Rheological characteristics of wheat-cassava composite flour and quality of the resultant bread

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I, Manano John, declare that this thesis is my own independent and original work and has not previously been presented either in whole or in part for any academic award at this University or elsewhere.

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Dedication

This piece of work is dedicated to my mother Angey-ango Theresa, my father Manano Saverio, my wife Thoduba Joy, and my children Ogenmungu Samuel, Bedayika Anna Hope, Yoacel Ruth Favour, and Manano Benjamin Nickolas for the suffering they have gone through in the course of my studies. Saverio passed away while I was still at Secondary school. I will miss him forever since he suffered a lot for my studies but did not enjoy the fruits of his efforts. May his soul rest in Eternal peace.

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List of Abbreviations/Acronyms

ACORD: Agency for Cooperation and Research in Development

AFARD: Agency for Accelerated Rural Development

CARITAS: International/National Catholic Humanitarian Organisation

C:AVA: Cassava: Adding Value for Africa

CBOs: Community-Based organisations

CN_p: Potential cyanide in a substance (total cyanide present in the tissues)

CRCoE: Cassava Regional Centre of Excellence

DFA: District Farmers' Association

DSIP: Development Strategy and Investment Plan

DFID: Department for International Development, U.K.

EAAPP: Eastern Africa Agricultural Productivity Project

HQCF: High Quality Cassava Flour

MAAIF: Ministry of Agriculture, Animal Industry and Fisheries

NAADS: National Agricultural Advisory Services

NaCRRI: National Crop Resources Research Institute

NADIFA: Nakasongola District Farmers Association

NARO: National Agricultural Research Organisation

NCP: National Cassava Policy

NGOs: Non Governmental Organisations

SG2000: Sasakawa Global 2000

UNBS: Uganda National Bureau of Standards

USAID: United States Agency for International Development

ZARDI: Zonal Agricultural Research and Development Institute

Abstract

Uganda is a major producer of cassava in Africa. Cassava has great potential as a raw material for agro-industry. Currently, utilization of cassava in Uganda is limited to semi-processed products through the informal sector. The overall objective of the study was to use rheological properties in assessing the potential of wheat-cassava composite flour as an industrial raw material for bread production in Uganda, and thus enhance cassava utilisation. Consequently, the study assessed the chemical composition of High Quality Cassava Flour (HQCF) from five selected cassava cultivars (NASE 3, NASE 14, NASE 19, *Nyamatia*, and *Nyarukeca*) used in the study. Proximate composition, starch content, minerals and anti-nutritional composition were also determined using standard methods. Minerals were determined by Atomic Absorption Spectrophotometry (AAS) and cyanogenic glucoside by Picrate Paper Kit. The HQCF from NASE 14 cassava cultivar was prepared and utilised as partial wheat substitute for product formulation. Rheological characterisation of the composite flour/dough was carried out using the Mixolab, Consistograph and Alveograph. Physical and sensory evaluation of bread produced from the composite flours was performed under standard procedures. Bread quality attributes were correlated with rheological characteristics of flour/dough in order to provide knowledge about the existence of relationships among properties and define groups of rheological parameters that could characterise and discriminate between dough samples, since rheological parameters might influence the quality of the final product. Quantitative data were subjected to analysis of variance (ANOVA) using the IBM Statistical Package for Social Sciences (SPSS), version 23. Results were presented as Means ± standard deviations. Least significant difference (LSD) test was used to separate means for the different cassava cultivars. Differences between means were considered significant at $p < 0.05$. The Mixolab Profiler indices were systematically rounded to the nearest unit. A difference of 1 point on the Profiler was regarded not a significant difference. The relationship between proportion of cassava addition (%), dough rheology and bread physical and sensory properties was evaluated by principal component analysis (PCA) using XLSTAT (v.2.2, 2019). Moisture content of the HQCF produced from the selected cassava cultivars was determined by weight difference before and after drying of the samples in a hot-air oven based on the AOAC official methods of analysis. It ranged from 5.43 to 10.87 %, with significant ($p < 0.05$) differences recorded except between NASE 3 and NASE 19; ash ranged from 1.05 to 2.39 %, with significant ($p < 0.05$) differences between improved and local cultivars; crude fibre from 1.06 to 1.18 %, with NASE 19 and *Nyamatia* significantly ($p < 0.05$) differing from the rest; crude protein ranged from 0.74 to 1.51 % with significant ($p < 0.05$) differences between some cultivars; crude lipid ranged from 0.39 to 0.63 %, with significant ($p < 0.05$) differences between some cultivars; and starch contents ranged from 66.72 to 84.42 %, with significant ($p < 0.05$) differences among the improved cultivars and between the local cultivars. The mineral contents (mg/kg): calcium ranged from 13.15 to 16.56; iron ranged from 0.002 to 0.01; zinc ranged from 0.56 to 0.87; magnesium ranged from 3.58 to 3.88; and copper ranged from 0.002 to 0.14. Content of minerals differed significantly ($p < 0.05$) among cassava cultivars. The mineral content of HQCF was generally low implying that cassava is a poor source of minerals. The contents of anti-nutrients (mg/kg): cyanogenic glucosides ranged from 30 to 800, and were significantly ($p < 0.05$) higher in the local cultivars; phytates ranged from 661.33 to 984.64, and were significantly ($p < 0.05$) higher in improved cultivars; oxalates ranged from 90.6 to 227.8 and were significantly ($p < 0.05$) different in all the cassava cultivars, though the levels were generally higher in improved cultivars; and tannins ranged from 0.18 to 0.33, with significant ($p < 0.05$) differences among some cultivars, with levels generally higher in improved cultivars. HQCF from all

the five cassava cultivars contained higher levels of cyanogenic glucosides than recommended by Ugandan and East African Standards of 10 mg/kg, making them unsafe in the primary form for direct utilization as food and food raw materials for industries. Proper methods to detoxify cassava roots have to be designed in order to utilise high-cyanogenic cassava roots. The standards on HCN should be revised, since Indonesia has 40 mg/kg as her standard without any detrimental consequences on the population. Content of tannins were lower than the permissible level of 1.72 mg/kg in HQCF from all cassava cultivars in the study. The low content of essential minerals, iron, zinc and copper necessitates fortification of HQCF used as human food. Fortification of HQCF with zinc and iron is recommended if it is to be used in bread, as is already done with wheat flour as a policy in Uganda. The high content of phytates and oxalates in cassava requires some form of processing before consumption to reduce their chelating effects on minerals. The high starch content in the cassava cultivars make them valuable raw materials for starch and starch-related industries. Bread quality, expressed as overall acceptability, was positively correlated with Mixolab parameters amylase activity ($r = 0.957$), dough stability ($r = 0.749$), C4 ($r = 0.941$), and C5 ($r = 0.945$); and Consistograph parameter PrMax ($r = 0.913$) and WAC ($r = 0.890$). Crumb texture was positively correlated with Mixolab parameters DDT ($r = 0.880$), C3 ($r= 0.819$), C2 ($r = 0.807$), viscosity ($r = 0.904$), mixing ($r = 0.843$), retrogradation ($r = 0.881$); Alveograph parameter P ($r = 0.803$); and bread volume ($r = 0.956$). Results showed that bread of acceptable quality can be processed using wheat-cassava composite containing not more than 30 % HQCF. There is thus, need for further research to design processes for higher inclusion levels of HQCF in the composite for bread making. Economic value of cassava can therefore, be realised through industrial processing of cassava-based products. Based on Mixolab parameters, DDT of not less than 1 min and dough stability of not less than 9 min are the parameter levels for wheat-cassava composite flour which are likely to produce bread of acceptable quality. Alveograph parameters P of not less than 78 mm H_2O and W of not less than 124 x 10^{-4} J; and Consistograph parameter PrMax of not less than 1675 mb are as well parameter levels for processing of high quality cassava-wheat composite bread of acceptable quality. Rheological properties of flour/dough can thus be used to assess the suitability of flour for bread making. Rheological parameters (dough stability, amylase activity, starch gelling, maximum pressure and water absorption capacity) can be used to predict specific volume, taste, and crust colour of the resultant bread. However, further research is required to assess the shelf-life of the composite bread, the suitability of flour from other cassava cultivars in composite with wheat for baking bread and the acceptability of the resultant bread by the general public/consumers.

Key words: composition**,** anti-nutrients, cassava cultivars, composite flour, bread, wheat, sensory, physicochemical, rheology, Mixolab, Alveograph, Consistograph, correlation

CHAPTER ONE: INTRODUCTION

1.1 Background

Cassava (*Manihot esculenta* Crantz) is a perennial woody shrub which is widely grown and consumed in tropical and subtropical areas. It has become the most important source of dietary energy in Sub-Saharan Africa (Goering, 1980; Scott et al., 2000; Hillocks, 2002; Sautter et al., 2006; Ndubuisi and Chidiebere, 2018; Gaffney et al., 2019; Matovu et al., 2022). It provides the food energy intake for nearly a billion people in 105 countries worldwide and is considered as the cheapest source of starch used in more than 300 industrial products (Zainuddin et al., 2018). Uganda is one of the major cassava producers in the world, with a production of 2,979,000 tonnes in 2019, ranking her 19th in the world (USDA, 2020). It provides 30 to 50 % of all calories consumed (Scott et al., 2000; Hillocks, 2002; Mbanjo et al., 2021).

Cassava is the second most important food crop in the country after plantain with production estimates of 2.842 million metric tonnes per annum (FAOSTAT, 2020). However, at the the moment, utilization of cassava is limited to semi-processed products such as flour, fermented sun-dried chips, roasted cassava, through the informal sector. Cassava is perceived as a low value famine reserve crop for the poor (Cock, 1985; Hillocks, 2002; Kleih, 2012; Vuong, 2012; Dixon et al., 2013; Zainuddin et al., 2018); and as such many have ignored the need to add value to it, unlike West Africa, Latin America, and Asia, where root crops are processed into a range of higher value products. Therefore, in Uganda, processing has not been developed as in other tropical countries (Westby, 2002; Kleih et al., 2012; Abass, 2013).

Much of the root harvested is consumed in the primary form after boiling or roasting or is used almost entirely for low value food products (Bokanga, 1995; Abass, 2013; Graffham

et al., 2000). Processing techniques are limited to traditional methods such as fermentation, open sun-drying, cooking/boiling, roasting, deep frying of chips. These generally produce low quality products that are constrained to low prices within the local marketing system. Cassava is processed into traditional products such as chips, flour, pancakes, beer, and gin (Ferris et al., 2002; Westby, 2002). The crop however, has technological potential as a raw material for agro-industrial products, such as flours in baked products, animal feeds and starch (Graffham et al., 2000; Westby, 2002).

Cassava is an important cash crop for smallholder farmers, middlemen as well as sellers in various markets and has become an important industrial crop (Mutyaba et al., 2016). Cassava tuberous roots are an excellent source of carbohydrates (38.6 % f/w) but contain very little protein (1.36 % f/w) (USDA, 2021). Cassava is traded either in the form of fresh roots or dried chips/flour. Kampala, with its expanding population, is Uganda's largest market for fresh cassava roots with supplies mainly from Masindi, Kiryandongo, Kigumba, Bweyale, Karuma, Lira, Apac, Luweero, Mityana, Masaka, Hoima, Kibaale, Mubende, Kyegegwa, Kyenjojo, Jinja, Kayunga, and Mukono. (Kleih, 2012; Waigumba et al., 2016).

Fresh cassava supplies from districts to the East of the capital (e.g., Busoga region) are still low. This is because former areas under cassava have been devoted to sugarcane production in much of Busoga region, which was a major cassava growing area in eastern Uganda (Waigumba et al., 2016). Kalerwe Market is the main wholesale market for fresh cassava in Uganda (Kleih, 2012; Waigumba et al., 2016). Other markets include St. Balikuddembe (Owino) and Kawempe. In addition to the capital Kampala, fresh cassava is traded in major towns/cities such as Jinja, Lira, Mbale and Mbarara. Although small quantities of roots are also traded in smaller towns, it can be assumed that a significant

proportion of the inhabitants of these rural towns are also cassava producers (Kleih, 2012; Ogwal-Omara et al., 2012).

One of the cassava products of superior economic value for farmers as well as various industries is cassava starch (Graffham et al., 2000; Westby, 2002). Industrial demand for high-value cassava products is at a small scale with dried cassava products (i.e., primarily dried chips or High Quality Cassava Flour (HQCF)) being the main products traded. HQCF is utilised mainly in the biscuits, paperboard, rural bakeries, agri foods (composite flours) sectors, with demand estimates of over 800 tonnes per annum (Kleih, et al., 2012). There is potential for substitution of wheat flour with HQCF in bakery sectors of up to 30 %, which may increase the demand for cassava (Kleih, et al. 2012). There is also potential for usage of HQCF or improved chips as adjuncts in beer brewing, as in the production of "*Ngule*" and "*Senator*" brands by Uganda Breweries Ltd. (Westby, 2002; Kleih, et al., 2012). Trials are underway at Nile Breweries Ltd. to produce "*Eagle Lager*" using cassava flour as an adjunct (Kleih, et al., 2012).

Wheat-cassava composite flour is used in confectioneries (substituting 30 % wheat flour) and bakeries (substituting up to 10 % wheat flour). The demand for High Quality Cassava Flour (HQCF) is low (9,000 MT) (Ogwal-Omara et al., 2012). The low level of utililisation of HQCF is attributed to its lack of availability in terms of quantity and quality (Westby, 2002). The composite flour sector represents an immediate market potential for more sales of either HQCF or improved cassava chips used in composite flour blending.

Cassava flour is already used in composite flour manufacture, especially for markets in the east of Uganda where a blend of 60 % cassava flour and 40 % millet is a common

staple food. As a result, more than 700 tonnes of cassava flour is currently consumed in composite flour manufacture (80 % as chips, 20 % as flour), and potential demand exists for at least a further 2000 tonnes per annum of HQCF (or high quality chips) (Kleih et al., 2012). Wheat-cassava composite flour is used in rural bakeries in local bread manufacture and as home-baking flour for production of non-bread products *chapattis*, *donuts*, *pancakes*, and *baghias*. There is however, a strong resistance in the urban bakery sector to consider substituting wheat flour with HQCF in bread manufacture (Kleih, et al., 2012).

Wheat flour posseses its intrinsic physical and chemical characteristics such as water absorption capacity, oil absorption capacity, swelling power/capacity, water solubility, pasting property, foaming capacity and foaming stability, emulsion stability, gel formation ability and maximum/peak viscosity. Non-gluten (gluten-free) flours have pasting temperatures greater than that of wheat flour, and are more resistant towards swelling. Low protein flours, e.g., cassava, have higher peak viscosities (Oladunmoye et al., 2010; Etong et al., 2014; Novie et al., 2018). Variations in physicochemical properties may cause variations in functional properties of flours from different sources (Schopf and Scherf, 2021).

The rheological properties of wheat are affected by inclusion of non-wheat flours to form composite flours. Composite flours have lower dough development time than pure wheat, lower dough stability (dough weakening), lower dough mixing time, lower resistance to dough extension, and increase the energy needed to work the dough. Non-wheat flours lower the volume and specific volume of the composite bread. The non-wheat flours increase the density and firmness of the composite bread produced. The bread colour may also be affected when the added flour does not have high protein content to react with available sugars to cause Maillard browning (Callejo et al. 2009; Chilungo, 2013).

The current study characterised selected cassava cultivars based on chemical compositions, physico-chemical properties of HQCF produced from the roots, assessed the rheological characteristics of the wheat-cassava composite dough, and formulated and evaluated quality properties of the composite bread to provide the basis for large-scale utilisation of cassava in bread making.

1.2 Problem Statement

Cassava is a major crop grown in Uganda, with production of 2.842 million tones per annum (FAOSTAT, 2020). Despite the high volume of the crop produced, it is not being used adequately to drive the economy of the country through value addition. Traditional cassava processing largely provides cassava flour of low quality targeting small scale local industries. Cassava-based products include flour, chips, pancakes, ethanol, *kwete* (a local opaque beer) and gin. Over 60 % of cassava produced in Uganda is consumed locally either in fresh or flour forms (Ogwal-Omara et al., 2012). According to Buyinza and Kitinoja (2018), about 88 % of cassava produced in Uganda is consumed by humans. Information on the composition of flour obatined from different cassava varieties is limited. Cassava breeding programmes in Uganda do not currently select materials based on flour making quality (Nanyonjo et al., 2021). The commercial utilisation of bitter cassava cultivars in Uganda is low.

Wheat flour contains proteins that interact with each other when mixed with water, forming gluten (Schopf and Scherf, 2021). It is this elastic gluten framework which stretches to contain the expanding leavening gases during rising, and provides structure to the bakery product (Monteiro et al., 2021; Schopf and Scherf, 2021). Cassava flour does not contain gluten-forming proteins. Therefore, partial replacement of wheat with HQCF would provide better utilisation of cassava for value addition.

The percentage of wheat flour replaced depends on the quality and quantity of the wheat gluten. Wheat and cassava blend in bread, cake and biscuit production are plausible means for wider utilisation of cassava. Value addition to the crop requires quality assessment of the composite flour/dough for high quality products. The East African Standard (EAS 741:2010) requires that the levels of substitution of wheat flour with cassava flour in composite flour shall have a minimum of 10 % weight of cassava in the composite flour. This therefore, is the government approved cassava flour substitution level into wheat flour as a basis for any food formulation in Uganda at the moment.

Investigations into the incorporation of HQCF into wheat flour for bread making have mainly concentrated on the overall acceptability of the composite bread to consumers (Oladunmoye et al., 2010; Tajudeen, 2013; Edwardo et al., 2013; Nwanekezi, 2013; Ayele et al., 2017; Agunbiade et al., 2017; Abass et al., 2018; Sampson, 2020) and the toxicity of the bread (Owuamanam, 2007; Eleazu et al., 2014; Iwe et al., 2017). In Uganda, production and utilization of HQCF in bread is still low. Information on the technological behaviour of HQCF-wheat flour composite is as well limited.

Few studies have focused on the rheological characteristics of the wheat-cassava composite flours in relation to the quality of the corresponding finished products (bread, *chapatti*, and pasta) (Jensen et al., 2015; Olubunmi et al., 2015). Dough rheology plays an essential role in baking products' quality prediction and may give information about dough behaviour during processing and therefore eliminate time delays between unit operations in correcting faults, and the use of final product quality as input to a feedback control loop (Amjid et al., 2013). Rheology is also important in understanding the bread staling process and how to control it (Barros and Franco, 2021).

Therefore, in this study, mixolab, alveograph and consistograph were used to investigate the influence of wheat flour substitution with 10 to 60 % of HQCF on the composite dough rheological properties. The effects of the flour blend on the properties of the resultant composite bread were as well investigated. The composite dough rheological properties were then correlated with the composite bread properties in order to establish relationships between them.

HQCF was produced from the roots of five selected improved (NASE 3, NASE 14, NASE 19), and local (*Nyamatia* and *Nyarukeca)* cassava cultivars. The HQCF were subjected to chemical analysis and physicochemical tests. HQCF from NASE 14 cassava cultivar was incorporated into wheat flour to form composite flour, and the composite flour subjected to rheological analysis. The composite flour was used to bake bread. Bread was chosen because of its increasing demand, convenience and commercial value. Rising urbanization and increase in the number of consumers shifting towards western lifestyles and food habits (diets) provide ready market for bread (Florkowski et al., 2012; Noorfarahzilah et al., 2014; Sibanda et al., 2015). Production of wheat-cassava composite bread can be a suitable alternative to utilize the potential of lowly exploited cassava.

1.3 Justification

The Ministry of Agriculture, Animal Industry and Fisheries (MAAIF)'s Development Strategy and Investment Plan (DSIP) (2013), objective 3, stressed the importance of value addition to agricultural produce to drive the economy of Uganda. Uganda received $30 million under the World Bank's East Africa Agricultural Productivity Project (EAAPP, 2010, 2015), and was chosen to lead research on cassava and share new technologies with Kenya, Ethiopia and Tanzania. This has led to a rise in cassava production in the country.

The Agricultural Technology Transfer (AgriTT) Cassava Value Chain Development Project (2013 – 2017), the trilateral cooperation project through which the Government of the Republic of Uganda (GoU), Department for Foreign and International Development (DFID), United Kingdom (UK), and the Government of the People's Republic of China through Ministry of Agriculture have jointly supported the development of cassava production, productivity and value addition in 4 districts (Masindi, Kiryandongo, Bullisa and Hoima) in Mid-Western Uganda. The project addressed three main areas: cassava production; cassava processing; and cassava value addition (Africa Innovations Institute, 2018). Cassava needs to be utilised industrially to provide market for the increased production.

The level of industrialization of Uganda is likely to increase demand for cassava products for the pharmaceutical, food, plywood, and textile industries. Total world production/use of cassava is projected to increase from 172.7 million tonnes to 275 million tonnes in the period 1993 to 2020 (Scott, Rosegrant and Ringler, 2000), and Africa is anticipated to account for 62 % of total world production/use (Scott et al., 2000). According to Sowcharoensuk (2020): Industry Outlook 2022 – 2024: cassava industries, in 2019, a total of 304 million tonnes of cassava were produced globally, and Africa contributed 63.3 % of the total, and was therefore the most important cassava-producing region, followed by Asia (28.0 %), the Americas (8.6 %) and Oceania (0.1 %).

By individual country, Nigeria was the world's biggest producer, with 19.5 % of all outputs globally, followed by the DR Congo (13.2 %), Thailand (10.2 %), Ghana (7.4 %), Brazil (5.8 %) and Indonesia (4.8 %). According to Scott (2021), between 1961– 63 and 2016 –18, cassava output increased from 75 to 282 million tones, more than that of any roots, tubers and banana in the emerging economies. Practically, all of that increase took

place in sub-Saharan Africa (SSA) with 138 million tonnes and South East Asia (SEA) (26 %) with 58 million tonnes in response to growing demand for both food and processed products.

In 2019, Uganda was the 22nd biggest producer of cassava in the world at 2.979 MMT (FAOSTAT, 2020). In Uganda, cassava produced is largely used for human food while nearly half of the production is used in processed products. Cassava: Adding Value for Africa (CAVA) II Uganda had the intention of creating an anual demand for 69, 030 tonnes of fresh cassava roots by 2019 (CAVA, 2018). This argument was based on demands of HQCF during the period 2014 to 2016: breweries - 22,020 tonnes; composite flour millers - 5, 859 tonnes; biscuits and bakery - 2, 934 tonnes; gari - 868 tonnes and paperboard - 51 tonnes.

Partial substitution of wheat with HQCF will likely lower production costs for bakery products, result in lower retail prices of the bakery products and hence make them affordable for the majority of the rural communities. Researches in some countries have demonstrated that cassava flour can partially substitute wheat flour in the baking of bread and cakes (Shittu et al., 2007; Adeniji, 2013; Aryeetey et al., 2019). This study was intended to contribute to the reduction in overreliance on wheat flour as the only raw material for bread and cake making.

1.4 Objectives

1.4.1 Overall Objective

To use rheological properties of wheat-cassava composite flour in assesing the potential of the flour blends as an industrial raw material for bread production in Uganda.

1.4.2 Specific Objectives

1. To determine the proximate composition, starch content, mineral content, and anti-nutritional compounds of HQCF produced from the roots of sweet NASE 3, NASE 14, NASE 19, and bitter *Nyamatia* and *Nyarukeca* cassava cultivars.
2. To determine the physico-chemical characteristics of HQCF obtained from the roots of the selected cassava cultivars in (1) above.
3. To determine the rheological characteristics of wheat-cassava composite flour/dough containing 0 to 60 % HQCF.
4. To assess the physico-chemical and sensory qualities of bread developed from the wheat-HQCF composite flour, and correlate these qualities with rheological characteristics of the composite flour/dough.

1.6 Hypotheses

1. There are no differences in proximate composition, starch content, mineral content and anti-nutritional content among the HQCF produced from the selected cassava cultivars grown in Nebbi district, and the HQCF conform to East African standard DEAS 779:2012.
2. There are no differences in the physico-chemical properties among the HQCF produced from the selected cassava cultivars.
3. Incorporation of HQCF into wheat flour does not affect the wheat's rheological characteristics and the quality of the resultant composite bread.
4. There are no correlations between the rheological characteristics of wheat - HQCF composite flour/dough and the quality of the resultant composite bread.

1.6 Significance of the study

The study will identify the impact of HQCF on wheat flour dough rheology in order to find the optimal values of the properties that give the best technological parameters and thus, provide evidence that HQCF can be used to substitute wheat flour in bread making. Results will provide millers and manufacturers with scientific evidence of an optimal substitution level of refined wheat flour to produce quality bread; provide information to help researchers develop cassava cultivars with the appropriate characteristics for the end users (low cyanogen level, high starch content); help cassava root processors produce HQCF which meets the regulatory requirements; and initiate the debate on the need to revise Uganda Standards on cyanogen content of cassava flour. The cassava cultivars used in the study are cultivated in Uganda hence, the research findings can be adapted in commercial bread production, leading to commercial cultivation of cassava.

CHAPTER TWO: LITERATURE REVIEW

2.1 Taxonomy of cassava

Cassava (*Manihot esculenta* Crantz), is a single species, belonging to the family *Euphorbiaceae*. Of the 98 species that belong to the genus *Manihot*, cassava is the only species that is widely cultivated for food production (Rogers and Appan, 1973; Onwueme, 1978; Mkumbira, 2002). Cassava cultivars have been classified according to morphology, e.g., leaf shape and size, plant height, stem and petiole colour, inflorescence and flower colour, root shape and colour, and levels of cyanogenic glucosides in the roots (Onwueme, 1978; Mkumbira, 2002).

Cyanogenic glucosides are used to place cassava cultivars into two major groups: bitter cultivars, in which the cyanogenic glucosides are distributed throughout the tuberous root, at levels higher than 100 mg/kg fresh root weight, and sweet/not bitter cultivars, in which the cyanogenic glucosides at low levels are mainly confined to the peel. The flesh of sweet/not bitter cultivars is therefore relatively free of cyanogenic glucosides (Mkumbira, 2002). Early literature on cassava therefore described the genus as having two edible species, *Manihot utilissima* Phol and *Manihot aipi* Phol delineating cultivars with high and low cyanogenic glucoside concentration, respectively.

Concerns about the possibility of poisoning led to cassava roots being classified according to their potential toxicity (Rosling, 1987). This procedure has been adopted by institutions involved in the growing and distribution of cassava cultivars. Initially, cassava plants were classified into three categories based on their potential cyanogenic content expressed in fresh pulp: a) harmless (less than 50 HCN_p kg^{-1}); b) moderately poisonous (between 50 to100 mg HCN_p kg^{-1}), and c) dangerously poisonous (above 100

mg HCN^-_p kg^{-1}) (Bourdoux et al., 1982; Jansz and Oluwaduge, 1997). Due to this classification the recommended limit for safe intake of cyanide from cassava was established by the FAO/WHO as 10 mg HCN_p kg^{-1} of body weight (FAO/WHO, 1991).

Only the released cyanide (CN) characterizes its toxic effects. The levels of CN in fresh cassava roots are indicative of toxicity, and after processing (by cooking or industrial uses) these levels are reduced due to release of enzymes, volatilization or entrainment by water (Tewe, 1992). Subsequently, the cassava plants are classified in Uganda as either “sweet”, e.g., NASE 3, NASE 14 and NASE 19 or "bitter", e.g., *Nyamatia, Nyarukeca* (Ameny, 1990; Nakabonge et al., 2018; Matovu et al., 2022). The term "bitter" cassava, as opposed to "sweet" cassava, refers to the taste of the root parenchyma. Since the morphology of the cassava plant or the bitterness of its roots cannot be used to assess the cyanide content (King and Bradbury, 1995), laboratory analysis is the only sure way of establishing the free and potential cyanide content (Brito et al., 2009).

2.2 Origin and spread of cassava to Africa

Cassava is an ancient crop species. The species *Manihot esculenta* are believed to have originated from the tropical regions of North and South America (Corbishley and Miller, 1984). It was domesticated before recorded history in Brazil and Paraguay, and forms of the modern domesticated species can be found growing spontaneously in the south of Brazil. Hillocks (2002) citing Purseglove (1968), reported that cassava was grown as a crop in Peru some 4000 years ago and 2000 years ago in Mexico. It is estimated that domestication of cassava started 5000 to 7000 years BC and archaeological findings using starch particles from the Amazon supported this idea (De Boer, 1975; Chiwona-Karltun, 2001).

The crop is known under a variety of names according to the region in which it is cultivated: cassava or sometimes cassada in the English-speaking countries of North America, Europe and Africa; manioc in French-speaking countries; tapioca in the English-speaking countries of Southeast Asia; mandioca or aipim in Brazil and yucca/yuka, manioc or rumu in the Spanish-speaking countries of South America; ubi ketella or kaspe in Indonesia; balinghoy or kamoteng kahoy in Phillippines (Corbishley and Miller, 1984).

The Portuguese first brought cassava to Africa in the form of flour or 'farinha'. The Tupinamba Indians of eastern Brazil taught the Portuguese techniques of *manioc* preparation and production, and they developed a liking for the various processed forms (Carter et al., 1997). The first mention of cassava cultivation in Africa dates back to 1558 (Silvestre and Arraudeau, 1983). Cassava was introduced to Uganda (Buganda) through present day Tanzania by Arab traders between 1862 and 1875 (Langlands, 1966). Following its initial introduction, cassava quickly spread to other areas of Uganda, as an anti-famine and anti-locust crop. In some places planting of a legal minimum of land under cassava was enforced by the colonial government (Ameny, 1990). It is currently one of the most important food crops in Uganda. It ranks second to bananas in terms of area occupied, total production and per capita consumption (Otim-Nape, 1990; Essers, 1995).

Cassava is called *muhogo/muwogo* in Swahili/Kiswahili, and this name (or a variation of the same, *mogo*) has been adopted by many communities in the western, central, and eastern parts of Uganda and in Lango sub-region. In Acholi and West Nile, cassava has been given local names. It is called *ongura* in Alur; *gbanda* in Madi, Kakwa and Lugbara (also called *olaa* in Lugbara); *gwana* (a mispronunciation of the word *gbanda*) in Acholi

(Ameny, 1990). However, based on the local names and traditional folklore of the Alur, the cassava plant grew wild in the thick forest of the Congo basin. It is believed that hunters brought back the stems from their hunting expeditions, which were used as planting materials in the different settlements before the cassava plant was finally domesticated.

According to Lugbara legends, cassava came to the current West Nile from Democratic Republic of Congo (DRC) through Vura County and then spread to other parts of Lugbara. The cassava plant was introduced to Acholi and Adjumani from across the River Nile. The local name *gbanda* also originated from DRC. *Olaa* was the name given to cassava by the Lugbara in Uganda. It is worth noting that West Nile was part of Belgian Congo before it was bought by the British to become part of Uganda Protectorate in 1914, and that the cassava names used in the West Nile sub-region do not derive their roots from Swahili.

Furthermore, resemblance of the climatic conditions of the Congo basin and Amazon basin can bring an insight into the possibility of cassava having grown in the two areas concurrently. According to Kizito et al. (2005), cassava is relatively recently introduced in Uganda. It is thought to have spread from Buganda to northwest (Bunyoro) around 1870, to the east in the early 19th century and to the north even later. Cassava generally had an extremely limited distribution in Uganda in 1900. The study does not, however, mention West Nile because by that time the region was not part of Uganda.

2.3 Cassava cultivars grown in Uganda

There are several cassava cultivars grown in Uganda. Farmers use traditional knowledge and preferences such as culinary attributes, storability in the ground, early maturity

(maturity period), taste, dry matter content, cyanide content (determinant of taste/flavour), diversity in forms of utilisation and cooking quality to select cassava cultivars. Farmers group local cassava cultivars into the bitter and sweet varieties. The bitter cultivars are believed to be more resistant to pests and diseases, are higher yielding and store better in the ground than the sweet cultivars (Mkumba et al., 2003; Nakabonye et al., 2018; Iragaba et al., 2021).

Bitternes is also a security measure as thieves cannot use/sell them. Cassava-breeding programmes have generated new cassava cultivars, commonly refered to as the improved cultivars. These breeding efforts have led to identification and deployment of cultivars with low HCN content which are safe for fresh consumption (Nanyonjo et al., 2021). The improved cultivars include the Namulonge Selection Series (NASE family) (NASE 1 to NASE 19), NAROCASS 1 and NAROCASS 2, NAM 130, Migyeera, Bukalasa 8, Bukalasa II. Local varieties include *Bao*, *Okonyoladak*, *Icilicil*, *Arawkirra*, *Tyeno*, *Oturolak Ayita*, *Ebwanatereka*, *Empologoma*, *Bintiminsi*, *Serere*, *Sukari*, *Kiwoko*, *Kulanabwana*, *Ongada*, *Akena*, *Ochide*, *Angaraba*, *Malukwa*, *Nyaraboke*, *Kakwale*, *Bamunanika* (Ameny, 1990; Kizito et al., 2005; Abele et al. 2007; Nuwamanya et al., 2010; Turyagyenda et al., 2012). In many parts of Uganda, farmers are mainly growing the improved varieties from the cassava breeding program and it is becoming difficult to find landraces (Nakabonge et al., 2018).

In Uganda, identification and collection of cassava germplasm has predominantly relied on vegetative characteristics. In traditional farming systems, the concept of a cultivar can encompass very diverse genetic entities. Traditional naming and classification systems are often based on traits that are perceived subjectively in so doing it is not uncommon to find confusion between cultivars or use of different names for the same cultivar (Kizito et

al., 2005). Ugandan farmers are actively involved in continuous testing and the adaptation of new planting materials to suite their situations and their need to keep genetic variation. The most widely adopted improved cultivars in Uganda are TME14, TMS30752 (NASE 3), MM96/4271 (NASE 14), I92/0067 (*Akena*), and I92/0057 (*Omongole*). TME 204 had by the time of this study been abandoned (Ogwal-Omara et al., 2012; Ntawuruhunga et al., 2013). The increasing growing of uniform crops may lead to vulnerability to diseases and pests; and local knowledge and crop diversity may be lost (Brush, 1995; Brush, 2000). It has been observed that farmers in Malawi, for example, continually replace and update cultivars (Moyo et al., 1999). The outbreak of a strange disease that attacked cassava gardens in Nebbi district was reported by Daily Monitor of 3rd December, 2020. It was suspected that the diseases could have been Cassava Brown Streak (CBSD) and Cassava Soft Rot. There was mass loss of cassava roots. If the farmers in the affected areas depended on a single vulnerable cassava cultivar, these losses could have resulted in untold suffering.

2.4 Cassava production trends in Uganda

In Uganda, cassava production is in substantial volumes with exceptional production in Northern and Eastern regions, in the districts of Lira, Apac, Gulu, Arua, Nebbi, Soroti, Kumi, Tororo, Pallisa, Iganga, and Kamuli (CAVA, 2007; UCA, 2008/2009). Cassava is the second most important food crop in Uganda (after bananas) and its production was estimated at 2,842 million metric tonnes per year, and sold 10, 223 tonnes in 2019, worth about US$ 2.80 million (FAOSTAT, 2020). Cassava production in Uganda has, however, not always been at that level. Higher production levels were recorded in the periods 1970 to 1978, 1981 to 1993, and 1998 to 2007 (the harvests reached an estimated 5.3 million metric tonnes per annum, worth about US$ 5.5 billion FAOSTAT, 2011), which ranked Uganda 6th in Africa (NCP, 2013), as depicted in Figure 1. Uganda has so far not

regained her lost position as one of the top ten cassava producers on the African continent. Though Uganda cassava production fluctuated substantially in recent years, it tended to increase through 2019 (6, 983,000 tonnes) to 2020 period ending at 4.21 million tonnes in 2020 (FAOSTAT, 2021).

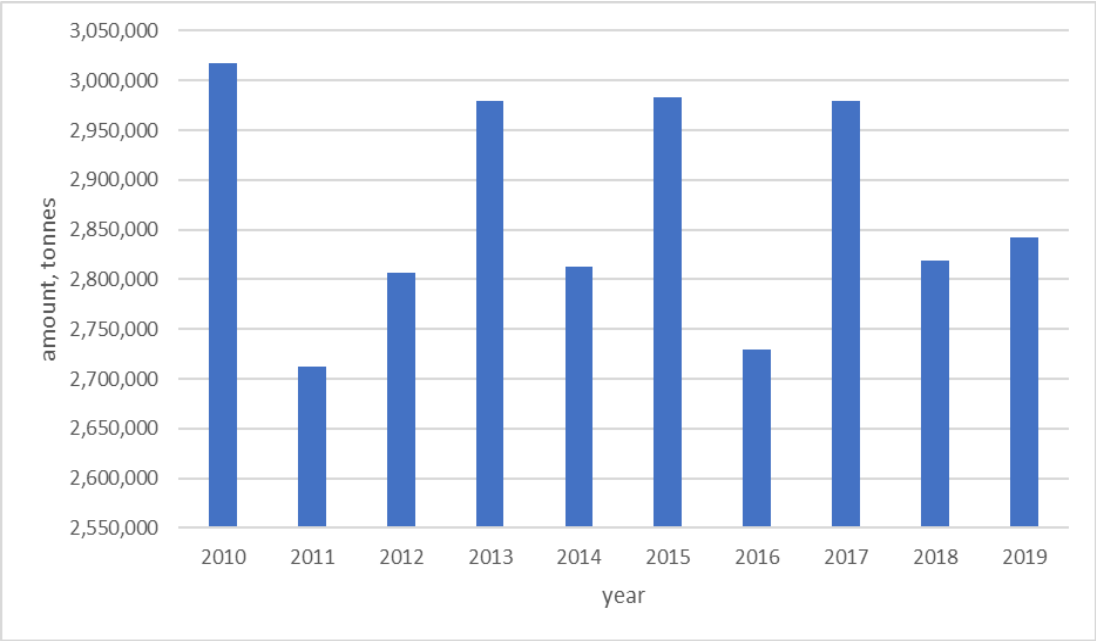


Figure 1: Trends in cassava producion in Uganda. Source: FAOSTAT (2020).

