

**EFFECT OF VARIETY AND MATURITY STAGE OF CASSAVA (*Manihot esculenta*)
ON FLOUR PROPERTIES AND SENSORY CHARACTERISTICS OF WHEAT-
CASSAVA COMPOSITE BREAD**

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**A Thesis Submitted to Graduate School in Partial Fulfillment for the Requirements of a
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DECLARATION

This thesis is my original work and has not been presented for the award of a degree in any other institution.

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RECOMMENDATIONS

This thesis is the candidate's original work and has been prepared with our guidance and assistance. Therefore, it has been submitted with our approval as the official university supervisors.

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DEDICATION

I dedicate this work to my family; my husband Joseph Karisa, my children Fatuma Joseph and Emmanuel Karisa, my mother Consolater Wambua, sisters; Joyce, Agnes and Jane, brothers; Samuel and Antony and friends for their love, support and encouragement.

ABSTRACT

Cassava (*Manihot esculenta*) grows well in Tropical and Sub-Tropical regions of Sub-Saharan Africa. It is used as a food and raw material for many industrial applications, including food, feed and starch. Cassava has several varieties whose physico-chemical properties and functional properties of flour are hardly known. The aimed at characterizing physico-chemical and functional properties of cassava flour from different varieties harvested at different maturity stages and to determine their suitability for baking. Cassava tubers from 5 improved varieties; MH95/0183, MH95/0193, MH96/093, MH95/6484 and *Migyera* and 2 indigenous varieties; *Selele* and *Merry go round* were obtained from farmers in Migori County. Dry matter content and cyanide content of the fresh tubers and functional properties of cassava flour were analyzed. Wheat- cassava composite flour blends were prepared from three cassava varieties; MH95/0183, MH95/0193 and *Selele* that had wheat: cassava ratios as 95:5, 90:10, 85:15, 80:20, 75:25 and 70:30 with baker's wheat flour as the control. These flour blends were analyzed for proximate composition, rheological properties and the physical properties of the bread. Sensory evaluation was done for every composite blend after baking using 25 semi-trained panelists and shelf life of the bread was determined. The roots that were harvested at 12 month had the highest dry matter, swelling power and low water binding capacity thus more suitable time for harvesting. *Selele*, MH95/0183 and MH95/0193 had the best dry matter content at 46.69%, 47.21% and 47.05% respectively at 12 months and functional properties for baking. The protein and gluten content of the blended breads reduced with increase in cassava substitution for all the cassava varieties with the 100% wheat flour having the highest content of 13.2 % and 63.20 % respectively. Composite flours with MH95/0183 variety were found to have better rheological properties while composite bread with *Selele* variety had the highest specific volume, form ratio of 3.07 cm³/g and 1.34 respectively and sensory properties. The sensory acceptability of composite bread made from 5%, 10% and 15% cassava flour didn't have significant (P<0.05) difference from that of the control Bread (100% wheat flour). The external loaf characteristics were the major factors the panelist used to rate the acceptability of the bread. Results of this study show that cassava flour from different varieties have different physico-chemical and functional properties and that it can be used in the substitution of bread wheat flour at 15%.

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ABBREVIATIONS

| | |
|-------|--|
| AACC | American Association of Cereal Chemistry |
| ANOVA | Analysis of Variance |
| AOAC | Association of Official Analytical Chemists |
| BU | Brabender Units |
| HQFC | High Quality Cassava Flour |
| KALRO | Kenya Agricultural and Livestock Research Organization |
| KEBS | Kenya Bureau of Standards |
| LSD | Least Significant Difference |
| MC | Moisture Content |
| MCA | MacConkey Agar |
| PCA | Principle Component Analysis |
| PDA | Potato Dextrose Agar |
| SAS | Statistical Analysis System |
| TVC | Total Viable Counts |
| WAI | Water Absorption Index |
| WBC | Water Binding Capacity |
| WHO | World Health Organization |

CHAPTER ONE

INTRODUCTION

1.1 Background information

1.1.1 Cassava production and utilization in Kenya

Cassava (*Manihot esculenta*) is a tuber crop native to tropical America (Allen, 2002; Olsen and Schaal, 2001) and grows well in tropical and subtropical regions especially Sub Saharan Africa. It is the basic staple food of many people in these regions and a raw material for numerous industrial applications, including food, feed and starch (Balagopalan, 2002). Kenya produces about 800,000 metric tonnes of cassava from an area of about 65,000 hectares and is utilized mainly as a subsistence food crop and only the surplus is sold in unprocessed form such as boiled or roasted tubers. Cassava is grown in many regions of Kenya with Western, Coastal and Eastern regions producing 60%, 30% and 10%, respectively (Karuri *et al.*, 2001). Traditional utilization of cassava in Kenya is limited to roasting and boiling of fresh roots for consumption. However, in Nyanza and Western regions, tubers are processed into flour to make *ugali*, a thick porridge like product when combined with maize or sorghum flour or both.

Cassava production in Kenya remains low compared to other Africa countries like Nigeria, Angola, Ghana, and Democratic Republic of Congo (FAO, 2013) among others who are producing cassava as a staple food and also as a commercial crop. Use of high quality cassava flour (HQCF) for partial substitution of wheat in baking of composite bread has been done in other countries like Nigeria whose interest was to reduce importation bills on wheat by stimulating local production and processing of non-wheat flours like cassava to be incorporated into wheat. Inclusion of cassava in bread, cakes and other bakery products reduces the cost of these products since cassava is a cheaper raw material and is available locally as compared to wheat which is mostly imported.

Cassava consists mainly of starch which is important in industries like bakery, confectionary and as an additive in most foods and beverages. It can also be used in textiles, paper and plywood industries and in the mining and construction industry. The use of cassava flour in some of these applications depends on the physicochemical and functional properties of its starch (Nuwamanya *et al.*, 2010) which are determined by its structure in terms of amylose-amylopectin ratios. These differences in amylose and amylopectin ratios are influenced by factors like cassava variety, period of harvesting and environmental factors like soil and climate. However, there has been no detailed study on the effect of these factors on the properties of cassava flour. By investigating

the effects of variety and maturity stage on the physicochemical and functional properties of starch will help differentiate and assign specialized uses of the various varieties of cassava in Migori County.

1.1.2 Bread utilization and production in Kenya

Bread is a fermented baked product that is made mainly from wheat and is widely consumed as part of breakfast and other meals. It is an important staple food in both developing and developed countries and is rich in carbohydrate, protein, fibre, vitamins and minerals. The most common types of bread in Kenya is white bread made from wheat flour which has been processed and the bran removed and whole grain bread commonly known as brown bread made from whole grain wheat without the removal of bran.

Due to the increasing population, industrialization and changing food habits in the country demand for wheat-based convenient food products has increased. Kenya only produces about 40% of its national requirement for wheat (Economic Review of Agriculture, 2010) this is because wheat is mostly adapted to temperate climate while 80% of Kenyan land lies in the Arid and semi-arid regions. The remaining 60% relies on expensive imports of wheat which is paid in foreign currency costing the government about 5.85 billion shillings on imports (Annon, 1997). Due to the increased cost of wheat, Kenya is looking for alternatives sources of baking flour. Such sources include maize, cassava, sweet potatoes and sorghum flour. Out of these, cassava flour has more potential to be used as an alternative to wheat flour in terms of agronomic aspects. Unlike wheat and other flour yielding crops, cassava requires low inputs like water, fertilizer and labour. Other advantages include flexibility in planting and harvesting time, tolerance to drought conditions giving reasonable yields where other crops do not grow well (Bradbury and Holloway, 1988).

There are new cassava varieties developed through research that are drought resistant, disease resistant, high yielding and low cyanide content making farmers to be interested in growing cassava for industrial purposes (Etiang *et al.*, 2012). However, these varieties have not been screened for flour yields and functional properties that will determine their application in the various industries. The functional properties of cassava starch vary depending on the variety, location, age and environmental factors such as weather, soil conditions, fertilization, irrigation, plant protection chemicals applied and time of planting and harvesting (Aberi *et al.*, 2012).

This study aimed at characterizing the physico-chemical and functional properties of cassava flour based on variety and maturity stage and development of cassava/wheat composite bread to determine its acceptability through sensory evaluation.

1.2 Statement of the Problem

Different cassava varieties have been developed which are high yielding, resistance to diseases and drought resistance in Kenya. However, they have not been screened for flour yields and functional properties that will determine their application in the various industries. Characterizing flour properties of these varieties will help pick the variety with best flour properties for bread making industry.

1.3 Objectives

1.3.1 General objective

To enhance cassava utilization in Kenya by characterizing flour properties of different cassava varieties for cassava flour and determine its suitability in bread baking industry.

1.3.2 Specific objectives

1. To assess cassava varieties grown in Migori County
1. To determine dry matter content and cyanide content of selected cassava varieties in Migori County.
2. To determine the functional properties of cassava flour obtained from the different cassava varieties.
3. To develop composite bread from cassava/wheat mixture, evaluate its acceptance through sensory evaluation and determine the shelf life.

1.4 Hypotheses

1. There are no cassava varieties grown in Migori County
2. There is no significant difference in dry matter content and cyanide content of cassava flour obtained from selected cassava varieties in Migori County.
3. There is no significant difference in the functional properties of cassava flour obtained from the different cassava varieties.
4. There is no significant difference in the acceptance and shelf life of composite bread made from cassava/wheat mixture compared to bread made from wheat.

1.5 Justification

Cassava, a crop that is neglected by many is becoming an important food crop in Africa. Its ability to produce high yields under poor soil conditions, low rainfall and its flexibility in harvesting makes it an important food security crop (Nweke, 2003). It is also a cheaper raw material for many industries e.g. bakery, confectionary, brewing and animal feed industry. In Kenya, despite the great potential of cassava, its utilization as cash crop remains very low. Manufacturing sector is still hesitant to use cassava as an ingredient for the manufacture of many food products such as bread, biscuit, cakes and a source of carbohydrates for the animal feeding industry. This might be attributed to the fact that there is no much information on the properties of cassava flour from different varieties which influence the functional properties in food system like pasting properties, swelling power and water binding capacity. Maturity stage also plays a major role on the chemical composition of starch which is the main constituent of cassava flour which in turn affects its functionality. It was therefore important to investigate the effect of variety and maturity stage on the physical and chemical properties that in turn affect the functional properties of cassava flour in food. Knowledge on the properties of cassava may therefore increase utilization of cassava in the industry thus increasing its production in Kenya. This will contribute substantially to poverty alleviation by making cassava a cash crop and improve livelihoods of the farmers.

1.6 Limitation of the study

It was not possible to analyze all the cassava varieties that are found in Migori County for their physico-chemical and functional properties due to cost and time constraints.

CHAPTER TWO

LITERATURE REVIEW

2.1 Origin and distribution of cassava

Cassava (*Manihot esculenta Crantz*) is a root crop and a very important staple food for over 500 million people in the developing countries (Falade and Akingbala, 2010). It was domesticated about 5000-7000 years BC in the Amazon, Brazil (Allen, 2002) and was introduced to Africa by Portuguese navigators in the 16th century (Nweke, 1994). It was brought to East Africa in the 18th century by Portuguese from Cape Verde. Cassava forms a staple food for over 500 million people around the world (FAO and IFAD, 2001). It is now grown widely in most countries in the tropical regions of Africa, Latin America and Asia.

2.2 Description of the plant

Cassava is a perennial woody shrub with edible roots (Heuze *et al.*, 2012) that grows best under high solar radiation, fertile and well drained soils and annual rainfall ranging from 500 mm to 3500mm. Completely mature cassava plant reaches a height of about 2-4 m and this can take a period of about 8-10 months after planting. Cassava can be harvested when required and its wide harvesting period enables it to act as a famine reserve (Stone and Sindel, 2004).

2.3 Nutritional profile of cassava

A mature cassava plant consists of the leaves, stem and the roots. The roots and the leaves of cassava plant are the most nutritional and edible parts. There is considerable variation in the chemical composition of cassava roots and leaves depending on the variety as observed by (Apea- Bah *et al.*, 2011), age of plant and the processing technology. Cassava roots are mainly high in carbohydrates especially starch. Cassava has nearly twice the amount of calories than potatoes and highest for any tropical starch rich tuber and root crops. Cassava is free from gluten, thus can be used in special food preparations for celiac disease patients. Cassava roots are very low in fats and proteins than cereals and pulses, but it has more protein than that of other tropical starchy food sources like yams and potatoes. Cassava proteins range between 1-3% proteins on dry matter basis (Montagnac *et al.*, 2009) and contain a low percentage of essential amino acids, such as lysine, methionine and tryptophan (Falade and Akingbala, 2010). Leucine, phenylalanine and threonine are also low in cassava compared to other tuber crops (FAO, 1990).

Young tender cassava leaves are a source of high and good quality dietary protein. Essential and non-essential amino acids can be found in substantial amount in cassava leaves. They are also

rich in calcium, vitamin A and vitamin K. The annual yield of cassava leaves has been reported to about 90 tonnes fresh leaves/ ha when harvested three times a year (IFAD and FAO, 2004).

2.4 Global production of cassava

Globally cassava production increased from 223 Metric tonnes in 2006 up to 230 Metric tonnes in 2010 (FAO, 2013). Africa contributed 52.8% of the global supply; Nigeria was the highest producer contributing 30.8 % of Africa production. In Africa cassava production is mainly for food while in Asia cassava is grown for industrial and energy purposes. In Latin America and the Caribbean, cassava production reduced from 36 M tonnes to 32 M tonnes between 2006 and 2010.

2.5 Cassava production in Kenya

Cassava is a staple food in most parts of Kenya, where the western, coastal and eastern regions produce 60%, 30% and 10%, respectively as reported by (Karuri *et al.*, 2001). Cassava is second to maize in importance in Western and Coastal region of Kenya (Njeru and Munga, 2003). Traditional utilization of cassava in Kenya is limited to roasting and boiling of fresh roots for consumption. However, in Nyanza and western provinces of Kenya, roots are also processed into flour to make a food product called Ugali (a thick porridge) when combined with maize or sorghum flour or both. In the Coast Province of Kenya, cassava is the second main staple food. However, cassava production in Kenya remains low compared to other countries. This can be attributed to the fact that cassava in Kenya is grown mainly as a food security crop rather than a commercial crop like in other countries.

2.6 Importance and uses of cassava

Cassava is the staple food of many people in the tropical and subtropical areas and is a source of raw material to numerous industries. In Africa, cassava is mostly grown as a subsistence crop by small-scale farmers who sell the surplus for cash crop to meet their needs. Cassava can be utilized in many different ways; the leaves can be eaten as a vegetable and the starchy root is eaten raw, cooked (through boiling) or processed into flour and other derivatives

2.7 Common cassava utilization in Kenya

2.7.1 Traditional fermented foods and beverages

The fermented cassava is grated and dried to produce flour which is mixed with other flour for Ugali or porridge. Although in Kenya fermentation is done particularly to reduce the cyanide content, the effect on the quality of flour and what would be the maximum substitution rate of this flour compared with unfermented flour and quality of resulting products has not been studied. There are different products that can be made from the fermented cassava and these include; *Ugali*, local brew (*chang'aa*), porridge and *chapatti*. Other products include boiled cassava roots, fried cassava chips or crisps, cassava flour and the leaves are consumed as vegetables.

2.7.2 Composite flour

A composite of wheat and cassava flour can be used to produce high quality bread, cakes, scones. Since cassava is cheaper and locally grown, it will be able to reduce the cost of the baked products since wheat is expensive. However, currently there is no bread made from wheat/cassava flour in the market, therefore more research needs to be done on the utilization of composite wheat/cassava flour in the bakery industry.

2.7.3 Alcoholic beverages

Cassava roots have been used to make alcoholic beverages in various countries, like Brazil, South America and Ghana. In Kenya it is made as a local brew by the natives but it is not commercialized.

2.7.4 Cassava starch

Cassava flour consists mainly of starch. Starch is composed of two polysaccharides, amylose, the minor component, has a linear structure of α -D- glucopyranose units joined by α (1 -4) D - glucosidic linkages, while amylopectin, with a higher molecular weight, has a branched structure due to the presence of α (1 -6) linkages. The structural differences of the two polymers give them different properties in food systems. Amylopectin is more stable due to its branched nature and amylose molecules have a tendency to precipitate spontaneously due to the formation of hydrogen bonds between aligned molecules thus affecting the retrogradation of starch.

Structure of amylose and amylopectin

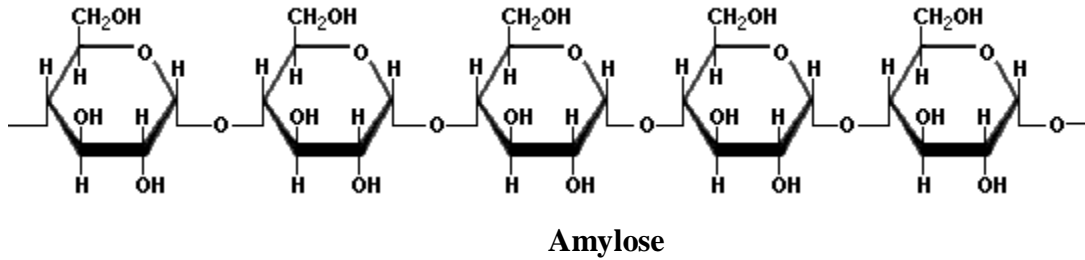


Figure 1: Chemical structure of amylose (Matheson, 1996)

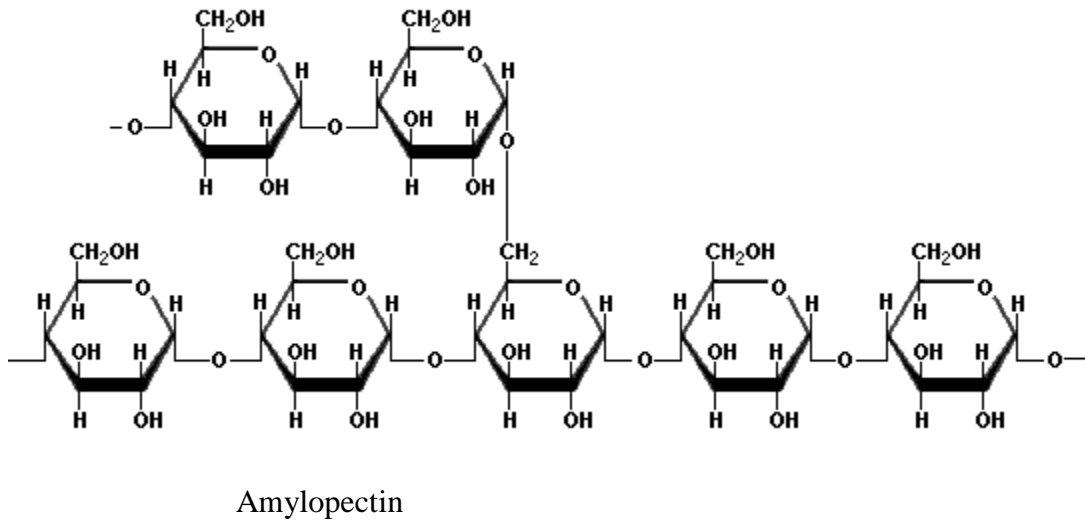


Figure 2: Chemical structure of amylopectin (Matheson, 1996)

The process of starch extraction from cassava is relatively simple as there are only small amounts of secondary substances, such as proteins, in the roots. When cassava roots are harvested or selected for starch extraction, age and root quality are critical factors. Cassava has many advantages for starch production, high level of purity, excellent thickening characteristics, a neutral taste, desirable textural characteristics, a relatively cheap source of raw material containing a high concentration of starch (dry matter basis) that can equal or surpass the properties offered by other starches such as maize (*Zea mays*), wheat (*Triticum spp*), sweet potatoes (*Ipomoea batatas*), and rice (*Oryza spp*). Cassava starch can be used in the food industry and in the non-food industry. However despite the fact that cassava can be used to produce starch, its production in Kenya remains low.

Food industry

It is used as a raw material in the food and beverage industry for the manufacture of pastries, biscuit, noodles, baby foods, alcoholic drinks and as binding and thickening agents in soups and stews.

Non-food uses

Cassava starch is mostly preferred in the adhesive production because it is more viscous, work more smoothly and provides stable glues of neutral pH and has a clear paste. Starch can be used in paper and cardboard, plywood and textile industry as an adhesive; it can also be used in the pharmaceutical industry for manufacture of drugs.

2.8 Problems associated with cassava

2.8.1 Hydrogen cyanide poisoning in cassava

Cassava consists of two cyanogenic glucosides compounds namely linamarin and lotaustralin, which upon hydrolysis release hydrogen cyanide (HCN) which is very toxic. The level of these compounds varies significantly between varieties, climatic and cultural conditions. The amount of cyanogenic glucosides in the tubers forms a basis for the classification of cassava varieties as either “bitter” or “sweet”. Bitter cassava contains over 50 mg HCN/ Kg fresh weight while sweet cassava contains less than 50 mg HCN/ Kg fresh weight basis. However the bitterness or sweetness could not be exactly correlated with the level of cyanogenic glucosides according to (IFAD and FAO, 2004). The safety limit for cyanide in cassava food is 10 mg/kg body weight. These poisoning has limited the utilization of cassava for fear of death and other diseases. Fear of cassava product safety due to presence of cyanide has led to unacceptability of products like starch, chips, flour and animal feed, a problem that can be easily solved through appropriate processing technologies.

2.8.2 Post harvest deterioration of cassava

The other constraint associated with cassava utilization after cyanide poisoning is the fact that the fresh roots deteriorates very fast. The fresh cassava roots have a shelf life of about 24-48 hours (Hillocks, 2002; Westby, 2002). The deterioration of cassava can take two ways: primary physiological deterioration that involves internal discoloration and secondary microbial spoilage. The primary deterioration is a complex process which is still not fully understood involving wound response due to enzymatic activity. Traditional method of preservation after harvest like

burial in soil or piling in shade is used for small quantities of cassava roots for about 3-7 days. The secondary microbial spoilage is caused by the high moisture content of cassava roots that favor the growth of spoilage microorganisms.

2.8.3 Nutritional composition

Cassava is considered an inferior food as the roots are low in protein, essential minerals and vitamins, so most people consider it as poor man's diet and will not want to be associated with it. Cassava also contains tannic acid in the root, which imparts dull colour to the processed products, which affects their market value and also acts as a growth-depressing factor by decreasing protein digestibility.

2.9 Processing of cassava tubers

There are various processing methods used to make different products depending on locally available processing resources, customs and preferences (Hillock, 2002). Cassava processing improves palatability of the product, increases shelf life, lowers the cost and ease of transportation and detoxifies hydrogen cyanide produced (Nweke, 1994). The most commonly used methods of cassava root processing are boiling of the fresh roots, sun drying and fermentation. Boiling of cassava is done in almost all countries where cassava is used as food. Only the sweet varieties with low cyanogenic glucosides levels are recommended for boiling to avoid incidences of cyanide poisoning. The efficiency of removal of cyanogens during boiling is influenced by the ratio of roots to water. Sun drying is more efficient in removal of cyanogens compared to boiling. Sun dried products are the most common types of cassava processed products in Africa (Westby, 2002). The process involves peeling the roots, grating and spreading on an open surface for sun drying. The efficiency of removal of cyanogens depends mainly on the rate of moisture loss. Drying can also be done using electricity or fuel depending on economic viability (Tewe, 1992). Fermentation is an important means of processing cassava to improve palatability, textural quality and to upgrade nutritive value and food safety. Fermentation process reduces the cyanide level from 10–49 mg HCN/kg raw cassava to 5.4 – 29 mg HCN/kg in the fermented products, which is well below the safe level of 10 mg HCN/kg body weight. Fermentation also extends the shelf-life (3 – 30 days) of the fermented food products in comparison to that of fresh roots (48–72 h). There are three types of fermentation of cassava roots; the grated root fermentation, fermentation of roots under water and mold fermentation of roots in heaps (Westby, 2002). Grating is important as it allows linamarin to

come into contact with linamarase enzyme thus being hydrolyzed to glucose and cyanohydrins and the converted to HCN which is highly volatile (Westby and Choo, 1994).

2.10 Baking of wheat–cassava bread

To evaluate the bread making quality of wheat/cassava composite flour, baking tests is the most reliable method.

2.10.1 Baking procedure

The essential ingredients in bread making are flour, yeast, salt and water. Flour for bread making is usually produced from hard wheat that has high protein content since the amount and the quality of protein in flour are important for its bread-making capacity and loaf volume. Gluten in wheat plays a major role for the rheological properties of the dough, forming a strong, elastic and cohesive network that retains gas during fermentation and eventually produces light and leavened bread. Starch undergoes swelling and gelatinization at baking temperatures, thus contributing to bread expansion (Nindjin *et al.*, 2011). Cassava flour does not contain gluten therefore there is a maximum substitution for wheat to ensure that there are no adverse effects on the properties of the final product.

2.10.2 Quality and sensory evaluation of bread

Loaf volume is widely used as a measure of bread- making capacity. The specific volume of a loaf of bread is the ratio between its volume and weight and has been adopted as a reliable measure of loaf size (Shittu *et al.*, 2007). Sensory evaluation is a useful tool in the food industry to assess acceptability of food products. It is therefore defined as “a scientific discipline used to evoke, measure, analyze and interpret reactions to those characteristics of foods and materials as they are perceived by the senses of sight, smell, taste, touch and hearing” (Stone and Sidel, 2004). For untrained panelists, they should be regular users of the product in order to be familiar with its sensory attributes. The 9 -point scale, also known as a degree-of -liking scale, is the most common hedonic scale as it is very simple to use and easy to implement. It is based on equal interval spacing which gives the responses numerical values that can be used for statistical analysis. In a hedonic test, panelists are not asked to give specific information about product sensory attributes since untrained subjects often exhibit more individual differences in their interpretation than trained panelists. The aim here is to predict consumer response and readiness to buy the product (Lawless and Heymann, 2010).

2.10.3 The microbiological shelf life of bread

The high moisture content of baked bread encourages quick spoilage by microorganisms like molds and bacteria. Molds are killed during the baking process but the spores present in the air of the bakery cause the spoilage after baking and hygiene of the food handlers can also cause contamination. In order to extend the shelf life of bread use of preservatives which inhibit growth of microorganism and atmospheric packaging can be of great help (Saranraj, 2012).

Factors that affect the growth of microorganism in bread

Factors that affect the growth of microorganism in bread include; temperature at which the product is stored, pH of the product, moisture content of the product, a_w value of the product, type of micro-organism that is present, initial contamination of the product, the presence of products which slow down the development of molds (typical example are raisins which contain a natural preservative) and whether or not the bread is packed (packed bread will get moldy quicker because the air in the packed gets moist)(Saranraj, 2012)

Types of Bacteria

Psychrophile bacteria grow at a temperature range of 5°C to 30°C . Some of them are even active below 0°C and can grow on food stored in the fridge. Mesophile bacteria grow at an optimum temperature between 15°C and 50°C . Most bacteria belong to this group including pathogenic bacteria. Thermophile bacteria grow best at temperatures ranging between 50°C and 60°C . Some of these bacteria can survive temperatures up to 90°C . Acidogenic bacteria are bacteria that produce acids therefore reducing the pH of the product. Lactic acid bacteria belong to this family. Acidophilic bacteria can grow at a higher pH but they prefer a neutral pH although they can tolerate a pH-range from 5 to 8 (Saranraj, 2012).

Yeast and mould spoilage of baked products

Fungi are divided into yeasts and moulds. Yeasts that spoil baked products are divided into two groups; visible yeast which grows on the surface of the bread as white or pinkish patches and fermentative spoilage that cause alcoholic and essence odours and osmophilic yeasts. Contamination of products by osmophilic yeasts usually results from dirty utensils and equipment. Therefore, maintaining good manufacturing practices will minimize the contamination by osmophilic yeasts. Moulds spoilage is serious and a costly problem for most bakeries (Hickey, 1998), however, the use of preservatives can be an attractive means to reduce

the spoilage and ensure safety. Most molds prefer high water activity values of >0.8 while a few xerophilic moulds prefer to grow at water activity values as low as 0.65. Moulds are generally killed by the baking process in fresh baked products (Knight and Menlove, 2006). Therefore contamination of baked products by moulds occur after baking either by the surrounding air, bakery surfaces, equipment or food handlers during cooling, slicing and packaging. The most common types of moulds found in bakery products are: *Rhizopus species*, *Aspergillus species*, *Penicillium species*, *Monilia species*, *Mucor species* and *Eurotium sp* (Saranraj, 2012).

CHAPTER THREE MATERIALS AND METHODS

3.1 Site

The study was carried out in Migori County (Suna east and Rongo sub-county) located in South - Western part of Kenya. The county is located between the Latitude of $0^{\circ}24'$ South and $0^{\circ}40'$ South and Longitude of 34° East and $34^{\circ} 500$ East. The county covers an area of $2,596.5 \text{ km}^2$ including approximately 478 km^2 of water surface. Migori County experiences two seasons of rain with an average rainfall of 1200 mm annually and a temperature range of 21°C to 35°C .

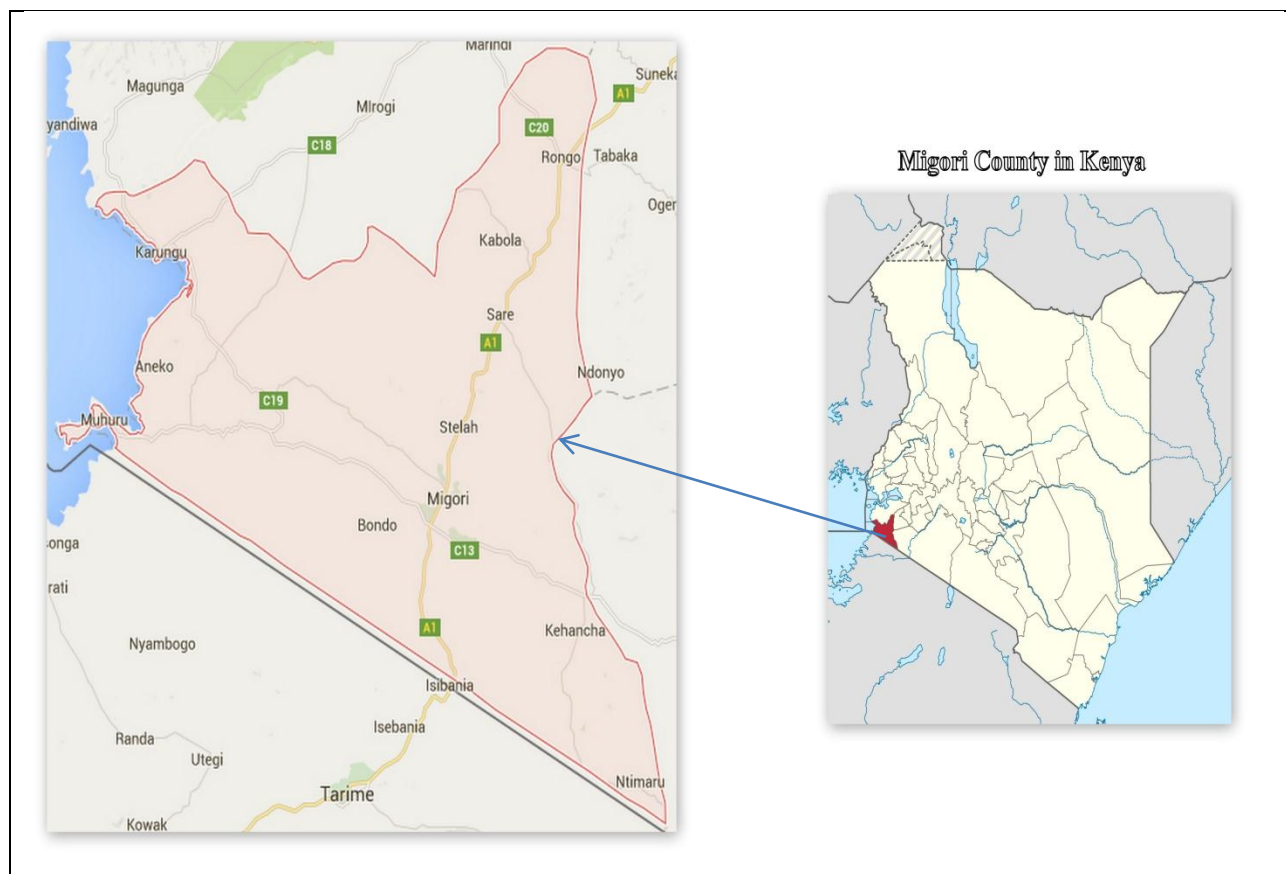


Figure 3: Map showing Migori County in Kenya (Kenya Google maps, 2016)

3.2 Data collection

Quantitative and qualitative methods of data collection were used in this study.

3.2.1 Questionnaire

A questionnaire was conducted using structured questionnaires interview to the randomly selected smallholder farmers (n= 90) who are involved in the growing of the cassava on their farms. Information on the common cassava varieties i.e. both indigenous variety and improved variety cultivated in the region, age at harvesting and post-harvest handling of cassava was collected. Improved cassava varieties referred to varieties that are modified to become high yielding, early maturing and resistance to diseases especially cassava mosaic virus, while indigenous varieties referred to those that are prone to diseases, low yielding and takes longer time to mature. Criteria used to select the varieties for analysis was based on the varieties that were mostly grown by most farmers and the ones which the farmers preferred based on resistance to diseases, root yield and cooking properties of the flour.

3.2.2 Sampling and preparation of cassava flour

From the data collected from questionnaires, seven cassava varieties were found to be the most grown varieties in Migori County. These included five improved varieties (MH95/0183, MH95/0193, MH96/093, MH95/6484 and *Migyera*) and two indigenous varieties (*Selele* and Merry go round). The seven cassava varieties were obtained from farmers selected at random and they were harvested at different maturity stages. For physicochemical properties analysis, the fresh cassava tubers were wrapped in polythene paper bags, labeled after harvesting and packaged in cool boxes maintained at low temperatures of 5°C to prevent spoilage and transported to University of Nairobi for analysis.

Cassava flour was processed using a method described by (Dziedzoave *et al.*, 2003). Cassava tubers were peeled by hand then washed with portable water. The washed tubers were grated using a motorized cassava grater to disintegrate cassava tissues. The mash was pressed to remove excess water then sundried to a moisture content of about 13% dry weight basis and milled into flour using a hammer mill. The milled flour was sifted using a sieve fitted with a 250 µm screen to remove as much fiber as possible in order to obtain fine flour with uniform particles size.

3.3 Experimental design

The second and third objectives were done in a completely randomized design in a Nested arrangement where there were two main clusters: cultivar of the cassava and the age at which the cassava tubers are harvested. Cassava tuber samples obtained for analysis were of different varieties (improved cultivars n=5 and local cultivars n=2) and of different ages at harvest. Therefore the main cluster was the cassava variety with seven levels and the other nested cluster within the variety was the age at harvesting and it had varying levels from variety to variety.

Statistical model; $Y_{ijk} = \mu + A_i + B_j(A_i) + R + \varepsilon_{ijk}$

Where Y_{ijk} was the different dependent variable responses due to i^{th} variety and j^{th} maturity period, μ was the overall mean, A_i was the effects due to variety, $B_j(A_i)$ was the effect due to maturity as it was nested in the variety, R was the replication and ε_{ijk} was the random error component. The following dependent variables were determined; Dry matter content, cyanide content, water binding capacity, swelling power and pasting properties.

The fourth objective was determined using a Completely Randomized Design in 3×6 factorial arrangement. Three cassava varieties namely MH95/0183, MH95/0193 and *Selele* were used for this specific objective since they had the best physico-chemical and functional properties for bread making.

Statistical model; $Y_{ijk} = \mu + V_i + S_j + VS_{ij} + R + \varepsilon_{ijk}$

Where Y_{ijk} was different dependent variable responses due to i^{th} variety and j^{th} substitution level, μ was the overall mean, V_i was the effect of variety, M_j was the effect of substitution level of baker's flour with cassava flour, VS_{ij} was the effect of interaction between variety and level of substitution of baker's flour with cassava flour and ε_{ijk} was the random error. Nineteen different samples of composite flour were prepared using baker's flour and cassava flour from the three varieties using different ratios of baker's: cassava flour as follows; Baker's flour: cassava flour, 100%: 0, 95%: 5%, 90: 10%, 85%: 15%, 80%: 20%, 75%: 25%, 70%: 30% with 100% baker's flour serving as the control. The composite flours were then packaged in polythene paper bags to await analysis. All the experiments were carried out in three replications.

3.4 Analysis of the cassava

3.4.1 Determination of dry matter content of fresh cassava tubers

Dry matter content was determined using AOAC Methods (2006). The fresh tubers were peeled and grated using a grater. Two grams of the grated fresh cassava mash was weighed in moisture dishes then placed in an oven (Electrolux, Sweden) at (105°C for 4 hours). The % Moisture Content (MC) was calculated as follows:

$$MC\% = \frac{W_2 - W_3}{W_1} \times 100$$

Where: W_1 was the weight of the sample,

W_2 was the weight of sample plus dish;

W_3 was the weight of the dry sample plus dish.

Percentage dry matter content was calculated as; 100% minus % MC.

3.4.2 Determination of hydrogen cyanide content of the fresh cassava tubers and cassava flour

Cyanide content of the fresh cassava tubers and flour was done using Alkaline Titration method AOAC methods (1980). Ten grams of the grated fresh cassava mash and flour were weighed using a weighing balance into distillation tubes and 100 ml of distilled water was added to the tubes and left to settle for 2 hours then 25 ml of 2.5% sodium hydroxide (NaOH) was weighed into a conical flask which was then connected to the distillation unit to collect the distillate. The distillation tubes with the sample were also connected to the distillation unit and distilled until 200ml of the distillate was obtained. Eight milliliters of 5% potassium iodide (KI) was added to each distillate and titrated using 0.02 M silver nitrate ($AgNO_3$). The end point was marked by a light blue colour. The amount of cyanide was then calculated as follows;

$$\text{Mg/100g HCN} = \frac{\text{Titre} \times 108}{\text{sample weight}}$$

Where 1 ml of $AgNO_3$ = 1.08 mg HCN

3.5 Functional properties of cassava flour

3.5.1 Determination of water binding capacity (WBC) of cassava flour

The WBC of the cassava flour from the different samples was determined as the Water Absorption Index (WAI) according to the method described by Ruales *et al.*, (1993). Exactly 2.5 g of the cassava flour was suspended in 30 ml of distilled water at 30 °C in a centrifuge tube

(Funke Gerber 3680-1983, Germany). The mixture was stirred for 30 minutes intermittently and then centrifuged at 3000 rpm for 10 minutes. The supernatant was decanted and the weight of the gel was recorded. The water binding index (WBI) was calculated as gel weight per gram dry sample.

$$\text{WAI} = \frac{\text{Bound water(g)}}{\text{Weight of dry sample(g)}}$$

3.5.2 Determination of swelling power

The swelling power of cassava flour from the different samples was determined using the method described by Takashi and Sieb (1988). One gram of the sample was weighed into 50 ml centrifuge tube, 50 ml distilled water was added and mixed gently. The slurry was heated in a water bath (Water bath-DSB-1000, Taiwan R.O.C) at 85°C for 15 minutes while stirring to prevent clumping of the starch. The tubes were centrifuged (Funke Gerber-3680-1983, Germany) at 3000 rpm for 10 minutes then decanted immediately. The weight of the sample was taken and recorded and moisture content of the gel then determined. The swelling power was calculated as follows;

$$\text{Swelling Power} = \frac{\text{Weight of wet mass of sediment}}{\text{Weight of dry matter in the gel}}$$

3.5.3 Pasting properties of cassava flour

Pasting properties were determined according to AACC methods 2000 using Brabender Amylograph type 800101(Duisburg, Germany). Fifty grams of sample was weighed into beakers of 1000 ml and then mixed thoroughly with 450ml of distilled water using a blender. Then the mixture was transferred to heating vessel in the Brabender and heated at a rate of 1.5° C/ minute up to 95 °C. The gelatinization temperature and peak viscosity were determined.

3.6 Bread making of composite wheat/cassava bread

3.6.1 Preparation of composite wheat/cassava flour

Baker's flour (wheat flour) for baking was obtained from a commercial Miller in Nakuru town and was used as the control. The wheat: cassava flour ratios of 100:0, 95:5, 90:10, 85:15, 80:20, 75:25, and 70:30 respectively were made. The composite flour was then packaged in polythene sealable paper bag.

3.6.2 Wheat-cassava flour proximate composition and rheological analysis

The composition of the composite flours (protein, gluten, moisture content and water absorption) was measured using Near Infra-Red (NIR) grain analyzer model 1241. The different blends of baker's flour and cassava flour were subjected to rheological analysis using Alveograph MA (Chopin, Tripette et Renaud, France). The length (dough extensibility), height (dough strength) of the curve and W (deformation energy) were determined. The height is the force required to blow the bubble of the dough. Deformation energy is the area under the curve and is a combination of dough strength and extensibility.

3.6.3 Bread production by pup-loaf method

The different composite flours formulated above were used to make the different bread samples of 100 grams weight using the Pup loaf method (AACC, 1993). The other ingredients used for baking were added to the composite flour at different percentage of the weight of the bread as follows; sugar 4%, salt 1%, shortening 3%, milk powder 3%, malted barley 0.2%. The amount of water used to prepare the dough was determined from farinograph water absorption. The ingredients were then mixed for 3 minutes using a dough mixer. The dough was rounded and placed into a fermentation cabinet at 30°C and 85% relative humidity for 105 minutes. First punch was done by passing the dough through a sheeter then folding twice and returning to the fermentation cabinet for 50 minutes. The second punch was treated the same way as first and the dough returned into the fermentation cabinet for 25 minutes. The dough was molded by passing it through a molding machine and folded it into a cylinder and proofed in a greased pan for 62 minutes. The dough was baked in an oven (Lincoln manufacturing Copp Lincoln NE) at 240°C for 24 minutes, weighed the loaves of bread then cooled to room temperature.

3.6.4 Physical properties of wheat-cassava composite bread

The volume of the bread was measured using rapeseed displacement method (AACC, 1993). The specific volume was calculated using the weight and the volume of the bread (volume /weight). The loaves were sliced into 10 mm thickness and the width and height of the central slice measured to determine the form ratio. The baked loaves were packaged in resealable polythene bags and stored at room temperatures for 12 hours.

3.6.5 Sensory evaluation of the bread

The loaves of bread were then cut into 2×3×5cm slices using a bread knife and labeled using different codes. The samples were then presented to 25 semi-trained panelists for sensory analysis. A five point hedonic scale was used to rate the different bread attributes where; 1 represented dislike extremely and 5 represented like extremely. The external bread characteristics included; shape, crust color, aroma and texture while the internal loaf characteristics included; crumb color, crumb softness and taste. Overall acceptability of the bread was also determined.

3.7 Shelf life determination of wheat cassava bread

3.7.1 Biochemical analysis

Water activity

The water activity of the bread samples was measured using durometers (Aw Messer- