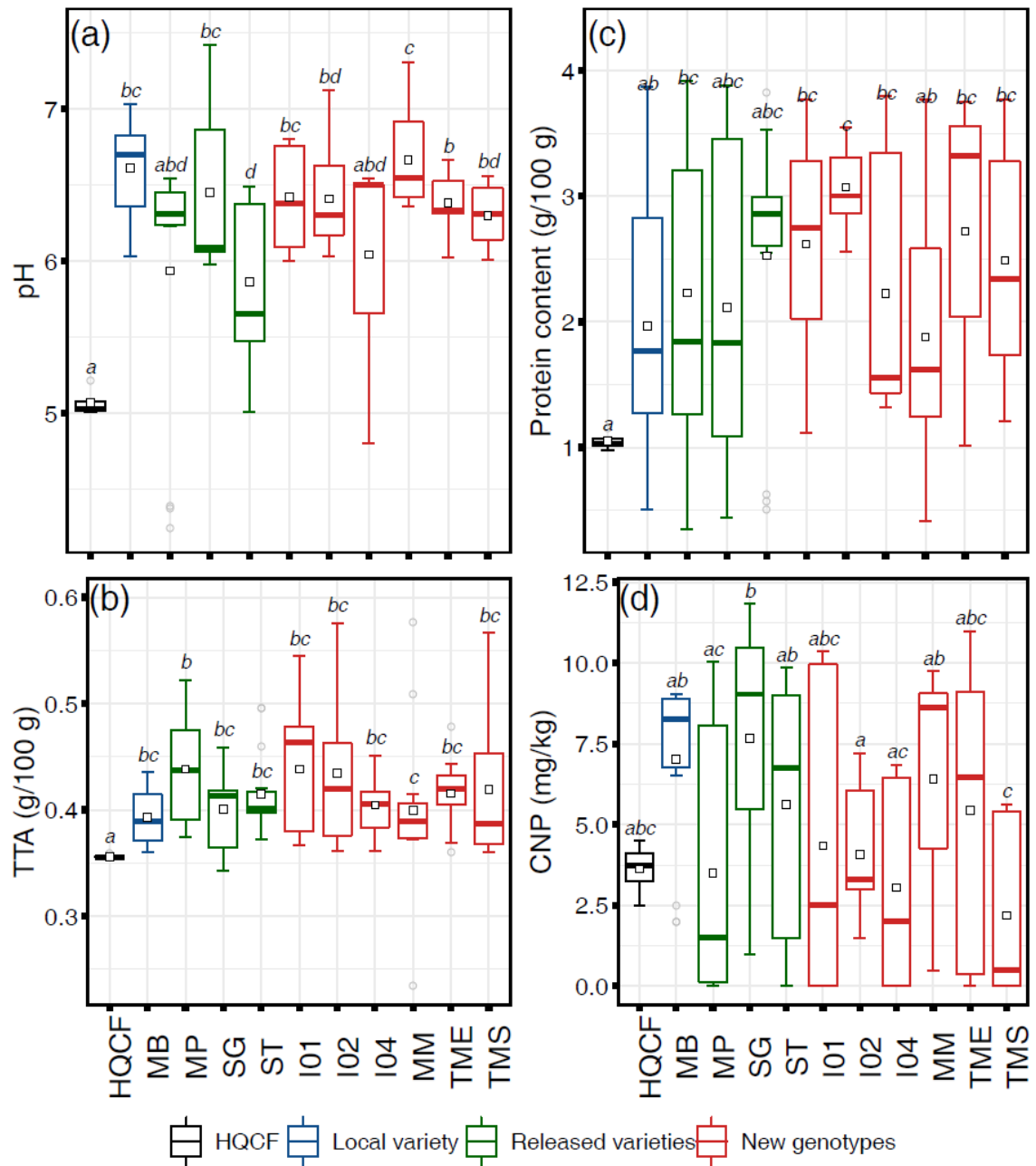


Figure 4.2: Distribution levels of (a) pH, (b) TTA, (c) protein content and (d) cyanogenic potential (CNP), expressed on dry weight basis, for the studied cassava varieties, genotypes and HQCF. Sample types with the same letter have insignificant differences ($p = 0.05$). The small square in the boxplots indicates an average for each sample type. The dots, line-ends and upper and lower ends of the box represent outliers, spread, and first and third quartiles, respectively. Genotype and variety codes: SG = Sagonja; ST = Sauti; MB = Mbundumali; MM = MM06/0045; I01 = I010085; TME = TMEB419; MP = Mpale; TMS = TMSL110080; I04 = I010040; I02 = I020452

As such, bakery products to be made from sagonja, sauti and mbundumali varieties, and MM06/0045, I010085, TMEB419, TMSL110080, I010040 and I020452 genotypes maybe of desirable flavour and free from microorganism growth.

The mean values for protein content were low and ranged from 1.05 to 3.06 g/100 g (dry weight). The flours from I010085, I020452, I010040, Mpale, TMEB419 and TMSL110080 genotypes registered significantly higher ($p < 0.05$) protein content values (Figure 4.2) than the HQCF sample (average: 1.05 g/100 g), however, they were in the same range as reported by Aryee et al. (2006) who found that protein ranged from 0.87 g/100 g to 4.59 g/100 g in cassava flours. As such, genotypes I010085, I020452, I010040, TMEB419, TMSL110080 and mpale variety are best suited for food, bakery and confectionery industries. Cassava is known to have a low protein content of about 1–4 g/100 g (Aryee et al., 2006) thus these low protein levels registered by most genotypes and varieties were expected.

4.2. Chemical properties of cassava flours

The average values of total cyanogenic potential (CNp) of the flours for each genotype ranged from 2.19 mg/kg to 7.66 mg/kg (as HCN, dry weight basis). Therefore, all genotypes had an average CNp lower than 10 mg/kg (as HCN, dry weight basis) recommended for all cassava products as food or feeds (FAO/WHO, 1991). In addition, there were no significant differences ($p > 0.05$) in CNp values between genotypes and HQCF samples. The flour from Sagonja variety had significantly higher ($p < 0.05$) CNp

values than I020452, I010040 and TMSL110080 genotypes (Figure 4.2). All genotypes also had CNp levels below the Codex Alimentarius Commission (CAC) of definition of 'sweet' cassava of a total CNp of below 50 mg/kg as HCN on a fresh weight basis (FAO/WHO, 1991) hence could be used in food, bakery and confectionery industries.

The total carbohydrates content on a dry matter basis ranged from 79.82 g/100 g to 91.58 g/100 g (Figure 4.3). Only I010085 had significantly ($p < 0.05$) lower mean carbohydrate content than the HQCF sample (Figure 4.3). The mean levels of total carbohydrate content in the flour samples under this present study agree with those of previous studies (Chisenga et al., 2019; Oyeyinka et al., 2019). Starch is the main constituent of cassava, and one of its main attractive attributes. Starch content on a dry basis ranged from 76.49 g/100 g to 84.17 g/100 g, which is within the range of 67.92 g/100g to 88.11 g/100g reported in similar studies (Aryee et al., 2006). In general, the genotypes flour samples had comparable starch content to the HQCF sample, except TMEB419 genotype, Mpale and Sauti varieties which had lower starch content ($p < 0.05$). Cassava genotypes and varieties with high starch content have potential for production of many products such as industrial starch, alcohol, and glucose (Yajima, 2010). Genotypes I010040 and I020452, and sagonja variety had high starch content hence could be used to produce commercial products like starch, alcohol and glucose in chemical industries. The flour samples had amylose content mean values ranging from 14.58 g/100 g to 17.01 g/100 g. Besides, I010085, I010040, MM06/0045 and TMSL110080 had significantly higher ($p < 0.05$) amylose content than HQCF (Figure 4.3). The amylose content of the cassava genotypes significantly varied ($p < 0.05$) but fell within the range reported in the literature (Defloor et al., 1998; Gu, Yao,

Li, & Chen, 2013). Amylose content determines to a large extent the stability of the viscous solution formed when heat is applied. Genotypes I010085, MM06/0045 and TMSL110080 had high amylose contents hence could be used for industrial alcohol, and glucose and high fructose syrups in chemical industries since they will not be suitable for products in which adhesion will be required (Aryee et al., 2006).

On the other hand, the amylopectin content was significantly ($p < 0.05$) lower in flour samples of I010085, MM06/0045, Sauti and Mpale genotypes than in HQCF, but there were no significant differences ($p > 0.05$) between HQCF and the rest of the genotypes. Amylose content determines the stability of the viscous solution formed under the application of heat. In addition, the proportion of amylose to amylopectin is important for gelatinization and retrogradation of starch, and hence affect functional properties such as swelling power and viscosity (Charles, Chang, Ko, Sriroth, & Huang, 2004; Hasmadi et al., 2020). Genotypes I010040 and I020452; sagonja and mbundumali varieties had high starch and amylopectin contents with low amylose, hence could be used to produce thickeners, glue, adhesives, insecticides, and industrial alcohol in chemical industries.

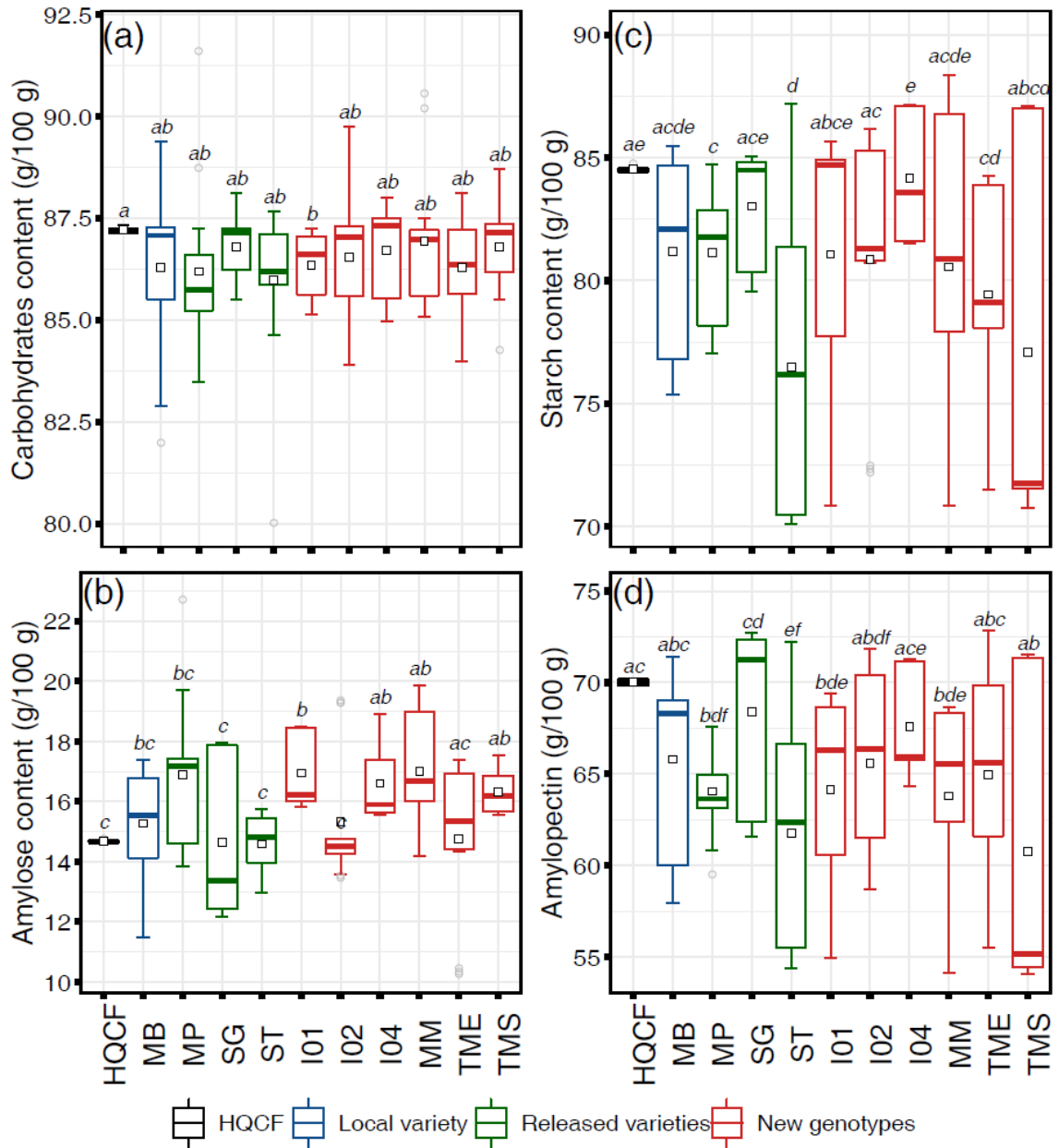


Figure 4.3: Distribution levels of chemical parameters, (a) carbohydrates, (b) amylose, (c) starch and (d) amylopectin), expressed on dry weight basis, for the studied cassava varieties, genotypes and HQCF. Sample types with the same letter have insignificant differences ($p = 0.05$). The small square in the boxplots indicates an average for each sample type. The dots, line-ends and upper and lower ends of the box represent outliers, spread, and first and third quartiles, respectively. Genotype and variety codes: SG = Sagonja; ST = Sauti; MB = Mbundumali; MM = MM06/0045; I01 = I010085; TME = TMEB419; MP = Mpale; TMS = TMSL110080; I04 = I010040; I02 = I020452.

4.3. Functional properties of cassava flours

Functional properties such as swelling power, water binding capacity and oil absorption capacity are important parameters for bioprospecting of flour from root crop, and in this study they were compared among the advanced genotypes and improved varieties (Figure 4.4). The genotype average values of swelling power of the flour samples ranged from 8.81 (Mpale) to 10.05 g/100 g (I010040). All varieties gave significantly higher ($p < 0.05$) values of swelling power than HQCF (Figure 4.4). I010040 had significantly higher ($p < 0.05$) swelling power than MM06/0045, Mpale, Sagonja and Sauti, but was comparable to Mbundumali and the other genotypes. However, all the genotypes had lower ($p < 0.05$) solubility, the average values ranging from 2.81 g/100 g (Sagonja) to 5.54 g/100 g (MM06/0045), than HQCF (average: 7.31 g/100 g). High swelling accompanied by low solubility obtained is due to high starch and amylopectin with strong associative forces in the starch granules of such cassava genotypes and varieties. Genotypes I010040 and MM06/0045; Mpale, Sagonja Mbundumali varieties had high swelling power implying high starch and amylopectin contents, hence could be used as thickeners in food, textile and confectionery industries.

The results presented in Figure 4.4 show that the values of water binding capacity of the tested genotypes flour samples ranged from 143.56 g/100 g (I00452) to 171.04 g/100 g (Mbundumali variety). In general, the genotypes' flours gave superior water binding capacity ($p < 0.05$) compared to HQCF (apart from genotypes I020452, I010040, TMEB419 and Sauti variety). Sauti, I020452 and I010040 genotypes gave a wide variation in water binding capacity (Figure 4.4). The values of water binding capacity reported here

are in the same range as reported by Aryee et al. (2006) who found that water binding capacity ranged from 113.66 g/100 g to 201.99 g/100 g. The results of water binding capacity indicate a good capacity of starch granules to hold water and further point to the extent of starch gelatinization (Padhan, Biswas, & Panda, 2020). Water binding capacity is used to describe the hydration properties of flour. Water tends to associate with hydrophilic substances in the flour. Genotype MM06/0045, TMSL110080 and mbundumali variety had highest water binding capacity which implies that they had weak associative forces between the starch granules, which allows for more molecular surfaces to be available for binding with water molecules, hence best suited for food and bakery industries.

The genotypes I010085, I010040, Mbundumali, Mpale and Sagonja had oil absorption capacity values comparable to HQCF (Figure 4.4). The average oil absorption capacity of the genotypes flour samples was between 130.01 g/100 g (MM06/0045) to 158.70 g/100 g (TMSL110080). Among the flour samples, TMSL110080 and I010040 genotypes had the highest values of oil absorption capacity (Figure 4.4). Oil absorption capacity entails components of cassava flour (and starch) to bind oil at hydrophobic sites. Oil absorption capacity of cassava flour (and starch) is important in industrial applications because it influences stability and uniformity of pastes and emulsions in confectionery, paints, textiles and adhesives industries, the stickiness of pasta during cooking and retention of flavour in the food industry (Kaur, Kaushal, & Sandhu, 2013) hence TMSL110080 and I010040 genotypes would be the best choice for adhesive, paints and textile industries as well as confectioneries.

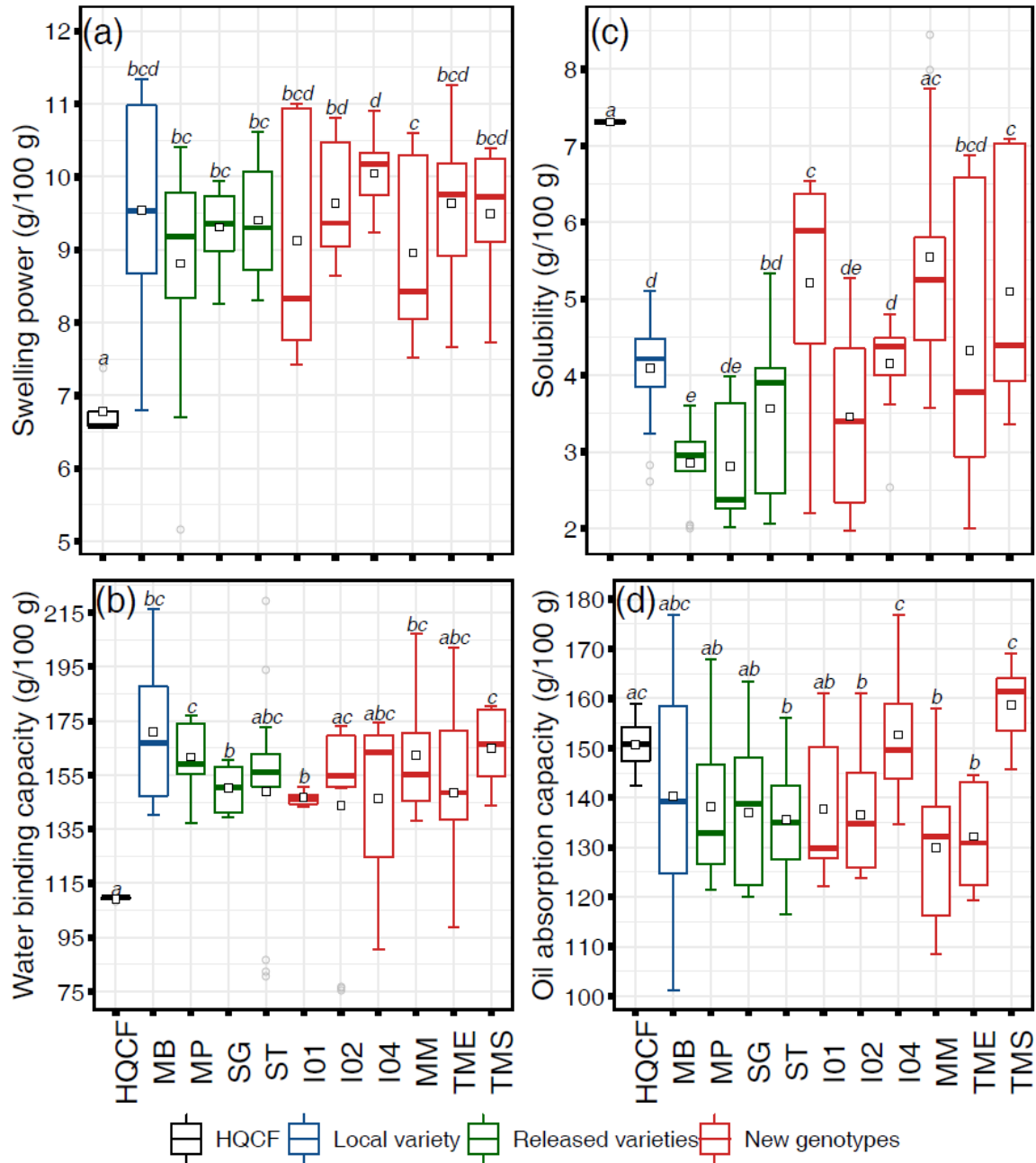


Figure 4.4: Distribution levels of functional properties, (a) swelling power (SP), (b) water binding capacity (WBC), (c) solubility and (d) oil absorption capacity (OAC)) for the studied cassava varieties, genotypes and HQCF. Sample types with the same letter have insignificant differences ($p > 0.05$). The small square in the boxplots indicates an average for each sample type. The dots, line-ends and upper and lower ends of the box represent outliers, spread, and first and third quartiles, respectively. Genotype and variety codes: SG = Sagonja; ST = Sauti; MB = Mbundumali; MM = MM06/0045; I01 = I010085; TME = TMEB419; MP = Mpale; TMS = TMSL110080; I04 = I010040; I02 = I020452.

The functional properties of improved cassava varieties and advanced genotypes flours vary according to their chemical parameters (Lu & Lu, 2012). Correlation analysis (Spearman) was conducted to elucidate further the relationship between the physico-chemical parameters and the functional properties. In addition, PCA was used to link correlations among chemical and functional parameters to the improved cassava varieties and advanced genotypes. The PCA and correlation analysis results indicate that starch and amylopectin content are the major determinants of variability in the functional parameters of the genotypes flours, such as water and oil absorption capacities, solubility, and swelling power. The other compositions seem to have a nominal contribution to the variability. Amylopectin content had a significant positive correlation with swelling power, total titratable acidity (TTA), pH, oil absorption capacity (OAC), bulk density (BD), solubility and water binding capacity (WBC) and ash. This was anticipated because the associative forces increases with high amylopectin (low amylose), decreasing intermolecular bonds leading to increase swelling. Also, short glycosidic linkages at branch points prevents intermolecular associations of carbohydrate polymers which allow water molecules to penetrate easily into the intermolecular spaces which enhances solubility (Moorthy, 2002).

In contrast, a negative correlation was found between the whiteness index and dry matter content (Figure 4.5). The functional properties and physicochemical parameters can also be split into four hierarchical clusters: carbohydrates, starch, amylopectin (alongside TTA, OAC and BD); ash and pH (alongside solubility and WBC); protein and amylose; dry matter (DM) and WI. Protein content showed negative correlations with oil absorption capacity, pH and dry matter (Figure 4.5).

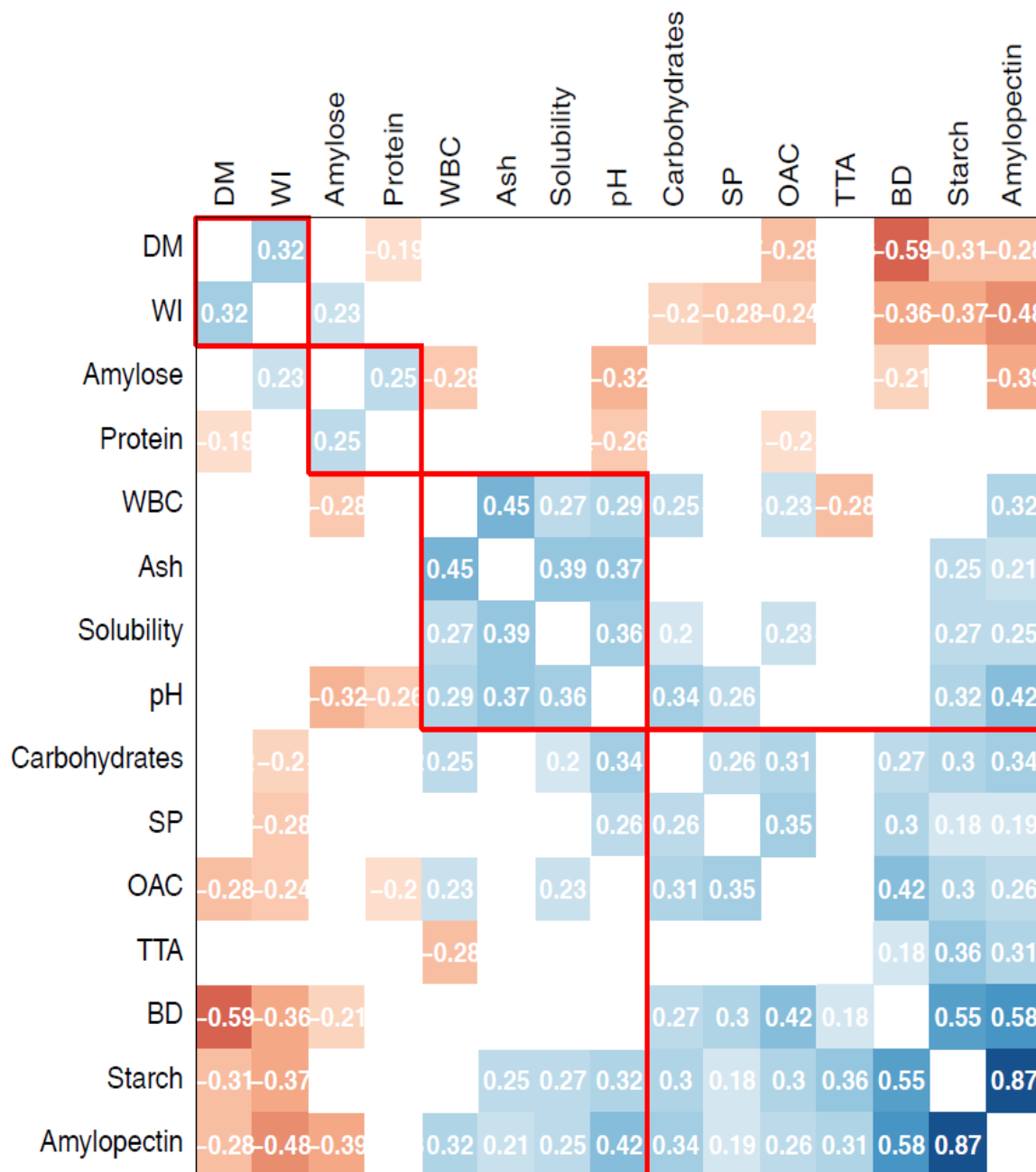


Figure 4.5: Correlation matrix of functional properties and physicochemical parameters for all genotypes and trials. Only significant Spearman's ρ values ($p < 0.05$) are shown. Variables are ordered by hierarchical clustering and red squares enclose four clusters, showing closely related functional properties and physicochemical parameters.

PCA gave four principal components (PCs), with eigenvalues ≥ 1 (Kaiser, 1958), which accounted for 66.89% of the total variance. In PC1, starch and amylopectin content, bulk density, total titratable acidity, and the functional properties (oil absorption capacity, water binding capacity, solubility and swelling power) are the dominant variables with the highest positive value, whereas dry matter content and whiteness index had the highest negative values. Positive sample scores along PC1 are typically high for flour samples for I020452 (I02), I010040 (I04), Sagonja (SG), whereas TMSL110080 (TMS) had a negative score (Figure 4.6). This implies that genotypes I020452, I010040, and Sagonja variety had high starch and amylopectin content, bulk density, total titratable acidity, swelling power, water binding and oil absorption capacities, and solubility. Since these parameters positively correlate with each other, this confirms the influence of high starch and amylopectin contents on the swelling power, water binding and oil absorption capacities, and solubility of cassava flours. As such, flours and starch from genotype I020452, I010040, Sagonja variety are best suited for bakery and confectionery industries as texture modifier, stabilizer and thickeners due to their high starch and amylopectin contents, swelling power and water binding capacity.

High starch will also enable genotype I020452, I010040, Sagonja variety to be used in pharmaceutical industries as fillers in making pills and tablets; binders and adhesives in paper making and plywood industries; and bio-fuel in energy industries. On the other hand TMSL110080 (TMS) had a negative score and dry matter content and whiteness index had the highest negative values, this means that TMSL110080 had highest fresh root dry matter content as well as whiteness index (WI), as such, TMSL110080 genotype may be used in

ethanol production in energy industry; chips and pellets in feed industry; and thickeners in food and confectionery industries. PC2 constituted mainly effects of approximate composition and has positive correlations with dry matter and ash content, and functional properties of solubility and water binding capacity, but was negatively correlated to protein content. PC2 is mainly associated with positive scores for Mpale (MP) and MM06/0045 (MM) genotypes (low protein content, Figure 4.6), and negative scores for Sauti (ST) (with high protein content, Figure. 4.6). Since high protein is associated with high water binding capacity, this implies that Sauti had high water binding capacity which is essential attribute for its application in food, bakery and cofenctionery industries.

PC3 has positive correlations with pH and water binding capacity and negative correlations with titratable acidity and amylose content. The dimension is positively related to Mbundumali variety implying that it had low amylose content (Figure 4.6) and negatively to HQCF and Mpale (MP) variety implying that they had high amylose content (Figure 4.6). Amylose is swelling inhibitor, the lower it is, the higher the swelling power, essential for texture of food products, hence Mbundumali variety is best suited for food and bakery industries due to its low amylose content. Mpale had high amylose content which provide stability, shape and integrity to confenctionery products such as candy pieces, sweet and gums. Therefore, Mpale is best suited for biscuit and confenctionery industries.

The last dimension has positive scores for I010085 (I01) genotype but negative scores for HQCF and varieties of Mbundumali (MB) and Sagonja (SG) (Figure 4.6). It is characterized by high values of ash, protein content and water binding capacity, associated with low oil absorption capacity. Protein content influences the water binding capacity of cassava flour and starch and this is confirmed by their positive correlations for PC4. This implies that I010085 had high protein content and water binding capacity, essential traits in food industry for bakery products.

Protein content may affect the rates and extent of hydration (water absorption) of flours. On one hand, hydrophilic polar or charged side chains may improve hydration (Lu & Lu, 2012). The entanglement of protein and starch could restrict swelling of starch granules (Chisenga et al., 2019), as shown by the negative correlation between protein and swelling power in PC2 (Figure 4.6). Amylose and amylopectin influence functional properties in cassava flour and starch such as crystallinity, gelatinization, retrogradation, gelling, and pasting (Julliano et al., 1987). However, amylopectin plays a more significant role in the hydration and pasting properties of cassava (Julliano et al., 1987). Amylopectin molecules can readily bind to water molecules using hydrogen bonds. High hydration increases the swelling, viscosity, and gelatinization ability of starch granules. (Babu et al., 2018; Singh, Dartois, & Kaur, 2010). This is consistent with the score along PC1. Cassava starch's swelling power is functionally beneficial in use as a thickener in the food industry for soups, gravies, baby foods, and breakfast gruels. Cassava flour with lower amylose content and higher amylopectin may produce better quality bread and noodles (Zi et al., 2019).

4.4. Variation of fresh root dry matter and cassava flour quality parameters with genotype and location

Phenotypic correlations of physicochemical parameters and functional properties revealed high significant ($p < 0.05$) positive correlations for starch content with amylopectin content, bulk density with swelling power (SP), and dry matter (on fresh root weight basis) with water binding capacity (WBC) (Figure 4.7). This implies that within the same trials (phenotypic correlations) genotypes and varieties with high starch content will definitely also register high amylopectin content. Such information is very important to plant breeders who will be certain that improvement in one parameter will simultaneously result in changes in other parameter. On the other hand, genotypic correlations showed high significant ($p < 0.05$) positive correlations for bulk density with oil absorption capacity (OAC), and dry matter content (on fresh root weight basis) with solubility and OAC (Figure 4.7). This implies that across all trials (genotypic correlations), bulk density influences OAC, and that solubility as well as OAC are influenced by fresh root dry matter content.

Both correlations showed relationship between physical parameters (bulk density and dry matter) with the functional properties (OAC, WBC, SP and solubility). The phenotypic correlations were more sensitive than the genotypic correlation for starch parameters (starch and amylopectin content) considered in this study. Starch and amylopectin content were the major determinants of variability in cassava flours' functional properties, such as water and oil absorption capacities, solubility, and swelling power (Figure 4.5). However, the study further looked at the expression of the crop in terms of functional properties where phenotypic correlation may be more appropriate (Benesi et al., 2008).

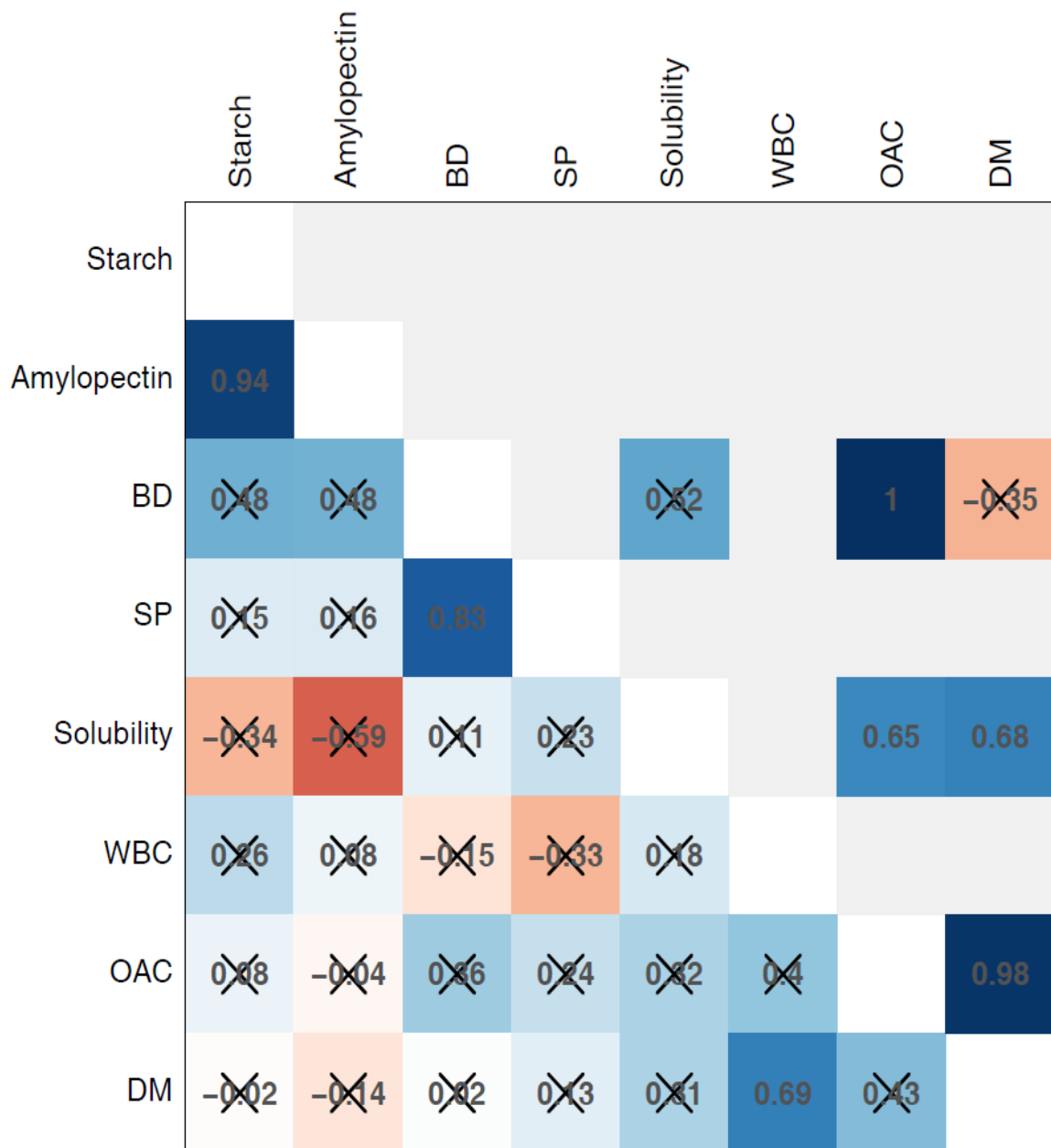


Figure 4.7: Phenotypic (below diagonal) and genotypic (above diagonal) correlation matrices for dry matter (on fresh root weight basis), bulk density, starch and amylopectin content and functional properties of cassava flours. $p = .05$, insignificant correlations are crossed out. Dark blue: $r^2 = 1$; dark red: $r^2 = -1$.

Variations of for bulk density, dry matter (on fresh root weight), chemical parameters (starch and amylopectin content) and functional properties (swelling power, solubility, water binding capacity and oil absorbance capacity) were highly significant for main effects (genotypes and locations) and their interaction (Table 4.2). Location played a major role in influencing dry matter (on fresh root weight basis), bulk density and solubility with contributions of 49.82 %, 85.10 %, and 85.10 %, respectively, of the total sum of squares (Table 4.2). The influence of location on dry matter (on fresh root weight basis) follows suggestions of CIAT (1995) that the performance of genotypes on root dry matter content strongly depend on on the edaphic-climatic and agronomic conditions (Benesi et al., 2008).

Genotype and environment interaction played a major role in influencing starch content, amylopectin content, swelling power, and WBC, with contributions of 49.97 %, 45.07 %, 58.42 %, and 50.17 %, respectively, of the total sum of squares (Table 4.2). OAC was influenced by genotype, location and their interaction with contributions of 22.19 %, 30.49 %, and 30.82 %, respectively, of the total sum of squares (Table 4.2).

Table 4.2: ANOVA for physicochemical parameters (bulk density, dry matter on fresh roots), chemical parameters (starch and amylopectin content) and functional properties (swelling power, solubility, water binding capacity (WBC) and oil absorbance capacity (OAC)) for cassava flours.

Source of variation	DF	Total	Location (L)	Rep (L)	Genotype (G)	G x L	Residual
		127	3	10	9	23	82
Starch content (g/100 g)	Sum of squares (SS)	3772.88	1206.13**	15.72**	620.1***	1885.17***	45.76
	Contribution to total SS (%)		31.97	0.42	16.44	49.97	1.21
Dy matter FWB (%)	Sum of squares (SS)	8135.60	4053.40***	150.50*	2176.70***	1147.60***	607.4
	Contribution to total SS (%)		49.82	1.85	26.76	14.11	7.47
Bulk density (g/mL)	Sum of squares (SS)	0.544	0.463***	0.006*	0.019***	0.030***	0.026
	Contribution to total SS (%)		85.10	1.16	3.54	5.47	4.73
Amylopectin (g/100 g)	Sum of squares (SS)	4154.01	1615.79***	10.25	595.55***	1872.01***	60.41
	Contribution to total SS (%)		38.90	0.25	14.34	45.07	1.45
Swelling power (g/100 g)	Sum of squares (SS)	156.60	11.76*	6.43	14.47***	91.47***	32.46
	Contribution to total SS (%)		7.51	4.11	9.24	58.42	20.73
Solubility (g/100 g)	Sum of squares (SS)	0.54	0.46***	0.006*	0.019***	0.030***	0.026
	Contribution to total SS (%)		85.10	1.16	3.54	5.47	4.73
WBC (g/100 g)	Sum of squares (SS)	95302	30486***	1291*	10454***	47810***	5261
	Contribution to total SS (%)		31.99	1.35	10.97	50.17	5.52
OAC (g/100g)	Sum of squares (SS)	34507.20	10523***	1576**	7657***	106370***	4115
	Contribution to total SS (%)		30.49	4.57	22.19	30.82	11.93

In general, the results from this study were in agreement with similar studies that reported variations in starch and dry matter content across genotypes (or varieties) and locations (Benesi et al., 2004; Ngendahayo & Dixon, 2001; Sanni & Olubamiwa, 2003; Sriroth, Pitachomkwan, & Wanlapatit, 2000). Variations in dry matter (on fresh root weight basis), starch and amylopectin content and bulk density were highly significant for genotypes and locations (Figure 4.8). Mkondezi was the best site for dry matter (on fresh root weight basis) followed by Njuli, with TMEB419, Mpale and Mbundumali as the highest yielding varieties and genotypes (Figure 4.8). Chitala was the best site for bulk density, starch and amylopectin content (Figure 4.8). Cassava genotypes and varieties that gave the highest starch content, amylopectin content and bulk density at Chitedze were Sagonja, I020452, TMSL110080, TMEB419 and I010040 for bulk density, I010040, TMSL110080 and I010085 for starch content and amylopectin content (Figure 4.8).

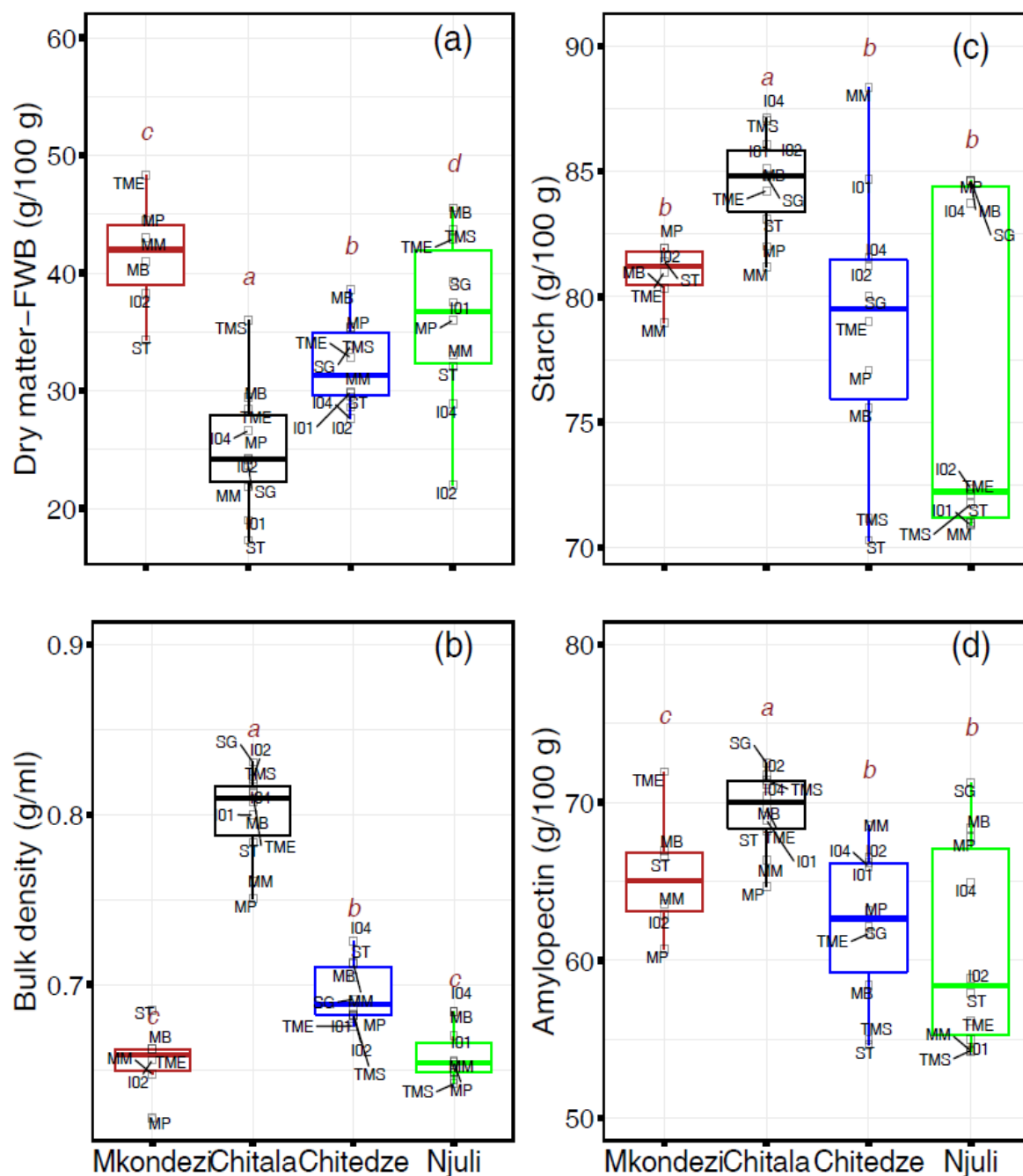


Figure 4.8: Dry matter (on fresh root weight basis), bulk density, starch, and amylopectin content (of cassava flours) of cassava genotypes and varieties for different trial sites. Research sites with the same letter have insignificant differences ($p = 0.05$). Genotype and variety codes: SG = Sagonja; ST = Sauti; MB = Mbundumali; MM = MM06/0045; I01 = I010085; TME = TMEB419; MP = Mpale; TMS = TMSL110080; I04 = I010040; I02 = I020452

In terms of functional properties, Mkondezi was the best site for WBC, whereas Njuli was the least performing site. Chitala was the best site for oil absorption capacity, swelling power and solubility (Figure 4.9). Cassava genotypes and varieties that gave high values at Chitedze were Mbundumali, Mpale, Sagonja, TMSL110080 and I010040 for OAC; Mbundumali, MM06/0045, I020452, TMSL110080, I010040 and I010085 for swelling power; and starch content and TMEB419, I010085, Mbundumali, MM06/0045, I010040 for solubility (Figure 4.9)

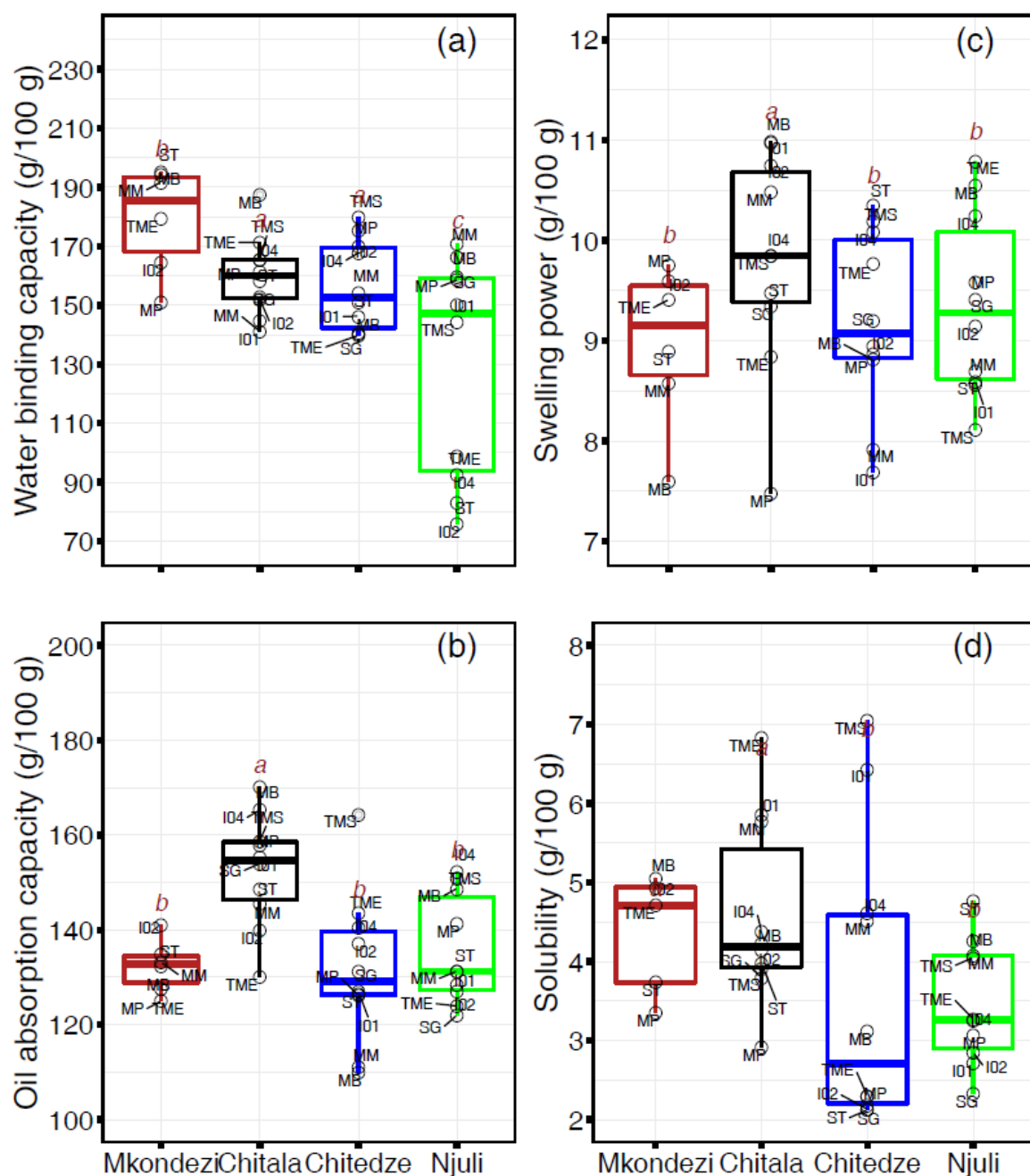


Figure 4.9: Functional properties (water binding capacity, oil absorption capacity, swelling power and solubility) of cassava flours from cassava genotypes and varieties for different trial sites. Research sites with the same letter have insignificant differences ($p = 0.05$). Genotype and variety codes: SG = Sagonja; ST = Sauti; MB = Mbundumali; MM = MM06/0045; I01 = I010085; TME = TMEB419; MP = Mpale; TMS = TMSL110080; I04 = I010040; I02 = I020452

The results show that the best sites for bulk density, dry matter (on fresh root weight), chemical parameters (starch and amylopectin content) and functional properties (swelling power, solubility, water binding capacity and oil absorbance capacity) were Chitala and Mkondezi study sites located in the Lake Shore agro-ecological zone. Mkondezi was the best site for dry matter content. Dry matter content is closely related to soil moisture content during 6-18 months of plant growth, which is a function of amount and distribution of rainfall (Ngendahayo & Dixon, 2001) as well as soil properties (IITA, 1990). Most of the sites have sandy loam and sandy clay loam soils (Table 3.1), which are the most preferred soil types for tuber development (Asher, Edwards, & Howler, 1980; Bationo et al., 2012; Rubatzky and Yamaguchi, 1997; Van Vlugt, Francke, & Giller, 2017) and also provide better soil nutrient retention (Byju and Suja, 2020). Mkondezi research station receives higher (> 1500 mm per year) (Figure 3.1) and well distributed rainfall than other stations (Benesi et al., 2008).

For optimum growth and production, cassava requires an annual rainfall of more than 1000 mm (Ekanayake, Osiru, & Porto, 1998; El-Sharkawy & Cadavid, 2002), although it can survive in a wide variation of rainfall conditions ranging from <600mm in semi-arid tropics to >1600mm in subhumid/humid tropics (Allem, 2002). High temperatures are also known to accelerate growth and formation of tubers of cassava (IITA, 1990; Nassar & Ortiz, 2006; Onwueme, 1978) and cassava is adapted to tropical semi-arid condition. The Lake Shore agro-ecological zone is characterized by relatively higher annual temperature than the mid-elevation agro-ecological zone. In Malawi, cassava is mainly grown along the lake shore areas of the central and northern regions (Kambewa & Nyembe, 2008). Optimum annual

mean temperatures for growth and tuber production are in the range 25–30°C for cultivars adapted to cool climates and 30–36°C for cultivars that come up well in hot-climate (Cock, 1985; El-Sharkawy, 2006; Keating & Evenson, 1979). The higher dry matter (on fresh root weight basis) and water binding capacity at Mkondezi site corresponds to higher rainfall and soil organic matter content (Howeler, 2002) than the other sites.

Potassium content in soil influences bulk density, starch and amylopectin content, solubility, swelling power and oil absorption capacity (Benesi et al., 2008). In Table 3.1, many researchers confirm what Benesi (2008) found that Chitala has high potassium content. Chitedze also has high soil potassium content (Benesi et al., 2008; Table 1), giving high levels of bulk density, starch and amylopectin content, solubility, swelling power and oil absorption capacity (Fig. 4.8 & 4.9). The critical level of soil exchangeable potassium for cassava is reported to be in the range 0.15-0.25 cmol/kg (Howeler, 1996). Potassium is important for starch synthesis and translocation, and tuber initiation and bulking (Howeler, 2002). Therefore, potassium is associated with total starch yield, root diameter and weight, storage cell size and number, and dry matter (Chua et al., 2020; Fernandes, Gazola, da Silva Nunes, Garcia, & Leonel, 2017; Kang, 1984; Kasele, Hahn, Oputa, & Vine, 1983; Malavolta, Graner, Courty, Brasile, & Pacheco, 1955; Obigbesan, 1977; Spear, Edwards, & Asher, 1979).

4.5. Stability of genotypes and varieties for fresh root dry matter and cassava flour quality parameters

Stability analysis was required since the interaction of genotypes by locations (Table 4.2) for bulk density, dry matter (on fresh root weight), chemical parameters (starch and amylopectin content) and functional properties (swelling power, solubility, water binding capacity and oil absorbance capacity) of the cassava flours were highly significant (Crossa, 1990). AMMI analysis of dry matter content (on fresh root weight basis) for G x E interaction, indicated that the first interaction principal component (IPC1) explained 62.4% of the variation from G x E interaction. IPC scores revealed that the most stable genotypes and varieties were MM06/0045, Mpale, TMEB419 and TMSL110080, with Chitedze as the most stable site, but Mkondezi as the most suitable for high yield of dry matter content (on fresh root weight basis). The most unstable genotype was I020452 and was most suited at Chitala (Figure 4.10). Chitala and Njuli were the most unstable sites for growing cassava intended for high dry matter content (Figure 4.10).

AMMI analysis for bulk density of cassava flours from the genotypes and varieties for interactions of genotype by location indicated that IPC1 explained 75.20% of the G x E interaction. IPC scores revealed that the most stable genotypes and varieties were I010085, I010040 and Mbundumali. Mkondezi was the most stable site, whereas Chitala was the most unstable site for bulk density (Figure 4.10). For amylopectin and starch, the most stable genotypes and varieties were TMSL110080, I010040, Sauti, TMEB419 and I020452. Mkondezi and Chitala were the most stable sites, with Chitala presenting a better opportunity for higher starch and amylopectin content.

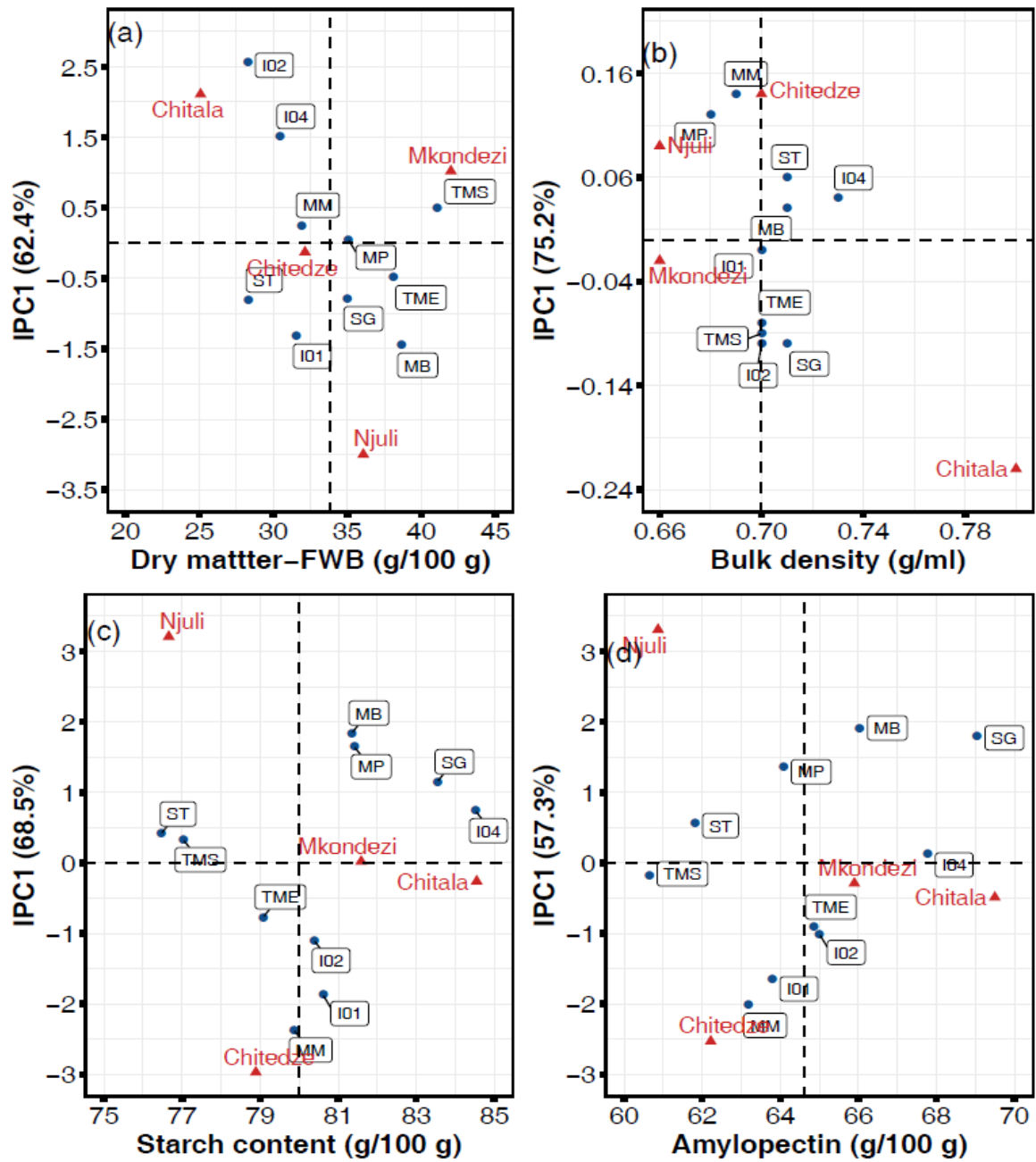


Figure 4.10: Biplot for AMMI IPC1 scores of the interaction term (GxE) against means of (a) dry matter on fresh root weight basis, (b) starch content, (c) bulk density and (d) amylopectin content of 10 advanced genotypes and varieties, and four environments. Genotype and variety codes: SG = Sagonja; ST = Sauti; MB = Mbundumali; MM = MM06/0045; I01 = I010085; TME = TMEB419; MP = Mpale; TMS = TMSL110080; I04 = I010040; I02 = I020452

MM06/0045 and I010085 were the most unstable genotypes and were best suited at Chitedze (Figure 4.10). In AMMI analysis for swelling power and solubility, IPC1 explained 58.80% and 59.80% of the variance from G x E interaction, respectively. TMSL 110080, I010040, I020245, Sauti and Sagonja as the most stable genotypes for swelling power (Figure 4.12). Mpale was best suited at Mkondezi and Chitedze, whereas Chitala was the most unstable location for swelling power (Figure 4.11). For solubility, IPC scores show that the most stable genotypes were I010040, Sagonja, MM06/0045, Mpale, I020452 and Mbundumali. TMEB 419 and Sauti were best suited at Chitala and Njuli, respectively. Chitedze was the most unstable location for solubility (Figure 4.11). For OAC and WBC of flours from the cassava genotypes and varieties, IPC1 explained 83.1% and 71.5.80% of the variance from G x E interaction, respectively. TMSL110080 was most stable genotype for WBC, followed by I010040 and TMEB 419 and the varieties of Mpale and Mbundumali. Njuli was the most unstable location for water binding capacity (Figure 4.11). IPC scores showed Sauti, Sagonja, I010085 and I010040 as the most stable genotypes and varieties for OAC, with Mkondezi as the most stable location. Chitala and Chitedze were the most unstable location for oil absorption capacity (Figure 4.11). This is attributed to Mkondezi's higher rainfall and soil organic matter content (Howeler, 2002) than the other sites which influence water binding and oil absorption capacities and fresh root dry matter content.

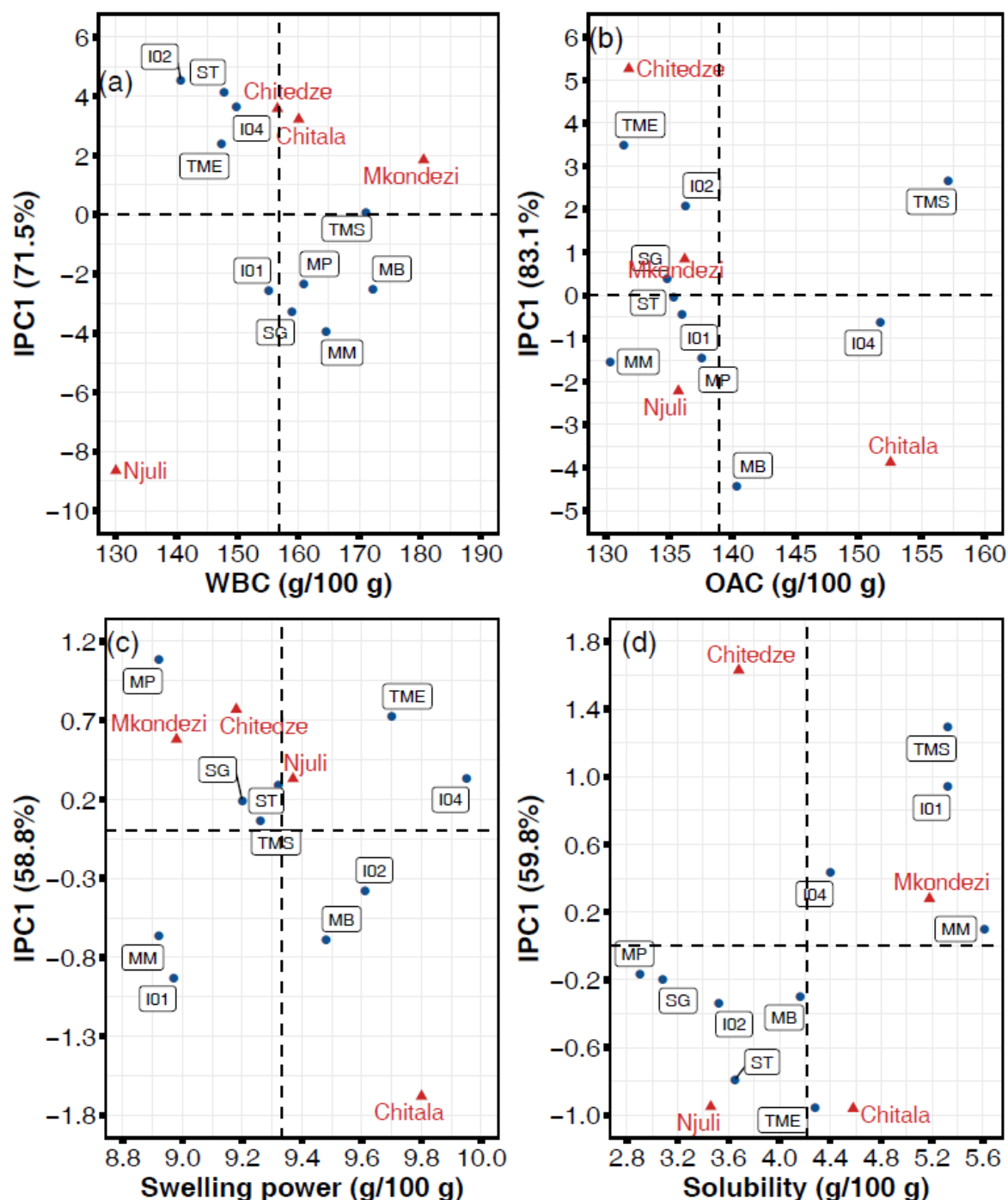


Figure 4.11: Biplot for AMMI IPC1 scores of the interaction term (G x E) against means of functional properties (a) Water binding capacity, (b) Swelling power, (c) Oil absorption capacity and (d) solubility of 10 advanced genotypes and varieties, and four environments. Genotype and variety codes: SG = Sagonja; ST = Sauti; MB = Mbundumali; MM = MM06/0045; I01 = I010085; TME = TMEB419; MP = Mpale; TMS = TMSL110080; I04 = I010040; I02 = I020452

Rankings of genotypes for the fresh root dry matter and cassava flour qualities varied from one location to the other and with the particular quality parameter. In order to obtain a summary of the rankings of stability for genotypes and varieties, Shukla's stability variance (Shukla, 1972) and Kang's yield-stability statistics (Kang, 1993) were calculated to identify stable genotypes and varieties by simultaneous selection for stability and response variable. The selected genotypes and varieties were then ranked according to AMMI stability value (ASV) and Yield stability index (YSI) to identify crops with better response and improved stability (Purchase, 1997; Sabaghnia, Sabaghpour, & Dehghani, 2008). The most stable genotype or variety is the one with the lowest ASV score and it is ranked 1. The YSI is based on the sum of the ranking due to ASV scores and yield or performance ranking. Genotype with low YSI value is the most stable with high mean yield performance, hence ranked 1.

The results of this selection and ranking are shown in (Figure 4.12). Mbundumali and I010040 were the most selected for both improved stability and better yield performance according to AMMI. I010040 showed higher levels of starch related properties (starch and amylopectin content, bulk density, OAC, solubility and swelling power) whereas Mbundumali yielded higher dry matter content (on fresh root weight basis) and also WBC (Figure 4.12). They are followed by Mpale and Sagonja varieties and MM06/0045 and TMSL110080 genotypes (Figure 4.13), with TMSL110080 as the highest yielding in dry matter content (on fresh root weight basis) (Figure 4.12).

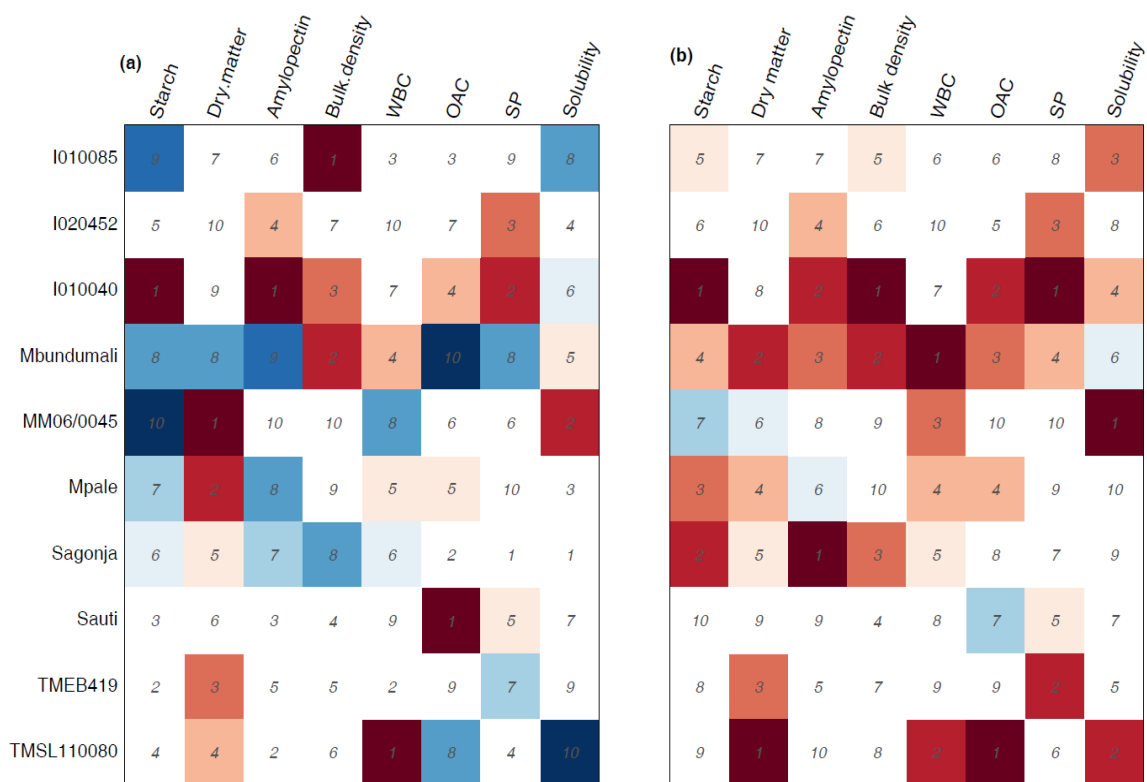


Figure 4.12: Summary of results of stability analysis for genotypes and varieties (a) with improved stability according to AMMI, (b) with better response and improved stability according to AMMI. Only genotypes and varieties that were selected according to Shukla's stability variance (Shukla, 1972) and Kang's yield-stability statistics (Kang, 1993) have been shaded. The number denotes a rank according to AMMI (a) stability value (ASV) and (b) Yield stability index (YSI) to identify crops with better response and improved stability (Purchase, 1997; Sabaghnia et al., 2008).

CHAPTER 5: CONCLUSION

5.1 Conclusion

This study has established the differences in physicochemical and functional properties of flours prepared from improved cassava varieties and advanced genotypes and evaluated their suitability for various industrial applications. The results of this study show that most of the varieties and advanced genotypes have particularly good physicochemical parameters and functional properties, which could be exploited for industrial uses. The PCA shows that genotype effect on overall industrial quality characteristics (as opposed to specific quality traits), was significant, which allowed genotypes with the potential to produce required quality characteristics to be identified.

The PCA and correlation analysis results indicate that starch and amylopectin content are the major determinants of variability in the cassava flours' functional properties, such as water and oil absorption capacities, solubility, and swelling power. The other compositions seem to have a nominal contribution to the variability. Overall, Sagonja, I020452 and I010040 show high starch and amylopectin content, bulk density, and functional properties (oil absorption capacity, water binding capacity, solubility, and swelling power). These genotypes show comparable-to-superior functional properties in comparison with currently used HQCF. Therefore, Sagonja, I020452 and I010040 can be processed into High Quality Cassava Flour (HQCF) and starch for processing of a wide range of products, including

food, beer, textile, paper, adhesives, chemicals, glucose, detergents, ethanol, cosmetic powders, pharmaceuticals and insecticides. As such, the results of the study contribute to the efforts of fast-tracking the selection of adaptable and preferred cassava genotypes for industrial applications.

The results of the effect of interaction of genotype with location on functional properties of cassava flours showed that location played a major role in influencing dry matter (on fresh root weight basis), bulk density and solubility. On the other hand, genotype and environment interaction played a major role in influencing starch content, amylopectin content, swelling power, and WBC. The appreciable influence of location supports the suggestion that the performance of genotypes and varieties on root dry matter (on fresh root weight basis) content strongly depend on the edaphic-climatic and agronomic conditions. In addition, dry matter content (on fresh root weight basis), high starch and amylopectin content, and their associated functional properties can be achieved by selection of suitable varieties and growing cassava in appropriate cassava production areas. In general, Mkondezi and Chitala, showed high levels of dry matter (on fresh root weight basis), starch and amylopectin content, bulk density and the functional properties, probably because of the role of high temperatures, rainfall and soil organic matter and potassium content in accelerating cassava growth and bulking of tuberous roots. Based on interaction principal component analysis of G x E, MM06/0045, Mpale, TMEB419 and TMSL110080 were the most stable genotype and variety for dry matter content (on fresh root weight basis) and Chitedze was the most stable site. I010085, I010040 and Mbundumali were the most stable genotypes and varieties for bulk density, and Mkondezi was the most stable

site. For amylopectin and starch, the most stable genotypes and varieties were TMSL110080, I010040, Sauti, TMEB419 and I020452. Mkondezi and Chitala were the most stable sites, with Chitala presenting a better opportunity for higher starch and amylopectin content. MM06/0045 and I010085 were the most unstable genotypes and were best suited at Chitedze. Mbundumali and I010040 were the most selected for both improved stability and better response according to AMMI. I010040 showed higher levels of starch related properties (starch and amylopectin content, bulk density, OAC, solubility and swelling power) whereas Mbundumali is high yielded higher dry matter content (on fresh root weight basis) and also WBC. They are followed by Mpale and Sagonja varieties and MM06/0045 and TMSL110080 genotypes, with TMSL110080 as the highest yielding in dry matter content (on fresh root weight basis).

5.2 Recommendations

Further research into the nature of stability of performance of cassava genotypes destined for industrial applications is required since most high-yielding improved genotypes in the trial did not completely outperform the released and local varieties in terms of stability. However, the study has identified I010040, MM06/0045 and TMSL110080 genotypes and Mbundumali, Mpale and Sagonja varieties as the most stable with high yield performance hence would be highly recommended for cultivation in wide range of environments for production of high quality cassava flour (HQCF) and starch for various industrial applications such as production of ethanol and industrial alcohol, adhesives, starch and glucose syrup, insecticides, sweeteners in chemical industries; thickeners, stabilizers, texture modifiers, and moulding powder in food, bakery and confectionery industries; pills and

tablets' fillers in pharmaceutical industries; binders and adhesives in paper making and plywood industries; fillers and stiffeners in textile and packaging industries; and chips and pellets in feed industries.

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