2.5 Main utilisation of cassava

2.5.1 Human food

Both cassava roots and leaves can be used as food, although economically the roots are more important. In parts of some African countries, e.g., the Democratic Republic of Congo, the leaves may be as important as or more important than the roots, accounting for approximately 68% of all vegetable output in the country (Westby, 2002). The major limitations to the utilization of cassava roots are low protein contents, naturally occurring cyanogens and short shelf-life due to rapid postharvest physiological deterioration (PPD) following harvest (Ospina and Wheatly, 1992; Essers, 1995; Kajuna et al., 2001; Westby, 2002; Ndubuisi and Chidiebere, 2018; Zainuddin et al., 2018; Ewebiyi et al., 2020).

Cassava roots are susceptible to spoilage and can only be stored for a short time (24 to 48 h). The short shelf-life of harvested cassava roots limits market potential and discourages all stakeholders in the value chain. PPD causes reduced incomes for cassava farmers and lowers the reliable supply of cassava as raw material for industry (Zainuddin et al., 2018). Processing is undertaken to primarily detoxify the cassava product, to improve its palatability and to convert it to a storable form (Oyewole, 1992; Essers, 1995; Kajuna et al., 2001; Cardoso et al., 2005). Processing yields products that have different characteristics, which creates variety in cassava diets. Most of the cassava roots produced is used as human food. About 88 % of cassava produced in Uganda is consumed by humans, 50 % of which is processed. In addition to the starchy root, the leaves of the cassava plant are edible and rich in protein (Buyinza and Kitinoja, 2018).

2.5.1.1 Fresh roots

Cassava roots are a good source of energy (Montagnac et al., 2009). In Africa and some other countries where cassava is grown, it is a common practice to eat cassava raw, after removing the skin and rind (Essers, 1995; Balagopalan, 2002; Matovu et al., 2022). Sweet cassava cultivars are grown for this purpose, close to the homestead to discourage theft. Because of their perishability, most roots are usually consumed or marketed close to the centres of production. Fresh peeled roots from sweet cultivars are consumed as a vegetable in the raw form as a snack between meals or while harvesting or peeling the roots or when travelling (Lamcaster et al., 1982).

The fresh peeled roots may also be boiled, steamed, roasted or fried. Peeled cassava roots may be boiled and mingled to produce a thick paste called *mugundi* in Alur, *kwon but* in Acholi or *kwon omyeno* in Lango. Peeled cassava roots may also be chopped and cooked together with beans, peas, pounded groundnuts or meat to produce a complete meal called

aputa in Lango, *katogo* in Lusoga and Luganda. Peeled or unpeeled roots from sweet cassava cultivars are roasted in hot ash or charcoal. The roasted product is mainly eaten as a snack. Peeled roots from sweet cassava cultivars are also cut in small pieces and deep fried in oil. The fried product is popular in trading centers and towns/urban areas (Ameny, 1990).

In Southeast Asia, especially in the Phillipines, cassava rice or *landang*, as it is locally called, is prepared from cassava pulp. *Landang* is prepared by shredding the roots and pressing the grated pulp in a cloth until most of the juice is squeezed out (Asiedu, 1989). Pellets are formed after whirling the drained mass in a winnowing basket. Pellets of more or less uniform size are isolated by sifting. They are steamed and then sundried for several days. Alternatively, the roots are soaked in water in earthenware jars for 5 to 7 days to ferment or until when they begin to soften. The soaked roots are then macerated, the fibre is removed by hand and the mass is air-dried then placed in a winnowing basket and swirled until pellets are formed (Sanchez, 2008; Lebot, 2009). The pellets are steamed and dried. The end product is a substitute for rice or maize, and has a long shelf life of up to six months (Asiedu, 1989; Sanchez, 2008; Lebot, 2009).

2.5.1.2 Flour

Sweet cassava is generally consumed as a vegetable, whereas the bitter cultivars are usually processed into chips or pulp, as this type of cassava is potentially toxic. Bitter cassava contains cyanogenic glucosides, substances capable of producing hydrogen cyanide if the roots are consumed in the unprocessed form. Cassava flour in Uganda is traditionally produced by two major methods: fermentation and/or open sun-drying. Fermentation may either be wet or dry (heap, solid-state) (Lamcaster et al., 1982; Oyewole, 1992; Hahn, 1997; Kajuna et al., 2001; Ferris et al., 2002; Lebot, 2009).

Although the roots of sweet cassava cultivars can be processed into flour without undergoing fermentation, some communities typically consume cassava products arising from flour processed through fermentation (Nanyonjo et al., 2021).

2.5.1.2.1 Wet fermentation

The wet fermentation/detoxification requires a lot of water, and is thus practiced where water is readily available (Kasese, Maracha, and Koboko districts) (Oyewole, 1992; Hahn, 1997; Kjuna et al., 2001). Soaking in water could have been used originally as a means of storing the roots while others are being processed, and the technique was later incorporated as a normal processing procedure (Lamcaster et al., 1982). Taste may be another factor, where some groups preferred the flavour of flour made from slightly fermented roots. The soaking also makes the roots softer and easier to grate. The roots may be fermented when peeled or unpeeled.

The unpeeled roots are fermented at the banks of streams or in swamps or ponds (as practiced in Maracha and Koboko districts). The peeled roots are fermented in bins/containers filled with water. The end of fermentation is determined subjectively (when the cassava roots become soft or when the roots have stayed in the water for 3 to 5 days). When the roots have softened sufficiently, they are removed from the water, and broken into pieces or pounded into a pulp to increase the surface area for easy drying, and then sun-dried (Lebot, 2009). The flour produced has a bland taste (Lamcaster et al., 1982; Ospina and Wheatly, 1992; Oyewole, 1992).

2.5.1.2.2 Dry/heap fermentation

Dry (heap/solid-state/solid substrate) fermentation is practiced in drier places, i.e., areas with limited water supply (Kumi, Soroti, Nebbi, Arua, Adjumani, Kiryandongo, Masindi

districts). The process is traditionally applied on bitter cassava roots to reduce toxicity and bitterness, and give the flour a white colour, nice odour and taste and the ability to endour elasticity or stickiness to the final *ugali* produced from the flour (Essers, 1995; Hahn, 1997; Buyinza and Kitinoja, 2018; Nanyonjo et al., 2021).

Heap fermentation is also a preparative step in the production of the potent gin *waragi*. After harvesting, the roots are peeled and sun dried or withered (preliminary drying) to reduce the moisture content of the roots without complete drying of the roots (Nanyonjo et al., 2021). The first sun-drying phase is a sort of curing serving to inhibit bacterial growth (Lebot, 2009). Large roots are split into halves to ease the process of moisture removal. Slicing into smaller pieces is avoided because this will lead to increased surface for mould growth and hence increased labour demand during the cleaning procedure, during which the surface moulds are removed (Essers et al., 1995; Kajuna et al., 2001).
The peeled roots are heaped into a pile that is covered with dry leaves, grass, or sacks, which extends the time for enzymatic degradation of the available cyanogens (Lebot, 2009). Over a period of 5 to 7 days, molds grow on the roots and facilitate the fermentation/detoxification process by using the cyanide as a source of nitrogen for their growth (Murugan et al., 2012). The fermented cassava imparts a flavour that appeals to some consumers (Essers, 1995; Hahn, 1997).

The major fungal species involved are *Rhizopus, Cladosporium, Fusarium* and *Aspergillus, Curvularia, Penicillium* and *Mucor, Neurospora sitophila and Geotrichum candidurn* (Essers, 1995; Kajuna et al., 2001). The mycotoxigenic mould *A. flavus* has been isolated from dried cassava chips from Kumi district in Eastern Uganda (Kaaya and Eboku, 2010). Yeasts are also involved in cassava fermentation (Chacha and Mamiro, 2019). After fermentation, roots are uncovered and the surface molds are scraped away

with a knife (Essers, 1995; Nanyonjo, et al., 2021). The cleaned, fermented roots are then broken down into smaller pieces by pounded using a mortar and pestle, into a coarse pulp, to increase the surface area for faster drying. The pulp or flakes are spread on mats or tarpaulin under the sun until they are dry. This is also the time when the woody pith of the roots and the remains of the roots' attachment to the stalk are removed.

2.5.1.2.3 Sun-drying

The sun-drying method is practiced during the dry, hot seasons and in areas where water for fermentation is scarce (Essers, 1995; Hahn, 1997; Kajuna et al., 2001). This process involves peeling, grating and drying of roots mostly of sweet cassava varieties, to produce a product called e*muhogo*, similar to *amukeke*, in Ateso and *mutete* in Alur (Buyinza and Kitinoja, 2018), or *makopa and chinyanya* in Tanzania (Kajuna et al., 2001; Ndubuisi and Chidiebere, 2018). Once grated the cassava is easy to sun-dry, but depending on the location sun-drying can be a fairly slow process. The method is commonly practiced during times of famine/food inscurity as a shortcut in processing, and is supposed to produce flour of high quality. However, a certain degree of fermentation occurs during the relatively long drying period (and alternating drying and storage during the cool night), and during storage when conditions are humid imparting a dark colour and an acid taste to the product. Hence, long drying times must be avoided (Asiedu, 1989; Kajuna et al., 2001; Lebot, 2009). The growth of moulds may occur during the wet season and may lead to the development of mycotoxins (Kajuna et al., 2001; Lebot, 2009), which may render the product non-marketable.

The dried product from the detoxification processes is sold as "local chips," a mixture of small granules and large coarse pieces (Kajuna et al., 2001; Tivana et al., 2007; Nakabonge et al., 2018). The dried chips or pulp are taken to local hammer mills for

milling into fine flour that is most desirable. Cassava chips or flour are used by the household or sold. Consumers rehydrate the flour in boiling water by mingling to produce the food, locally known as *posho* or *ugali* as a single type of flour or the cassava flour is blended with finger millet/sorghum/maize flour to make *ugali*, known by different names in different communities: *kwon/kwen* in Lwo, *atapa* in Atesoo, *anyasa* in Lugbara, *ocada*/*chawda* in Lusoga, *buwunga* in Luganda and *owusima* in Lunyole. Special names are given to *ugali* made from a blend of cassava flour and millet flour in Lusoga called *obuita* and *ugali* from a blend of cassava flour and sorghum flour called *mutama* (Ameny, 1990; Hahn, 1997; Kajuna et al., 2001; Kleih et al., 2012).

2.5.1.2.4 Other fermented cassava products

In some West African countries, cassava is boiled and pounded with boiled plantain to form an elastic dough called *fufu* which is consumed with vegetable or meat soups/stew (Hahn, 1997; Lebot, 2009). The main or most popular form in which cassava is eaten in West Africa is however, a product prepared from cassava roots known as *gari* (Asiedu, 1989; Hahn, 1997). In the production of *gari*, harvested cassava roots are peeled, washed and then grated. The resulting pulp is put into a cloth bag and subjected to pressure by heaping stones on the bag. After pressing, the bag is left for 2-3 days, during which time the pulp ferments. During this period most of the cyanide-containing juice from the cassava pulp is expressed from the bag. The fermented pulp is then removed from the bag, sieved and roasted or fried in wide, shallow metal pans until dry. *Gari* is customarily consumed in the form of meal, which is prepared by soaking the *gari* in water to swell the starch, and by making the swollen meal into dough. The dough is then made into a ball using fingers and is dipped into a stew containing ingredients such as palm oil, vegetables, meat or fish. *Gari* may also be eaten by soaking it in cold watet and adding sugar or milk (Asiedu, 1989).

In South America and Latin America, a product similar to *gari* called *farinha de mandioca*, is the most common form in which cassava is consumed (Lancaster et al., 1982; Asiedu, 1989; Hahn, 1997). The major difference between *gari* and *farinha de mandioca* is that the pressing and fermentation time for the latter are considerably shorter. Another product, *couac* (bread), is also produced from the drained, fermrnted pulp. The preparation of *farinha de mandioca* consists of peeling, grating, pressing, mixing, heating and roasting. The drained pulp is mixed with 3-day old prefermented pulp, pounded together and rubbed through a sieve, to form a slifgtly damp meal which is then heated in an open pan for 3 to 4 hours, with continuous stirring, to yield a granular, slightly roasted product. It is usually eaten with meat and gravy. *Couac* (bread) is obtained by heating the drained pulp more intensively, without stirring, until the mass is slightly brown on one side. After treating the other side, the product is then dried in the sun, giving it a longer shelf life. This cassava bread (*couac*) is very hard and is usually eaten after being dipped in gravy (Lancaster et al., 1982; Asiedu, 1989).

In Indonesia, modified cassava flour, *mocaf*, is produced using submerged fermentation. Harvested roots are peeled, washed, chipped and soaked (submerged fermentation for 48 to 72 h) (Seveline et al., 2020). The fermented mass is drained, dried, milled, sifted and stored or dispatched to the end users. The fermentation is carried out by selected biostarter, a single culture, co-culture or mixed culture. Spontaneous or natural fermentation is not encouraged in large-scale *mocaf* production due to the competitive activities of various micro-flora which results in different products and hence not meet mocaf standards. Lactic acid bacteria (LAB) are the starters mainly used, especially *Lactobacillus plantarum*; *L. plantarum* and *Bacillus subtilis*; *L. plantarum* and *Aspergillus oryzae*; *B. subtilis* and *A. oryzae* (Firdaus et al., 2014).

Mocaf is claimed to have better physical characteristics compared to ordinary cassava flour on viscosity, gelatinization ability, hydration capacity and solubility (Triyono et al., 2019). Besides, *mocaf* has the preferred aroma and sensory characteristics arising from the fermentation due to volatile organic compounds such as lactic acid, acetic acid and/or alcohol, which impart distinctive aroma and taste that overpower the original aroma and taste of cassava that tend to be unpleasant to customers. *Mocaf* is different from regular cassava flour which is produced without fermentation in that it has higher protein content and better physicochemical properties (odourless, smoother, whiter and longer-lasting). The quality of *mocaf* is determined by, among others, genotype/variety of cassava, and the age at harvest, duration of soaking /fermentation and the starter culture. *Mocaf* flour is commonly used as a substitute to wheat flour for foods such as instant noodles, cookies, biscuits, and in bakeries for bread making (Firdaus et al., 2014; Triyono et al., 2019).

2.5.2 Animal feeds

Pellets and chips from cassava roots are a source of energy in animal feeds. Chips are produced from fresh roots which are cut into slices not exceeding 6 cm in length, with a starch content of not less than 70% (Tewe, 2004; Raji et al. 2008). The roots are prepared, i.e., trimmed, peeled and washed. Because peeling operations require time, alternative methods to produce chips and pellets without peeling have been developed. One such method consists in grating and chopping unpeeled tubers, mixing them with cassava foliage in a 4:1 ratio and passing the mixture through a pelletizer (Tewe, 2004). The roots are sliced, and the slices are then dried, mostly in the open air, on special drying areas or concrete flours, turning them periodically for even drying until a storable moisture content of not more than 12 %. The dying period should not be long to avoid attacks by bacteria and moulds. Pellets are produced by grinding dried roots and compressing the powder into cylindrical shapes 2 to 3 cm long and 0.5 to 1 cm diameter

(Hahn et al., 1992; Tewe, 2004). Pelletizing results in a product that is 25 to 40 % denser, more uniform, more durable, less dusty and easier to handle (Hahn et al., 1992).

The most prominent exporting countries of cassava chips and pellets are Thailand, Malaysia, Viet Nam and Indonesia (Henry et al., 1998; Sowcharoensuk, 2020). In Uganda, there is a chip/pellet processing plant at Nakasongola, Nakasongola district (opened in 2006), by Farm-Africa, a UK based non-governmental organization (URN, accessed at www.radionetwork.com, 2021). It was intended to benefit among others, a local farmers' association – the Nakasongola District Farmers Association – (NADIFA). The amount sold and the location of the market is however, unclear. Another group in the district which has benefited from cassava projects in the country is Agaliawamu Cassava Processing and Marketing Cooperative Society Ltd (ACAPROMA).

2.5.3 Industrial cassava-based products

Cassava is an important raw material for the non-food industries. Cassava root is also a raw material for starch production, production of glucose syrup, and a potent gin, locally known as *waragi or enguli* or *lira lira*, *kwete* and *malwa*/*ajono* (Otim-Nape and Zziwa, 1990; Ogwal-Omara et al., 2012). Ethanol is now commercially produced at the Kamtech Logistics Ethanol Plant in Lira. There is also potential for usage of HQCF or improved chips as adjuncts in beer brewing, as in the production of "Ngule" and "Senator" brands by Uganda Breweries Ltd. Trials are underway at Nile Breweries Ltd. to produce "Eagle Lager" using cassava flour as an adjunct.

The low amylose, high amylopectin content of cassava starch give it the necessary viscosity for high quality adhesives and for use in the paper and textile industries (Kleih et al., 2012). Cassava starch is also used for the production of dextrins, which are utilized

in glues (Kleih et al., 2012). However, the starch industry in Uganda is not developed. Lira Starch Factory, which produced 5 tonnes of native starch per day, closed down in the 1980s (Kleih et al., 2012; Ferris et al., 2002). Another starch factory was established by Yogi Agro Industries Limited at Busoba Trading Centre, Mbale, in 2013. It is yet to become operational. By 2019 the factory was defunct (URN, accessed at ugandaradionetwork.net, 2021). It is understood that amongst other reasons for her collapse, the factory did not invest in developing a raw material supply chain and was therefore unable to acquire sufficient amounts of raw material (i.e., fresh cassava roots) from farmers (Graffham et al., 2017). As a result of the lack of a starch factory in the country, all the starch used by industries is imported.

Apart from the tuberous roots, cassava leaves are consumed in many countries, including Uganda. Cassava leaves are a good source of protein, vitamins A, C, B_1 and B_2 and E, B_1 and minerals (USDA, National Nutrient database, 2021) www.ars.usda.gov/nutrientdata, 2021). In the Demcratic Republic of Congo (DRC) and Central African Republic (CAR), the leaves may be consumed as a green vegetable or fermented to produce what is locally called *ntoba mbodi* (Kobawila et al. 2005; Ouoba et al., 2015; Jackson et al., 2020). In areas along the Uganda - DRC border the leaves are referred to as *sombe* (or *chombe*). The nutrient composition of cassava leaves varies in both quality and quantity depending on the cultivar of cassava, the age of the plant, geographical location, environmental conditions, processing methods and the proportional size of the leaves and stems (Gil and Buitrago, 2002).

2.6 Chemical composition of cassava roots

The chemical composition of cassava roots differs depending on variety, environmental conditions (soil type and climate) and maturity. Storage environment, region of

cultivation and post harvest/cultural practices (chopping, grating, soaking, drying, frying, boiling, and fermentation) further affect nutrient retention in the roots (Burns et al., 2012).

2.6.1 Nutritional value of cassava

The nutritional value of cassava roots is important because they are the main part of the plant consumed. The fleshy edible portion, consisting of parenchyma cells, makes up 80 to 90 % of the tuberous root and is composed of 60 to 65 % water, 30 to 35% carbohydrate, 1 to 2 % protein, 0.2 to 0.4 % fat, 1.0 to 2.0% fibre, and 1.0 to 1.5% mineral matter (Onwueme, 1978; Asiedu, 1989; Sarkiyayi and Agar, 2010). Most of the carbohydrate fraction contains starch which makes up 20 to 25 % of the tuber flesh (Purseglove, 1968). The roots also contain other carbohydrates (maltose, sucrose, fructose and glucose) (Montagnac et al., 2009; Salvador et al., 2014). The root is relatively rich in vitamin C (35 mg/100 g fresh weight), and contains traces of niacin and vitamin A, but low amounts of thiamine (vitamin B1) and riboflavin (vitamin B2) (Onwueme, 1978).

Cassava roots are rich in calcium, phosphorus, potassium, magnesium, iron, copper, zinc, manganese and sodium (Table 1). Calcium contributes to the development of bones and teeth, muscle contraction, the transmission of nerve impulses, blood pressure regulation and clotting of blood. Magnesium is an active component of several enzyme systems in which thymine pyrophosphate is a cofactor. Iron functions as haemoglobin in the transport of oxygen and as essential component of enzymes involved in biological oxidation. Copper is necessary for the growth and formation of bone and myelin sheaths in the nervous systems. Zinc functions as a cofactor and is a constituent of many enzymes; the primary roles of zinc appear to be in cell replication and gene expression and in nucleic acid and amino acid metabolism. Sodium is needed for proper fluid

balance, nerve transmission, and muscle contaction. Potassium is needed for proper fluid balance, nerve transmission, and muscle contraction. Phosphorus is important for healthy bones and teeth, found in every cell as an energy source, and forms part of the system that maintains acid-base balance in the body. Thus, deficiency or excess amount of these microelements can alter enzyme activities and influence important biological processes in the body (Maru et al., 2013).

Table 1: Nutritional composition of cassava root

Variable	Unit	Fresh root
		Proximate composition/100 g
Food energy	Kcal	110 – 149
Food energy	Kj	526 – 611
Moisture	g	45.9 – 85.3
Dry matter	g	29.8 –39.3
Protein	g	0.3 – 3.5
Lipid	g	0.03 – 0.5
Total carbohydrate	g	25.3 – 35.7
Dietary fibre	g	0.1 – 3.7
Ash	g	0.4 -1.7
Vitamins		
Thiamin	mg	0.03 – 0.28
Riboflavin	mg	0.03 – 0.06
Niacin	mg	0.6 – 1.09
Ascorbic acid	mg	14.9 - 50
Vitamin A	µg	5.0 – 35.0
Minerals		
Calcium	mg	19 – 176
Total phosphorus	mg	6 – 152
Ca/P		1.6 – 5.48
Iron	mg	0.3 – 14.0
Potassium	%	0.25 – 0.72
Magnesium	%	0.03 – 0.08
Copper	Ppm	2.00 – 6.00
Zinc	Ppm	14.00 – 41.00
Sodium	Ppm	76.00 – 213.00
Manganese	Ppm	3.00 – 10.00

Source: Asiedu (1989); Sarkiyayi and Agar (2010); USDA National Nutrient database (2021)

2.6.2 Toxic and anti-nutritional substances in cassava

Cassava, though an important food, contains toxic and anti-nutritional substances that interfere with digestion and uptake of nutrients (Table 2).

Table 2: Anti-nutritional composition of cassava root

Item	Unit	Dry Root
Anti-nutrients	mg	Contents per 100 g of Product
Cyanogenic glucosides	mg	0.46 – 0.65
Phytates	mg	216.0 – 304.0
Oxalates	mg	22.0 – 44.0
Tannins	mg	0.4 – 0.6
Trypsin inhibitor	mg	1.0 – 4.0

Source: Asiedu (1989); Sarkiyayi and Agar (2010).

2.6.2.1 Cyanogenic glucosides

Cyanogenic glucosides are bioactive natural products, sometimes reffered to as secondary metabolites, present in crop plants, forage plants, and trees (Heldt, 1997). More than 3,000 plant species are cyanogenic, including cassava, apricot, cherry, clover, flax, barley, sorghum, wheat, bamboo, eucalyptus, and poplar (Vetter, 2000; Ndubuisi and Chidiebere, 2018). The main purpose of the cyanogenic glucosides is to offer chemical defense response in order to protect the plant from being eaten by herbivores (animals) and from damage by pathogens (Moller, 2010; Ndubuisi and Chidiebere, 2018).

Cyanide is the most toxic factor restricting the consumption of cassava roots and leaves. It is associated with Tropical Ataxic Neuropathy (TAN) and glucose intolerance. All cassava varieties contain the toxic cyanogenic glucosides Linamarin and Lotaustralin (Figure 2), at levels of 150 to 300 ppm in peeled root or 300 to 900 ppm of the dry matter

(Asiedu, 1989; Aalbersberg and Limalevu, 1991; Wheatley et al., 1993; Cardoso et al., 2005, CIAT 2007). Cyanide in cassava can be found as bound glucosides, cyanohydrins, and free cyanide (Cooke and de La Cruz, 1982; Cooke, 1983; Heldt, 1997; Coultate, 1999; Ndubuisi and Chidiebere, 2018). Each of the three forms has different toxicity and reacts differently to processing techniques that remove cyanide (Cooke and Maduagwu, 1978). Cassava roots also contain other anti-nutriens such as phytates, oxalates, tannins, saponnins, fibre, nitrate, polyphenols and trypsin inhibitors that can reduce nutrient bioavailability (Sarkiyayi and Agar, 2010).

Linamarin Lotaustralin

Figure 2: Cyanogenic glucosides in cassava (Adapted from Nyirenda, 2020)

Cyanogenic glucosides are distributed in all parts/tissues of the cassava plant (White et al., 1998; Tivana, 2012; Bolarinwa et al., 2016; Ndubuisi and Chidiebere, 2018). The leaves and root peels contain generally higher concentrations of the glucosides than the parenchyma (the edible part of the cassava root), as depicted in Table 3.

Table 3: Total cyanogenic glycosides (CNp) in different tissues of the cassava plant

Plant tissues	**CNp**	**Reference**
	(mg HCN/kg) dry weight)	
Roots parenchyma	30-1200	Barrios and Bressani (1967)
(pulp)	30-1300	Wheatley and Cruzel (1993)
	160-700	Burns et al. (2012)
	81-500	Nambisan and Sundaresan (1994)
Roots cortex (peel)	50-770	Barrios and Bressani (1967)
	60-550	Wheatley and Cruzel (1993)
	800-1600	Burns et al. (2012)
	1800-1900	Nambisan and Sundaresan (1994)
Leaves	10-940	Barrios and Bressani (1967)
	400-800	Burns et al. (2012)
	1700-1900	Nambisan and Sundaresan (1994)

Cyanide content of cassava increases during periods of droughts and or prolonged dry weather due to water stress on the plant (Bokanga et al. 1994). For example, in Mozambique, about 55 % of the sweet fresh roots were found to be extremely toxic and the remainder moderately so during drought conditions (Cardoso et al. 2005). Similar observations were recorded in The Democratic Republic of Congo (Gitebo et al. 2009), and various citations in Africa (Cardoso et al. 2005). Splittstoesser and Tunya (1992) reported that cassava grown in wet areas contains lower amount of cyanide than that grown in drier areas. The effect of harvesting method on cyanide content is not clear, although injuring the roots increases rate of post harvest deterioration.

The cyanogen content of the roots of some cassava cultivars has been shown to reduce with the age of the root. *Migyera* cultivar (TMS 30572) has high levels of the glucosides from 3 to 9 months but relatively safe levels when the roots are 12 months old (Cooke and Elba, 1982; Hidayat et al., 2002; Chotineeranati, et al., 2006; Kolijin et al., 2007); *Nyarukeca* and *Nyamatia* (from Nebbi district) are very toxic/bitter before 12 months

after planting, but the bitterness becomes manageable after about 13 months after planting.

High content of cyanogenic glucosides and their breakdown products may constitute a nutritional problem in regions where cassava is the dominant or staple food. During processing, disruption of cassava tissues initiates hydrolysis of cyanogenic glycosides (Figure 3). Cyanogenic glycosides are leached from vacuole and come into contact with linamarase, a β-glucosidase, to produce acetone cyanohydrin from linamarin and 2-butanone cyanohydrin from lotaustralin (Conn, 1994). These cyanohydrins are unstable and decompose spontaneously to the corresponding ketones and hydrogen cyanide (HCN) at pH values above 5 and temperatures above 30 ºC. Cyanohydrin degradation can also be catalysed by α-hydroxynitrile lyase, located in apoplastic space (White et al., 1994).

The glucosides and their breakdown products are all toxic and should not be consumed in excessive amounts (Rosling, 1987). They are associated with diseases such as Tropical Ataxic Neuropathy (TAN) - a progressive disorder that mainly affects older adults, *konzo*, characterized by irreversible paralysis of the legs and other developmental disorder, goiter, and spastic paraparesis (Rosling, 1987; Banea-Mayambu et al., 1997). These disorders are apparent when there is excessive cyanide consumption and a poor dietary intake of sulphur-containing amino acids.

Mkumbira (2002) observed that cassava farmers in Malawi could examine and differentiate with ease 167 (92 %) of 181 cassava cultivars. Malawian farmers accurately classified cassava into sweet and bitter cultivars, and linked them to safety levels of cyanogenic glucosides (Chiwona-Karltun, 2001; Mkumbira et al., 2001). Processing methods are adopted based on toxicity levels of cassava. Since cassava in Africa is mostly

produced by smallholder farmers in marginal environments, they use a relatively large number of crops and crop varieties in trying to reduce risks in terms of food security and balancing their diet (Brush, 1995; Chiwona-Karltun et al., 1998). Sweet cassava cultivars have a high concentration of free sugars but it does not always follow that they have low concentrations of cyanogenic glycosides (King and Bradbury, 1995).

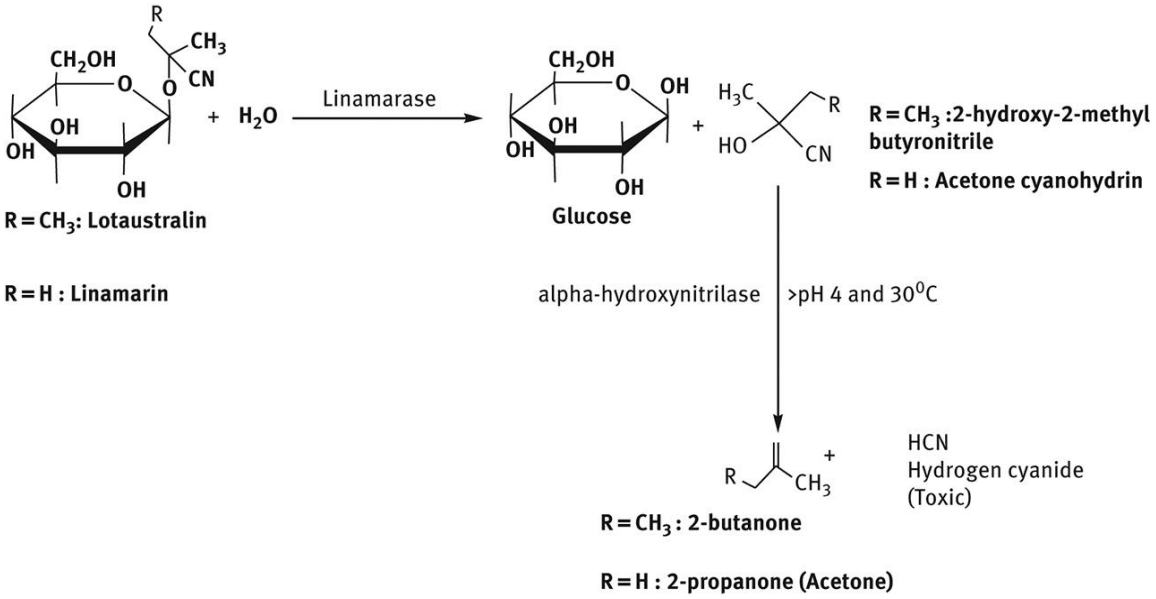


Figure 3: Hydrolysis of cyanogenic glucosides by β-glucosidase (Linamarase) (pH = 5.5) and α-hydroxynitrile lyase (pH ≥ 4, Temp. ≥ 30). Cited by Tivana (2012). Adapted from Conn (1994)

Bitterness in (and the general taste of) cassava roots does not, however, depend on the quantity of linamarin alone. Other substances associated with bitterness include apiosyl glucoside (isopropyl-β-D-apiofuranosyl-(1→ 6)-β-D-glucopyranoside), phenylalanine, tryptophan, citrate and malate (King and Bradbury, 2006; Jansz, and Uluwaduge, 1997). Consequently, bitterness of cassava roots is not always correlated positively with cyanide potential; neither can it be considered a guide to the relative toxicity of tubers anymore. Sundaresan et al. (1987) however, in a sensory evaluation of cassava cultivars found that bitterness in cassava was related to the cyanoglucoside content of the roots.

2.6.2.1.1 Management of bitterness and toxicity by cassava farmers

The forefathers of cassava consuming communities that domesticated cassava plants solved the problem of toxicity by selecting cultivars with negligible toxin levels or developing processing methods that reduced toxins to negligible levels (Chiwona-Karltun et al., 1998; Chiwona-Karltun et al., 2004). During domestication and evolution of cassava, both ways were employed leading to the availability of both bitter and sweet cultivars. The cyanogenic glycoside levels can be reduced to acceptable levels that would not impose hazard to the consumer when the cassava roots are adequately processed (Rosling, 1987; Banea-Mayambu et al., 1997; FSANZ, 2004).

The prussic acid (HCN) in particular is lethal if more than 0.1 g of it is contained in a food consumed by an individual at any one time (Asiedu, 1989). According to Borges, Fukuda and Rossetti (1989) the acceptable limit of cyanide content of fresh cassava roots for human consumption is less than 100 mg/kg of raw pulp. The FAO/WHO (1991) recommended limit value for the safety of consumption of cassava products is 10 mg HCN/kg and the acceptable limit in Indonesia is 40 mg HCN/kg (DSN, 1996; Djazuli and Bradbury, 1999; Ginting and Widodo, 2013). The diet or eating pattern influences the risks of cyanide poisoning. These limits may be confusing to adhere to. It is worth noting that when cassava is eaten with other foods balancing the nutritional value by being rich in sulphur amino acids, there is only a limited risk of intoxication.

Cassava roots with high contents of cyanogenic glucosides are detoxified through microbial fermentation for 3 to 7 days (to reduce the cyanogenic glucoside content), dried and ground into flour from which *ugali* is made (Otim-Nape and Zziwa, 1990). Different communities have adopted different methods of cassava detoxification, i.e., dry or wet

fermentation, yet others apply both depending on the season. In some communities, both sweet and bitter varieties are fermented to produce the desired flour quality.

Roots from both sweet and bitter cassava cultivars may be cut into chips which are dried in the sun for a number of days to reduce their moisture content to 15 to 20 % (Asiedu, 1989). The maceration allows the enzyme Linamarase to act on the cyanogenic glucosides, linamarin and lotaustralin, since the enzyme is not located in the same cell compartments as the cyanogenic glycosides (Nweke, 1994; Heldt, 1997). This reduces product toxicity. Attempts to utilise immobilized enzymes extracted from micro-organisms for detoxification of cassava roots are being made, although the cost may be too high for local producer in the developing world (Narinesingh et al., 1988; Ajayi et al., 2012; Murugan et al., 2012; Behera and Ray, 2016; Latif et al., 2019). The dried chips can be stored or ground into flour for immediate consumption.

Knowledge of the distribution of cyanogenic glycosides in cassava roots is important for the sampling of roots, by reference to the taste of fresh roots, which partly correlates with cyanogen concentrations (Chiwona-Karltun et al., 2004). However, bitter taste can also be related to environmental stress conditions, such as drought, low soil fertility and pest attack (Burns et al., 2012; Imakumbili, 2019). The liberated HCN through the hydrolysis of cyanogenic glycosides is toxic. The HCN blocks the reduction of oxygen in the respiratory pathway, i.e., cessation of aerobic cell metabolism by reversibly binding to the ferric ions cytochrome oxidase, thus effectively halting cellular respiration by blocking the reduction of oxygen to water, leading to acute cyanide intoxication or death (Leavesley, et al., 2008; Tivana, 2012; Ndubuisi and Chidiebere, 2018; Traylor and Graham, 2022). Chronic cyanide intoxication may lead to the development of certain

conditions such as disturbance of thyroid function (goiters), and neurological disorders (ataxia, partial paralysis).

2.6.2.2 Phytate

Phytate (Figure 4) also known as phytic acid or myo-inositol hexaphosphate (IP6), binds divalent metals (Figure 5), and is commonly considered as an antinutrient. Enzymes can dephosphorylate phytate and release the bound minerals (Figure 6). 3- and 6-phytase hydrolyze the molecule at the 3 and 6 positions first and are presumed to continue at other positions (Zeller, et al., 2015). An acid phosphatase may exist in the fermenting bacteria that can hydrolyze phosphates at nonspecific positions.

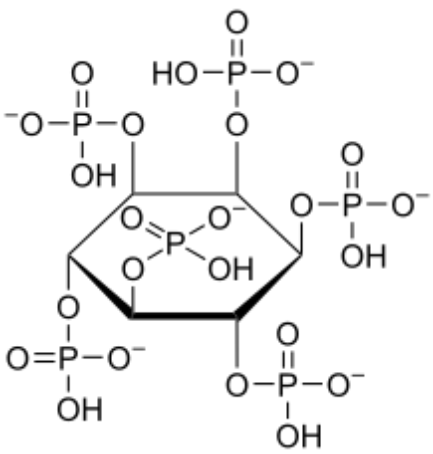


Figure 4: Chemical structure of the phytate anion (Adapted from Schlemmer et al., 2009)

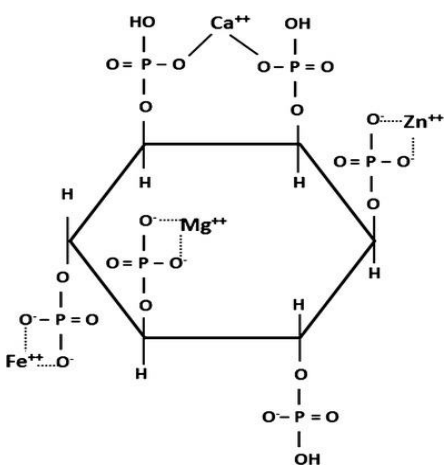


Figure 5: Chemical structure of phytate-metal complex (Adapted from Oh et al., 2004)

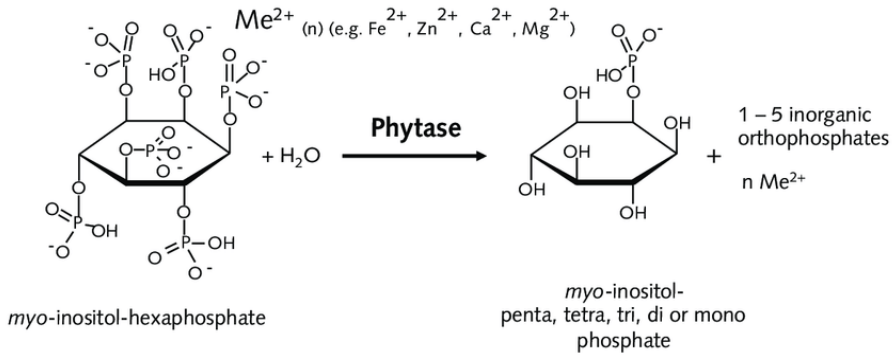


Figure 6: Hydrolysis of phytate-metal complex into inositol, phosphate and divalent Metals (Adapted from Shivange and Schwaneberg, 2017).

Phytate is widely distributed in the plant kingdom. It primarily serves as storage for plant phosphorus, as an energy source, and antioxidant for germinating seeds. Phytate is produced during seed, roots and tuber development (Mitsuhashi et al., 2005). Structurally, phytate (IP6) is made up of six phosphate groups, attached to an inositol ring, with the ability to bind up to 12 protons total. These phosphate groups act as strong chelators, readily binding to mineral cations, particularly Cu^{2+}, Ca^{2+}, Zn^{2+}, and Fe^{3+}, hence adversely affecting their bioavailability (Eleazu et al., 2014). These complexes are insoluble at neutral pH values (6–7), and cannot be digested by human enzymes, thus could decrease mineral bioavailability in high-phytate, homogenous diets (Graf et al., 1984; Idachaba, 2016; Popova and Mihaylova, 2019). Low-income, developing countries that predominantly rely on grains and legumes and roots and tubers as dietary staples are of special concern for zinc deficiency and/or insufficiency (Gibson et al., 2010; Popova and Mihaylova, 2019).

The chelating properties of phytate also allow it to act as an antioxidant, lending possible protective traits. Ensuring an appropriate phytate to mineral ratio minimizes the negative effects of phytate on mineral absorption in vulnerable populations. This can be achieved through enrichment with animal-source foods and/or fortification with the necessary

minerals (Gibson et al., 2010). Phytates can also bind proteins in the gastrointestinal tract hence preventing absorption and utilization by the body (Selle et al., 2000); affect amylase activity and decreases carbohydrate utilization (Kumar et al., 2020).

During food processing, phytate can be dephosphorylated which releases the minerals and makes them available for absorption. Therefore, cassava processing can minimize the antinutritional value of phytate. Processing techniques such as soaking, fermentation, sprouting, germinating, oven-drying and cooking can significantly alter phytate content in grains, legumes, and roots and tubers, allowing for increased mineral availability (Bhandari, 2006). Fermentation of cassava appears to be the most efficient processing technique to remove phytate (Montagnac et al., 2009).

2.6.2.3 Oxalate

Oxalate, or oxalic acid (Figure 7), is a substance that can form insoluble salts with minerals, including sodium, potassium, calcium, iron, and magnesium. These compounds are produced in small amounts in both plants, and mammals. All major groups of photosynthetic organisms produce oxalate. It is suggested that plants manufacture oxalate for a variety of functions including calcium regulation, plant protection, and detoxification of heavy metals (Nakata, 2003; Franceschi and Nakata, 2005). In mammals, endogenous oxalate is a metabolite of ascorbate, glyoxylate, hydroxyproline and glycine (Kohlmeier, 2003). Urinary oxalate mostly consists of endogenous oxalate, as opposed to exogenous dietary oxalate. Plant-derived oxalate is available in several different forms; as either water-soluble oxalate (oxalic acid, potassium, sodium and ammonium oxalates) or insoluble oxalate salts (primarily as calcium oxalate) (Savage et al., 2000; Radek and Savage, 2008; Akhtar et al., 2009; Nguyễn and Savage, 2013).

Soluble (unbound) oxalates can chelate minerals, reducing absorption/bioavailability, or are absorbed through the intestines and colon (Eleazu et al., 2014). Absorbed dietary oxalates contribute to calcium oxalate kidney stone formation (Montagnac et al., 2009). Insoluble oxalates, on the other hand, are excreted in the feces. Due to their effects on nutrient absorption and possible role in kidney stone formation, oxalates are considered to be 'antinutrients'. Although events of toxicity have occurred in livestock chiefly grazing on oxalate-rich plants, a balanced human diet typically contains only small amounts of oxalates. Boiling is more effective in reducing soluble oxalates compared to steaming or baking (Chai and Liebman, 2005). Insoluble oxalate contents may increase in foods subjected to roasting, grilling, baking, or consuming low-calcium diet (Montagnac et al., 2009; Juajun, et al., 2012).

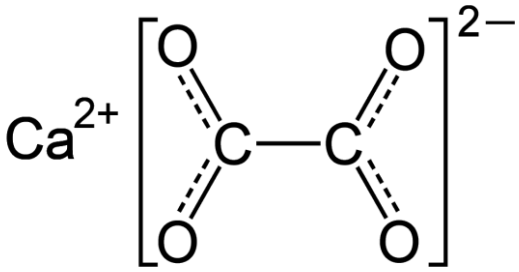
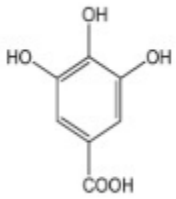


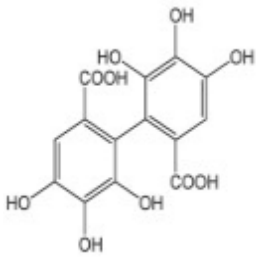
Figure 7: Chemical structure of oxalate (Adapted from Lo et al., 2018)

2.6.2.4 Tannins

Tannins are a broad class of polyphenol compounds of high molecular weight (500–3000 Daltons) ubiquitously present in commonly consumed plant foods and are responsible for the astringent taste of many fruits and beverages (Coultate, 1996; Chung et al., 1998; Serrano et al., 2009; Fraga-Corral et al., 2021). They can be chemically classified into two groups: hydrolysable tannins (Figure 8 a and b) and condensed tannins (also known as catechin tannins, flavanols, or proanthocyanidins) (Figure 9). (Coultate, 1996; Chung et al., 1998). Nowadays, the classification according to their chemical characteristic and

structural properties has been updated. Thus, tannins can be grouped into gallotannins, ellagitannins, CTs, complex tannins (CoTs) and phlorotannins (PTs, an exclusive class of tannins found in the algal species of the Phaeophyceae class) (Fraga-Corral et al., 2021).





(a) **(b)**

Figure 8 (a) and (b): Chemical structures of hydrolysable tannins (Adapted from Heldt, 1997)

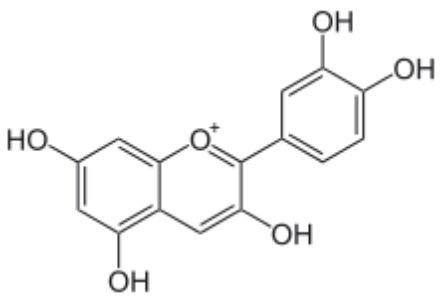


Figure 9: Chemical structure of condensed tannin (Adapted from Heldt, 1997)

Hydrolysable tannins, including gallotannins and ellagitannins, are selectively found in the diet. Condensed tannins, or proanthocyanidins, on the other hand, are the most abundant plant-derived polyphenols in the diet and include catechin, epicatechin (EC), epigallocatechin (EGC), epicatechin-3-gallate, and (-)-epigallocatechin-3-gallate (EGCG) (Heldt, 1997; Fraga-Corral et al., 2021).

Due to their phenolic nature, tannins are chemically reactive, forming intra- and inter-molecular hydrogen bonds with macromolecules like proteins and carbohydrates (Heldt, 1997; Chung et al., 1998; Fraga-Corral et al., 2021). This lends to their role in plant

defense, as well as to their antioxidant, anticarcinogenic, immunomodulatory, detoxifying, and cardioprotective activities. Tannins act as antioxidants by scavenging free radicals, although their ability to act as chelators have also been reported to inhibit the absorption of dietary minerals such as iron, copper, and zinc, thus, negatively impact on stores of these minerals in the human body (Chung et al., 1998; Afsana et al., 2004; Delimont et al., 2017).

The elucidated 'anti-nutritional' effects of dietary tannins have been suggested as a contributor to iron-deficiency anemia, particularly in developing and low-income countries who rely on tannin-rich foods (Chung et al, 1998). Other studies, e.g. Delimont et al. (2017) suggeste that iron status and absorption is not significantly affected by dietary tannin intake and is found to be highly variable between individuals.

In cassava roots, the specific polyphenols identified are 3 flavan-3-nols (gallocatechin, catechin, and catechin gallate) (Buschmann et al., 2000a), hydroxycoumarins (Buschmann et al., 2000b), and the flavone 3-glycosides known as rutin and kaempferol 3-rutinoside (Montagnac et al., 2009) (Figure 10). Polyphenols in the roots accumulate as the roots deteriorate in the process called "Post-harvest physiological deterioration" (PPD) (Rickard and Gahan, 1983; Rickard, 1985; Buschmann et al., 2000a; Alves, 2002; Salcedo and Siritunga, 2011; Uarrota et al., 2016). Visually, PPD is characterized by a blue/black or brown discoloration of the vascular parenchyma, which starts to appear within 24–72 h of harvest (Zainuddin et al., 2018).

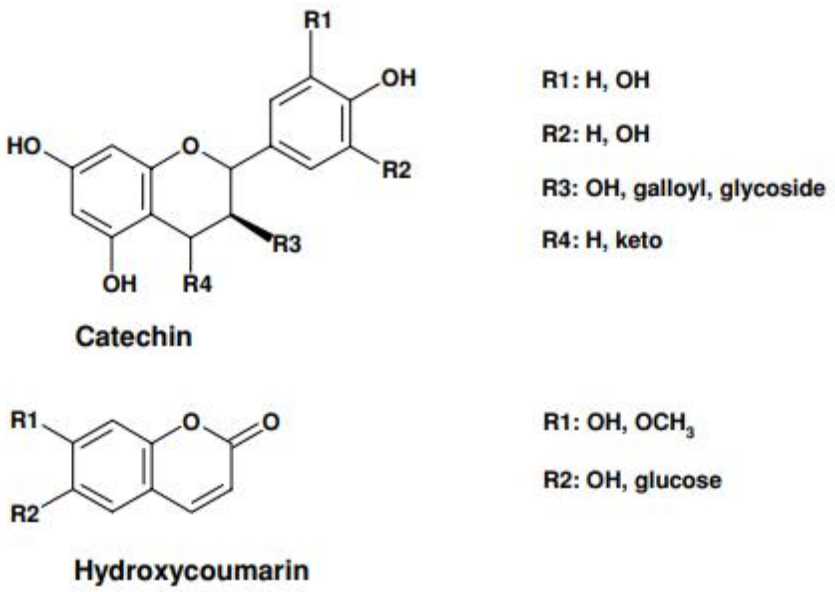


Figure 10: The basic structures of the polyphenols present in cassava roots (catechin and hydroxycoumarin and their derivatives) (Adapted from Buschmann et al., 2000a)

Tannins are not consumed alone, but in combination with thousands of other bioactive compounds, including ascorbic acid. Potential inhibitory effects of tannins may be offset by the inclusion of ascorbic acid in the diet, which chemically reduces iron and prevents the formation of less soluble iron-containing compounds (Coultate, 1996). Ascorbic acid facilitates iron absorption by forming a chelate with ferric iron at acid pH that remains soluble at the alkaline pH of the duodenum (Coultate, 1996). This may explain why human epidemiological studies investigating iron deficiency anemia are unable to demonstrate any correlations between dietary tannin intake and iron-deficiency anemia (Delimont et al., 2017; Petroski and Minich, 2020). Tannins may be reduced in the food by preparation processes such as fermentation, cooking, oven-drying and peeling skins of fruits and nuts (Montagnac et al., 2009). Cassava also contains antinutrients such as fibre (which decreases the absorption of carbohydrates), nitrate and saponins that can reduce nutrient bioavailability (Montagnac et al., 2009).

2.7 High Quality Cassava Flour

2.7.1 The concept of High Quality Cassava FLour

High Quality Cassava Flour (HQCF), also known as improved quality unfermented cassava flour, is produced from the roots of both sweet and bitter cassava varieties. HQCF is a relatively new concept in Uganda although it has been circulating around Africa since 1992 with trials in Nigeria and Ghana by National resources Institute, U.K. (CAVA, 2012).

2.7.2 Production of HQCF

The production process of HQCF, as depicted in Figure 11, was initially developed at the International Institute for Tropical Agriculture (IITA) in Nigeria as an alternative to imported wheat flour for the food and non-food industries and the technology is now used in some cassava-growing nations (Onabolu and Bokanga, 1995; Onabolu et al., 1998; Falade and Akingbala, 2008).

HQCF production differs from production of traditional fermented cassava flours such as *gari* and *agbelima* by the absence of extensive fermentation that gives a low pH and a sour taste, unsuitable for inclusion in industrial products (Dziedzoave et al., 2006). Commercial production of HQCF is relatively new in Africa and the technology is still being used mainly by small- and medium-scale processors. Most of the small-scale processors have difficulties in production during the rainy season meeting the quantity, quality and regularity of supply required by the industrial users because the technology has not been perfected yet (Ohimain, 2014).

HQCF may be produced in one of two ways. One method involves mechanical dewatering of the grated roots, producing a type of flour designated HQCFG, resulting

into a softer and finer flour with smaller particle size. The dewatering removes some starch from the cassava mash or pulp hence the final product has lower starch content. It is a favourite for bakery, composite and paperboard markets because of its qualities.

Another method does not involve pressing (the roots are chipped/sliced and immediately dried) producing a flour type designated HQCFC. This flour type has a relatively mild cassava flour scent, larger particle size than HQCFG and is mildly creamy in colour. Since there is no dewatering in the processing, HQCFC has higher starch content than HQCFG. This type of flour is a favourite for the breweries market for its high starch content (CAVA, 2018, accessed at www.academia.edu, 2021; www.afrii.org, 2021). According to Cassava Value Chain Analysis report (Ogwal-Omara et al., 2010), HQCF is commercially produced only in Eastern Uganda where the NGOs SOSPA, CAVA and SG2000 have established operational centres. The report estimated the quantity of HQCF produced to be 3.5 MT per day. The quantity of HQCF produced and sold in other regions of the country is difficult to establish because of lack of records.

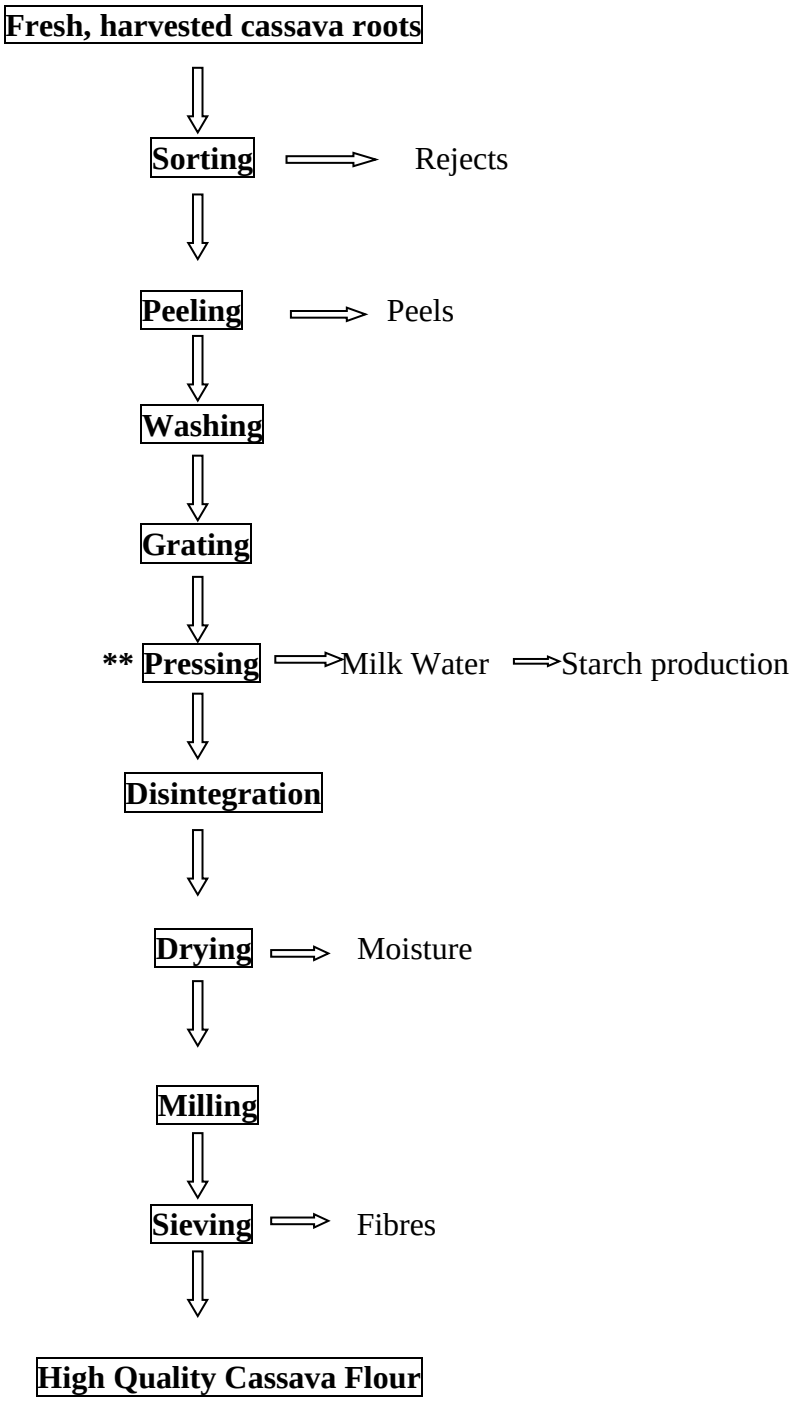


Figure 11: Production process for High Quality Cassava Flour (Adopted from Onabolu et al., 1998; Ohimain, 2014)

**Pressing is optional

2.7.3. Quality requirements for HQCF

The General Quality Requirements for HQCF according to the Draft East African Standard (DEAS 779: 2012) is that:

1. HQCF shall be produced from selected fresh cassava roots or from high quality intermediate products such as chips and grits conforming to EAS 738/ EAS 778/EAS 739.

2. High quality cassava flour shall be: free from extraneous matter; free of off flavours and odours; free from any living insects and foreign matter; safe and suitable for human consumption; of colour characteristic of the variety; give a blue-black colouration when tested with iodine; and have a pasting temperature less than 75.

2.7.3.1 Specific requirements

2.7.3.1.1 Compositional quality

HQCF shall conform to the compositional quality requirements shown in Table 4

Table 4: Compositional requirements for high quality cassava flour

S/N	Parameter	Requirement	Method of test
1	Total acidity, %, by mass, max.	0.25	AOAC 942.15
2	pH	5.5 – 7.0	AOAC 943.02
3	Acid insoluble ash, % m/m on dry matter basis, max.	0.35	EAS 82
4	Cyanide content, mg/kg, max.	10.0	EAS 744
5	Starch content by mass, %, by mass, min.	60.0	ISO 10520
6	Moisture content, %, by mass, max.	12	EAS 901
7	Crude fiber, %, by mass on dry weight basis, max.	0.2	ISO 5498

Source: DEAS 779: 2012

2.7.3.1.2 Physical properties

HQCF is required to have the following physical properties (DEAS 779: 2012): not less than 95 % by mass of HQCF shall pass through a sieve of 250 µm mesh screen; extraneous matter shall not be more than 10 specks/100 cm^3 (Table 4).

2.7.3.1.3 Chemical properties

HQCF shall: give a blue-black colouration when tested with iodine; have a pasting temperature less than 75 ^oC (DEAS 779: 2012).

2.7.3.1.4 Hygiene

HQCF shall be handled in hygienic manner in accordance with EAS 39 and shall conform to microbiological limits specified in Table 2.

Table 5: Microbiological limits for high quality cassava flour

S/N	Micro-organism(s)	Requirement	Method of test
1	*Escherichia coli*, CFU/g, max.	Absent	ISO 7251
2	*Salmonella spp per*, 25g, max.	Absent	ISO 6579-1
3	Yeasts and moulds, CFU/g, max.	10^4	ISO 21527-2

Source: DEAS 779: 2012.

The East African Standards on HQCF may be compatible with requirements from other regulatory bodies, e.g., Codex Alimentarius and Draft African Standards, High Quality cassava Flour - Specifications (CD-ARS 840, 2014).

Table 6: Physical and chemical quality characteristics and requirements of high quality cassava flour for food uses (CD-ARS 840, 2014).

Characteristics	**Quality levels**
Moisture content	8 – 10 %
Starch content	65 – 70 %
pH	6 - 7
Total titratable acidity (as lactic)	< 0.25%
Particle size	250 – 500 μm
Colour	L* > 99
	a* < 8
	b* < (- 4)
Total cyanogens (CNP)	< 10 mg/kg HCN eq
Pasting temperature	< 74 °C
Cook Paste Viscosity	> 750 BU

Source: Dziedzoave et al. (2006).

The FAO-WHO commission of the *Codex Alimentarius* defined the standard for edible cassava flour, Codex Stan 176 (Codex Alimentarius Commission, 1989; modified/amended 2019) (Table 6).

Table 7: Requirements for edible cassava flour (CXS 176-1989)

Factor		**Limit**
Moisture content (m.c., %)		Max 13
Hydrogen cyanide content (mg/kg)		Max 10
Crude fibre (%)		Max 2.0
Ash (%)		Max 3.0
Abnormal flavours and odours		Exempt
Impurities and contaminants		Exempt
Food additives		Conform with legislation of the Country in which the product is sold
Particle size	Fine flour	Min: 90 % shall pass through a 0.60 mm sieve (mesh 30)
	Coarse flour	Min: 90 % shall pass through a 1.20 mm sieve (mesh 16)

Source: Codex Alimentarius Commission (1989).

2.7.4 Challenges to the production and marketing of HQCF

Primary processing of cassava in Uganda is done at the household level using rudimentary tools (knives, pangas, axes, chippers, presses and graters) (Buyinza and Kitinoja, 2018). There is over-reliance on the sun for drying of chips and grates. The use of rudimentary tools and sun drying negatively affect quality of the products. Processors have found it difficultt to meet the safety standards set for cassava products by the national and international regulatory agencies regarding microbial load and cyanogens due to the absence of quality management systems (Adebayo, 2010). This limits their access to national as well as international export markets (Aristizábal et al., 2017; Graffham et al., 2017; CAVA, 2018).

The emerging markets for HQCF want deliveries in bulk yet the cassava value chain systems are not currently set up to accommodate commercial scale production and a large number of variable quality supplies. These challenges may be attributed to lack of information about improved processing technologies, adoption of the new technologies and the cost associated with it; the lack of policies and/or implementation of policies needed to link national agricultural research and extension systems with private-sector participants to support the commercialisation of technologies generated through research (Ewebiyi et al., 2020).

The major challenge of linking up cassava farmers to the large markets for HQCF has partly been solved by CAVA. The Cassava: Adding Value for Africa Phase Two (CAVA II) Project has supported value addition in cassava and commercialization of cassava in Africa. In Uganda, the CAVA II project has successfully developed value chains for HQCF by supporting investors to establish processing sites in Eastern, Northern and Central Uganda. The Project has established HQCF sites in the districts of Pallisa, Kibuku, Budaka, Bukedea, Kumi, Soroti, Ngora, Serere, Dokolo, Nakaseke, Nakasongola, Lira, Apac, Otuke, Oyam, Kole and Alebtong (CAVA, 2018, accessed at www.afrii.org, 2021). Examples of such a site is Matilong Community Development Organization (MACDO) in Bukedea district, Farming for Food and Development Program – Eastern Uganda (FADEP – EU), and VISION TERUDO (Adebayo et al., 2010). Other independent community-based organisations producing HQCF are NYAPRAC and NYADUKU farmers' groups in Nebbi/Pakwach district. Farm Uganda, a privately owned agro-processing company, located in Kigumba sub county in Kiryandongo district, Central Uganda, has benefited from the intervention of cassava projects: the Agricultural Technology Transfer (AgriTT) and Cassava Adding Value for Africa (CAVA) in improving the quality and quantity of HQCF sold to her customers,

especially Uganda Breweries Limited (UBL). Farm Uganda is the biggest supplier of cassava flour to the brewery that consumes about 3000 metric tons annually to brew the locally popular Ngule and Senator Beer brands (CAVA, 2018, accessed at www.afrii.org, 2021).

2.7.5 Potential sectors for the utilisation of HQCF

2.7.5.1 Food and beverage industries

High quality cassava flour can be used as raw material in the food and beverage industries for the manufacture of bread, pastries (noodles, spaghetti), sweets and hard dough biscuits, other agri foods, e.g., *chapattis*, doughnuts, *manadazi*, *baghias*, pancakes in rural bakeries, baby foods, glucose syrup, and alcoholic drinks as an adjunct in brewing of clear bear and as binding and thickening agent in soups and stews (CAVA, 2018; accessed at www.academia.edu, 2021). HQCF can be used to replace pure native starches as fillers and binding agents in extruded meats such as the meat fillings used in sausage rolls and as a partial replacement for wheat flour in bread and other bakery products (Graffham et al., 2017).

The possibility of using flours derived from cassava roots for producing bread has not been extensively explored in Uganda due to lack of innovation on processing technologies and lack of HQCF conforming to the grades and standards (Siyeni et al., 2005). Cassava flour incorporation into wheat flour has to be accepted in the bakery industry in order to serve as a suitable substitute to wheat flour. There is a need to sensitise local industries to start using cassava flour, and also embark on sensitising farmers and processors for processing HQCF that can meet the required standards (Kleih et al., 2012; Graffham et al., 2017).

2.7.5.2 Other industries

HQCF can substitute wheat or starch in the manufacture of paperboard adhesives. It is also suitable to be used as glue extender in plywood manufacture, in the manufacture of gum, textile sizing, paper, and pharmaceutical drugs (Graffham et al., 2017). HQCF can substitute starch as a source of carbohydrate for production of industrial alcohol and extra neutral alcohol (ENA) (Graffham et al., 2017). It can also be used as a carbohydrate source for production of feed for beef cattle and pigs and for production of cooked and extruded pellets for aquaculture feeds (Graffham et al., 2017).

2.8 The need to replace wheat flour with HQCF in bakery processes

2.8.1 Uganda wheat import

Ugandans consume large quantities of wheat products (*chapatti*, *manadazi*, bread, confectioneries, and pasta). However, Uganda's demand for wheat estimated at (500 000 MT per year) (Figure 12), is higher than its domestic supply of (20 000 MT) (USDA, 2020). This makes the country a net importer of wheat, which takes a significant amount of the country's foreign exchange reserve, hence making bread a luxury commodity for most communities. Partial replacement of wheat flour by cassava flour, a local raw material, in the manufacture of bakery and confectionery products is thus economically important in Uganda.

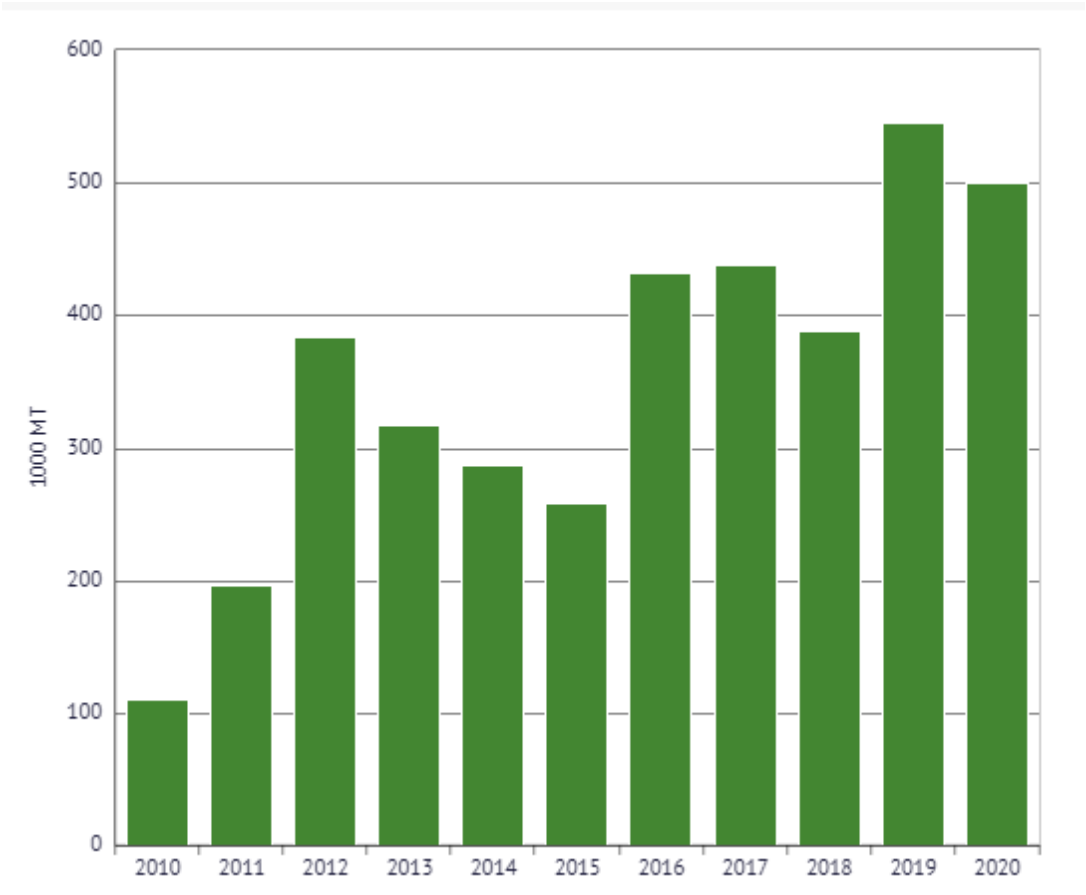


Figure 12: Uganda wheat imports by year. Source: USDA (2020)

The FAO composite flour programme of 1964 encouraged developing countries to save foreign exchange on wheat importation by replacing part of it with local products in bread making (Abass et al., 2018). Composite flour is a mixtute of several flours obtained from roots and tubers, cereals, legumes, etc with or without the addition of wheat flour (Adeyemi and Ogazi, 1985; Shittu et al., 2007; Olaoye and Omowaye, 2011; Chandra et al., 2015; Julianti et al., 2017). It can also be a mixture of different flours from cereals, legumes or root crops that is created to satisfy specific functional characteristics and nutrient composition (Adeyemi and Ogazi, 1985). The ingredients used in composite flours depend on the availability of raw materials in the country concerned (Olaoye and Omowaye, 2011). In selecting raw materials for use as alternatives to wheat flour one must consider factors such as (a) compatibility - that is to say, suitability for end use and

(b) availability and cost at point of use (Chandra et al., 2015). Cassava flour is best placed for that purpose in Uganda. HQCF is white/cream, odourless and has a bland taste and can thus easily blend with wheat flour at higher inclusion levels compared to banana/plantain flour, which produces acceptable bread at 10 % inclusion level (Bamidele et al., 1990).

Wheat (*Triticum aestivum*) is a cereal, the fruits of cultivated grass, a member of the monocotyledonous family Gramineae. Wheat is an important raw material in the production of aerated/fermented baked foods, and is an indispensable ingredient in leavened bakery products with the current technology (Nwanekezi, 2013; Julianti et al., 2017). Complete replacement of wheat flour with cassava flour for baking is challenging and not currently feasible in Uganda. Wheat contains glutenin and gliadin, the protein complex responsible for the dough-forming capacity of wheat flour on hydration. The suitability of flour for bread making depends on the contents of glutenins (Heldt, 1997; Julianti et al., 2017; Monteiro et al., 2021). The wheat proteins play a key role in quaranteeing the bakery quality of wheat (influencing water absorption, cohesion, viscocity, extensibility, elasticity, and resistance to deformation, tolerance to kneading, ability to gas/bubble retention and dough-strenghthening properties, mixing stability and the bread volume) (Noorfarahzilah et al., 2014; Julianti et al., 2017; Monteiro et al., 2021).

Incorporation of HQCF in wheat flour dilutes wheat gluten. HQCF can be incorporated in wheat at levels from 10 to 20 % in sweet and hard dough biscuits (Graffham, 2017). As the percentage is increased machinability problems are encountered on the production line and the biscuits lose volume, have reduced colour and become more brittle. In bread production, reduction in gluten results in reduced loaf volume and a heavy/dense cake

like structure, characteristics which are unacceptable for most consumers of bread (Abass et al., 2018; Monteiro et al., 2021).

2.8.2 Gluten chemistry

When hydrated the wheat proteins form gluten, in which gliadin behaves as a viscous liquid (viscous and extensible but lacks elasticity) and glutenin behaves as a cohesive solid (lacks extensibility but exhibits substantial strength and elasticity) (Kent and Evers, 1994). Gliadins and glutenins make different contributions to the viscoelasitcity of the gluten. While gliadin cannot retain gas due to its extensibility, glutenin can retain gas but the gas cannot expand or grow due to its strength (elasticity). It is therefore, the combination of the two groups of proteins that impacts the unique viscoelastic properties responsible for gas retention in wheat flour doughs (Kent and Evers, 1994; Nwanekezi, 2013).

According to the linear gluten hypothesis proposed by Schofield (1966) and explained in Kent and Evers (1994), the relationship between gluten structure and function is envisaged as a series of polymeric subunits joined head to tail by interchain disulphide bonds (Figure 13).

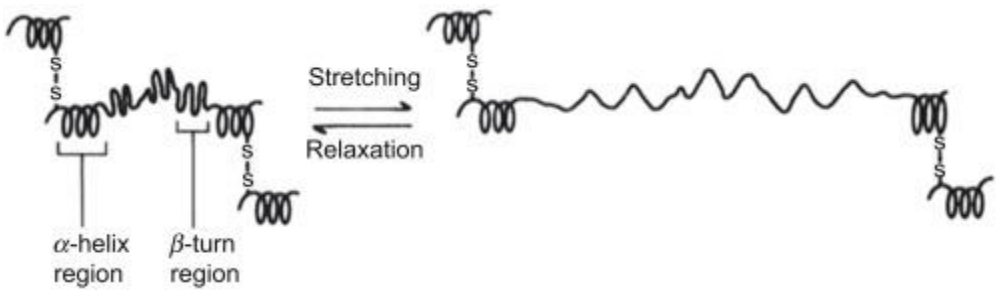


Figure 13: Schematic representation of a polypeptide subunit of glutenin within a linear concatenation (Kent and Evers, 1994)

The polypeptide subunits of glutenin are joined together via disulphide (S-S) bonds to form polymers. The subunits have a conformation that may be stretched when tension is applied to the polymers, but when the tension is released, the native conformation is regained through elastic recoil. Gluten proteins contain the amino acid cysteine with sulphydryl (-SH) side groups. Reaction between the sulphydryl (-SH) groups and the disulphide (S-S) bonds permits new inter- and intra-polypeptide/protein relationships to be formed via -SS- bonding. The disulphide bonds formed between adjacent polypeptides give rise to beta spiral structure (β-turns), which confers elasticity to wheat dough. An effect of this interchange is the relaxation of the dough by relief of stress induced by the mixing process (Kent and Evers, 1994).

The protein complex gluten in the dough is capable of retaining the air incorporated during mixing and the carbon dioxixie produced during proofing/fermentation and baking to form a typical aerated foam structure known as bread (Schopf and Scherf, 2021). Yeast leavened products, such as bread, loose their quality, especially volume and sensory appeal when their gluten quantity is reduced. Blending of non-wheat flour with wheat flour dilutes the quantity of gluten in wheat. The more the gluten is diluted by raising the ratio of non-wheat flour (e.g., cassava flour) in the composite flour, the more the quality of the yeast leavened baked product (bread) is reduced (Kent and Evers, 1994; Nwanekezi, 2013).

2.10 Dough rheological characteristics and their measurement

Rheology is the study of the deformation and flow of materials under applied force (Dickinson, 1992; Shaw, 1994). Rheological properties of dough are very important in bread baking quality. Knowledge of the rheological behaviour of bread dough is of technological importance in order to understand mechanical properties of the dough for

process efficiency and control of final products' quality (Hruskova et al., 2001; Mondal and Datta, 2008). Dough is a viscoelastic material (Shaw, 1994), which behaves as both a liquid and a solid. It exhibits a viscous (fluid-like) and an elastic (solid-like) behavior when a force is applied to it, i.e., it shows characteristics of viscous flow and elastic deformation (Dickinson, 1992; Zaidel, et al., 2010; Upadhyay and Mehra, 2012).

Viscoelasticity of dough is related to many factors such as nature of flour/flour composition, processing parameters, dough ingredients, temperature, water uptake, air incorporation and type of mixing (Mirsaeedghazi et al., 2008). When stressed, some of the energy is stored elastically while other parts of the dough are deformed into new non-equilibrium positions relative to one another (Shaw, 1994; Rao, 2014). In breadmaking, the dough undergoes some type of deformation in every phase of the process, i.e., during mixing, fermentation, sheeting and shaping, proofing and baking. Accordingly, the application of rheological concepts to the behaviour of doughs seems a natural requirement on the interrelationships among flour composition, added ingredients, process parameters and the characteristics of the loaf bread (Faubion and Hoseney, 1990). There are many techniques employed in the study of the rheological properties of dough, and as many instruments have been designed for the empirical measurement of dough rheological properties, such as penetrometer, texturometer, consistograph, amylograph, farinograph, mixograph, extensograph, maturograph, fermentometer, alveograph (Steffe, 1996).

Consistograph determines the water absorption capacities of different flour formulations and evaluates empirical rheology parameters related to mixing tolerances (dough consistency during mixing): the water absorption capacity, (WAC), maximum pressure (PrMax), tolerance to kneading (Tol), consistency of the dough after 250 s (D250) and

consistency of the dough after 450 s (D450) (Atuderei et al., 2021) The information produced by the consistograph and the mixolab is complementary, since the mixolab measures torque and the consistograph measures the pressure on a wall of the dough mixer (Mixolab Application Handbook, 2012).

In this study, the consistographic characteristics determined were: 1) water absorption capacity (WAC), which is the absorption of the flour (14 % moisture basis) calculated by the Chopin Software, to obtain a target consistency, and 2) maximum pressure (PrMax) at constant hydration (CH), which indicated the consistency of the dough used for the test (the higher the number the more resistance the dough offers the mixer).

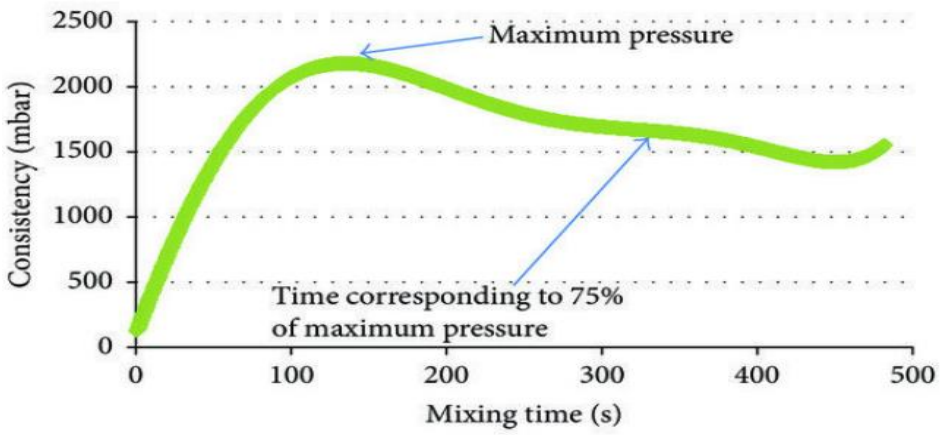


Figure 14: A typical consistograph curve (Adapted from Secchi et al., 2018)

Amylograph measures apparent viscosity, gelatinisation temperature, i.e., the viscosity of starch (or flour) and its changes during heating (Steffe, 1996). In other words, amylograph continuously measures the consistency of a starch (or flour) paste as a function of time and temperature. It determines the starch gelatinization properties, particularly the maximum viscosity achieved and also measures amylase activity (Mixolab Application Handbook, 2012). However, it should be noted that the mixolab works on a dough

(limited availability of water) and the amylograph on a suspension (excess of water). This difference may explain the slight differences observed between the mixolab and the amylograph results (Mixolab Application Handbook, 2012).

Farinograph estimates the water absorption of flours, the relative mixing time, and stability to overmixing, i.e., evaluates dough strength and dough stability (Steffe, 1996). Mixograph measures mixing time/torque, i.e., provides information on the mixing and absorption characteristics of flour (Steffe, 1996). Extensograph measures extensibility, i.e., the balance of the elastic and viscous properties of dough (Steffe, 1996). Maturograph measures volume change of complete dough, i.e., final proving time, proving stability, elasticity, dough level (Steffe, 1996). Oven rise recorder measures buoyancy of the dough, i.e., dough volume, baking volume, oven rise, final rise, peaks - when gas escapes (Steffe, 1996). Fermentometer determines the total CO_2 volume production (VT, ml), maximum height of gaseous production (H'm, mm), volume of the gas retained in the dough at the end of the test (VR, ml) and retention coefficient (CR, %) (Steffe, 1996; Atudorei et al., 2021). Simply, the (rheo) fermentometer measures dough height and its volume.

Alveograph measures biaxial extensibility thus simulates bubble growth during dough fermentation and in the beginning of baking process. The alveograph measures the parameters: maximum pressure (tenacity) (P), an index of resistance to extension (elasticity or tenacity); the average abscissa (L) at bubble rupture, an index of dough extensibility; the ratio of dough tenacity to extensibility (P/L); deformation energy/baking strength/work (W), an index of dough strength; flexibility (elasticity) index *Ie* and dough blowing (swelling) index (G) (Steffe, 1996; Atudorei et al, 2021). However, alveographic values are not very predictive of the dough properties during the Mixolab "hot" phase (C2, C3, C4 and C5) (Mixolab Application Handbook, 2012).

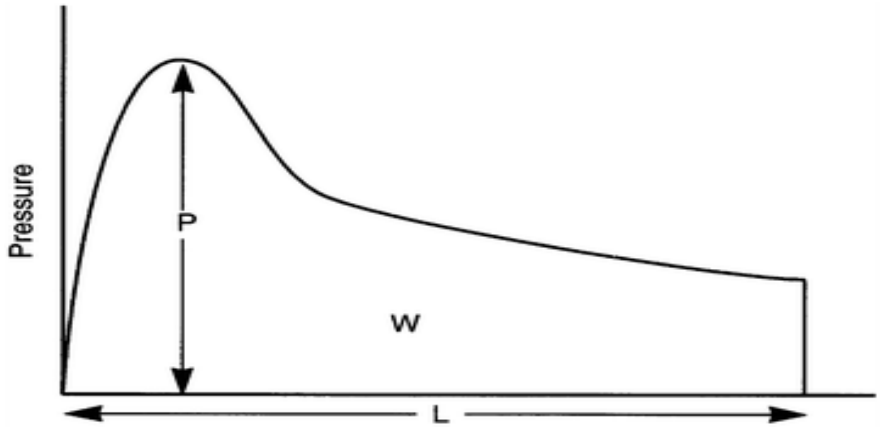


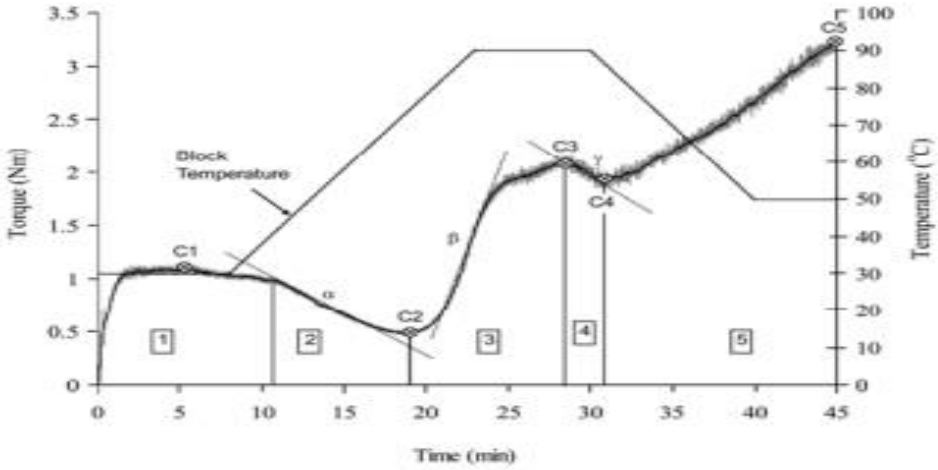
Figure 15: A typical alveograph curve (Adapted from Dubois et al., 2008; Jodal and Larsen, 2021)

The mixolab has been designed to eliminate some of the short-comings associated with farinograph and amylograph (Bakare, 2016). Mixolab measures the dough properties and the pasting behaviour of flour at the same time (Mirsaeedghazi et al., 2008; Dhaka et al., 2012; Macedo, et al., 2020). The moisture content of the flour should be known in order to configure the mixolab equipment before the determination of rheological properties of the flour/dough. In this study, the standard "Chopin +" protocol was followed, whereby analysis was performed on 14 moisture content basis as recommended by Mixolab Applications Handbook 2012).

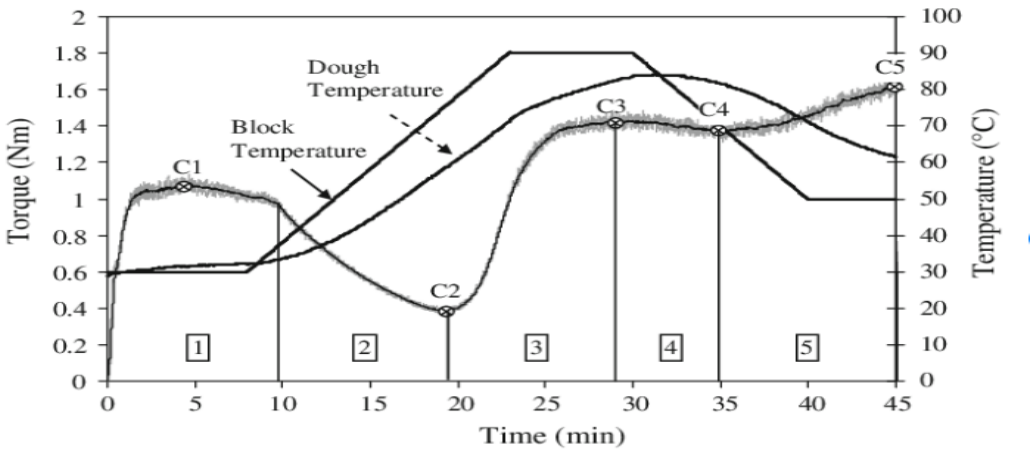
The HQCF had a moisture content of 12.46 % while wheat flour had a moisture content of 13.58 %. The "Chopin +" protocol was as follows: initial equilibrium at 30 °C for 8 min; heating to 90 °C over 15 min (at a rate of 4 °C /min); holding at 90 °C for 7 min; cooling to 50 °C over 5 min (at a rate of 4 °C/min); and holding at 50 °C for 5 min. The mixing speed was kept constant at 80 rpm. The parameters recorded were: water absorption, % (the percentage of water required for the dough to produce a torque of 1.1

± 0.05 Nm); dough development time, min (the time to reach the maximum torque at 30 °C); dough stability at mixing, min (the elapsed time at which the torque produced is kept at 1.1 Nm); C1 (determines absorption/dough development), C2 (measures the weakening of the protein/decrease in dough consistency based on the mechanical work and the temperature); C3 (measures starch gelatinization, i.e., starch granules swell and absorb water and amylose molecules leach out resulting in an increase in the viscosity); C4 (measures the stability of the hot-formed gel/amylase activity); and C5 (measures starch retrogradation/gelling during the cooling period) (Mixolab Applications Handbook, Chopin Technologies, 2012).

The difference between the C3 and C4 peak values (C3-4) corresponds to starch breakdown and the difference between the C5 and C4 peak values (C5-4) corresponds to starch retrogradation at cooling stage (Codina et al., 2019). The standard plots/curve (Figure 16) was converted into 6 indices (Figure 17) rated from 0 to 9, which profiled the flour on the basis of 6 fundamental criteria: absorption potential or water absorption index; mixing properties or mixing index; gluten strength or gluten+ index; maximum viscosity or viscosity index; amylase activity or amylolysis/amylase index and retrogradation index. Other parameters represented rates at which the process was taking place: α – represented the slope of the curve between the end of the period at 30 °C and C2 and gave an indication about protein weakening speed under the effect of heat; β - represented the slope of the curve between C2 and C3, and gave an indication about starch gelatinization speed; γ – represented the slope of the curve between C3 and C4, and gave an indication about enzyme degradation speed.



(a)



(b)

Figure 16 (a) and (b): Standard mixolab curves (Adapted from Mixolab Applications Handbook, 2012)

Based on the Mixolab Profiler standardised protocols for flour characterisation, results obtained were converted into 6 indices rated from 0 to 9. The flour was profiled on the basis of 6 fundamental criteria, i.e.:

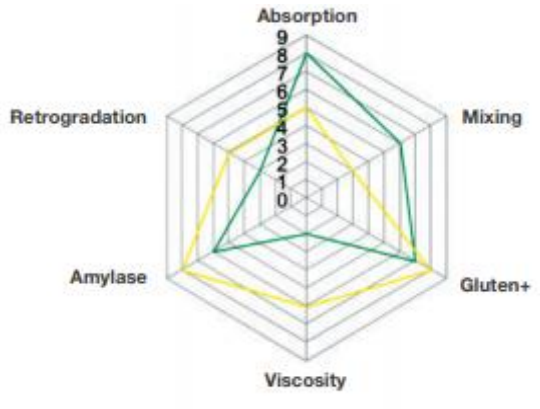


Figure 17: Mixolab Profiler indices (Adapted from Mixolab Applications Handbook, 2012)

Absorption potential or water absorption Index: this is dependent on the flour composition (protein, starch, fibre). It influences the dough yield (profit). The higher the index, the more water is absorbed by the flour.

Mixing properties or mixing Index: this represents the properties of the flour during mixing at 30 ^{0}C. It accounts for the stability, dough development time, weakening. The higher the index, the greater the stability of the flour during mixing.

Gluten strength or Gluten+ Index: this represents the properties of the gluten when the dough is heated. The higher the index, the higher the resistance of the gluten to heating.

Maximum viscosity or viscosity Index: this represents the increase in viscosity during the heating phase. It is dependent both on the amylase activity and the starch quality. The higher the index the higher the viscosity of the dough when hot.

Amylase activity or Amylase Index: this is dependent on the ability of the starch to resist "amylolysis". The higher the index the lower the amylase activity.

Retrogradation or Retrogradation Index: this is dependent on the characteristics of the starch and its hydrolysis during the test. The higher the index, the shorter the cooked product's shelf-life.

Empirical tests are purely descriptive and dependent on the type of instrument, size and geometry of the test sample and the specific conditions under which the test was performed. Many of these tests are used as single point tests. Since dough experiences a wide range of conditions of stress states and strain rates during processing and baking and the rheological properties of dough are dependent both on time and strain, there is often a discrepancy between such single point type tests and actual performance in the processing plant (Steffe, 1996). Instruments used in dough rheology measurements must be capable of measuring both viscous and elastic properties of dough due to its viscoelastic behavior (Mirsaeedghazi et al., 2008; Dhaka et al., 2012).

Results from empirical measurements may be used in quality control, correlation to sensory data, i.e., end-product quality such as bread loaf volume, texture, and sensory attributes or even serve as official standards of identity (Upadhyay and Mehra, 2012).

2.10 Sensory attributes of foods

Scientists and philosophers in ancient and modern times have tried to capture the essence of the notion of "quality" and five principal approaches have been identified: the transcendent, user-based, product-based, manufacturing-based and values-based (Kihlberg, 2004; Drake, 2007). Because quality is connected with human cognition and perception, it is linked to knowledge and variables in nature (Kihlberg, 2004). The notion of food quality in its full complexity is crucial for liking of food, food choice and food purchase (Kihlberg, 2004).

The five major sensory properties of materials – appearance, texture, aroma, taste and irritation – are perceived by the primary human senses – visual (sight), tactile (touch), olfactory (smell), gustatory (taste), auditory (hearing), and chemesthesis (common chemical sense) (Lee et al., 2008). These sensory attributes of foods are therefore, responses to products as perceived through the senses (Lawless and Heymann, 1999). Sensory evaluation is the scientific method used to evoke, measure, analyse and interpret sensory responses (Lawless and Heymann, 1999). The sensory properties of food determine the food quality and degree of of compliance with legal requirements and consumer habits (Lee et al., 2008).

In order to evaluate sensory qualities of food different test methods have been developed (Lawless and Heymann, 1999; Kihlberg, 2004; Drake, 2007). Discrimination test method (simple difference testing procedure) merely attempts to assess whether any differences exist between any two types of products. The panelists for the test are screened for acuity, oriented to the test method, and sometimes trained. Descriptive test method quantifies the perceived intensities of the sensory characteristics of the product. The selected and trained sensory panel describes qualitatively and quantitatively the product in terms of appearance, aroma, flavour and texture (Kihlberg, 2004; Drake, 2007; Isakar et al., 2021). The panellists in this test have to show how products differ in specific sensory characteristics. This creates a sensory profile of the product.

Affective or Hedonic/acceptance test method attempts to quantify the degree of liking or disliking of a product. The panelists therefore respond to the question “how much/well the products are liked or which products are preferred” (Kihlberg, 2004; Drake, 2007; Isakar et al., 2021). The test does not require trained panelists, but the panelists have to be screened for product use (Lawless and Heymann, 1999; Isakar et al., 2021). It is also

referred to as consumer acceptance test (Kihlberg, 2004). However, there are significant roles of non-sensory variables in the formulation of a hedonic judgement by the consumer (Guinard, 2001).

Sensory tests provide useful information about the human perception of product changes due to ingredients, processing, packaging, or shelf-life. Sensory attributes can influence a customer's preference towards a product (Isakar, 2021). The importance of sensory quality is ageless, with basic capitalism driving individuals to market and sell the best and freshest products because these products demand the top dollar (Drake, 2007).

Bread is a food product, and therefore evaluation of its sensory attributes/ qualities/ properties such as appearance, color, texture, flavor and overall acceptability does not differ from those of other food materials. The acceptability of the product by the consumer is the critical factor in the market growth. The present day consumer looks for new bakery products, better appeal, taste and convenience from bakery foods (Kihlberg, 2004).

It is well known that in bread making flour composition has a strong impact on bread quality (Kihlberg, 2004). Bread baked with flour from whole wheat, compared with bread baked with the same wheat sample, but with white flour, results in products that are perceived as very different in terms of comparable quality attributes such as colour, aroma, flavour and texure. This implies that flour's extraction rate and its ash content influence the sensory quality of bread (Kihlberg, 2004). Consequently, sensory evaluation of composite breads should consider the incorporation of the non-wheat component in the flour blends, which provides a new taste to the product. Every bread has its own

requirements and quality characteristics. The quality of bread is a function of its ingredients, yeast activity and processing conditions.

CHAPTER THREE: MATERIALS AND METHODS

3.1 Sample collection and preparation

3.1.1 Cassava

A purposive sampling approach was used for selecting the cassava cultivars based on a previous Baseline Survey conducted in the district of Nebbi, Nothern Uganda (Otim-Nape and Zziwa, 1990; Ogwal-Omara et al., 2012). The district was chosen because it is a place where cassava is commonly grown and consumed as a staple food (Ministry of Agriculture, Animal Industry and Fisheries, MAAIF, 2017) . Fresh roots from three improved cassava cultivars, the Namulonge Selection; NASE 3, NASE 14, and NASE 19, and two local varieties, *Nyamatia* and *Nyarukeca*, were purchased from farmers. The villages and farmers were purposively selected to ensure that they had the studied varieties. MAAIF encourages farmers to grow improved cultivars (NASE 3, NASE 14 and NASE 19) under the Agriculture Cluster Development Project (ACDP) in Nebbi district, because these cultivars are high yielding, disease resistant, less toxic, early maturing and have ready market (MAAIF, 2017).

All the roots were purchased during the rainy season, September and October, to eliminate changes in the roots due to water stress in the dry season. Cyanogen content increases during drought because of the “concentration effect” from reduced yields (which increases cyanide per mass), due to water stress (Ojo et al., 2013). The naturally high cyanogenic glucoside content of bitter cassava cultivars is further increased by water stress. Dry season (inter-seasonal dry spells) water stress, is similarly known to result in increased cyanogenic glucoside levels in cassava (Ojo et al., 2013). Negative correlations between free cyanide and moisture has been reported by Araújo et al. (2019), which

indicates that an increase in water contents promotes fast solubilization and volatilization of cyanide and higher amounts of water in the root may reduce cyanide concentration as well.

The roots, from the cassava plant at thirteen months after planting, were carefully harvested to avoid injuries, inspected for any signs of diseases, e.g., root rot, CBSD, or physiological deterioration, e.g., vascular streaking, and transported same day in plastic bags to Kyambogo University Food Science and Technology Laboratory for the preliminary preparation and analysis. The roots from each cultivar were separately washed in clean portable running water, weighed and peeled. The peeled roots were sliced using a grating hand-operated machine into smaller pieces, approximately 1 to 2 mm thick, dried at 50 ºC for 24 h in a hot- air oven (Carbolite, 4EKF63A – 251, Greiffer berger ABM, Korea) and cooled to ambient temperatures (18 to 22 ºC) for 3 h.

The dried cassava chips were packaged in clean, dry, new, high-density polyethylene (HDPE) bags (Afroplast Enterprises Ltd., Uganda) and stored at ambient temperature (18 to 22 °C) prior to analysis and utilisation. Before analysis, the dried cassava chips were ground to powder using Sunbeam grinder (model SCG-2012, NU WORLD IND, PTY Ltd., Southern Africa). The cassava powder was the High Quality Cassava Flour (HQCF). The analyses were carried out at Uganda Industrial Research Institute (UIRI), chemistry laboratory, Government Analytical Laboratories (GAL) and Kyambogo University Business Incubation Centre (BIC).

3.1.2 Wheat flour and other ingredients

Wheat flour (AZAM brand, home baking flour, Bakhresa Grain Milling, (U) Ltd.), sugar, shortening, salt, nutmeg and instant yeast (2 in1, Plus brand, Fabrique en France par, France) were purchased from local stores in Kampala City.

3.2 Chemical analyses of HQCF

3.2.1 Proximate analysis

3.2.1.1 Determination of moisture

The moisture content of HQCF was measured by weight difference before and after drying of the samples in a hot air oven. The method described by AOAC (1995) was adopted. Two crucibles were properly washed and allowed to dry in an oven (Memmert UF30 F-NRB117.2292, model 30-1060, Memmert GmbH, Schwabach, Germany) at 105 $^{\circ}$C for 5 h to a constant weight. The crucibles were allowed to cool in a desiccator for 30 min, labeled and weighed (W_1). Each sample (2.0 g) was weighed into crucibles and reweighed (W_2). The crucibles containing the samples were placed in an oven maintained at 105 $^{\circ}$C for 5 h. They were removed and transferred to desiccators to cool and finally weighed (W_3). Moisture content was calculated as the difference between weights W_3 and W_2, and expressed as a percentage.

3.2.1.2 Determination of crude protein

Micro Kjeldahl method as described by AOAC (1995) was used. Briefly, 0.6 g of sample HQCF was weighed and placed on nitrogen free filter paper, then folded and dropped into a Kjeldahl digestion tube. Three grammes of digesting mixed catalyst (96% $CuSO_4$ + 3.5 % Na_2SO_4 and 0.5 % selenium oxide) and 25 mL of Conc. H_2SO_4 were added to the sample in the digestion tube. The mixture in the digestion tube was transferred to the

Kjeldahl digestion apparatus (Kjeltec System HT 2, Foss tecator, Hoganäs, Sweden); the heater was regulated at a temperature below the boiling point of the acid until frothing ceased. The mixture was allowed to boil vigorously as temperature was increased, until clear (light) green color was obtained. The digest was allowed to cool then transferred into 100 cm^3 volumetric flask, diluted with distilled water to make up 100 cm^3. A 10 ml aliquot of the digest was introduced into the distillation jacket of the micro steam distillation apparatus that was connected to the mains, as the water in the distiller flask boiled. Twenty millilitres of 40 % NaOH was added to each digest in the distillation jacket.

Fifty millilitres of 4 % boric acid was measured into a 250 mL conical flask and four drops of a mixture of methyl blue and methyl red indicators were added. The conical flask containing the mixture was placed onto the distillation apparatus with the outlet tubes inserted into each conical flask and NH_3 was collected through the condenser. The distillation continued until 25 mL of the distillate was trapped into the boric acid solution and color changed from red to yellow. The distillate was then titrated with 0.02 M HCl and the titre value was recorded. Percentage nitrogen and crude protein was calculated using a correction (protein conversion) factor, F, of 6.25 using the formula:

% Protein = F x % N.

3.2.1.3 Determination of crude lipid

Lipid content was determined by Soxhlet extraction procedure using petroleum ether as described by AOAC (1990). Three grammes of sample (W_1) A and B were placed in two different extraction thimbles respectively then covered with cotton wool. The extraction thimbles containing the samples were placed in the extraction jacket. Two clean dried aluminium cups containing few anti-bumping granules were weighed (W_2) and 300 mL

of petroleum ether was poured into each flask fitted with sohxlet extraction units. The aluminium cups and the condenser were connected to the sohxlet extractor (Soxtec System, 1045 Extraction Unit, 4066, 0009, Hoganäs, Sweden), and cold-water circulation was put on. The heating mantle was switched on and the heating rate was adjusted until the solvents were refluxing at a steady rate. Extraction was carried out for 6 h. The solvents were recovered and the oil was dried in the oven at 70 ºC for 1 h. The aluminium cup and oil were cooled and then weighed (W_3). The percentage lipid was calculated.

3.2.1.4 Determination of starch

Total starch (anthrone reagent) content of the samples was measured colorimetrically based on the method of Oladayo and Joseph (2016). About 100 mg flour sample was first treated with hot 80:20 (v/v) ethanol/water to extract soluble sugars and the residual starch was hydrolysed with 52 % perchloric acid into monosaccharides (glucose), extracted and centrifuged and the supernatant saved. The supernatant was made up to 100 ml with distilled water. Then 0.1 ml of the supernatant was pipetted into tubes using micro-pipette and made up to 1.0 ml with distilled water.

To determine total starch content, calibration curves were made using anhydrous glucose, 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 which were also made to the mark of 1 ml volume and 0 served as a blank. Then 4 ml of anthrone (dissolved in ice-cold 98 % sulphuric acid) were added to the samples as well as to the standard solutions of glucose and heated for about 8 minutes in a boiling water bath. The mixture was then rapidly cooled. The glucose was dehydrated to hydroxymethyl furfural as a result. This compound forms a green coloured product with anthrone.

The glucose content in the standards and the samples was colorimetrically determined using a UV-VIS spectrophotometer (UV – 1601, Shimadzu, Japan) at a wavelength of 630 nm (Duboise et al., 1956; Kalenga et al., 1981; Ezeagu et al., 2011). Glucose content in the sample was found using calibration curve and the following equation:

$$100 \text{ ml of the sample} = \frac{\chi \times 100 \text{ mg}}{0.1 \text{ ml}}$$

where χ = concentration

The total starch content was obtained by multiplying the glucose content obtained from the sample using the standard graph by a factor of 0.9.

3.2.1.5 Determination of ash

Ash was determined using the incineration method as described by AOAC (1990). Samples were incinerated in the Muffle Furnace Nabertherm (model: B – 180, L – 15/12/B180), Nabertherm GmbH, Bremen, Germany. Three porcelain crucibles were washed and dried in an oven to a constant weight at 100 ºC for 10 min. They were allowed to cool in desiccators, then labelled A, B, C, and weighed. Three grammes of each sample (W_1) was weighed into each of the previously weighed porcelain crucibles and reweighed (W_2). The crucibles containing the samples were transferred into the furnace, which was set at 550 ºC and ashed for 8 h. They were then removed and allowed to cool in the desiccators then finally weighed (W_3). The percentage ash content was calculated using the following formula:

$$\% \text{ Ash} = \frac{G_2 - G_1}{W}$$

Where, G_2 = weight after ashing (sample + crucible)

G_1 = Tare weight of crucible

W = Original sample weight

3.2.1.6 Determination of crude fibre

The fibre content was determined using acid hydrolysis according to AOAC (1990), where 2.1 g of sample (W) was weighed into two separate round bottom flasks labelled A and B, respectively. Exactly 100 mL of 0.25 M sulphuric acid solutions was added to each sample in the flask, and the mixtures were boiled under reflux for 30 min. The hot solutions were quickly filtered under suction. The residues were thoroughly washed with hot water until acid free. Each residue was transferred into the labelled flasks and 100 mL of hot 0.3 M sodium hydroxide solution was added and the mixtures were boiled again under reflux for 30 min and filtered quickly under suction using Whatman filter paper No. 4 (Whatman^{TM}, GE Healthcare, UK Ltd.). Each insoluble residue was washed with hot water until it was base free. They were dried to a constant weight in an oven at 100 ^{o}C for 2 h, cooled in a desiccator and weighed (C_1). The weighed samples were then incinerated, and reweighed (C_2). The loss of weight on ignition was used as a measure of the fibre content. Percentage crude fibre content was calculated using the following formula:

$$\% \text{ Crude fibre} = 100 \times \frac{C_1 - C_2}{W}$$

3.2.1.7 Determination of total carbohydrate

The total carbohydrate content was determined by a difference method.

Total carbohydrate = 100 - (% moisture + % ash + % protein + % lipids + % fibre).

3.2.2 Anti-nutritional compounds

3.2.2.1 Determination of total cyanogenic components

Total cyanogen in the cassava products (HQCF) was determined according to the alkaline picrate method developed by Bradbury et al. (1999) using picrate paper kits, Protocol B2. One hundred milligrammes of cassava flour was poured on top of a round paper disc containing buffer at pH 6 and the enzyme linamarase, which was already placed in a flat-

bottomed plastic bottle. Half a millilitre of clean water was added to the cassava flour, and immediately a yellow picrate paper attached to a plastic strip was added. The bottle was immediately closed with a screw-capped lid. A blank was prepared the same way except that no cassava flour was added. To verify the method, a standard linamarin paper was used. The bottle was allowed to stand for 16 to 24 h at ambient temperature ± 5 ºC. The bottle was then opened and the colour of the picrate paper which developed was matched/compared with the shades of the colour chart. The total cyanogen content of the HQCF (mg/kg) was read off from the colour chart.

3.2.2.2 Determination of phytate

Phytate content was determined using the anion-exchange method according to Ma et al. (2005). Samples (1.00 or 2.00 g) were accurately weighed and transferred into 100 mL conical flasks. A total of 40 mL of Na_2SO_4 (100 g/L) and 50 ml of HCl (1.2 %) were added. Flasks were capped and shaken vigorously for 2 h on a rotator at ambient laboratory temperature. The above mixture was then centrifuged at 500 rpm for 20 min, after which the supernatant solution was filtered through Whatman qualitative filter paper No. 4 (Whatman^{TM}, GE Healthcare, UK Ltd.).

Ten mL of filtered extract was diluted to 30 mL with distilled water after mixing with 1 mL of 0.75 M NaOH and then passed through an anion resin column (resin, AG1-X4, 100-200 mesh, BioRad Laboratory, Inc., CA; column, 0.8 × 10 cm, Beijing Glass Instrumental Factory). The column was washed before use with 20 mL of 0.5 M NaCl solution and deionized water until no Cl^- could be detected. After sample application, the column was washed with 15 mL of distilled water and 20 mL of 0.05 M NaCl in order to remove inorganic phosphate. The retained phytic acid (the eluate) from the resin was eluted with 0.7 M NaCl to 25 mL. The post column reagent was made up as a 0.03%

$FeCl_3$ solution containing 0.3% sulfosalicylic acid. A total of 4 mL of the reagent was added into 5 mL of collected eluate and then centrifuged at 3000 rpm for 10 min. The absorbance of the supernatant was measured at 500 nm using a spectrophotometer (UV-1601, Shimadzu, Japan). A calibration curve for the colorimetric method was obtained by using sodium phytate standards (P-8810 Sigma Co., USA). The phytate content of samples was calculated using the standard curve.

3.2.2.3 Determination of oxalate

Total oxalate in each cassava cultivar was determined using the titrimetric method (Adeniyi et al., 2009). An accurately weighed amount of sample (2 g) was digested with 10 ml 6 M HCl for 1 h, cooled and made up to 250 ml in a volumetric flask and filtered using Whatman paper No.1 (Whatman^{TM}, GE Healthcare, UK Ltd.). About 125 ml of the filtrate was measured into a beaker and 3–4 drops of methyl red indicator were added.

The pH of the filtrate was adjusted with concentrated NH_4OH solution (being added drop wise to the test solution) until the colour changed from salmon pink to faint yellow and the pH of the solution was determined. Each portion was heated to 90 ºC, cooled and then filtered using Whatman Filter paper No.1 (Whatman^{TM}, GE Healthcare, UK Ltd.) to remove the precipitate. The filtrate was again heated to 90 ºC and 10 ml of 5 % $CaCl_2$ solution was added with continuous stirring to precipitate the insoluble oxalate. The solution was decanted and the precipitate completely dissolved in 10 ml of 20 % (v/v) H_2SO_4 solution. The filtrate was made to 300 ml mark and aliquot of 125 ml of the filtrate was heated until near boiling, then titrated against 0.05 M standardised potassium tetraoxomanganate (VII) to give pink colour (which persisted for 30 s) at end point. The

burette reading was then taken. The oxalate content was evaluated from the titre value using the formula:

$$\text{Oxalate content (g/100g)} = \frac{T \times Vme \times Df}{ME \times MF} * 100$$

where : T is titre value of $KMnO_4$; Vme is volume-mass equivalent (that is, 1 ml of 0.05 M $KMnO_4$ = 0.00228 g of anhydrous oxalic acid); Df is dilution factor; MF is mass of sample; and ME is molar equivalent of $KMnO_4$ in oxalate concentration (g/dm^3).

3.2.2.4 Determination of tannins

Total tannin content was determined using Folin–Denis method (Markkar et al., 1993). This is based on the non-stoichiometric oxidation of the molecules containing a phenolic hydroxyl group. Tannin-like compounds reduce phosphotungstomolybdic acid in alkaine solution to produce a highly coloured blue solution, the intensity of which is proportional to the amount of tannins. Absorbance was taken using spectrophotometer (UV-1601, Shimadzu, Japan) at a wavelength of 760 nm and concentration was estimated from the tannic acid standard curve prepared by taking absorbance of standard tannic acid solution with concentration range of 25 to 100 μg/L. Results were expressed as milligrams of tannic acid equivalents (TAE) per 100 g of cassava flour.

3.2.3 Determination of minerals

The mineral (Ca, Zn, Fe, Mg, and Cu) content was determined from the ash of the samples using an Atomic Absorption Spectrophotometer (AAS) (AAS system, AAnalyst 400, 2009, Perkin Elmer, Singapore) according to AOAC (1995). Uganda has adopted the harmonized Uganda standard/East Africa fortification standards on maize flour fortification (US: EAS 768), and fortified wheat flour (DEAS 767: 2017), under Uganda's Food and Drug Act (Regulations), 2011, to fortify flour with vitamins and minerals. The

minerals of interest are iron and zinc. Calcium and magnesium are normaly found in root crops; hence the selected minerals were determined. About 1g of the dry sample was weighed into a tarred crucible, which was then placed in a cool muffle furnace. The furnace was ignited for 4-6 h at about 550 °C. The furnace was turned off and left to cool to about 250 °C. The door was carefully opened to avoid losing ash that may be fluty (sample in crucible was completely white with no black spots). Using safety tongs, the crucible was quickly removed and transferred to a dessicator with a porcelain plate and desiccant. The crucible was covered, the dessicator was closed and the crucible allowed to cool.

The ash was dissolved in 5 ml of 20 % HCl, the solution was warmed to dissolve any un-dissolved particles in the residue. The solution was filtered through an acid washed filter paper into a 50 mL volumetric flask. The filter paper was washed and the solution diluted to volume with potassium chloride solution. The solution was transferred to a sample Vial, the AAS switched on, and the absorbance read. The mineral contents: Calcium (Ca), Zinc (Zn), Iron (Fe), Magnesium (Mg), and Copper (Cu) were determined at the respective wavelengths (λ) of 317.0, 213.9, 248.3, 285.2, and 324.8 nm. The mineral content was calculated using the calibration curves for the specific minerals.

3.3. Production of high quality unfermented cassava flour

The roots were processed into HQCF using the method described by IITA (2006). The roots were separately washed, weighed and peeled manually using a stainless knife. The peeled cassava samples were then washed with portable water and weighed to determine percentage yield after peeling. The cleaned tubers were grated manually using stainless steel kitchen grater to form slurry/mash. The mash was dewatered using hydraulic presser

to about 40 % moisture. The cake was pulverized and subjected to drying in a hot air oven (Carbolite, 4EKF63A – 251, Greiffer berger ABM, Korea) set at 50 ^{o}C and dried to constant moisture level. After drying, the cake was removed from the oven and left in the open to cool to room temperature (22 ^{o}C). The cooled samples were packaged in new polythene bags and stored at room temperature for analysis. Before analysis the samples were ground to powder using a mortar and pestle. The fine HQCF thus obtained was packed in clean, dry, high-density polyethylene bag, awaiting use.

3.4 Physico-chemical analysis of HQCF

3.4.1 Determination of pH

Ten grammes of HQCF was weighed into a 250 ml beaker. Ninety ml of distilled water was added and the content mixed homogeneously, and then left for 1 h at room temperature. The pH was measured using a pH meter (Mettler Toledo, Switzerland). The pH meter was first calibrated with buffers of pH 4, pH 7, and pH 10. pH values were measured in triplicate, meanwhile, the meter was washed thoroughly using distilled water and calibrated after each measurement.

3.4.2 Determination of total titratable acidity

Titratable acidity was determined using AOAC (2000) method, where 25 ml of the filtrate obtained from 5 g of HQCF dissolved in 50 ml of distilled water was collected in a 100 ml flask. Three drops of 1 % phenolphthalein indicator were added to the solution in the flask and thoroughly mixed. Titration was carried out by adding 0.1 M NaOH until end point identified by a color change to pink which persisted for about 10 s. The volume of NaOH added was multiplied by 0.09 to obtain the % titratable acidity as lactic acid (1 mL of 0.1 M NaOH = 0.009 g of lactic acid).

3.4.3 Determination of swelling power

Swelling power and swelling volume were determined based on the method of AACC (2000) on all the flours, where 0.25 g of HQCF was transferred into a weighed graduated 25 ml centrifuge tube. Ten millilitres of distilled water was added to the centrifuge tube. The suspension in the tube was stirred gently by hand and then heated at 85 ºC in a water bath (Grant Instruments Ltd., Cambridgeshire, UK) for 30 min with constant shaking. After cooling to room temperature (18 - 22 ºC), the suspension was centrifuged for 15 min at 2200 rpm (Hermle, Hermle Labortechnik GmbH, Germany). The supernatant was transferred into a can, dried at 105 ºC for 3 h in a hot air oven (BS Gallenkamp, England) and the dry residue was weighed. The sediment paste (pellet) was weighed. The swelling volume was obtained by directly reading the volume of the sediment in the graduated tube. The swelling power was recorded as the ratio in weight of the wet sediment to the initial weight of the HQCF.

$$Swelling\ power = \frac{W_{pellet}}{W_{sample\ dry\ basis} - W_{dried\ residue}}$$

3.4.4 Determination of water-binding capacity

Water binding capacity was determined according to the method AACC (2000), where 0.5 g of HQCF was mixed with 10 ml of distilled water in a centrifuge tube. The suspension was agitated for 1 h at room temperature (22 ºC) on a shaker (Grant instruments) and centrifuged for 10 min at 2200 rpm (Hermle, Hermle Labortechnik GmbH, Germany). The free water was decanted from the pellet and drained for 10 min. The pellet was weighed and water-binding-capacity of the HQCF was calculated, expressed as percent water bound per gramme of HQCF.

$$WBC = \frac{W_{bound\ water}}{W_{sample}} * 100$$

Where: $W_{bound\ water}$ is the weight of the pellet after centrifugation – weight of the initial HQCF and W_{sample} is the weight of the initial HQCF

3.4.5 Determination of water solubility

Water solubility was determined based on a modification of the method of AACC (2000) on all the blends, where 0.25 g of HQCF was transferred into a weighed graduated 25 ml centrifuge tube. Distilled water was added to give a total volume of 10 ml. The suspension in the tube was stirred gently by hand and then heated at 85 ºC in a water bath (Clifton, Model 85390, Nickel – Electro Ltd., England) for 30 min with constant shaking. After cooling to room temperature, the suspension was centrifuged for 15 min at 2200 rpm (Hermle, Hermle Labortechnik, Wehingen GmbH, Germany). The supernatant was transferred into a can, dried in a hot air oven at 105 ºC for 3 h (Memmert UFE - 600) and the dry residue was weighed. The solubility was calculated using the formula:

$$Solubility\ (\%) = \frac{W_{dried\ residue}}{W_{sample\ dry\ basis}} * 100$$

W_{pellet} is the weight of the sediment paste after centrifugation, $W_{sample\ dry\ basis}$ is the weight of the initial HQCF on dry basis, $W_{dried\ residue}$ is the weight of the residue of supernatant after drying.

3.5 Formulation of wheat-cassava composite flour

Researchers have reported up to 40 % cassava inclusion levels in wheat–cassava composite flour and bread (Siyeni et al., 2004; Jensen et al., 2015). The East African Standard (EAS 741:2010) recommends a minimum of 10% HQCF by weight in composite flour. NASE 14 is the most grown cassava cultivar in Nebbi district (Omony, 2017), and proximate analysis showed that it had the highest protein content among the studied cultivars. HQCF from NASE 14 was therefore chosen to form the composite with wheat. The wheat flour and HQCF composites were consequently formulated in the

ratios: 100:0, 90:10, 80:20, 70:30, 60:40, 50:50, and 40:60 respectively and stored in clean, dry high-density polyethylene (HDPE) bags prior to utilisation and analysis.

3.6 Analysis of wheat-cassava composite flour

3.6.1 Rheological characterisation of the composite flour

Evaluation of the suitability of wheat-cassava composite flours for baking was carried out using the Mixolab Profiler, a feature of the Mixolab 2 equipment. The wheat and cassava flours used in the experiment were of known moisture content (13.58 % for wheat flour and 12.46 for cassava flour) and water absorption capacities (57.5 % for wheat flour and 68.5 % for cassava flour). Moisture content of the wheat and cassava flours used in the research were measured and calculated using the oven drying method according to AACC (2000) method (section 3.2.1.1). Water absorption capacitiy of each flour and composite was measured using Mixolab 2 (Chopin Technologies v 4.1.2.5, Villeneuve-Garenne, France) (Mixolab Applications Handbook, 2012). The water absorption capacity of the flour being defined as the hydration required to bring dough to a given maximum consistency. This consistency is equal to 1.1 Nm (+/- 0.05 Nm) with the "Chopin+" protocol (equivalent to 500 Farinograph units) (Mixolab Applications Handbook, 2012). It is generally expressed on a 14 % hydration base.

3.6.2 Mixolab Properties

Dough mixing and pasting characteristics of flour composites were studied using the mixolab (Chopin Technologies, v 4.1.2.5, Villeneuve-la-Garenne, France). Mixolab has the capacity to determine qualitative profile of flour and plot the torque produced by the passage of the dough between two kneading arms when subjected to both shear stress and temperature constraints. Mixolab analysis was carried out using the water absorption

level determined by the consistograph according to the method of AACC (2000). The standard "Chopin +" protocol was followed. The evaluated parameters were: Water absorption (WA), which corresponded to optimum dough consistency of 1.1 Nm; Dough Development Time (DDT), which was the time taken to reach the maximum torque at 30 ºC); dough stability at mixing, which was the elapsed time at which the torque produced was kept at 1.1 Nm; C1 torque, which determined absorption/dough development; C2 torque, which corresponded to the weakening of the protein/decrease in dough consistency as induced by mechanical work and temperature; C3 torque, represented the starch gelatinization; C4 torque, represented the stability of hot starch paste; C5 torque, represented the final starch paste viscosity after cooling at 50 °C.

3.6.3 Consistograph properties

The effect of cassava flour substitution on the rheological characteristics of wheat flour was studied using Consistograph (AlveoLAB, Chopin Technologies, Villeneuve-la-Garenne, France), according to AACC (2000) method. Consistographic characteristics determined were: 1) water absorption capacity (WAC), which was the absorption of the composite flour (14 % moisture basis) calculated by the Chopin Software, to obtain a target consistency, and 2) maximum pressure (PrMax) at constant hydration (CH).

3.6.4 Alveograph properties

Alveograph test (the determination of viscoelastic behaviour of flour blends) was carried out according to AACC (2000) method using Alveograph (AlveoLAB, Chopin Technologies, Villeneuve-la-Garenne, France) at constant hydration. Alveograph parameters automatically recorded by a computer software were: the maximum overpressure (P) needed to blow the dough bubble; the average abscissa (L) at bubble

rupture; the ratio of tenacity to extensibility of the dough, P/L; the deformation energy (W); and flexibility/elasticity index, *Ie.*

3.7 Baking of the composite bread

A modification of the recipe´ for bread production proposed by Sanni et al. (2006) was used to formulate the bread loaves in this study. The amount of water (57.5 to 64.0 %) used was determined by the Mixolab. The straight dough method was used. Bread was prepared with the following ingredients: flour (200 g), water (57.5 to 64.0 %), margarine (45 g), sugar (25 g), salt (1.5 g), dried instant yeast (2.5 g) and nutmeg (0.5 g). Bread baked with 100 % wheat flour (100:0 ratio) was used as control. Mixing was done manually for 15 min prior to kneading. The dough was kneaded manually for 3 to 5 min until smooth dough of uniform consistency (silky and elastic) was obtained. To assess if the dough was properly developed, the gluten film test was performed (Hamer et al., 2009; MacRitchie, 2010), whereby a small portion of dough was stretched between the hands into a thin, smooth, translucent film to test its extensibility and elasticity. The dough was moulded into loaves, transferred immediately into greased baking cans, and proofed for 30 min at ambient temperature. The loaves were baked in an electric oven (Gallenkamp, London, UK) at 200 ± 2 ºC for 30 min, removed after baking, cooled to ambient temperature and wrapped in polyethylene bags ready for analysis.

3.8 Analysis of bread quality attributes

3.8.1 Physical evaluation of bread

3.8.1.1 Determination of loaf weight

Loaf weight was determined using the AACC method (2000). Loaf weight was determined after sufficient (2 h) cooling using a digital balance RADWAG (0.001 g

accuracy) (RADWAG Wagl Elektroniczne, WTC 3000, Poland). Independent measurements were recorded in triplicates.

3.8.1.2 Determination of bread volume and density

The weight (W) of the loaf was measured as in 3.8.1.1. Loaf volume (VL) was determined by the Rapeseed Displacement Method according to the AACC method (2000) as modified by Greene and Bovell-Benjamin (2004). Millet seeds were used instead of rapeseeds. The loaf was put in a baking can of known volume (VB) and the baking can filled to the brim with millet seeds. The edge of a meter rule was used to cut off all seeds above the can rim such that the seeds formed a plateau with the rim of the can. The loaf was removed and the volume of the millet seeds (VM) was measured with a measuring cylinder. Loaf volume (VL) was determined according to the following formula: VL (cm^3) = VB−VM; Specific volume (SV) was calculated using the equation: SV (cm^3/g) = VL /W; Density was calculated using the equation: DL (g/cm^3) = W/VL

3.8.1.3 Determination of water loss in bread

The weight of the bread loaf was measured 2 h after baking and the percentage water loss was calculated as the difference between the weight of dough before baking and the weight after baking, i.e., the weight of loaf, and expressed as a percentage.

Water Loss = weight of dough – weight of loaf

3.8.2 Sensory evaluation of bread

Bread loaves were allowed to cool for 12 h and cut into slices of uniform thickness and transferred onto white coloured plates coded with random 3-digit codes. A sensory panel consisting of 20 randomly selected semi-trained staff members and graduate students at Kyambogo University, Deparment of Food Science and Technology, who are familiar

with the sensory attributes of local bread, was employed to evaluate the coded products. A 9-point Hedonic scale was used to rate the bread for crust colour, taste, crumb texture and overall acceptability. A score of 1 represented “dislike extremely”, 5 - neither like nor dislike, and 9 represented “like extremely” (Lawless and Heymann, 1999). An atmosphere of complete quietness, conventional lighting and privacy was provided for each panelist. Seven bread samples were evaluated simultaneously and served randomly and individually to the panelists along with water to cleanse/rinse their palate between tasting. Each panelist was presented separately with a loaf of bread for external quality assessment and a slice of about 3 cm thick from the same sample for crumb assessment.

3.9 Statistical data analysis

Quantitative data were subjected to analysis of variance (ANOVA) using the IBM Statistical Package for Social Sciences (SPSS), version 23. Results are presented as Means ± standard deviations. Least significant difference (LSD) test was used to separate means for the cultivars and cassava inclusion levels. Differences between means were considered significant at $p < 0.05$. The Mixolab Profiler indices were systematically rounded to the nearest unit. A difference of 1 point on the Profiler was regarded not a significant difference (Mixolab Applications Handbook, 2012). The relationship between proportion of cassava addition (%), dough rheology and bread physical and sensory properties was evaluated by Principal Component Analysis (PCA) using XLSTAT (v.2.2, 2019).

CHAPTER FOUR: RESULTS AND DISCUSSION

4.1 Chemical composition of HQCF from the selected cassava cultivars

4.1.1 Proximate composition

Moisture content differed significantly ($p < 0.05$) among the HQCF from the selected cassava cultivars except between NASE 3 and NASE 19 (Table 8). The moisture content of HQCF from the local cassava cultivars was lower than that of the improved cultivars (5.43 % for *Nyamatia* and 8.03 % for *Nyarukeca* as compared to 10.07 % for NASE 3, 8.65 % for NASE 14, and 10.87 % for NASE 19). The lower the moisture, the higher the dry solids content (protein, starch, fat, sugars, and ash). The higher the water content, the lower the value of the flour as less solids can be used to produce value-added products. This may affect processing and handling conditions (Posner, 2011).

Sarkiyayi and Agar (2010) reported much lower moisture values of 0.82 % for sweet and 0.14 % for bitter Nigerian cassava cultivars. Baah et al. (2005) reported moisture contents between 6.68 and 10.96% in peeled, washed, grated and de-watered cassava roots. Charles et al (2005) reported moisture contents between 9.2 and 12.3 % among cassava cultivars grown in Thailand. The differences in the moisture content of HQCF from the cassava cultivars in this study compared with literature values might be due to their differences in initial moisture content, textural structures (which cause differences in the effective diffusitivities of the chips), the thickness/depth of the drying layers and constituent solutes (Kajuna et al., 2001; Usman and Idakwo, 2011). Resistance to moisture movement that creates gradients within the material being dried cannot be neglected (Kajuna et al., 2001).

Moisture content of flour is a shelf-life and stability indicator, i.e., the moisture content of flour is important for storage stability and longer shelf-life of the flour (Kent and Evers, 1994; Nasir et al., 2003; Posner, 2011). High moisture content (above 14 %) encourages the growth of microorganisms present in the flour, which encourages the production of off flavours and off odours (Posner, 2011). Mustiness may develop in the flour due to mould growth. Low moisture levels inactivate enzymatic activity and microbial growth (fungal and bacterial) (Posner, 2011). At moisture content lower than 12 %, the risk of fat oxidation and development of rancidity increases (Kent and Evers, 1994). The moisture content of the HQCF in this study (5.43 to 10.78 %) showed that the flour was safe for storage. During bread making, flours with high moisture content absorb less water, since the flour is already "water–rich" and has less room to add more**.** Conversely, the drier the flour, the more water it will absorb (Zghal, 2001; Puhr and D'Appolonia, 1992). The East African Standards for HQCF has put the moisture content limit of 12 % (DEAS 779: 2012). A negative correlation between cyanide and moisture has been reported by Araújo et al. (2019). This finding might be interesting for the manufacturing of products derived from cassava root.

The ash contents differed significantly ($p < 0.05$) between the improved and local cultivars. The improved cassava cultivars had a higher content of ash (2.27 % for NASE 3 and NASE 19 and 2.39 % for NASE 14) compared to the local cultivars (1.5 % for *Nyarukeca* and 1.05 % for *Nyamatia*), an indication of a higher mineral content in the improved cultivars. Sarkiyayi et al (2010) reported values of 2.71 % for sweet and 1.85 % for bitter cassava cultivars. Safo-Kantanka and Acquistucci (1996) reported ash contents between 1.2 and 1.6 % among Ghanaian cassava cultivars harvested at 13 months and between 1.2 and 2.1 % among cassava cultivars harvested at 6 months. Baah et al. (2005) reported ash contents between 1.15 and 1.2% in sone Ghanaian cassava cultivars. Charles

et al. (2005) reported ash contents between 1.3 and 2.8 %. Higher ash contributes to the flavour of the bread and the nutrient quality but also compromises the gluten strength (thebakingnetwork.com, accessed at www.theartisan.net, 2021).

Despite not being regarded as a flour quality parameter in some bakers' specifications, ash in flour is still relevant for bakers and millers (Kent and Evers, 1994; Posner, 2011; Carson and Edwards, 2009). The following are some of the reasons: firstly, ash content is a good indicator of bran contamination in white or refined flours. As flour extraction rate is increased, the amount of contamination with non-endosperm increases and the ash content increases. For most breadmaking applications, bakers look for excellent quality flours with high protein levels and highest purity in terms of endosperm content (Carson and Edwards, 2009). Secondly, it is a good method to differentiate patent flours from clear flours. The ash content of patent flours is lower compared to clear flours. Thirdly, during storage the rate of increase in acidity of flour increases with fall in flour grade, i.e., with increase in ash residue. Therefore, the shelf-life of brown and wholemeal flours is shorter than that of white flour (Kent and Evers, 1994).

In the present study all the investigated cassava cultivars showed low fibre contents (approximately 1 %), majority of which were not significantly ($p > 0.05$) different from each other except between NASE 19 and *Nyamatia*. However, the fibre content was higher than recommended by the East African Standards (0.2 %). Sarkiyay et al. (2010) reported fibre contents of 4.40 % for sweet and 4.61% for bitter cassava cultivars. Safo-kantanka et al. (1996) reported fibre contents of between 5.4 and 8.3 % among cassava cultivars harvested at 13 months; and between 3.9 and 6.1 % among cultivars harvested at 6 months. Baah et al. (2005) reported fibre contents between 2.9 and 3.64 %. Charles et al. (2005) reported higher fibre contents than obtained in this study, ranging from 1.5 to

3.5 %. In bread baking, fibre has a negative impact on bread volume and crumb texture. High fibre content results in lower volume and harder and more cohesive crumb structure (Jensen at al., 2015). Fibre imparts colour variability, giving a browner or darker colour to the bread. Howver, fibre imparts a sweet nutty flavour to baked products, improves texture by providing structure to bread during the leavening process, and increases dough water-holding capacity (Kurek et al. 2015). The type, content and particle size of fibre can also influence the hydration properties of flours (Boye et al., 2010; Farooq and Boye, 2011).

Protein contents differed significantly ($p < 0.05$) among the cultivars except between NASE 3 and NASE 19, and between NASE 19 and *Nyarukeca*. The protein contents were higher in the improved cultivars (1.32 % for NASE 3, 1.51 % for NASE 14, and 1.19 % for NASE 19) compared to the local cultivars (0.74 % for *Nyamatia* and 1.04 % for *Nyarukeca*). Sarkiyayi et al. (2010) reported higher protein contents (2.69 % for sweet and 3.37 % for bitter cultivars). Safo-kantanka et al. (1996) reported lower protein contents of between 0.6 % and 1.0 % among six cassava cultivars investigated. Baah et al. (2005) reported protein contents of 0.24 and 0.42 %. Charles et al. (2005) reported protein contents between 1.2 and 1.8 %, similar to the findings in this study. Araújo et al. (2019) reported protein contents in 'bitter' cassava varying from 0.46 to 2.27% and from 0.18 to 1.10% for 'sweet' cassava cultivars. Nyakaisiki (2016) reported protein content between 0.5 and 1.0% on a fresh weight basis in some cassava varieties in western Uganda harvested at 12 months after planting. Differences in the protein content of cassava cultivars in this study might be due to genotype rather than environment, since the cassava roots were collected from the same agro-ecological zone. The study shows that cassava is not a major source of protein in the human diet; therefore it has to be

consumed with other foods with high protein contents. Cassava flour could be chemically fortified with proteinous foods, or biofortified to enhace its protein content.

Proteins serve several functions in foods and bakery systems such as foaming, emulsifying, viscosity building, gelling and water-binding, flavor and color development through Maillard reaction (Hoseney, 1986; Kent and Evers, 1994; Coultate, 1999; Boye et al., 2010; Farooq and Boye, 2011). In baking, proteins perform as dough conditioners, structuring agents and moisture controllers. The higher the concentration of the proteins, the stronger the gluten bond in the flour. Gluten bonds influence the cell structure or "crumb" of bread and the ability of the dough to rise or trap gas produced from yeast activity. Bread wheat flours contain higher levels of gluten resulting in a tough and chewy bite while cakes have a light and airy texture due to lower amounts of gluten in the flour. The bakery industry relies on the utilisation of the unique properties of the wheat endosperm proteins to create a variety of baked goods. HQCF does not contain gluten, hence dilutes the wheat gluten when incorporated into wheat flour.

Table 8: Proximate composition (%) of high quality cassava flour from improved and local cassava cultivars grown in Uganda

Parameters	**NASE 3^{S}**	**NASE 14^{S}**	**NASE19^{S}**	***Nyamatia ^{B}***	***Nyarukeca^{B}***
Moisture	10.69±0.07^{a}	8.65±0.19^{b}	10.87±0.16^{a}	5.43±0.22^{c}	8.03±0.04^{d}
Ash	2.27±0.03^{a}	2.39±0.08^{a}	2.27±0.39^{a}	1.05±0.02^{b}	1.5±0.05^{b}
Crude fibre	1.08±0.03^{ab}	1.07±0.00^{ab}	1.18±0.03^{a}	1.06±0.02^{b}	1.08±0.01^{ab}
Crude protein	1.32±0.08^{a}	1.52±0.05^{a}	1.19±0.05^{a}	0.74±0.04^{b}	1.04±0.15^{c}
Crude lipid	0.48±0.05^{a}	0.57±0.01^{abd}	0.63±0.01^{b}	0.39±0.03^{acd}	0.48±0.01^{acd}
Starch content	84.42±1.98^{a}	75.25±1.40^{b}	66.72±3.65^{c}	78.44±2.22^{ab}	71.75±0.07^{bc}
Total carbohydrate	85.27±1.20^{bc}	85.83±0.43^{bc}	83.86±0.91^{c}	91.33±0.47^{a}	87.87±0.37^{b}

S: Sweet cultivar, B: Bitter cultivar

The values are means ± SD of triplicate determinations expressed on dry weight basis.

Values in the rows with different superscripts are significantly different at $p < 0.05$.

Lipid contents were low in both improved (0.48 to 0.63 %) and local (0.39 to 0.48 %) cultivars with signicant ($p < 0.05$) differences among some cultivars (Table 8). Sarkiyayi et al. (2010) reported high lipid values (3.92% for sweet and 3.82 % for bitter cassava cultivars), while Safo-kantanka et al. (1996) reported lipid values of 1.5 % and 2.2 % for the Ghanaian cassava cultivars they investigated. Charles et al. (2005) reported lower lipid contents of between 0.1 and 0.8 % in cassava. Difference in lipid content of cassava cultivars may be due to the genotype, since these cultivars are grown in different countries on the African continent and in Thailand (Asia). In the Ugandan context, cassava is not a good source of lipids in the human diet. The low content of lipids implies that the HQCF produced from the different cassava cultivars in this study was stable during storage with respect to rancidity. Lipids may act as foam stabilizers, amplifying their effects on loaf volume. Flour lipids is believed to exert their action in baking

through their role as surfactants in stabilizing or destabilizing the gas bubble structure during expansion of the loaf (MacRitchie, 1977).

Starch contents differed significantly ($p < 0.05$) among the improved (84.42 % for NASE 3, 75.25 % for NASE 14, and 66.72 % for NASE 19) and between the local cultivars (78.44 % for *Nyamatia* and 71.75 % for *Nyarukeca*). Nuwamanya et al. (2010) reported starch contents ranging between 70.36 and 93.85 % (dry basis) among local and improved cassava cultivars grown in Uganda. Safo-kantanka et al. (1996) reported starch contents ranging from 69 to 71 % in cassava roots from Ghana and Nigeria, while Baah et al. (2005) reported starch yields of 68.89 % and 79 % in cassava cultivars grown in Ghana. Aryee et al. (2006) reported starch content ranging from 67.92 to 88.11 % in 31 selected cassava varieties grown in Ghana, similar to the results obtained in this study. Araújo et al. (2019) reported that bitter cassava had higher starch and sugar contents than sweet cassava cultivars. Nyakaisiki (2016) reported starch contents ranging from 14 to 18 % on fresh weight basis. According to this study, Ugandan cassava cultivars are richer in starch than their counterparts in West Africa. The high starch content of cassava roots is an important characteristic that makes the crop a potential industrial cash crop.

An initiative on cassava which aims at industrialising the cassava sector through starch extraction could create jobs and improve livelihood. This could in turn spur research to identify cassava cultivars that can yield more starch (75 % or more) per total dry weight to feed the starch factories for sustainable production. The roots of “bitter” cassava cultivars tend to have higher starch contents (Corbishley and Miller, 1984), which has not been the case in this study. Araújo et al. (2019) reported that starch was positively correlated with total cyanide, where the researchers found that a group of ‘sweet’ cassava

had lower starch contents in their roots than that of 'bitter' cassava cultivars of local cassava cultivars found in Brazilian Amazona Region.

Starch is known to exhibit high swelling power in water, and when this power is combined with the swelling abilities of proteins and fiber, starch can contribute superior swelling ability (Boye et al., 2010; Farooq and Boye, 2011). Thus, the quantity and composition of starch in flours and fractions can influence their water binding capacity and swelling properties (Boye et al., 2010; Farooq and Boye, 2011). In bread making, starch in flour contributes to the development of optimal bread crumb and crust texture. It is also responsible for the physical deterioration of bread quality through staling (Onyango, 2016).

Total carbohydrate content was between 83.86 and 91.33, with significant difference between the local cultivar *Nyamatia* and all the other cultivars both improved and local. Charles et al. (2005) reported a lower range of carbohydrates between 80.1 and 86.3 %. This makes cassava an important source of carbohydrate in Uganda. Hence, cultivar but not environment or maturity of the roots may be of significance at the time of harvesting since the cassava cultivars were harvested at the same age of thirteen months after planting, and were grown in the same agro-ecological zone. However, Sarkiyayi et al. (2010) reported total carbohydrate content of 85.46 % in sweet cassava cultivars and 86.21% in bitter cassava cultivars, showing a significant difference between the two cultivars. The results of current study might mean that there is a difference in the potential of different cultivars to accumulate carbohydrates in their cells since belonging to either improved or local cultivar does not show a particular trend in total carbohydrate content of roots.

During bread making, reducing carbohydrates and dextrins react with amino acids in the non-enzymic browning reaction, Millard type, to form melanoidins which give bread crust the brown colour (Kent and Evers, 1994; Coultate, 1999). The interaction of reducing sugars and amino compounds produce aldehydes and alcohol, which give bread its aroma and flavour (Kent and Evers, 1994; Coultate, 1999).

Overall, the cassava cultivars in this study conformed to the compositional requirements for cassava flour of the Uganda and East Africa Standards (crude ash content, max. 3.0 %; moisture content, max. 13 %; crude fibre content, max. 2.0 %; and acid insolub le ash, max. 0.35 % and starch content, min. 60 %) (US 347: 2007; EAS 779:2012).

4.1.2 Mineral content

Mineral content differed significantly ($p < 0.05$) among cassava cultivars (Table 9). Zinc, magnesium, copper, and calcium contents were particularly different in the cultivars. The mineral contents were generally low, compared to what has been reported except for calcium (13 to 18 mg/100g), and magnesium (3.6 to 3.9 mg/100g). The composition of cassava roots depends on several factors, such as geographic location, cultivar, age of the harvested plant, soil conditions and environmental conditions (Chavez et al., 2000; Montagnac et al., 2009; Bayata, 2019). Limited content of these minerals in most soils where cassava is grown may limit their content in the roots (Bechoff, 2017). Cassava plant is poor at concentrating mineral micronutrients at nutritionally significant levels, since there is no genetic diversity for these traits within the existing germplasm (Ihemere et al., 2012; Narayanan et al., 2019; Narayanan et al., 2020; Okwuonu, et al., 2021).

Table 9: Mineral content of high quality cassava flour from improved and local cassava cultivars (mg/100g) grown in Uganda

Element	**NASE 3^{S}**	**NASE 14^{S}**	**NASE 19^{S}**	***Nyamatia ^{B}***	***Nyarukeca ^{B}***
Calcium	16.56±1.19^{a}	14.28±0.02^{b}	14.88±0.01^{c}	13.15±0.03^{d}	18.09±0.01^{e}
Iron	0.01±0.00^{a}	0.01±0.00^{a}	0.01±0.00^{a}	0.01±0.00^{a}	0.002±0.00^{b}
Zinc	0.87±0.00^{a}	0.64±0.00^{b}	0.64±0.00^{b}	0.56±0.00^{c}	0.60±0.00^{d}
Magnesium	3.73±0.00^{a}	3.67±0.01^{b}	3.58±0.00^{c}	3.65±0.00^{b}	3.88±0.01^{c}
Copper	0.14±0.00^{a}	0.05±0.01^{b}	0.08±0.00^{c}	0.002±0.00^{d}	0.03±0.00^{e}

S: Sweet cultivar, B: Bitter cultivar

The values are means ± SD of triplicate determinations expressed on dry weight basis; Values in the rows with different superscripts are significantly different at 8$p < 0.05$.

Sarkiyayi et al. (2010) reported higher values for calcium (30 mg/100g in bitter and 33 mg/100g in sweet cassava cultivars) and for iron (18 mg/100g in bitter and 30 mg/100g in sweet cassava cultivars) than levels in this study. Charles et al. (2005) reported much higher levels of calcium (136 to 369 mg/100g) and for magnesium (31 to 43 mg/100g) in cassava cultivars grown in Thailand. The copper contents in this study were between 0.002 and 0.14 mg/100g, which are within the limits required by the Ugandan Standards (US 347:2007) or East African Standards (EAS 740:2010). Charles et al. (2005) reported higher levels of copper (between 0.037 and 0.057 mg/100g), zinc (between 13 and 19 mg/100g), and iron (between 29 and 40 mg/100g). Thai cassava cultivars are thus richer in minerals than Ugandan cultivars, implying that cassava roots may be a reasonable source of calcium and magnesium in the diet of Ugandans compared to other minerals evaluated, despite the fact that the mineral content is below the Recommended Dietary Allowance (USDA, accessed at www. usda.gov, 2021). At low moisture content of flour

(below 12 %), metal ions such as Cu^{2+} may catalyse reactions leading to oxidative rancidity of the flour (Kent and Evers, 1994).

4.1.3 Anti-nutritional compounds

4.1.3.1 Cyanogenic glycosides

Contents of cyanogenic glycosides were significantly ($p < 0.05$) higher in the local varieties compared to the improved varieties (Table 10). The cyanogenic glycosides ranged from 30 mg/kg in NASE 3 and NASE 19, to 800 mg/kg in *Nyamatia*. Sarkiyayi et al. (2010) reported much lower cyanogenic glucoside values of 4.6 mg/kg and 6.5 mg/kg for sweet and bitter cassava varieties, respectively. Nyakaisiki (2016) reported cyanogenic glucoside contents ranging between 28 and 53 mg/kg on fresh weight basis. Charles et al. (2005) reported lower values for cyanide potential in cassava varieties from Thailand, ranging from 8.33 to 28.8 mg/kg on dry weight basis (the cyanide potential ranged between 26.9 and 28.8 mg/kg in bitter cassava varieties and between 8.33 and 12.5 mg/kg in sweet cassava varieties).

Bakayoko et al. (2009) reported total cyanogen content of 9.1 to 13.3 mg/kg (FW) of improved cassava cultivars and 36.2 mg/kg (FW) in a local cultivar. Aryee et al. (2006) reported cyanogenic potential ranging from 0.58 to 20.0 mg/100g (DW) from 31 selected Ghanaian cassava varieties. Oloya et al. (2017) reported cyanogenic contents between 101. 84 and 116.54 mg/kg (D/W) among improved varieties (NASE 3, NASE 9, NASE 14, NASE 19, TME 14, and TME 204), and between 88.50 and 316.69 mg/kg among local varieties (*Nyar-anderiano*, *Nyar-papoga*, *Nyar-udota*, *Nya-pamitu*, *Bisimwenge*, and *Nya-matia*) harvested 13 months after planting from Zombo District, Uganda. These researchers reported lower cyanide contents in two local varieties (*Nyar-udota* and *Nyar-anderiano*) compared to the improved varieties investigated. They also reported a much

lower level of cyanogenic contents in *Nya-matia* grown in Zombo district compared to the one grown in the neighbouring Nebbi district, insinuating the influence of soil and weather on the composition of cassava.

The World Health Organisation (WHO) set a safe limit of 10 mg/kg total cyanide for cassava flour (FAO/WHO, 1995). This maximum limit of 10 mg/kg has been adopted in Uganda. According to Uganda Standards, (US 347: 2007), the total hydrocyanic acid content of cassava flour shall not exceed 10 mg/kg, while 40 mg/kg is the limit in Indonesia (Djazuli and Bradbury, 1999). Internationally, the Codex Standard for 'sweet cassava' (those cultivars with low levels of cyanogens) is 50 mg/kg (fresh weight basis, FAO/WHO, 2005). The local cultivars are also bitter, implying that the level of cyanogenic glucosides in a cassava root is directly related to bitterness. Since cyanogenic glucosides release the toxic cyanide as a breakdown product, bitterness of a cassava root is directly related to its toxicity. All cultivars require detoxification before consumption, and reduced consumption is necessary to limit the ingestion of toxic levels of cyanide.

Table 10: Anti-nutritional composition of high quality cassava flour from improved and local cassava cultivars (mg/kg) grown in Uganda

Parameter	NASE 3^{S}	NASE14^{S}	NASE 19^{S}	Nyamatia^{B}	Nyarukeca^{B}
Cyanogenic glucoside	30.00 ± 0.00^{d}	50.00 ± 0.00^{c}	30.00 ± 0.00^{d}	800.00 ± 0.00^{a}	200.00 ± 0.00^{b}
Phytate	984.64 ± 0.00^{a}	959.57 ± 0.80^{a}	877.14 ± 19.60^{b}	773.92 ± 8.04^{c}	661.33 ± 20.2^{d}
Oxalate	227.80 ± 4.05^{a}	181.60 ± 0.11^{b}	161.40 ± 2.02^{c}	140.40 ± 1.21^{d}	90.60 ± 0.21^{e}
Tannins	0.325 ± 0.01^{a}	0.29 ± 0.04^{b}	0.26 ± 0.02^{b}	0.24 ± 0.03^{b}	0.18 ± 0.00^{c}

Sweet cultivar, B: Bitter cultivar. The values are means ± SD of triplicate determinations expressed on dry weight basis; Values in the rows with different superscripts are different at $p < 0.05$.

4.1.3.2 Phytate

Phytate contents were high for all cassava cultivars compared to the recommended 4 to 9 mg/100 g (Udousoro, 2014) and differed significantly ($p < 0.05$). The phytate content ranged from 661.33 (*Nyarukeca*) to 984.64 (NASE 3). It was higher in improved varieties compared to local varieties (Table 10). Sarkiyayi et al. (2010) reported much higher phytate values of 2,160 mg/kg and 3, 040 mg/kg for sweet and bitter cassava varieties respectively. Charles et al. (2005) reported phytate levels ranging between 950 and 1, 360 mg/kg in cassava with no significant difference ($p > 0.05$) between sweet and bitter varieties. Phytate may impair the bioavailability of iron, calcium, magnesium, and zinc in the diets of people dependent on cassava as a staple food (Popova and Mihaylova, 2019).

However, phytate may play the role of an antioxidant by sequestering iron and thus hinder the formation of free radicals (Popova and Mihaylova, 2019). By virtue of forming a unique iron chelate it suppresses iron-catalyzed oxidative reactions and may serve a potent antioxidant function in the preservation of seeds (Graf and Eaton, 1990). By the same mechanism dietary phytic acid may lower the incidence of colonic cancer and protect against other inflammatory bowel diseases. Its addition to foods inhibits lipid peroxidation and concomitant oxidative spoilage, such as discoloration, putrefaction, and syneresis. A multitude of other industrial applications are based on the antioxidant function of phytic acid (Graf and Eaton, 1990). The findings of this study indicated that all the cassava cultivars investigated would require processing to reduce phytate content before consumption.

4.1.3.3 Oxalate

Oxalate contents differed significantly ($p < 0.05$) among the cassava varieties in this study. The oxalate content ranged from 90.60 (*Nyarukeca*) to 227.80 (NASE 3). The

oxalate contents were generally higher in improved varieties compared to local varieties (Table 10). Sarkiyayi et al. (2010) reported oxalate contents of 220 mg/kg in sweet and 440 mg/kg in bitter cassava varieties. Oxalates may chelate minerals such as calcium, zinc and iron and therefore prevent their absorption and utilization by the human body (Akter et al., 2020; Petroski and Minich, 2020). The oxalate content in this study was lower than the maximum dose (250 mg/100g) recommended by USDA (Udousoro and Akpan, 2014), implying that the cassava cultivars investigated are safe to consume with regard to oxalate content.

4.1.3.4 Tannins

Tannin content differed significantly ($p < 0.05$) among the cassava cultivars studied except among NASE 14, NASE 19, and *Nyamatia* (Table 10). The tannin contents were generally higher in improved cassava cultivars than in the local varieties. The tannin content in this study ranged from 0.18 (*Nyaukeca*) to 0.325 (NASE 3). The tannin content in this study was lower than that reported by Sarkiyayi et al. (2010), 0.40 mg/100g in sweet and 0.60 mg/100g in bitter cassava cultivars. The recommended content of tannin in foods by USDA is 1.7 mg/100g. In cassava, wound responses may lead to the formation of condensed tannins in the roots which cause a discolouration of the vascular tissue and storage parenchyma (Rickard and Gahan, 1983; Buschmann et al. 2000b; Alves, 2002; Karim et al., 2009; Zainuddin et al., 2018; Uarrota et al., 2015).

4.1.3.5 Strategies for reducing anti-nutritional compounds in foods

4.1.3.5.1 Reduction of cyanogens in foods

Cyanogens are found in 3 forms in cassava: cyanogenic glucoside (95% linamarin and 5% lotaustratin), cyanohydrins, and free cyanide. Different processing techniques exist to remove cyanogens. Residual levels of cyanogenic glucosides and their toxic degradation

products, cyanohydrins and free cyanide, in processed cassava depend upon the original levels of cyanogenic glucosides, and on the nature and duration of the method used for processing (Nambisan, 1994; Ndubuisi and Chidiebere, 2018). Effectiveness of processing method depends on the processing steps and the sequence utilized, and it often is time-dependent (Montagnac et al., 2008; Ndubuisi and Chidiebere, 2018)

Cyanide content of foods may be significantly reduced by processing of cassava roots. Cassava is processed in many different ways to be used in a variety of snacks and main dishes. Many times the processing techniques are combined to either improve the end product or further reduce the amount of cyanide. Pounding or crushing of the fresh roots and subsequent sun-drying is the most effective for cyanogenic glucoside removal because it ruptures cell compartments, thus allowing direct contact between linamarin and the enzyme linamarase that catalyzes the hydrolytic breakdown (Nambisan, 1994; Montagnac et al., 2008; Ginting and Widodo, 2013).

Boiling is not an effective method for cyanide removal (50%). The inefficiency of this processing method is due to the high temperatures. At 100 °C, linamarase, a heat-labile β-glucosidase, is denatured and linamarin cannot then be hydrolyzed into cyanohydrins. However, using small-sized cassava pieces or increasing the volume of water in which cassava roots are boiled can increase the efficiency of the boiling method (Nambisan, 1994; Montagnac et al., 2008; Ginting and Widodo, 2013; Ndubuisi and Chidiebere, 2018). The loss of cyanide resulting from steaming, baking, or frying is small due to processing temperatures of over 100 °C and to the stability of linamarin in neutral or weak acid conditions. These methods are only suitable for sweet cassava cultivars, because they contain low cyanide content (Nambisan, 1994; Montagnac et al., 2008; Ginting and Widodo, 2013; Ndubuisi and Chidiebere, 2018).

Drying peeled cassava roots removes some cyanogens. In the drying process, endogeneous linamarase controls the cyanogenic glucoside removal, and thus is responsible for cyanohydrin and free cyanide accumulation in dried cassava (Essers, 1995; Alves, 2002; Montagnac et al., 2008; Ginting and Widodo, 2013; Ismaila et al., 2018; Ndubuisi and Chidiebere, 2018).

Cyanogens could be reduced by fermentation (Oyewole, 1992; Essers, 1995; Alves, 2002; Ginting and Widodo, 2013; Abdullahi and Saba, 2014; Ismaila et al., 2018; Ndubuisi and Chidiebere, 2018); and mechanical pressing-fermentation (Abdullahi and Saba, 2014); soaking of cassava chips for at least 24 h prior to sun drying was reported to have significantly reduced cyanogens content of the resultant cassava flour (Montagnac et al., 2008; Nebiyu and Getachew, 2011; Ginting and Widodo, 2013); fermentation and soaking or soaking and fermenting and roasting as in the production of *gari* and *farina,* are reported to detoxify cassava roots, and hence render the processed roots safe for human consumption (Montagnac et al., 2008; Ginting and Widodo, 2013; Ismaila et al., 2018; Ndubuisi and Chidiebere, 2018). Bradbury (2006) reported reduced cyanide content of flour soaked for about 5 h before cooking.

Other strategies to reduce toxicity include development of low-cyanogen cassava cultivars and cassava transgenic lines with accelerated cyanogenesis during processing or increased expression of hydroxynitrilelyase in tuber (Siritunga and Sayre, 2003; Montagnac et al., 2008; Nambisan, 2011; Ginting and Widodo, 2013). Narinesingh et al. (1998), Somphit et al. (2010), Ajayi et al (2012), Murugan et al. (2012) and Latif et al. (2019) reported successful detoxification of cassava using immobilised enzymes.

Addition of exogenous linamarase increased the rate of breakdown of linamarin in cassava flour soaked for about 5 h before cooking (Bradbury, 2006)

4.1.3.4.2 Reduction of phytates in foods

Phytate content could be reduced by processing techniques such as soaking, fermentation, sprouting, germinating, and cooking which can significantly alter phytate content in grains and legumes, allowing for increased mineral availability (Montagnac et al., 2008; Fernandes et al., 2010; Gibson et al., 2010; Ismaila et al., 2018; Petroski and Minich, 2020; Samtiya et. al., 2020). Soaking cereals and legumes reduce phytate content by a combination of passive diffusion of water-soluble sodium or potassium phytate and hydrolysis of phytate by endogenous phytases.

Fermentation, such as the natural leavening of bread, has also been found to significantly reduce phytate. It is elucidated that along with activity of bacterial phytases, lactic acid bacteria activate endogenous cereal phytates by lowering the pH of the dough to 4 to 5 (Gibson et. al., 2010; Petroski and Minich, 2020). Ismaila et al. (2018) reported significant reduction in phytate content of the roots of bitter cassava cultivar by sun-drying, roasting, frying, partial fermentation and sun-drying, submerged fermentation and solid-state fermentation. Germination induces enzymatic hydrolysis of phytates through the action of endogenous phytase enzymes (Gibson et. al., 2010).

Home pounding can be used to reduce phytic acid content of unrefined cereals that have phytic acid localized in the outer aleurone layer (rice, sorghum and wheat) or in the germ (maize). Dephytinization at the commercial level can be achieved by milling or the addition of exogenous phytase enzymes (Gibson et. al., 2010; Samtiya et. al., 2020). The inhibitory effect of phytate on mineral bioavailability in cereal- and legume-based foods

can also be attenuated by the addition of animal protein or ascorbic aci-rich foods to enhance absorption of zinc and nonheme iron (Gibson et. al., 2010).

4.1.3.4.3 Reduction of oxalates in foods

Oxalate content of foods could be reduced by cooking, preparation, and processing of food, thereby rendering minerals availabile from food items (Ismaila et al., 2018; Petroski and Minich, 2020). Traditional and industrial cooking methods such as soaking overnight and boiling or autoclaving, microwaving, is reported to significantly reduce total and soluble oxalate content in legumes. Due to oxalate's solubility in water, wet processing methods such as boiling, and steaming seem to be the most efficient solutions to decreasing oxalate content (Ismaila et al., 2018; Petroski and Minich, 2020). Ismaila et al. (2018) reported significant reduction in oxalate content of the roots of bitter cassava cultivar by sun-drying, roasting, frying, partial fermentation and sun-drying, submerged fermentation and solid-state fermentation.

4.1.3.4.4 Reduction of tannins in foods

Tannin content of foods could be reduced by Cooking and processing (Petroski and Minich, 2020), soaking and cooking (Serrano et. al., 2009; Fernandes et al., 2010; Samtiya et. al., 2020). Although a majority of catechin-rich foods, like fruits, are consumed raw. Removing the skins from nuts may reduce phenolic content by up to 90% (Petroski and Minich, 2020). Tannins are not consumed alone, but in combination with thousands of other bioactives, including ascorbic acid. Potential inhibitory effects of tannins may be offset by the inclusion of 30 mg of ascorbic acid (Petroski and Minich, 2020). Ismaila et al. (2018) reported significant reduction in tannin content of the roots of bitter cassava cultivar by sun-drying, roasting, frying, partial fermentation and sun-drying, submerged fermentation and solid-state fermentation. Hawashi et al. (2019)

reported significant reduction in the tannin content of cassava leaves during solid-state fermentation using *Saccharomyces cerevisiae*. Samtiya et. al. (2020) also reported reduction in tannin content during fermentation.

4.2 Physico-chemical and functional characteristics of HQCF

4.2.1 pH

The pH of HQCF ranged between 5.71 and 6.22 (Table 11), which was acceptable according to the quality requirements (East African Standard, DEAS 779:2012). pH is a good quality indicator for cassava flour since flour with a pH of 4 or less indicates significant degree of fermentation, and thus some starch breakdown, which imparts characteristic sour aroma and taste, making the flour undesirable for use in bakery products (Apea-Bah et al. 2011). However, pH of 8.0 has been reported in traditionally processed cassava flour using heap/solid substrate fermentation (Essers et al., 1992). Negative correlations of glucose and sucrose with pH has been reported by Araújo et al. (2019), who concluded that higher levels of pH reduce sugar accumulation in cassaca roots and root products.

4.2.2 Total titratable acidity

HQCF had total titratable acidity (TTA) ranging between 0.037 and 0.043 % (Table 11). Any increase in TTA (above about 2 %) is a result of fermentation. The level of acidification increases with increasing period of fermentation (Oyewole and Ogundele, 2001). The lowering of pH and the concomitant increase in TTA is attributed to the accumulation of organic acids such as lactic, acetic and formic acids in the fermenting cassava produced by activities of bacteria (*Bacillus* sp., *Leuconostoc* sp., *Klebsiella* sp., *Corynebacterium* sp., *Lactobacillus* sp) and yeasts/moulds (*Candida* sp., *Aspergillus* sp.,

and *Geotrichum* sp.), which contribute the dominant specific microflora (Oyewole and Odunfa, 1988; Essers et al., 1992).

pH is a critical factor in developing flavour and aroma characteristics of foods (Tetchi et al., 2012). Lactic acid bacteria are implicated throughout the duration of fermentation of cassava into fermented products, their activity imparting the typical sour fermented taste to these products (Oyewole and Odunfa, 1988). TTA is a better indicator of fermentation activity than pH because it continues to increase after pH levels off. Although the TTA of *Nyamatia* and *Nyarukeca* cassava cultivars were not significantly diffefent ($p > 0.05$) from those of other cassava cultivars studied, these cultivars contain high amounts of cyanogenic glucosides and are therefore not recommended to be processed into HQCF using the method described in this study. The result proved that content of cyanogenic compounds did not affect TTA. Araújo et al. (2019) reported similar findings from the roots of bitter and sweet cassava cultivars grown in Brazilian Amazon region. Total titratable acidity was negatively correlated with free cyanide and starch, as reported by Araújo et al. (2019).

4.2.3 Water-binding capacity

HQCF had water-binding capacity (WBC) between 142.89 (*Nyamatia*) and 166.57 % (NASE 3), while wheat flour had water-binding capacity of 87.33 % (Table 11). Flour functional properties are important in product development, as they indicate the quantity of water needed to develop optimum dough and also indicate the properties and behavior of flour with the addition of water (Stear, 1990). WBC is important in bulking and consistency of products as well as in baking applications. WBC or water absorption can influence the following parameters in bread and baking: proofing, fracture stress of bread

crumb, loaf volume, bread yield, final products' attributes, machinability and shelf-life (Zghal et al., 2001; Puhr and D'Appolonia, 1992; Awuchi et al., 2019).

The results of the present study showed that cassava flours absorb a larger ($p < 0.05$) amount of water than wheat flour (Table 11). Similar results were observed by Erikson et al. (2014), who reported WBC for three cassava cultivars between 151. 97 and 166.87 %, and 88.43 % for wheat flour. Defloor et al. (1993) and Eduardo et al. (2013) reported increase in WBC of wheat flour with incorporation of cassava flour in making composite bread. Aryee et al (2006) reported water binding capacity ranging from 113.66 to 201.99 %. Oladunmoye et al. (2010) reported water absorption of 221.8 % for cassava flour and a much lower value of 31.9 % for wheat flour. Eke et al. (2010) reported water absorption capacity ranging from 415.13 to 595.26 %, while Agbemafle (2019) reported water absorption capacity ranging between 122.10 and 151.26 % for cassava flour.

The variability in the values may be due to seasonal, maturity, environmental and processing factors for cassava cultivars used in the different studies respectively, as reported by Defloor et al. (1993); Apea Bah et al. (2007); Eke et al. (2010); Agbemafle (2019). The WAC of flour affects the profitability of the bakery. The water WAC cannot be viewed independently from the viscoelastic characteristics of the dough**.** It would be useless to produce flour with a very high absorption rate if it can't be used for bread making. Hence, the balance between WAC and rheological propertiesof flour must be ensured**.**

Water-binding capacity (WBC), water-absorption capacity (WAC) or water hydration and water-holding capacity (WHC) are the terms often used to describe the hydration properties of flours and refer to the amount of water that can be absorbed per gram of sample material

(Zayas, 1997). WAC is the amount of water taken up by flour to achieve the desired consistency and create a high quality end-product (Awuchi et al., 2019; Onyeneke, 2019). It is the optimal amount of water that can be added to dough before it becomes too sticky to process (Zghal et al., 2001). Different flours absorb different amounts of water and therefore make doughs of different consistencies.

WA is related to protein quantity and quality, damaged starch, and wheat polysaccharides such as pentosans and β-glucans (Farooq and Boye, 2011; Okuda et al., 2016). WA in baking and bread production may influence parameters such as loaf volume, fracture stress and bread crumb, bread yield, machinability, proofing, final product attributes and shelf life (Henao and Aristizábal, 2009; Okuda et al., 2016). The amount of water absorbed by flour influences the mobility of gliadins and glutenins during dough preparation and thus network development, which is dependent on the formation of intra- and intermolecular disulfide bonds and the hydration properties of starch and gluten (Schopf and Scherf, 2021). Materials that have a low WBC may not be able to hold water effectively (Boye et al., 2010; Farooq and Boye, 2011). Materials that have a high WBC or WAC may render food products brittle and dry, especially during storage (Srikiatden and Roberts, 2007; Boye et al., 2010; Farooq and Boye, 2011). Water absorption results in swelling of flour. The swelling power of flour depends on the concentration of starch, protein, pentosans, fibre and any addidive (vital wheat gluten, eggs, bran and hydrocolloids (gums) intended to increase water absorption and impart greater stability to the dough (Schopf and Scherf, 2021).

4.2.4 Swelling power and solubility

The swelling power of cassava flour ranged between 6.62 (NASE 19) and 7.64 % (*Nyamatia*), and solubility between 5.99 (*Nyamatia*) and 8.04 % (NASE 14); while wheat flour exhibited swelling power of 6.19 % and solubility of 10.28 % (Table 11). The flour

from the five cassava varieties had a significantly higher (p <0.05) swelling power than wheat flour. Eke et al. (2010) reported higher swelling power of cassava flour ranging from 20.76 to 29.01 %. Agbemafle (2019) also reported higher swelling power of cassva flour than obtained in this study, ranging between 9.233 and 12.513 %. Aryee et al. (2006) reported swelling power ranging between 5.87 to 13.48 %. Erikson et al. (2014) reported swelling power of three cassava culivars between 10.32 and 12.04 % and a swelling power of wheat flour of 7.65 %.

Uptake of water by flours results into swelling. The swelling power of flour depends on the concentration of protein, starch and fiber (Farooq and Boye, 2011). Swelling power is an important parameter especially in characterisation of flours from different botanical origins which display different swelling powers at a given temperature (Moorthy, 2002; Charles et al., 2007; Farooq and Boye, 2011). It also affects both the eating quality of cassava roots and the use of flour in a number of industrial applications (Moorthy, 2002). High swelling power results into high digestibility and ability to use flour in solution suggesting improved dietary properties and the use of flour in a range of dietary applications. Swelling power and solubility properties may influence the characteristics of bakery products. Flours which have lower swelling power and solubility may cause the bakery products not to swell appropriately (Boye et al., 2010; Farooq and Boye, 2011).

The significantly (p < 0.05) higher SP and WBC of HQCF than wheat flour (Table 11) might have been a result of the differences in amylopectin content of the various flours since SP has been described as an amylopectin property (Eriksson et al., 2014). Furthermore, proteins and lipids, which are present in higher amounts in wheat compared to HQCF, inhibit SP (Eriksson et al. 2014). Also, the higher SP in HQCF than wheat flour reflects the weaker bonding forces in the starch granules of HQCF (Eriksson et al., 2014).

Cassava flour does not have gluten, therefore, it cannot provide a stabilizing network for retention of gas during baking (Edwardo et al., 2013; Chisenga et al., 2020).

The solubility of flour differed significantly ($p < 0.05$) among the cassava cultivars, ranging from 5.99 % (*Nyamatia*) to 8.04 % (NASE 14), and with the solubility of wheat flour (10.28 %). Solubility of wheat flour was significantly ($p < 0.05$) higher than the solubility of cassava flour. This however, contradicts the findings of Eriksson et al., (2014), in which they reported significantly higher solubility of flour from the Ghanaian cassava cultivars they studied compared to wheat flour. Eke et al. (2019) reported solubility of cassava flour ranging from 4.04 to 20.42 %, which encompass the solubility obtained in this study. Agbemafle (2019) reported higher cassava flour solubility between 10.883 and 15.533 % than obtained in this study.

Table 11: Physico-chemical characteristics of high quality cassava flour from different cassava cultivars grown in Uganda, and wheat flour

Parameter	NASE 3[S]	NASE 14[S]	NASE 19[S]	*Nyamatia* [B]	*Nyarukeca* [B]	Wheat
pH	6.01±0.01[a]	6.22±0.01[b]	5.95±0.05[a]	5.71±0.03[c]	6.1±0.10[ab]	6.01±0.03[a]
Water-binding capacity, %	166.57±1.01[a]	151.57±8.26[a]	161±7.80[a]	142.89±7.50[a]	156.19±18.72[a]	87.33±22.19[b]
Swelling Power, %	7.47±0.17[a]	6.98±0.11[ab]	6.62±0.43[ab]	7.64±03[a]	7.51±0.71[a]	6.19±0.39[b]
Solubility, %	7.16±0.91[a]	8.04±0.24[b]	6.52±0.80[c]	5.99±0.22[d]	7.01±1.32[a]	10.28±1.57[e]
Titratable Acidity, %	0.04±0.01[a]	0.04±0.00[a]	0.04±0.00[a]	0.04±0.00[a]	0.04±0.00[a]	0.03±0.00[a]

S: Sweet cultivar, B: Bitter cultivar

The values are means ± SD of triplicate determinations expressed on dry weight basis. Values in the rows with different superscripts are significantly different at $p < 0.05$.

Eriksson (2014) reported swelling power of Ghanaian cassava flour between 10.48 and 12.04, while Apea-Bah et al. (2007) reported much higher values for four Ghanaian cassava flours they studied, and they noted that the parameter varied with the age of the cassava root (Months After Planting). Kusumayanti et al. (2015) reported higher value of swelling power (13.80) of some Indonesian cassava flour. Eriksson (2014) reported solubility between 11.0 % and 20.8 % in some Ghanaian cassava flour while Apea-Bah et al. (2007) reported solubility ranging between 7.81 % and 18.80 % among the Ghanaian cassava cultivars, depending on variety and age of the roots. Eriksson (2013) reported water-binding capacity of some Ghanaian cassava flour between 152.0 % and 166.9 %, similar to the findings in this study. Kusumayanti et al (2014) reported lower values of solubility in some Indonesian cassava flour at 3.02 %.

The solubility of the wheat flour used in this study (10.28 %) was significantly ($p < 0.05$) higher than for all the five cassava cultivars. The wheat used by Eriksson (2013) had a solubility of 5.15 %, much lower than the solubility of wheat used in the current study. Nilusha et al. (2021) reported very low (1.95 %) water solubility of commercial wheat flour in Sri Lanka compared to solubility of cassava flour, which ranged between 1.92 and 4.08 %. The differences in the studied parameters were due to higher starch content in cassava flour and a loose association of starch polymers in the cassava starch granule (Eggleston, 1993). The significantly higher SP and WBC of cassava flour than wheat flour may be a result of the differences in amylopectin content of the various flours since SP has been described as an amylopectin property (Erikson et al., 2014). Furthermore, proteins and lipids, which are present in higher amounts in wheat compared to cassava, inhibit SP. Also, the higher SP in cassava flour than wheat flour reflects the weaker bonding forces in the starch granules of cassava flour (Erikson et al., 2014).

There were no significant differences in swelling power between the cassava cultivars in this study. Apea-Bah et al. (2011) reported higher values for *Afisiafi* than found in this study. Starches with a high swelling power are less resistant to break down (Apea-Bah et al., 2011). Eggleston (1993) reported similar results for water-binding capacity to the results in this study. Solubility is an important parameter in baking since flour with a high solubility may give a soggy and less cohesive dough (Apea-Bah et al., 2011; Erikson et al., 2014). Solubility of NASE 14 flour was significantly ($p < 0.05$) higher than that of the other four cultivars.

4.3. Rheological characteristics of wheat-cassava composite flour/dough

NASE 14 is the most grown cassava cultivar in Nebbi district (Omony, 2017), and proximate analysis showed that it had the highest protein content and highest solubility among the cultivars in this study. HQCF from NASE 14 was therefore chosen to form the composite with wheat.

Wheat flour possesses unique bread-making properties due to the ability of wheat storage proteins, glutenin and gliadin, to form viscoelastic dough when hydrated and kneaded (Kent and Evers, 1994; Heldt, 1997), therefore Mixolab results obtained for the wheat flour were used as the standard.

4.3.1 Mixolab indices of wheat-cassava composite flour

Mixolab indices (Table 12) indicated that water absorption increased significantly ($p < 0.05$) with increase in cassava flour at more than 40 % inclusion level. Addition of cassava flour to wheat flour increased the starch and fibre content, thereby increasing water absorption potential (Nilusha et al., 2021; Khan et al., 2019). According to Kent and Evers (1994), flour contains protein, undamaged starch granules and damaged starch

granules, all of which absorb water, albeit to differing degrees. Khan et al. (2019) reported an increase in water absorption in wheat flour on addition of pumpkin flour, which was attributed to the increase in fibre content of the resulting flour. However, the cassava flour diluted the wheat gluten thus reducing the dough stability (Khalil et al., 2000; Edwardo et al. 2013).

Mixing index decreased significantly ($p < 0.05$) with blends containing 40 % and more cassava flour. Mixing index represents the properties of the composite flour during mixing at 30 $^{\circ}$C and accounts for stability, dough development time and protein weakening (Mixolab Applications Handbood, 2012). The low mixing index observed at 40 % and higher inclusion levels of cassava flour in this study indicated that the composite flour became less stable to mixing and kneading as more cassava flour was added to the wheat flour.

The gluten index or gluten strength was not significantly ($p < 0.05$) affected by the addition of cassava flour, which might imply that starch behaves as gluten in food. This property represents the property of gluten when the dough is heated. The higher the index, the higher the resistance of the gluten to heating. The results in this study might indicate that even when diluted with non-gluten flours, gluten behaviour during heating is not affected.

The viscosity index decreased significantly ($p < 0.05$) with the addition of up to and more than 40 % cassava flour. This property represents the increase in viscosity during the heating phase. It is dependent both on the amylase activity and the starch quality. The higher the index, the higher the viscosity of the dough when hot. The decrease in this

property might mean that cassava flour had a higher amylase activity which degraded some of the starch responsible for hot dough viscosity.

The amylase index decreased significantly ($p < 0.05$) with the addition of up to and more than 20 % cassava flour. This property is dependent on the ability of the starch to resist "amylolysis". The higher the index, the lower the amylase activity. The results in this study therefore, indicated that cassava flour had a higher amylase activity than wheat flour. Wheat flour contains small amounts of fermentable carbohydrates. Amylase acts on starch, with the formation of maltose used by yeast for the production of CO_2, ethanol and other fermentation products, which leads to a rapid and uniform dough fermentation (Kent and Evers, 1994; Atudorei et al., 2021).

The retrogradation index reduced significantly ($p < 0.05$) with the addition of more than 30 % cassava flour. This property is dependent on the characteristics of starch and its hydrolysis during the test. The higher the index, the shorter the cooked product's shelf-life. The results in this study indicated that the retrogradation tendencies in the composite dough was lower than in the pure wheat dough, probably due to hydrolysis of some starch, therefore, products produced from the composite flours would have a longer shelf-life than their counterparts from pure wheat flour.

Table 12: Mixolab indices of wheat-cassava composite flour (14 % moisture basis)

	Proportion (%) of cassava flour in blend							
Mixolab indices	0	10	20	30	40	50	60	100
Water absorption	4^{a}	4^{a}	3^{ab}	5^{a}	7^{c}	8^{cd}	8^{cd}	9^{d}
Mixing	4^{ab}	4^{ab}	5^{a}	3^{b}	1^{c}	1^{c}	1^{c}	3^{b}
Gluten+	4^{a}	4^{a}	4^{a}	3^{ac}	3^{ac}	4^{a}	5^{ab}	7^{d}
Viscosity	8^{a}	8^{a}	8^{a}	7^{ab}	6^{bc}	5^{c}	4^{c}	1^{d}
Amylase	8^{a}	7^{a}	5^{b}	4^{bc}	3^{cd}	2^{d}	3^{cd}	5^{b}
Retrogradation	8^{a}	8^{a}	7^{ab}	6^{bc}	6^{bc}	5^{c}	5^{c}	4^{cd}

The indices are from 0 to 9.

Values with different superscripts in the rows have means which are significantly different at $p < 0.05$

4.3.2 Mixolab properties of wheat-cassava composite flour

The Mixolab results showed that water absorption (WA) ranged from 57.5 to 64.0 %; dough development time (DDT) ranged from 0.90 to 1.46 min; dough stability ranged from 4.60 to 9.30 min; C1 ranged from 1.088 to 1.158 Nm; C2 ranged from 0.401 to 0.492 Nm; C3 ranged from1.373 to 1.945 Nm; C4 ranged from 1.150 to 1.788 Nm, and C5 ranged from 1.906 to 3.001 Nm. (Table 13 and Figure 17).

There was a significant ($p < 0.05$) increase in WA by wheat-cassava blend at a substitution level of 30 % and above. Increase in WA might be due to the high amount of starch, (and broken starch) and fibre in cassava flour as reported by Kumar et al. (2015) and as discussed by Khalil et al. (2000). WA influences dough yield, therefore these results suggest that inclusion of cassava flour in bread making could be profitable, since WA of flour increases dough yield (Kent and Evers, 1994; Awuchi et a., 2019). An increase in WA of wheat flour with the addition of wheat bran to the flour was reported by Xhabiri et al. (2016), which could have been due to the increased amount of fibre (bran increased the amount of fibre in the wheat flour) in the dough. The increase in WA of wheat-cassava blend could be due to the high amount of starch and fibre in cassava flour (Khalil et al., 2000; Kumar et al., 2015). Girma et al (2014) observed that WA levels, DDT, the mixing tolerance index, and the farinograph quality level of cassava–wheat composite dough differed from 100 % wheat dough beyond 20 % substitution level of wheat flour. The researchers attributed the increase in the values of WA in the composite flour dough with an increase of cassava flour and the higher fibre and carbohydrate contents in cassava flour, since the ability of cassava flour to absorb water has a significant correlation with its carbohydrate content. This might also have been as a result of the decrease in gluten content and weakening of the protein network due to proteolytic activity of composite flours, as discussed by Girma et al. (2014)

Table 13: Mixolab parameters of wheat-cassava composite flour (14 % moisture basis)

	Proportion (%) of cassava flour in blend							
Mixolab properties	0	10	20	30	40	50	60	100
WA (%)	57.50±0.10^{a}	57.50±0.10^{a}	57.00±0.10^{a}	58.50±0.20^{b}	60.50±0.20^{c}	62.50±0.20^{d}	64.00±0.20^{e}	68.50±0.20^{f}
DDT (min)	1.46±0.04^{a}	1.37±0.02^{a}	1.28±0.19^{a}	0.84±0.06^{b}	0.80±0.02^{b}	0.79±0.03^{b}	0.90±0.02^{b}	1.96±0.82^{a}
Stability (min)	9.30±0.10^{a}	9.45±0.05^{a}	9.35±0.05^{a}	9.03±0.35^{a}	4.867±1.05^{b}	3.90±0.40^{b}	4.60±0.20^{b}	7.100±1.10^{c}
C1 (Nm)	1.14±0.02	1.10±0.00	1.16±0.06	1.14±0.03	1.13±0.05	1.09±0.02	1.17±0.02	1.15±0.02
C2 (Nm)	0.49±0.01	0.48±0.00	0.49±0.03	0.46±0.03	0.42±0.01	0.43±0.06	0.40±0.01	0.49±0.00
C3 (Nm)	1.91±0.05^{a}	1.93±0.08^{a}	1.95±0.02^{a}	1.92±0.02^{a}	1.81±0.02^{ab}	1.69±0.01^{b}	1.58±0.05^{b}	1.37±0.02^{c}
C4 (Nm)	1.79±0.09^{a}	1.68±0.15^{ab}	1.61±0.03^{ab}	1.55±0.03^{b}	1.42±0.02^{bc}	1.31±0.01^{bcd}	1.25±0.05^{ef}	1.15±0.00^{f}
C5 (Nm)	3.00±0.29^{a}	2.79±0.28^{a}	2.64±0.08^{ab}	2.51±0.02^{bcd}	2.24±0.04^{bcd}	2.05±0.01^{de}	1.99±0.08^{cde}	1.91±0.01^{ebcd}

The values are means ± SD of triplicate determinations. Values with different superscripts in the rows have means which are significantly different at $p < 0.05$ level.

WA-water absorption; DDT - dough development time;

C2 - protein weakening; C3 - starch gelatinisation; C4 - amylase activity/hot gel stability; C5 - starch gelling/retrogradation.

Dough development time (DDT) of the pure wheat flour was low (1.46 min), signifying a weak flour (Dhaka et al., 2012; Dhaka and Khatkar, 2013). The DDT reduced significantly ($p < 0.05$) on addition of more than 20 % cassava flour. Xhabiri et al (2016) reported increased dough development time of wheat flour with the addition of wheat bran. Pomeranz et al. (1977) reported that the increase in dough development time (the time from first addition of water to the time the dough reaches the point of greatest torque) with increased level of cassava flour substitution might be due to higher fibre content of cassava flour, which picks up water slowly.

The dough stability of the pure wheat (9.3 min) exhibited the characteristics of strong flour. The longer the mixing time, the more the flour is said to be "strong" (Sabovics et al. 2011). Koksel et al. (2009) reported stability of 6 min 39 s for a medium strong bread wheat flour. Dough stability, which is a measure of dough resistance to mixing and kneading, reduced significantly ($p < 0.05$) above 30 % substitution of wheat with cassava flour. Girma et al. (2014) also reported decreased dough stability with increase in the level of cassava flour incorporation in wheat-cassava composite flour, due to decreased gluten content of the composite flour. Xhabiri et al. (2016) reported increased dough stability with addition of bran to wheat flour in some wheat varieties and decreased stability in other wheat varieties. The increase in dough stability was attributed to the increased interaction between hydroxyl groups of the fibre molecules and water to form hydrogen bonds.

Girma et al. (2014) reported that the mixing tolerance index and dough development time of cassava-wheat composite flour/dough was significantly higher than that of the wheat

flour/dough. According to Girma et al. (2014), increase in DDT with addition of cassava flour might be due to the higher fibre content of cassava flour which picked up water slowly. It might have also been due to the decrease in the gluten contents and weakening of protein network as a result of proteolytic activity of composite flours.

The protein quality, represented by C2 (the maximum torque attained when the dough undergoes mixing as well as heating), is an indicator of the quality and stability of wheat protein network to thermal weakening (Dhaka et al., 2012). At the beginning of heating, decrease in dough consistency was the result of protein weakening. The greater the decrease in consistency, the lower the protein quality (Lacko-Bartošová et al., 2019). The C2 values were greater than 0.4 Nm in all the blends, indicating that all the flour blends were moderate and similar in gluten strength (Sabovics et al., 2011) and the subsequent doughs produced were moderately tolerant to mixing (Koksel et al. 2009). C2 values below 0.4 Nm indicates that dough from that flour can be less tolerant to mixing. Good quality proteins are represented by C2 0.5–0.6 Nm (Lacko-Bartošová et al., 2019). It is worth noting that the inclusion of cassava flour in the wheat flour did not significantly ($p > 0.05$) influence this parameter. Xhabiri et al. (2016) reported increased values of C2 for wheat flour with the addition of wheat bran.

The viscosity of the dough (C3) was not significantly ($p > 0.05$) different for pure wheat and composite flours up to 40 % substitution level but decreased with the addition of more than 40 % cassava flour. Xhabiri et al. (2016) reported general decrease in the value of C3 with the addition of wheat bran to the wheat flour. This might indicate that the amylase activity

and starch quality was not significantly ($p > 0.05$) affected by the addition of up to 40 % cassava flour to wheat flour (Sabovics et al., 2011). Maximum value of torque in the heating phase C3 averaged 1.913 Nm for wheat flour and the lowest was 1.583 Nm for cassava flour, indicating higher starch gelatinisation temperature and higher dough viscosity for both the wheat and composite flours (Lacko-Bartošová et al., 2019).

The measure of hot gel stability (C4), decreased with the addition of cassava flour to wheat flour from 1.788 Nm for wheat flour to 1.546 Nm at 30 % cassava flour incorporation. However, the decrease was significant ($p < 0.05$) at 30 % substitution level and above. This might imply low amylase activity of the composite flours on heating since the decrease in consistency was a result of amylolytic activity (Sabovics et al., 2011; Lacko-Bartošová et al., 2019).

The measure of starch gelling and retrogradation in the cooling phase (C5) significantly ($p < 0.05$) decreased with the addition of more than 30 % cassava flour. This might indicate low starch retrogradation due to low amylolytic activities. Since starch is the main origin of staling in cereal products, decreased C5 during the cooling phase corresponds to a long shelf life of the end product (Sabovics et al., 2011; Lacko-Bartošová et al., 2019). Xhabiri et al. (2016) reported decreased value of C5 with the increase of bran in wheat flour.

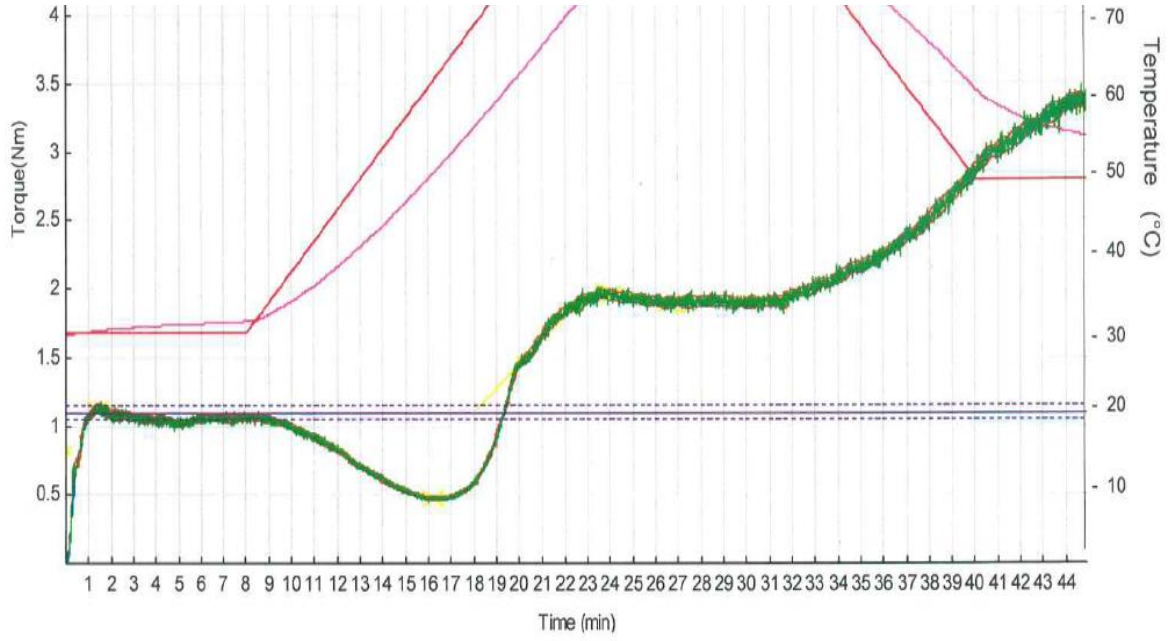


Figure 18 a: Mixolab curve for composite flour with 0 % cassava flour

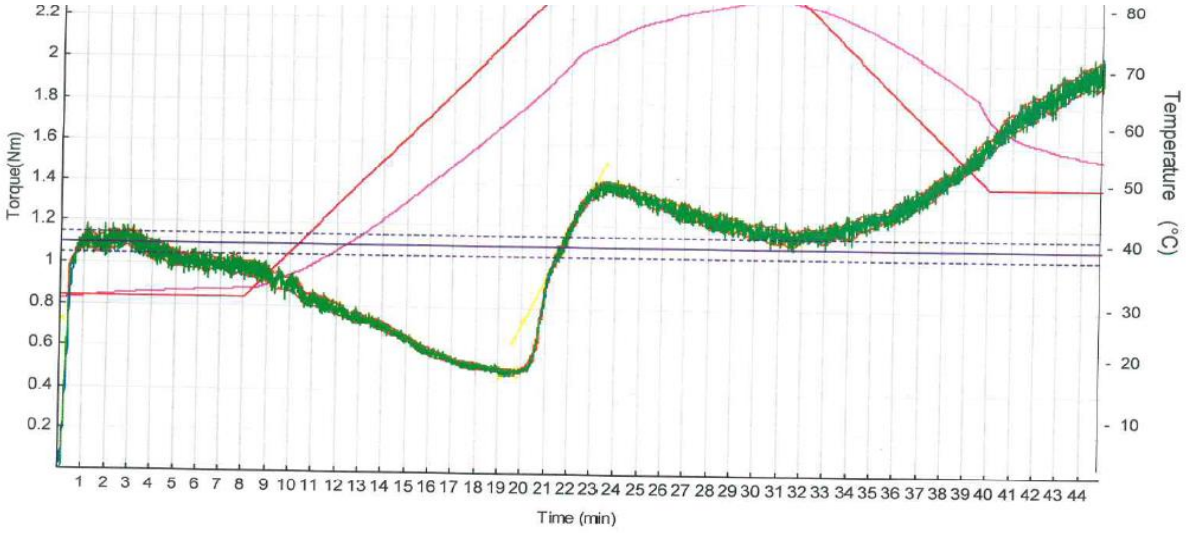


Figure 18 b: Mixolab curve for composite flour with 100 % cassava flour

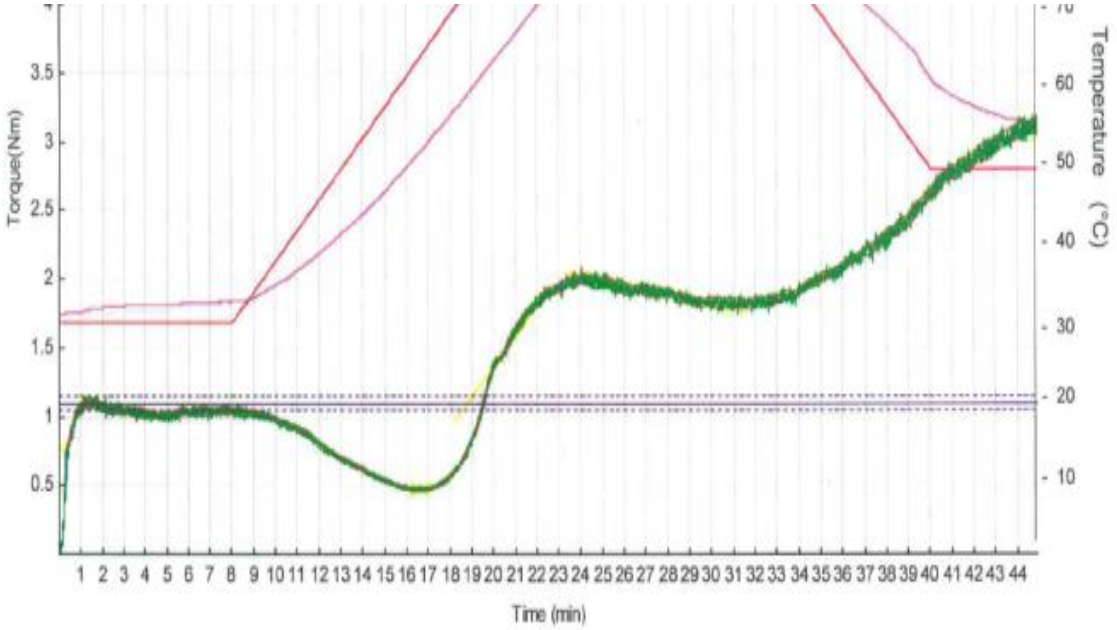


Figure 18 c: Mixolab curve for composite flour with 10 % cassava flour

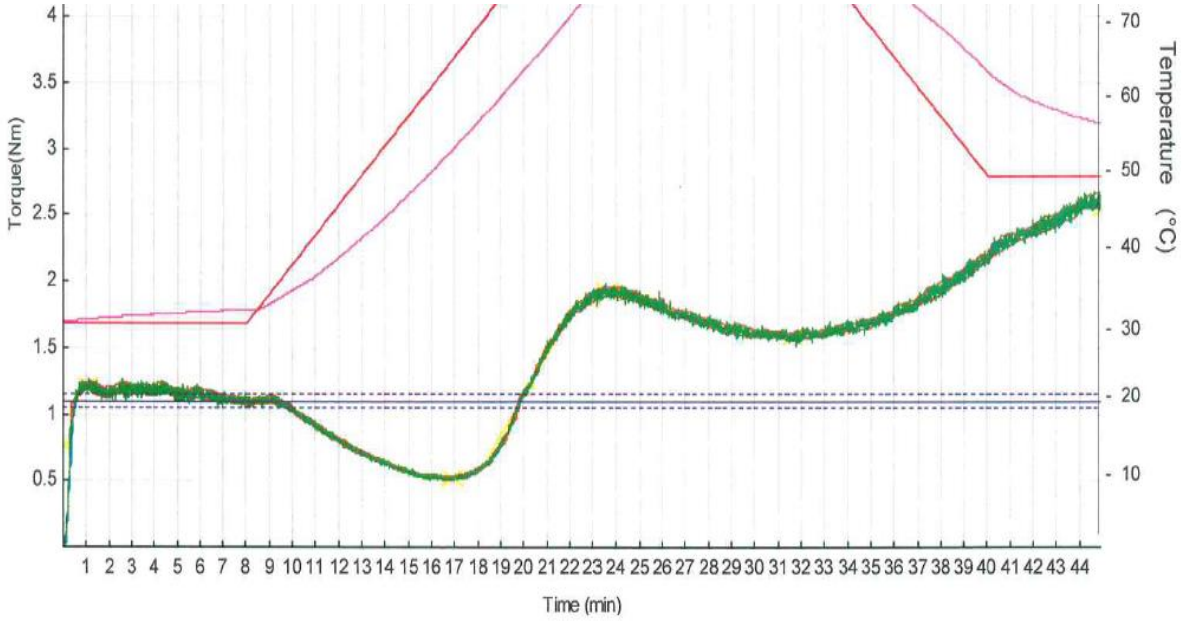


Figure 18 d: Mixolab curve for composite flour with 20 % cassava flour

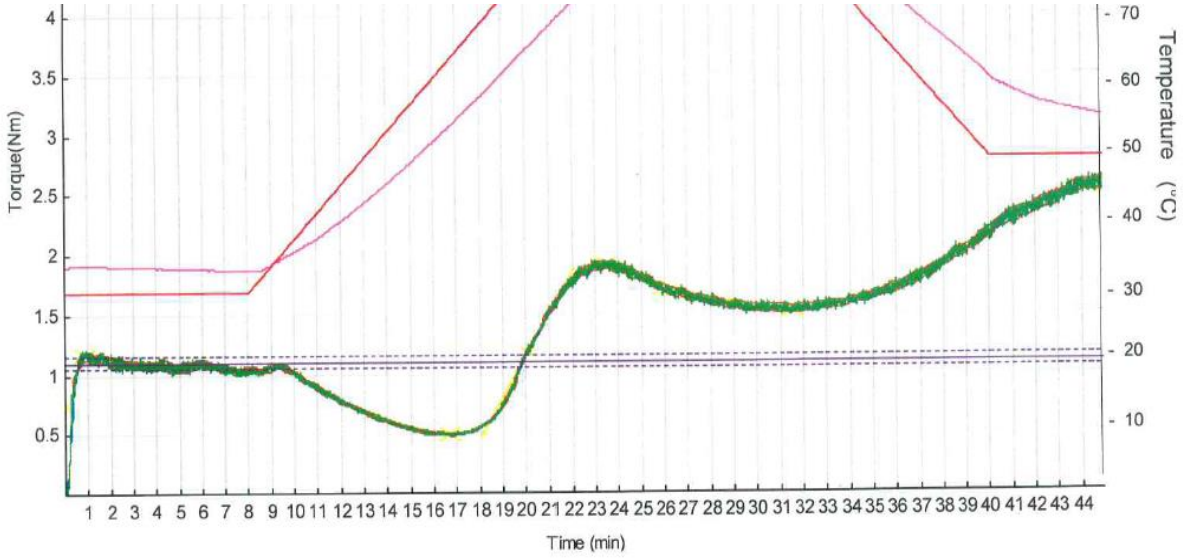


Figure 18 e: Mixolab curve for composite flour with 30 % cassava flour

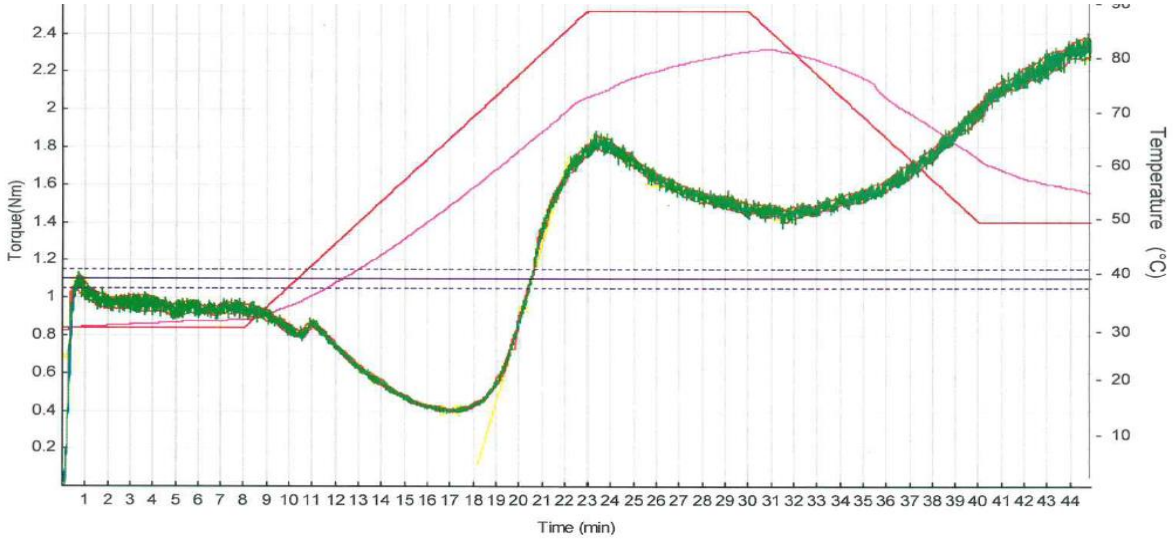


Figure 18 f: Mixolab curve for composite flour with 40 % cassava flour

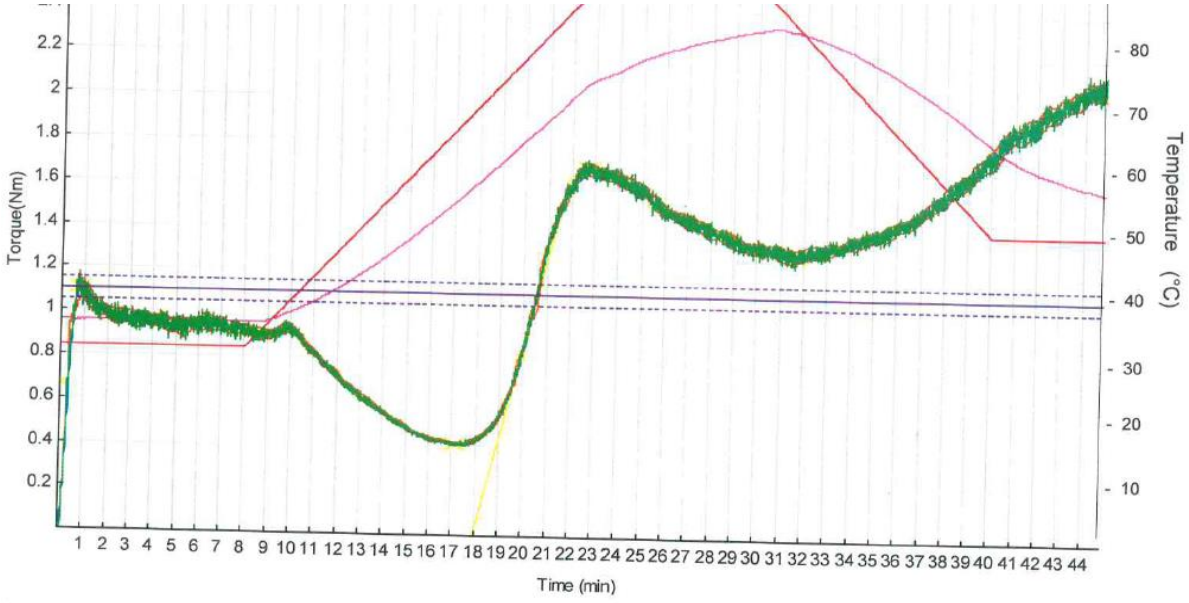


Figure 18 g: Mixolab curve for composite flour with 50 % cassava flour

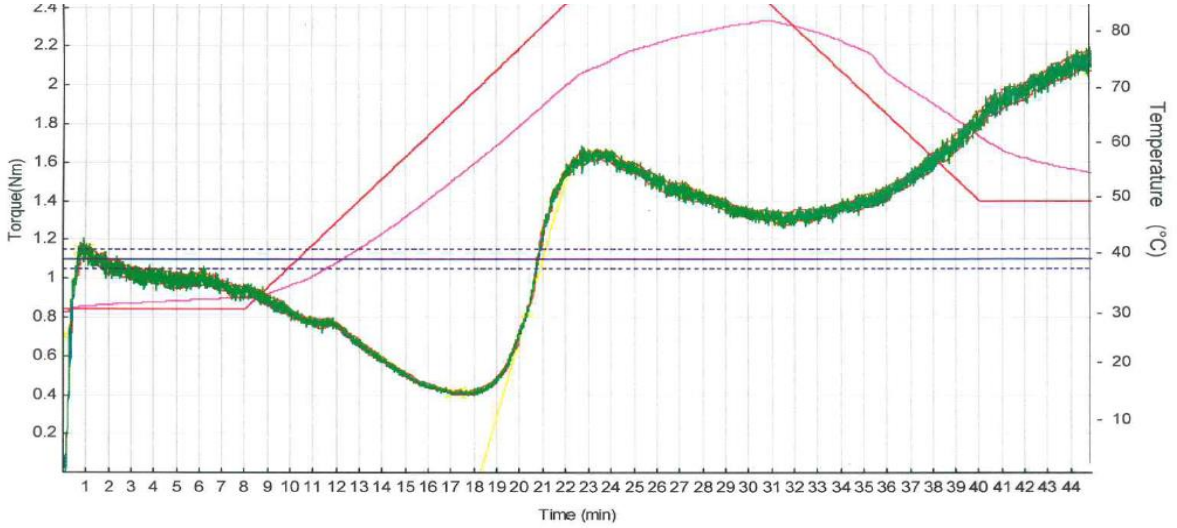


Figure 18 h: Mixolab curve for composite flour with 60 % cassava flour

4.3.3 Alveograph properties of wheat-cassava composite flour

The Alveograph showed that tenacity ranged from 29 to 117 mm H_2O; dough extensibility ranged from 32 to 120 mm; P/L ranged from 0.40 to 3.340; dough blowing index ranged from 12.60 to 24.30; energy ranged from 63 to 210 x 10^{-4} J and flexibility index ranged from 0 to 50.5 % (Table 14).

Addition of cassava flour to wheat flour up to 30 % inclusion level significantly ($p < 0.05$) increased dough elasticity/tenacity (P), after which the elasticity significantly reduced. The presence of high level of fibre in cassava flour could have interfered with wheat proteins during mixing, which caused an increase in dough resistance to deformation as maximum pressure. Khan et al. (2019) reported an increase in tenacity (P) of pumpkin/wheat flour blends. The blends, however, had increased content of fibre than carbohydrates. Based on flour classification according to Koksel et al. (2009), the tenacity observed in the current research (78 mm H_2O) at 40 % cassava flour inclusion was similar to that of medium strong wheat (73 mm H_2O).

Cassava flour addition did not show a particular pattern on dough extensibility (L) (first a decrease up to 20 % inclusion level, then an increase and a leveling off). Dough extensibility is an indicator of dough handling characteristic. The decrease in L could have been due to cassava flour interference with gluten network formation (Atudorei, et al., 2021). Khan et al. (2019) reported a decrease in the extensibility of the dough from pumpkin–wheat flour composite.

The P/L ration showed significant increase ($p < 0.05$) at 10 % and 20 % inclusion levels of cassava flour in the wheat flour, then a decrease and leveling off at 30 % and more cassava inclusion levels. The decrease of baking strength, which is a predictor of dough strength, indicated it's weakening by cassava flour addition. This could have been due to the "gluten dilution" effect induced by cassava flour addition, yet cassava flour does not contain gluten-forming plroteins, and cannot therefore form gluten (Atudorei et al., 2021). The P/L ratio observed in this research at cassava inclusion level of 50 % (0.93) was similar to the P/L ratio of a medium strong wheat reported by Koksel et al. (2009). P/L of 0.70 to 1.0 seems to provide a good balance of tenacity and extensibility (Papageorgiou and Skendi, 2014; Chopin "Alveolab Graph", 2018). Flour with good strength may lack extensibility in the constant hydration alveograph and may exhibit ratios from 1.2 to 1. According to Papageorgiou and Skendi (2014), strong wheat flour has P/L of 0.7, intermediate wheat flour: 0.3 – 0.7, and weak wheat flour: 0.3.

Table 14: Alveograph parameters of wheat-cassava composite flour (14 % moisture basis)

	Proportion (%) of cassava flour in blend						
Alveograph properties	0	10	20	30	40	50	60
P (mmH_2O)	96 ± 2.00^{a}	107 ± 3.00^{b}	117 ± 3.51^{c}	104 ± 3.00^{ab}	78 ± 2.00^{d}	54 ± 2.00^{e}	29 ± 2.00^{f}
L (mm)	58 ± 2.00^{a}	32 ± 2.00^{b}	46 ± 2.00^{c}	120 ± 2.00^{d}	98 ± 2.00^{e}	58 ± 2.00^{a}	73 ± 1.00^{f}
P/L	1.43 ± 0.38^{a}	3.34 ± 0.02^{b}	2.54 ± 0.02^{c}	0.87 ± 0.02^{d}	0.80 ± 0.02^{d}	0.93 ± 0.02^{d}	0.40 ± 0.02^{e}
G	16.90 ± 0.02^{ab}	12.60 ± 0.20^{b}	15.10 ± 0.02^{ab}	24.30 ± 0.20^{c}	22 ± 0.20^{c}	16.90 ± 0.20^{ab}	19.00 ± 2.00^{a}
W $x10^{-4}$ J	205 ± 2.00^{a}	142 ± 2.00^{b}	124 ± 2.00^{c}	189 ± 2.00^{d}	210 ± 2.00^{a}	93 ± 2.00^{e}	63 ± 2.00^{f}
Ie (%)	50.5 ± 0.02^{a}	0 ± 0.00^{b}	0 ± 0.00^{b}	16 ± 0.20^{c}	42.2 ± 0.20^{d}	30.4 ± 0.02^{e}	39.9 ± 0.02^{f}

The values are means ± SD of triplicate determinations. Values with different superscripts in the rows have means which are significantly different at $p < 0.05$ level.

P – maximum over pressure (resistance to extension, elasticity, tenacity); L – dough extensibility; G – dough blowing (swelling) index; W – work (deformation energy) (10^{-4} J); *Ie* – flexibility (elasticity) index;

The swelling index (G) reduced with the addition of 10 and 20 % but increased at higher levels of incorporation of cassava flour (> 20 %). Swelling index is an indicator of the dough handling characteristics. Reduced G on addition of cassava flour could have been caused by gluten solidification and dough solidification (decreased extensibility) (Atudorei et al., 2021). The swelling indices for pure wheat and wheat-cassava blends in this research (12.6 to 24.3) were similar to that of medium strong bread wheat (23.1) reported by Koksel et al. (2009). Moradi et al. (2016) reported a reduction in deformation energy (W), swelling index (G) and elasticity (*Ie*) with increasing bran (fibre) content of flour.

The deformation energy/baking strength (W) showed significant decrease with the addition of cassava flour to wheat flour except at 40 % cassava flour inclusion level. Khan et al. (2019) reported a decrease in baking strength with addition of pumpkin flour to wheat flour. The deformation energy of 210 $x10^{-4}$ J observed in this research at 40 % cassava flour inclusion was similar to that reported for medium strong bread wheat (214 $x10^{-4}$ J) (Koksel et al., 2009). According to Papageorgiou and Skendi (2014), strong wheat flour has W > 230, intermediate wheat flour: 140 – 230, and weak wheat flour: < 120. This would imply that the wheat flour and composite flours up to 40 % cassava inclusion in this study are similar to intermediate wheat flour, based on W.

Callejo et al. (2009) reported increase in P, while L and W decreased with increase in rye content of wheat-rye flour blends, probably due to the decrease of gluten content of the blends. Macedo et al. (2020) argued that from the P/L ratio values, it is possible to know about the elastic resistance of the dough to biaxial extension and the potential to produce bread. When the value is within the range of 0.40 to 0.80, dough has balanced gluten and is suitable to produce breads, while a P/L value lower than 0.40 indicates a very

extendable dough. The P/L values in this research ranged from 1.433 for pure wheat flour to 2.450 at 20 % substitution level and 3.340 at 10 % substitution level with cassava flour, suggesting that these composite flour doughs were too strong and not suitable for bread production. The P/L values for composite flour doughs at 30 %, 40 %, 50 % and 60% substitution levels were 0.870, 0.80, 0.93 and 0.40 respectively, suggesting that the latter were suitable for bread making. This may be contradictory to what Hruškova and Faměra (2003) reported, that alveograph parameter (P/L) of commercial wheat flours should range from 0.60 to 1.12, and energy (W) range from 161.10^{-4}J (weak flour) to 271.10^{-4}J (strong flour). Doughs prepared from flours with higher P/L do not have optimal viscoelastic behaviour (Hrušková and Šmejda, 2003). Based on these ranges, wheat-cassava composite flours studied in this research were equivalent to very weak, weak and moderately strong flours, depending on the level of wheat substitution, implying that bread could be produced from composite flours containing 30, 40 and 50 % cassava flour, based on P/L ratios, and composite flours containing 30 and 40 % cassava flour based on W. This therefore, would make composite containing 30 and 40 % the best choices based on recommendations of Hruškova and Faměra (2003).

4.3.4 Consistograph properties of wheat-cassava composite flour

Results from consistograph showed that water absorption capacity (WAC) of flours ranged from 48 to 55.6 % and maximum pressure ranged from 917 to 2819 mb (Table 15). According to the consistograph, addition of cassava flour above 30 % substitution level significantly decreased ($p < 0.05$) the WAC of the wheat flour. The reduction in WAC may be attributed to dilution of the wheat gluten by starch and fibre in the cassava flour, which inhibits the gluten network structure as reported by Puhr and D'Appolonia (1992), and Zighal et al. (2001). The presence of added starch and fibre also leads to increased competition for the available water among the different moieties of the flour

(Chisenga et al., 2020). Kumar et al. (2015) observed reduction in WAC of wheat on addition of multigrain premixes.

The reduction in WAC of wheat – cassava composite flour recorded by the consistograph contrasted with the increased WA recorded by the mixolab. This implies that the consistograph is not suitable for measuring WA in flours with increased starch and fibre contents. Maximum pressure (PrMax) decreased significantly ($p < 0.05$) with the addition of cassava flour to wheat flour. The decrease in PrMax might mean a decrease in the resistance of the dough to mixing (dough elasticity and extensibility is not high enough). The dough may not be good enough for bread making. Dough with high elasticity and extensibility produces loose bread with less developed volume and a core containing pores with thin walls (David et al. 2014). As cassava substitution levels increase, the bread volume is likely to reduce. Callejo et al. (2009) reported a decrease in WAC and PrMax in composite wheat-rye flours.

Table 15: Consistograph parameters of wheat-cassava composite flour (14 % moisture basis)

	Proportion (%) of cassava flour in blend						
Consistograph properties	0	10	20	30	40	50	60
WAC (%)	55.6±1.00^{a}	54.6±1.00^{ab}	54.2±1.00^{ab}	53.4±1.00^{b}	51.3±1.00^{bc}	49.2±1.00^{c}	48.0±1.00^{c}
PrMax (mb)	2819±1.00^{a}	2396±2.00^{b}	2315±2.00^{c}	2136±2.00^{d}	1675±2.00^{e}	1198±2.00^{f}	917±3.00^{g}

The values are means ± SD of triplicate determinations. Values with different superscripts in the rows have means which are significantly different at $p < 0.05$.

PrMax - maximum pressure; WAC - water absorption capacity;

4.4. Quality evaluation of wheat-cassava composite bread

4.4.1 Physico-chemical parameters of bread

For the different proportions of wheat-cassava composite flour, loaf volume ranged from 516.667 to 631.0 cm^3, loaf weight ranged from 306.0 to 331 g, bread density ranged from 0.49 to 0.636 g/cm^3, specific volume ranged from 1.571 to 2.065 cm^3/g and moisture loss ranged from 6.38 to 14.98 % (Table 16).

The volume of bread made from wheat - cassava composite flour decreased significantly ($p < 0.05$) with proportions of cassava flour more than 20 %. The density of bread made from wheat - cassava composite flour increased significantly ($p < 0.05$) with increasing proportion of cassava flour in the composite. Water loss was highest in 100 % wheat bread and significantly ($p < 0.05$) differed from water loss from composite breads.

In loaf bread consuming communities, loaf volume is the principal component of, and is of paramount importance to, bread quality evaluation. This is a vital aspect of the consumers' perception of value, because in bread and other leavened goods, higher volume (for the same weight) is invariably associated with more aerated crumb/gas retention and superior texture, proper formulation and quality of ingredients, dough handling, as well as processing conditions (Rozyo and Laskowski, 2011; Anderson et al., 2014; Bojnanaska and Mocko, 2014; Sahi et al., 2014). Higher loaf volume has positive economic effect on bread at the retail end since consumers falsely get attracted to bread loaf with bigger volume with the belief that it has more substance for the same price (Iwe et al., 2017).

For a baker or bread manufacturer, each batch of flour has to be evaluated by test baking to ensure that bread is made to a uniformly high quality. One component of this quality control is measurement of the volume of the bread, which can give information relating to the density of the bread crumb and the strength of the gluten in the flour. Lack of volume generally indicates the use of weak flour, or one low in enzyme activity. A very strong flour may also produce a loaf of small volume, which would indicate the need for a longer fermentation period during which the gluten would become ripened and so more extensible. This volume information can then be used to modify the dough mix used in bread production, in order to produce bread of the appropriate quality (Anderson et al., 2014; Sahi et al., 2014).
In Uganda, loaf bread is rated by the consumer in terms of appearance, weight, volume and brand. This may be attested by the fact that different bakeries in Uganda produce loaf breads with different appearances (crust colour), volumes, and crumb texture, yet all are able to survive in the competitive market.

To aid in the comparison of loaves of different weights, the concept of specific volume or volume per unit weight is used. This is done by dividing the volume measured by the weight of the bread sample in grams (cm3/g). Temperature of product should be registered when measuring volume and weight as bread loses moisture during cooling (Anderson et al., 2014; Sahi et al., 2014).

Table 16: Physical evaluation of bread made from cassava-wheat composite flour

	Proportion (%) of cassava flour in bread						
Parameter	0	10	20	30	40	50	60
Bread volume, cm3	631.0 ± 6.6^{a}	627.7 ± 2.5^{a}	657.7 ± 2.5^{b}	532.3 ± 2.6^{c}	552.3 ± 2.5^{d}	520.7 ± 1.2^{c}	516.7 ± 2.9^{e}
Loaf weight, g	306 ± 0.3^{a}	324.3 ± 1.4^{b}	330.6 ± 2.2^{c}	326.1 ± 1.6^{b}	328.9 ± 1.2^{bc}	331.0 ± 0.5^{c}	329.2 ± 0.5^{c}
Bread density, g/cm^3	0.49 ± 0.003^{a}	0.52 ± 0.002^{b}	0.51 ± 0.002^{c}	0.61 ± 0.003^{d}	0.60 ± 0.003^{d}	0.64 ± 0.002^{e}	0.64 ± 0.005^{e}
Specific vol, cm^3/g	2.07 ± 0.02^{a}	1.94 ± 0.01^{a}	1.66 ± 0.57^{b}	1.63 ± 0.01^{b}	1.62 ± 0.01^{b}	1.57 ± 0.00^{c}	1.57 ± 0.01^{c}
Moisture loss, %	14.98 ± 0.50^{a}	6.38 ± 2.52^{b}	9.87 ± 2.00^{c}	8.43 ± 1.11^{d}	9.63 ± 1.25^{c}	8.56 ± 0.80^{d}	9.62 ± 0.49^{c}

The values are means ± SD of triplicate determinations. Values in the rows with different superscripts are significantly different at $p < 0.05$.

The volume and specific volume (SLV) of bread made from wheat and HQCF composite flour decreased significantly ($p < 0.05$) with increasing concentration of HQCF (Table 16). The variation in loaf volume in this study could be attributed to the substitution of wheat with HQCF, which leads to dilution of the wheat gluten, since HQCF does not form gluten structures (Edwardo et al., 2013; Iwe et al., 2017; Chisenga et al., 2020).

Volume and specific volume of bread are also affected by the quantity and qualiy of protein in the flour. The low gluten level of cassava flour negatively affected the visco-elastic properties of the dough, and thus a reduction in loaf volume (Edwardo et al., 2013; Wambua, 2017; Iwe et al., 2017; Chisenga et al., 2020). Flour with high gluten produces bread with more volume and structure and a soft crumb since it has the ability to extend and trap carbon dioxide produced during fermentation, and/or slow the rate of carbon dioxide diffusion. Despite gluten dilution, good composite dough can be developed if the composite flours are carefully hydrated and optimally mixed and developed into dough (Othira et al., 2004).

The WAC of HQCF was higher than that of wheat flour (Eke et al., 2010; Oladunmoye, et al., 2010; Agbemafle, 2019), therefore extra water must be added to a blend of HQCF and wheat flour to form a dough of acceptable consistency (Edwardo et al., 2013). High amount of water results in gluten dilution. Physical interactions and biochemical reactions among cassava components during mixing, fermentation and baking steps may affect gluten matrix formation (www.ifst.org). HQCF contains about 1.1 % crude fibre (Manano et al., 2018) compared to approximately 0.5 % crude fibre in wheat (Khan et al., 2019). The elevated fibre content in the wheat-cassava composite flour due to incorporation of cassava flour may puncture expanding gas cells during fermentation leading to reduced loaf volumes (Oladunmoye et al., 2010).

The density of bread made from wheat and HQCF composite flour increased significantly ($p < 0.05$) with increasing proportion of HQCF in the composite. The variation in loaf density could be attributed to different rates of gas evolution and retention and moisture that diffused out of the loaf during baking. HQCF has high water absorbing capacity and resulted in heavy dough due to low air entrapment. Eriksson et al. (2014) observed decrease in bread volumes made with 20 % and 30 % cassava-wheat composite flours from some Ghanaian cassava cultivars. As the bread volume decreased with increasing level of wheat substitution with cassava flour, the bread density increased.

Studies showed that loaf volume is affected by the quantity and quality of protein in the flour used for baking and also by proofing time, baking time and baking temperature (Bushuk et al., 1969; Veraverbeke and Delcour, 2002; Shittu et al., 2007; Rózylo and Laskowski, 2011). Cassava flour lacks gluten and is therefore unable upon hydration to form the cohesive visco-elastic dough capable of forming the typical fixed open foam structure of bread (Aboaba and Obakpolor, 2010). Consequently, an increase in proportion of cassava flour in the composite reduces the gluten content as a percentage of the total flour. Gluten is responsible for dough elasticity (Kent and Evers, 1994; Nwanekezi, 2013; Noorfarahzilah et al., 2014), thus, an increased substitution with cassava flour would result in weaker and less elastic dough and a reduction in the leavening ability, resulting in bread with lower loaf volume and higher density.

According to Aboaba and Obakpolor (2010), an inclusion of cassava flour beyond 20 % significantly reduces the leavening profile of dough made from composite wheat-cassava flour. Besides the quality attributes of fresh bread, such as loaf volume, crumb texture and gas cell distribution, unfavorable changes in sensory features, like texture and flavour

during product storage, comprising the phenomenon of staling, are also important determinants of bread acceptance by consumers (Vouris et al., 2018).

4.4.2 Sensory evaluation of wheat-cassava composite bread

Results from sensory evaluation showed that crust colour ranged from 4.13 to 6.88, taste ranged from 4.25 to 7.13, crumb texture ranged from 4.50 to 6.63 and overall acceptability ranged from 4.13 to 8.13 (Table 17).

The results of sensory evaluation showed that incorporation of HQCF influenced the quality of bread. Bread made from 100 % wheat flour was superior in all the quality attributes assessed**.** The scores for all attributes decreased with increase in proportion of HQCF in the blend. The breads containing up to 20 % HQCF were not significantly different ($p > 0.05$) from the 100 % wheat flour bread in the sensory attributes. Bread which received overall acceptability score higher than 5 (neither like nor dislike) on the 9 – point hedonic scale was considered as acceptable. This implies that bread baked from 40 % cassava flour which recorded an overall acceptability of 5.75, slightly better than the assessment scale of "neither like nor dislike" although significantly ($p < 0.05$) different from bread made from 100 % wheat flour, was considered acceptable. This implies that any wheat – HQCF composite containing more than 40 % cassava will fail in overall acceptability.

Edwardo et al. (2013) reported that addition of pectin to cassava flour made it possible to bake bread with acceptable bread quality even at concentration as high as 40%. The researchers further reported that in addition to cassava proportion in the composite flour, the type of cassava flour (roasted, sun-dried or fermented) had the biggest effect on bread quality. At high inclusion of cassava, bread with roasted cassava had a higher volume

compared with sun-dried and fermented. They also observed that the level of pectin addition had a significant effect on improving the volume of roasted cassava bread at high proportions of incorporation of cassava flour in the composite. Crumb firmness similar to wheat bread was obtained with flours from sun-dried and roasted cassava. Bread from flour of roasted cassava was the only bread with crust colour similar to wheat bread.

Crust colour, taste and crumb texture of the bread samples in this study differed significantly ($p < 0.05$) with addition of more than 20 % cassava flour in the composite (Table 15). These parameters scored less and less as more cassava flour was incorporated in the flour composite. For consumers, taste/flavour and texture of bread are two of the most important factors in sensory evaluation (Kihlberg, 2004; Gellynck, 2008). Average scores for bread taste was significantly ($p < 0.05$) lower in bread containing more than 20 % cassava flour in the composite, which showed that addition of cassava flour at levels more than 20 % had a negative effect on final product in terms of taste. However, scores for taste did not seem to have determined the overall acceptability of bread in this study.

Table 17: Sensory scores of breads made from wheat-cassava composite flour

	Proportion (%) of cassava flour in bread						
Parameter	0	10	20	30	40	50	60
Crust colour	6.88 ± 1.96[a]	6.38±1.41[a]	6.63±1.41[a]	4.88±0.99[c]	5.38 ± 0.92[b]	4.13 ± 1.25[d]	4.63 ± 1.77[c]
Taste	7.13± 1.55[a]	6.38±1.85[a]	6.25±1.75[a]	4.88±1.46[b]	4.63 ± 1.69[b]	4.5 ± 1.0	4.25 ± 1.58[b]
Crumb texture	6.50± 1.69[a]	6.63±1.85[a]	6.50±1.93[a]	5.13±1.89[b]	5.63 ± 1.85[b]	4.50 ± 2.20[c]	4.63 ± 2.13[c]
Overall Acceptabilty	8.13 ±0.84[a]	7.13±1.36[ab]	6.00±1.51[b]	5.50±1.60[b]	5.75 ± 1.28[b]	4.13 ± 1.73[c]	4.50 ±1.31[c]

The values are the averages of twenty independent determinations ± SD. Values in the rows with different superscript letters are significantly different at $p < 0.05$

A number of studies have been conducted to show the potential of cassava flour in bread making, and the production of other baked products such as cakes and pastries. The studies revealed that wheat flour can be replaced by cassava flour at different substitution levels, without significant effects on processing and the quality of bread. Some researchers recommended 5 to 10 % substitution of wheat flour (Dendy et al., 1972; Giacco and Appolonia, 1977; Almazan, 1990; Defloor et al., 1995, Eddy et al., 2007; Aristizábal et al., 2017). Others recommended substitution levels of up to 30 % (Defloor et al, 1993 and Jensen et al., 2015). Siyeni et al. (2004) suggested a 33 % inclusion of HQCF in wheat flour for bread baking, arguing that at this level of substitution the quality and taste of the bread is not affected according to Malawian consumers. Eduardo et al. (2014) studied cassava–maize–wheat composite flour at cassava inclusion level of 40 %. The bread obtained had a perceived overall quality which was not significantly different from commercial wheat bread. Researchers from IITA and partners successfully baked bread with 40 % cassava flour and 60% wheat flour, showing bakers a window of possibilities (IITA, 2014).

The quality of bread produced from composite flours is influenced not only by the level of substitution of wheat by cassava flour but also by factors such as the cultivar of cassava (Eggleston et al., 1993; Eduardo et al., 2013; Eriksson et al., 2014; and Shittu et al., 2007), maturity of the cassava roots of the same cultivar (Defloor et al., 1994; Defloor et al., 1995). Linus-Chibuezeh et al. (2017) however, found out that bread made from 10 – 30 % cassava–wheat composite flours, irrespective of the cassava cultivar used, was of acceptable qualities. The quality of the bread produced from cassava-wheat composite flours may also depend on the type of bread. Aryeetey et al. (2019) observed that cassava substitution levels of 20 % for

sugar bread and 10 % for tea bread were deemed culturally acceptable. Therefore, the taste to which a community is accustomed may play a big role in determining acceptability of the levels of substitution of wheat with cassava in the products consumed.

Generally, addition of HQCF at levels greater than 20 % had significant effects ($p < 0.05$) on sensory attributes and overall acceptability of bread. Addition of HQCF up to 30 % did not have negative effect on crumb texture and overall acceptability. At 40 % substitution HQCF did not have negative effect on crust colour, crumb texture and overall acceptability but had a low score (4.63) for taste. This might be attributed to reduced flour strength and gas retention capacity due to a reduction in gluten content, thereby reducing bread volume and the sensory appeal of the baked composite bread (Khalil *et al*., 2000). This means that an appropriate additive to improve on cassava bread taste incorporated in the composite would make 40 % cassava bread acceptable to consumers.

The Federal Government of Nigeria (FGN) released a 40 % cassava inclusion in wheat flour policy in 2012, mandating flour mills and bakers to incorporate 40 % cassava flour into wheat flour meant for bread making, pasta and other confectionaries (IITA, 2014; Ohimain, 2014; Adikwu et al., 2017). This followed the successful development of bread containing 40 % cassava flour by the International Institute of Tropical Agriculture (Eleazu et al., 2014). Such a policy can be introduced in Uganda based on findings of this type of study, to encourage the use of cassava in the bakery and confectionery industry. There is however, the need to design appropriate processes and technologies to manufacture the products of choice.

Bread is a dietary staple in North Africa, Northeastern Africa, and the Horn of Africa, and it is important in the diet of many Saharan and sub-Saharan people (Lyons, 2016). African breads are made from the flours of a variety of cereals, tubers, and corms that are baked, steamed, and sometimes fried to produce pancake, flat bread, leavened or slightly leavened loaves, or cakes using ovens, hearths, griddles, pots, and molds. Technical choice in bread baking is associated with the baking properties of bread ingredients and social group's culinary practices and preferences and does not represent evolutionary stages.

Wheat and barley are Near Eastern cereals that were introduced into northeastern Africa in ancient times. Only Near Eastern cereals contain gluten, an elastic protein formed when their flours are mixed with liquid to produce dough (Lyons, 2016). Leavened bread made from wheat flour has been used in the Mediterranean for at least three thousand years. This staple subsequently spread all over the world (CTA, 1987; Adeniji, 2013). The fascination for Western food habits, increasing populations and urbanisation has encouraged bread consumption in most of the large cities in developing countries (Adeniji, 2013). Over the last few decades wheat flour and the American loaf of bread have become familiar products on markets of all continents (CTA, 1987).

Loaf bread was introduced in Uganda by colonial masters, probably in the 19th century. Later, the native elites also started eating bread. The natives accepted whatever quality attributes came with the bread and had to tame their taste buds to accept those quality attributes. The bread they got used to is made of 100 % wheat flour, which was brought from the temperate climatic zones of the colonialists. This research has shown that some wheat can

be substituted with other flour types, including cassava flour. The new product has a different flavour, which Ugandans have to be introduced to. It may take to tame the taste buds of the natives (Ugandans) to accept this flavour. Afterall, tastes change over time. After adaptation, tastes can be changed and people can learn to like foods of different tastes.

During this study, I made some critical analytical observations, that Ugandans prefer sweet bread. Therefore, the sensory evaluation was performed on sweet breads only, since breads with reduced sugar levels were rated as poor in taste and thus unacceptable by panelists at the start of this research. I have also observed that Ugandan consumers choose particular bread types for various reasons.

Price: the price of a 1 kg loaf bread ranges between Ushs 4,000 = and 7,000 =, depending on brand and place of sale. Thus, the amount of disposable cash will determine what the customer can afford. *Brand*: there are a number of bakeries in Uganda, most of them around Kampala. Each bakery produces bread with some perculiar attributes. Besides, what an individual was first introduced to as a child may determine the future preference of the individual. *Purported shelf life of the bread:* some bread type seem to grow moulds on their surfaces faster than others despite the "sell by date" period. Consumers want bread which lasts for some days before they can buy another loaf. *Flavour*: most customers will buy sweet bread as opposed to salty bread or bread with reduced sugar levels. The degree of sweetness may also be a factor in buying a particular bread. *Size/volume of the loaf*: some customers are interested in bread loaves that look bigger although weigh the same with a smaller loaf. The visual satisfaction may be the deciding factor. *Texture*: this plays a role to some customers.

The brand “Supa” is especially popular for the soft texture of the crumb. *Colour of the crust*: most Ugandans interviewed during this research were not influenced by the brownness of bread crust. It is, therefore, not a deciding factor in the choice of bread type. *Health and nutrition*: some people who are diabetic prefer to buy and consume “salty” bread as opposed to the common sweet bread most ugandans are used to. For some reasons, nutrition or otherwise, some ugandans are attracted to wholemeal bread.

Kusasira and Manano (2014) evaluated the level of wheat substitution with (HQCF) in the production of cookies using the “Rubbing-In” method, where 5 % milk was included in the recipé, and reported that up to 40 % wheat flour can be substituted with HQCF and produce cookies with acceptable sensory characteristics or even better characteristics than cookies from pure wheat flour. A 50 % wheat substitution with HQCF produced cookies which were neither liked nor disliked. Since swelling is not a prerequisite in cookies, the HQCF, with its low protein content is sufficient in the production of the said goods.

The use of cassava flour in bread and cake making is a convenient alternative for promoting the use of a local crop as well as reducing imports of wheat flour, promoting the production of HQCF, offering gluten-free products and developing biofortified and fortified foods (Oladunmoye et al., 2010; Kleih et al., 2012; Tonukari et al., 2015; Aristizábal et al., 2017; Lu et al., 2019; Irakiza et al., 2021). Although the substitution level of cassava flour is limited, in some products, the incorporation of additives or flours from other crops improve the nutritional value and bread/cake making quality of the baked foods (Eggleston, 1993; Chilungo, 2013; Edwardo et al., 2013; Noorfarahzilah et al., 2014; Owuamanam et al., 2014;

Umelo et al., 2014; Gioia et al., 2017; Lu et al., 2019; Akintayo et al., 2020). Several limitations have hindered the success of initiatives to promote, in some cassava producing countries, the intensive use cassava flour in bread making. Among these include the costs and efficiency of processing technologies, standards of the quality of cassava flour and lack of favourable policies (Pasqualone, 2010; Adeniji, 2013; Ohimain, 2014; Abass et al., 2016; Adikwu et al., 2017). Further studies about bioavailability and retention of nutrients in baked foods and evaluation on the effects of processing cassava flour in relation to increasing the resistant starch are required to provide scientific evidence for the health benefits of this flour.

In this study, the quality of composite bread was compromised at higher levels of HQCF substitution. It is however, possible to increase the substitution level by incorporating additives/dough improvers to composite flour containing cassava flour. Therefore, better quality parameters may be achieved with the use of 40 % HQCF substitution in bread-making if better chemical additives (reducing agents, oxidants, improvers, malt, hydrocolloids such as zanthan gum) (Edwardo et al., 2013; Owuamanam et al., 2014; Edwardo, 2015; Idowu et al., 2015) are identified and the bread-making process better optimized, e.g., by delaying the addition of the cassava flour until near the end of the mixing process, or by increasing the amount of sugar and fat in the recipe (Kent and Evers, 1994). HQCF may also be suitable for use as composite in the production of other baked goods which do not require any or tremendous increase in the volume of the finished product, like cookies and pasta. Different products, however, may require different cassava flour substitution for optimum results, and these need further investigation.

4.4.3 Cost implications of substituting wheat flour with HQCF

Taking a base of 10 kg of flour

Wheat (AZAM Brand)

1 kg costs 3 500 UShs

10 kg costs 10 kg x 3 500 Shs = 35 000 Shs

Cassava flour

1 kg costs 1 500 UShs **(at most)**

At a substitution level of 30 %

Cassava flour: 3 kg x 1 500 Shs = 4 500 Shs

Wheat flour: 7 kg x 3 500 Shs = 24 500 Shs

Total cost: (24 500 + 4 500) Shs = 29 000 Shs

Savings: (35 000 – 29 000) Shs = 6 000 Shs

At a substitution level of 40 %

Cassava flour: 4 kg x 1 500 Shs = 6 000 Shs

Wheat flour: 6 kg x 3 500 Shs = 21 000 Shs

Total cost: (21 000 + 6 000) Shs = 27 000 Shs

Savings: (35 000 – 27 000) Shs = 8 000 Shs

A total saving of 8 000 UShs for every 10 kg of composite flour used to bake bread will be realised at 40 % cassava incorporation in the composite flour. Bulk purchase may bring down the cost of cassava flour thus increasing the profit margin of the bakery. This implies that it would be economic to partially substitute wheat flour with HQCF in bread-making. However, a full cost analysis must be made in order to study the viability of any project involved in utilisation of composite four.

4.4.4 Correlation analysis

4.4.3.1. Correlation between rheological parameters of dough, and sensory and Physico-chemical properties of bread

Correlations were determined between Mixolab, Alveograph, and Consistograph parameters of the dough at constant hydration and bread quality characteristics to identify the instrumental parameters which could demonstrate the suitability of the instruments for estimating bread making quality of composite flour samples (Figure 19). The first two principal components (PC) F1 and F2 accounted for 78.72 % of the variance (64.26 and 14.46 % respectively) (Figure 19). The third PC accounted for 13.29 % of the variance. The fourth PC accounted for 5 % of the variance. The biplot using PC 1 and 2 is presented in Figure 19. The first PC (64.26 % total variability) contrasted bread volume and bread specific volume with bread density. The second PC (14.46%) contrasted loaf weight with moisture loss in bread during baking and bread taste.

From the Principal Component Analysis (PCA) score plot, panellists' overall acceptance of bread was positively influenced by sensory characteristics crust colour and taste. The physical properties of bread, i.e., specific bread volume (which is dependent on porosity/level of aeration of dough), and level of moisture loss (quadrant A) were positively correlated with bread acceptability. Bread weight and bread density were negatively correlated with bread acceptability. Bread weight was also negatively correlated with moisture loss in bread. The more the moisture loss, the lighter the bread loaf. Bread volume was also negatively correlated with bread density.

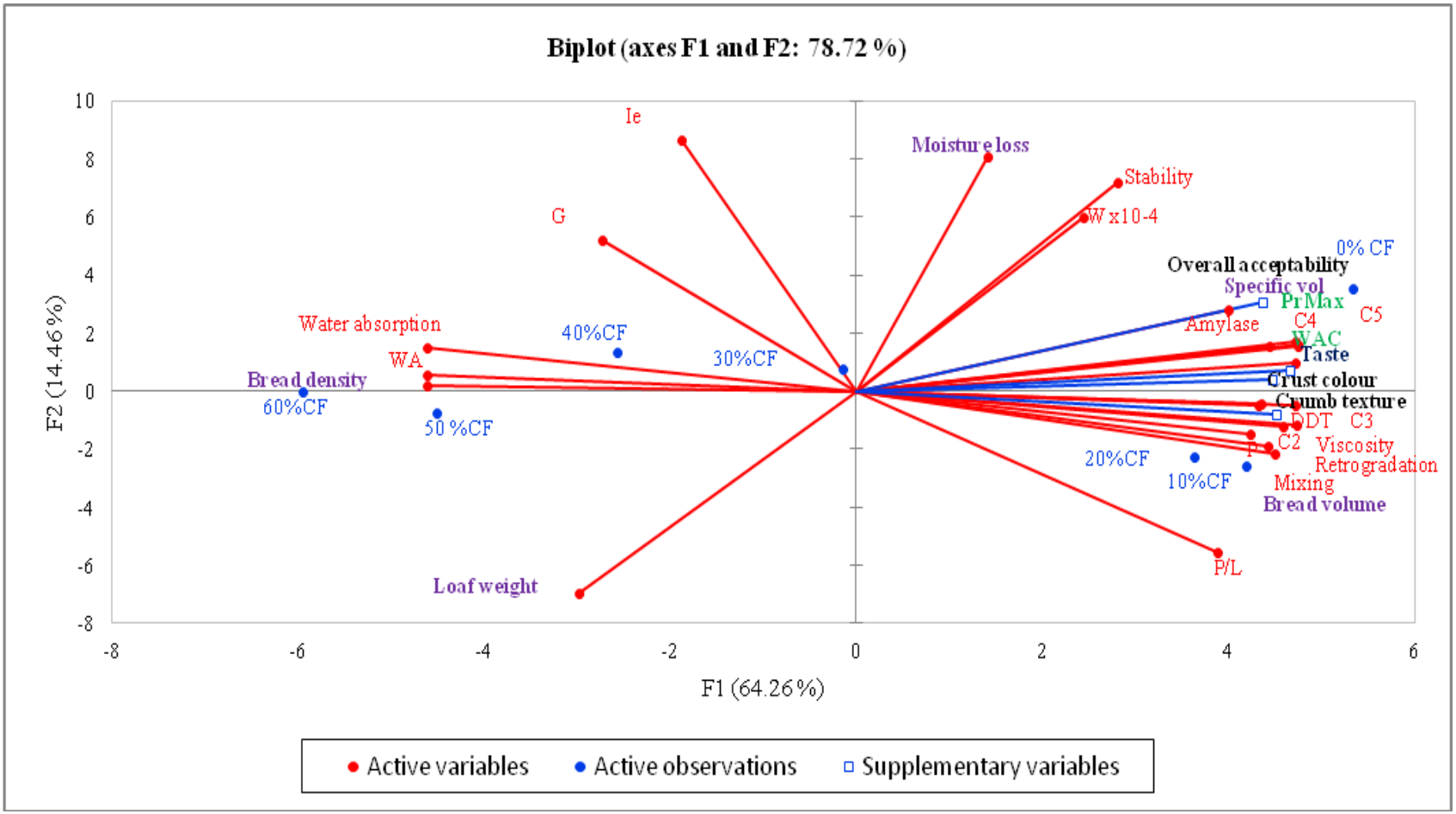


Figure 19: Principal component analysis of rheological parameters of dough and sensory characteristics and physicochemical properties of wheat-cassava composite bread: WA-water absorption, G - dough swelling index, Ie - flexibility (elasticity) index, L - dough extensibility, W- deformation energy, P - tenacity, DDT - dough development time, WAC - water absorption capacity, C2 - protein weakening, C3 - starch gelatinisation, C4 - amylase activity, C5 - starch gelling/retrogradation.

As the volume of the bread increased (for the same weight of dough) the density of the resultant bread decreased. The proportion of cassava in the composite flour showed varying influence on bread acceptability. At 10 % substitution (with a PC1 score of about 4) and 20 % substitution (PC1 score of about 3.5), cassava flour proportion did not have any negative influence on bread acceptability. At about 30 % cassava inclusion, the effects were not noticeable (PC1 score of about 0). At cassava inclusions higher than 30 %, it had negative influence on bread acceptability (40 % had a PC1 score of about -2.5, 50 % had PC1 score of about - 4.5, and 60 % had PC1 score of - 6). Cassava proportion was positively associated with density of the bread (noticeable at proportions more than 30 %). The higher proportions of cassava in composite flour (more than 30 %) were also positively associated with WA of the flour. For the panellists' overall acceptance, cassava proportion should not exceed 30 %. Loaf weight was positively correlated with cassava proportion in the composite.

According to the PCA (Figure 19), the correlations (F1 64, 26 %) between specific loaf volume (SLV), crust colour and bread taste were positive with Mixolab parameters dough stability, amylase index, amylase activity (C4) and starch gelling (C5) (quadrant A). In order to form dough, water was added to flour. The right amount of water was added to the flour to form dough of good consistency (Awuchi et al., 2019; Schopf and Scherf, 2021). This was the stage associated with dough mixing characteristics such as dough development time, stability, elasticity, and dough weakening and water absorption of the flour (Koksel et al, 2009; Codina et al., 2010; Mironeasa and Codina, 2017). The proteins glutenin and gliadin found in flour were hydrated and formed gluten (Hoseney, 1976; Bushuk, 1985; Hoseney, 1985; Hoseney, 1986; Stauffer, 1998; Awuchi et al., 2019). During the mixing and kneading stages of the dough air was incorporated in the dough (Bushuk, 1985; Hoseney, 1985; Hoseney, 1990; Hamer et al., 2009; MacRitchie, 2010).

The dough was thoroughly mixed so that the gluten became more developed. Mechanical energy input during kneading leads to a redistribution of the flour particles and finally to the formation of a viscoelastic protein network (Scopf and Scherf, 2021). Discrete masses of gluten proteins are destroyed during kneading, causing large glutenin molecules to be extended beyond their equilibrium conformation (Schopf and Scherf, 2021). This caused the dough to become elastic and stretchy. Developing gluten to the proper level is essential to obtaining the desired baked good. The gluten was formed during kneading of the bread dough. Kneading caused the gluten strands to get stronger and longer.

During fermentation, the carbon dioxide produced diffused into the air cells in the dough, which were trapped within the dough during mixing. This caused the dough to rise. During baking the carbon dioxide expanded and caused the bread to rise further. The alcohol produced during fermentation evaporated during the bread baking process. The sugar fermented by the yeast was partly added as an ingredient and partly released from starch present in the flour by the action of the enzyme amylase.

In baking, amylases play a double role. On the one hand they continuously supply the fermentable carbohydrates for yeasts and, in this way, ensure the continuous production of CO_2, and on the other hand, they contribute to the improvement of the properties of the dough (decrease in viscosity and dough consistency), and therefore to the high quality of the bread (Atudorei et al., 2021). The yeast cells, which released CO_2, had a major influence on dough rheology, volume, texture and taste of final bread product (Munteanu et al., 2019). The consistency of the dough at this time decreased as a result of amylolytic activity (demonstrated by stability of gelatinized starch granules), represented as C4 of the Mixolab (Codina et al., 2010; Koksel et al., 2014; Singh et al., 2019). The intensity of the decrease depended on amylase activity. The higher the enzyme activity the more

sugar was released from the starch for the yeast and more carbondioxide released, leading to a higher SLV of the dough.

During the heating phase and stabilisation of the bread, the gas expanded further (oven spring), and escaped at higher temperatures leaving a porous, well aerated crumb. Starch underwent gelatinization at temperatures above 60 °C, while the gluten network retained the gas generated by yeast during dough preparation and stabilized the characteristic, foamy structure of wheat bread crumb (Scopf and Scerf, 2021). The amylase was deactivated by heat, just as the proteins were denatured, forming a set crumb.

The liberated simple sugars from the starch reacted with proteins and amino acids in the Maillard reaction, and also caramelised, to form the brown colour of the crust (Ndubuisi and Chidiebere, 2018). The Maillard reaction and caramelisation produced flavour compounds which gave the bread its characteristic taste and aroma (Coultate, 1999; Mondal and Datta, 2008; Ndubuisi and Chidiebere, 2018). The cooling stage of the Mixolab (C5) was associated with retrogradation of the gelatinised starch, which increased consistency due to gel formation (Codina et al., 2010; Koksel et al., 2014). In practice, the retrogradation gave dough the structure we know as bread.

The Mixolab parameters C3, C4, and C5 were positively correlated with bread volume as reported by Koksel et al. (2014). With regard to bread making quality, Mixolab C2, dough stability and DDT were found to be highly associated with SLV (Dhaka et al., 2012). Dough stability and C2 indirectly refer to the protein quality (Dhaka et al., 2012). According to Lacko-Bartošová et al. (2019), the only Mixolab parameter strongly correlated with SLV was the slope α, showing the importance of protein weakening speed in bread volume. Medium strong correlation between C5 and SLV indicated the role of

starch phase – retrogradation and amylolytic activity for bread making quality, as reported by Lacko-Bartošová et al. (2019).

Positive correlation existed between bread volume and Mixolab parameters DDT, C2, C3, mixing index, viscosity index and retrogradation index (quadrant B). In the straight dough method of bread making, also known as no time dough or rapid dough processing, all ingredients (dry and liquid) are mixed together to full development of the dough (Sievert et al., 2017). Dough mixing is a process in which flour and water are mixed until gluten is developed, a result of the enhanced interaction between dispersed and hydrated gluten-forming proteins (Hamer et al., 2009; MacRitchie, 2010). The goal of mixing was to: incorporate air, hydrate dry ingredients, homogenize the dough by evenly distributing all the ingredients, knead the dough and develop the gluten.

The mixed dough consisted of continuous (gluten) and discontinuous or dispersed (air cells) phases. Ideally, this mechanical process created a visco-elastic mass that had optimum dough handling properties and gas retention capacity, essential for product expansion during proofing and oven spring (Hamer et al., 2009; MacRitchie, 2010). Mixing is a crucial step in all dough systems used for the manufacture of yeast-leavened baked goods. It was critical to obtain the right rheological properties and consistency of the dough for the production process to run smoothly, as well as achieve the desired finished product quality (Hamer et al., 2009; MacRitchie, 2010). DDT and C2 were directly influenced by the protein quality and quantity of the flour.

According to Lacko-Bartošová et al. (2019), slope α was the Mixolab parameter correlated with bread volume, showing the importance of protein weakening speed in estimation of direct baking parameter. Zhang et al, (2016) reported that Mixolab C2 could

be used in prediction of loaf volume, bread appearance, structure, and total sensory score of bread qualite attributes. For bread texture and elasiticity, the Mixolab stability, C2, parameters of starch pasting properties, C3, C4, and C5, were more important. The effects of protein property and starch quality on bread baking quality were well explained with Mixolab parameters thus, Mixolab was particularly applicable to determine wheat quality property due to the different contributions to the evaluations of bread volume (Zhang et al, 2016).

Bread density was positively correlated with Mixolab parameter water absorption index (quadrant D). Moisture loss in bread, bread specific volume and bread taste were positively correlated with Alveograph parameter deformation energy (W) and Consistograph parameter PrMax (quadrant A). Bread volume was positively correlated with Alveograph parameter P/L.

A positive correlation also existed between SLV and Mixolab parameters dough DDT (r = 0.867), dough stability (r = 0.791), C4 (r = 0.871) and C5 (r = 0.886), and Consistograph parameter PrMax (r = 0.812) and WAC (r = 0.778). Positive correlations between SLV and Mixolab parameters C2, DDT and dough stability, and Alveograph parameter P/L) were reported by Dhaka et al. (2012). Koksel et al. (2009) observed significant negative correlations between bread volume and SLV with Mixolab parameters starch gelatinisation (C3), amylase activity (C4) and starch gelling (C5).

Positive correlations were observed between bread volume and Mixolab parameters DDT (r = 0.914), C2 (r = 0.850), C3 (r = 0.754), C4 (r = 0.836), and C5 (r = 0.841); Alveograph parameters P (r = 0.763), P/L (r = 0.855) and Consistograph parameters WAC (r = 0.828) and PrMax (r = 0.822). PCA results also indicated a close relationship

between overall acceptability of composite bread and taste, crust colour, crumb texure, bread volume and the level of substitution of wheat with cassava flour. The results of this study, therefore suggest that Mixolab, Alveograph and Consistograph have combined potential which can be used to determine the best possible composition of wheat-cassava composite flour for bread making.

Based on the Mixolab parameters dough stability, C2 and DDT, which were indirectly related to the protein quality of flour, starch properties such as C3, C4 and C5 (Zhang et al., 2016; Dhaka et al., 2012; Torbica et al., 2019), the Mixolab has potential as a standalone instrument that can be used to determine the appropriate composition of wheat-cassava composite flour for bread making as demonstrated by the results in sections 4.3.1, 4.3.2 and 4.4.4.

Results of the study also showed that positive correlation existed between rheological characteristics of dough, Alveograph parameters P, P/L and W and Consistograph properties PrMax and WAC, bread making quality of flour/dough and bread quality parameters. Dough rheological properties can therefore, be used as predictors of bead quality characteristics, an ability that could be used to modify dough formulations depending on the characteristics desired in the final composite bread.

Inclusion of cassava flour in wheat flour at varying levels negatively affected both physical and sensory qualities of bread. Despite effects on physical and sensory quality, sensory evaluation, supported by the PCA analysis, indicated that bread of acceptable quality can be produced from wheat-cassava composite flour containing not more than 30 % cassava. Based on Mixolab parameters, DDT of not less than 1 min and dough stability of not less than 9 min are the parameter levels for wheat-cassava composite flour which

are likely to produce bread of acceptable quality. Alveograph parameters P of not less than 78 mm H_2O and W of not less than 124 x 10^{-4} J; and Consistograph parameter PrMax of not less than 1675 mb are as well parameter levels for processing of high quality cassava-wheat composite bread of acceptable quality.

CHAPTER FIVE: CONCLUSIONS AND RECOMMENDATIONS

5.1 General conclusions

1. The mineral content of HQCF was generally low implying that cassava is a poor source of minerals.
2. HQCF from all cassava cultivars had total cyanide and phytate concentrations above permissible limit.
3. HQCF from sweet and bitter cassava roots had higher water absorption capacity than wheat flour.
4. Partial substitution of wheat by 20 to 30 % HQCF is feasible based on the Mixolab measurements.
5. Up to 40 % substitution of wheat by HQCF is feasible based on sensory evaluation.

5.2 Recommendations

1. HQCF in combination with wheat flour can be used in bakery.
2. Fortification of HQCF with zinc and iron is recommended if it is to be used in bread, as is already done with wheat flour as a policy in Uganda.
3. Proper methods to detoxify cassava roots to be designed in order to utilise high-cyanogenic cassava roots. The standards on HCN should be revised.
4. Mixolab parameters could be used to predict the quality and quantity of wheat–HQCF composite in bread making.
5. There is need for further research to design processes for higher inclusion levels of HQCF in the composite for bread making.
6. There is need to establish the shelf-life stability of wheat-HQCF composite bread, and its acceptability by the general public/consumers.

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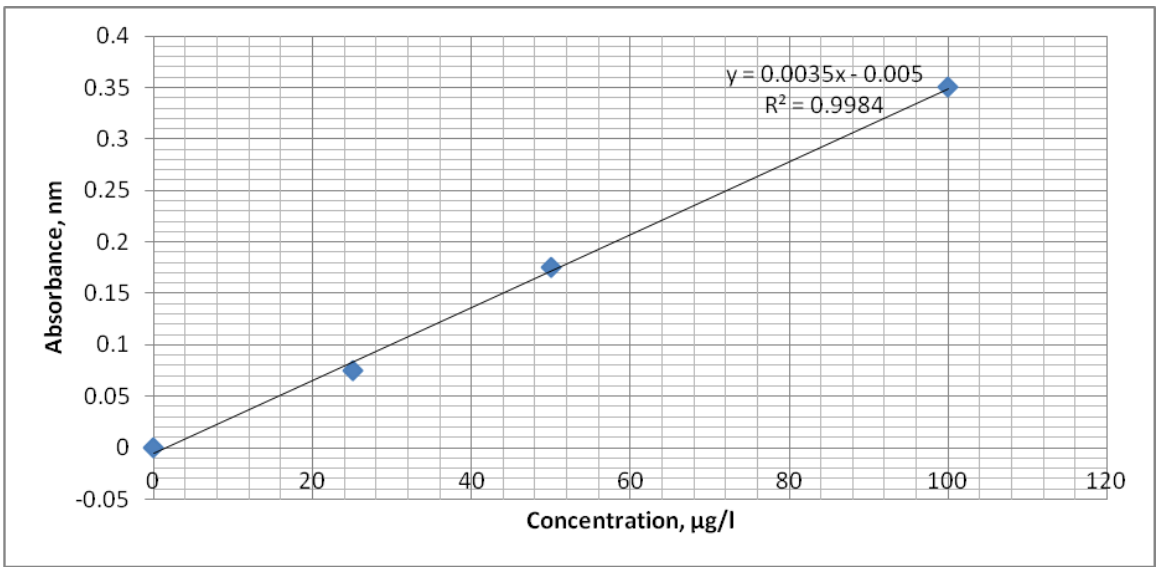
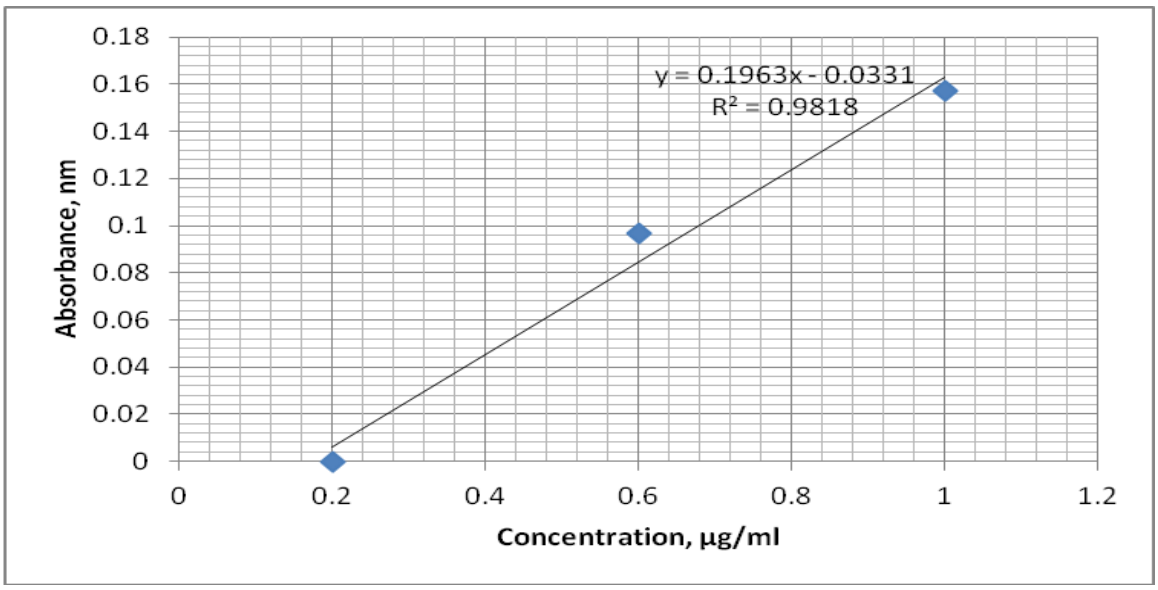
APPENDICES

APPENDIX I: Calibration curves

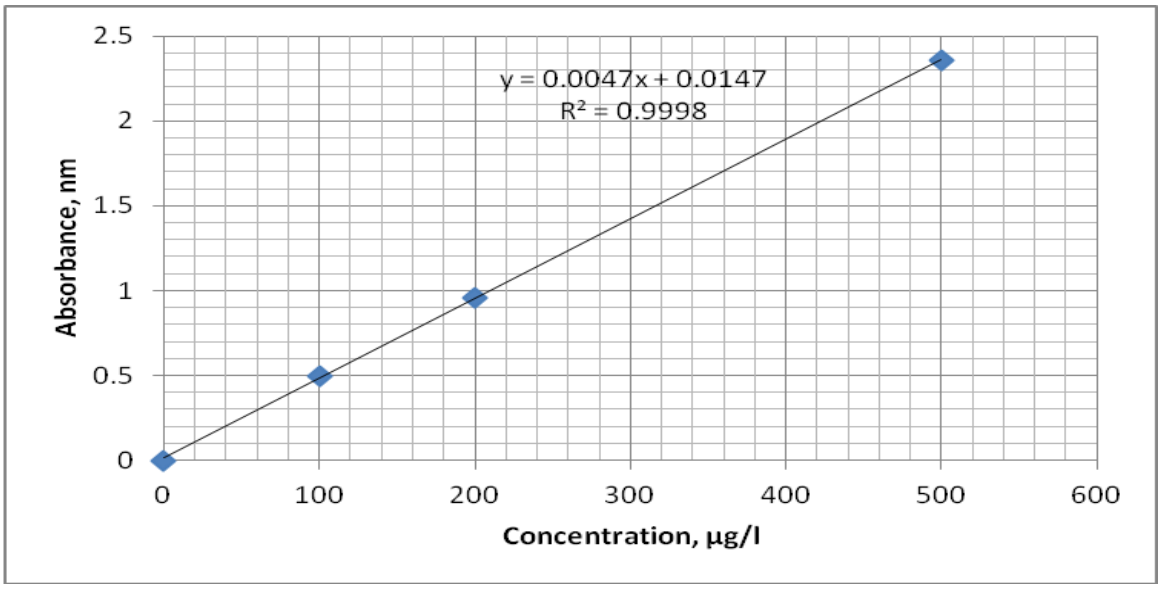
Glucose standard curve



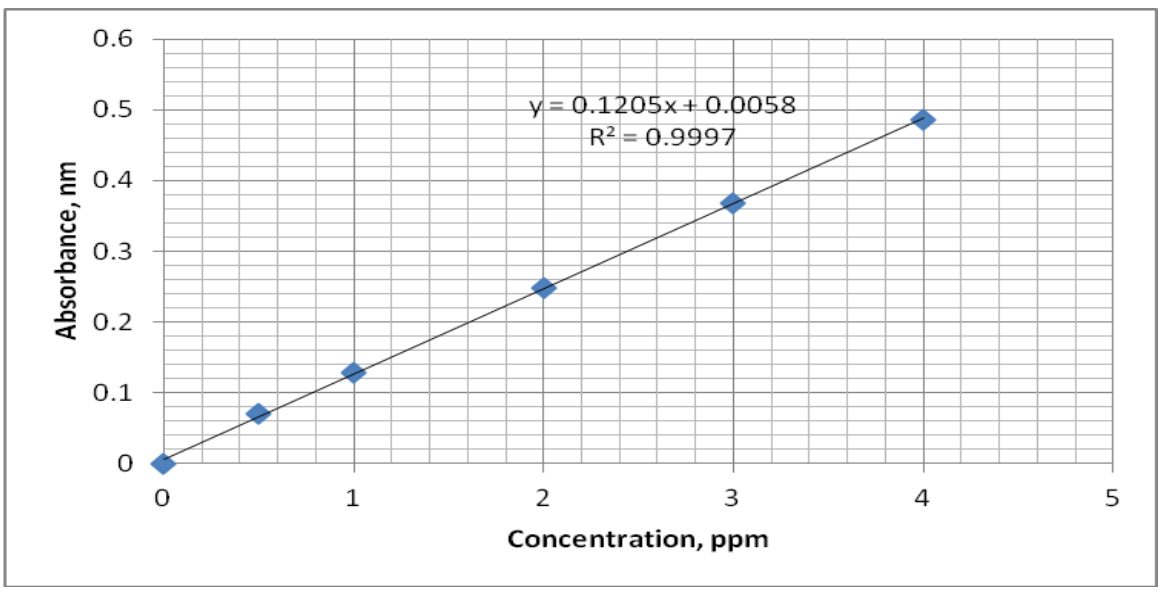
Zinc standard curve



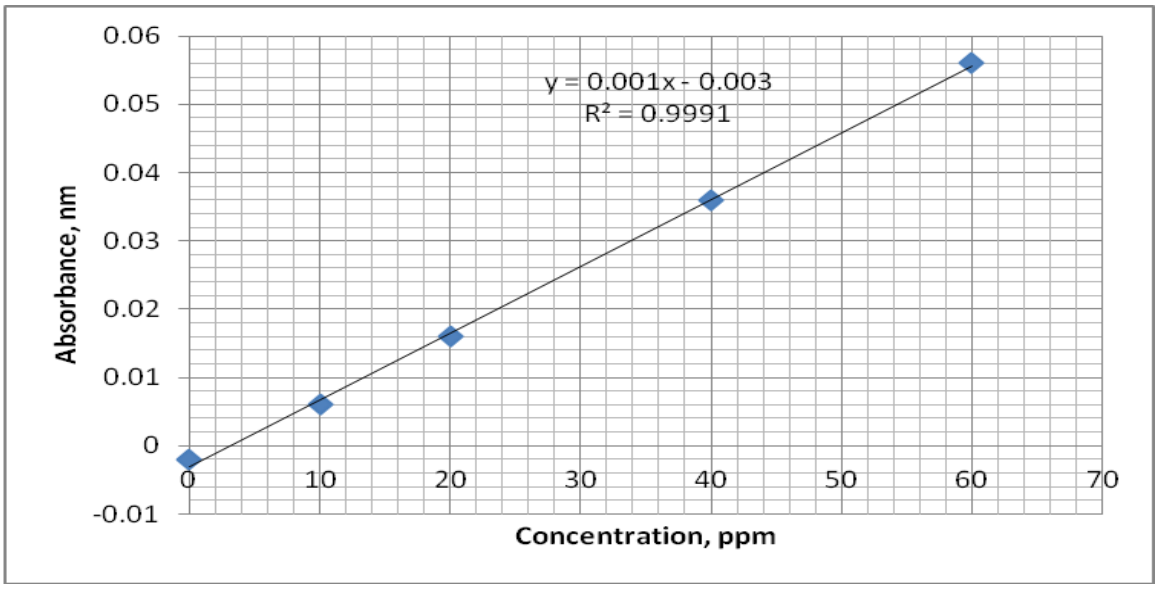
Copper standard curve



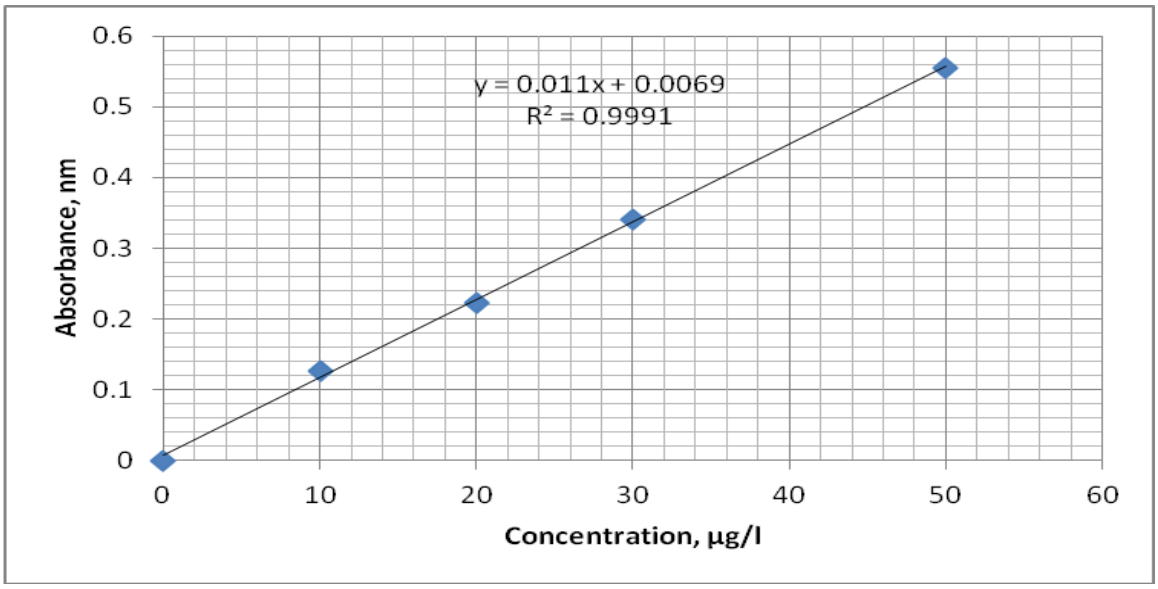
Magnesium standard curve



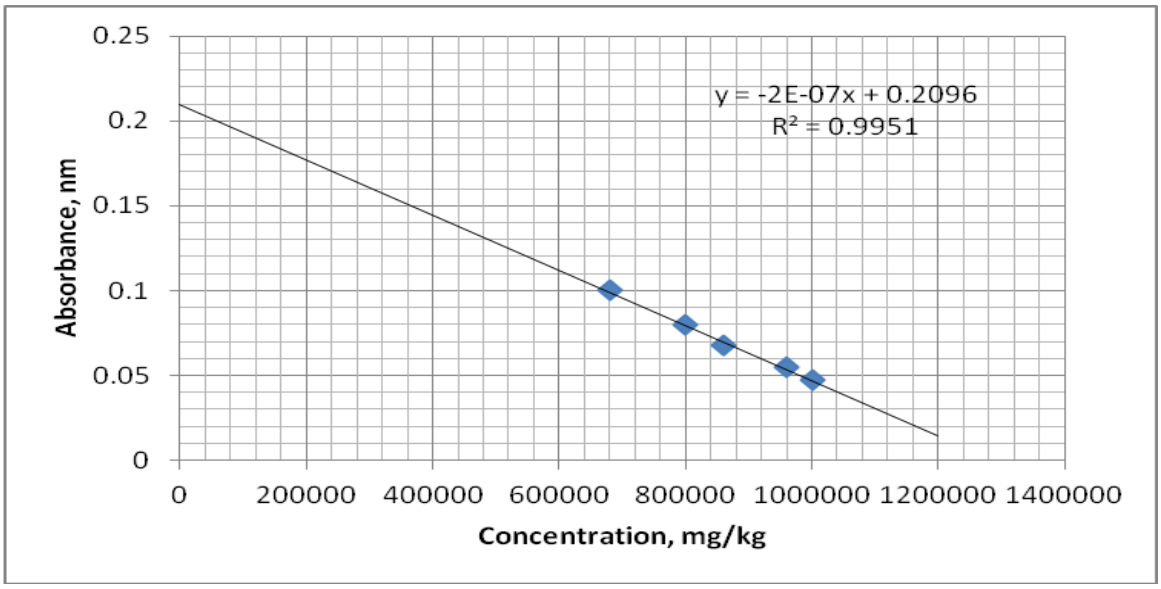
Calcium standard curve



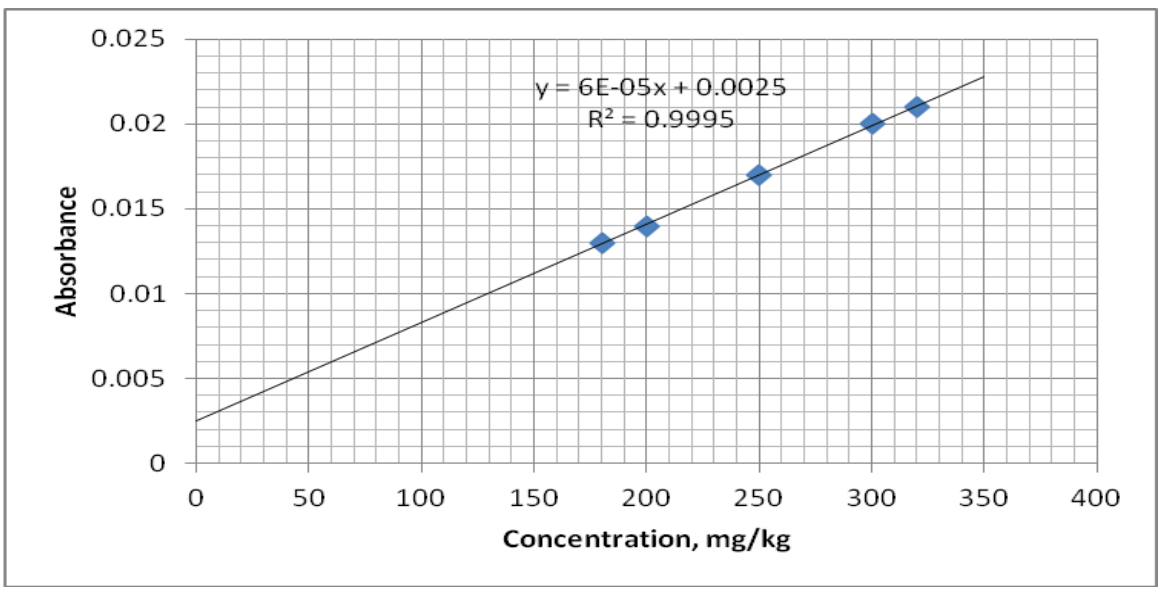
Iron standard curve



Phytate standard curve



Tannin standard curve



APPENDIX II: Published papers in peer-reviewed journals

Journal of Food Research; Vol. 7, No. 1; 2018
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Chemical Composition of Major Cassava Varieties in Uganda, Targeted for Industrialisation

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Rheological, Baking and Sensory Characteristics of Bread from Wheat-Cassava Composite Dough

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APPENDIX III: Map of Uganda, showing Nebbi district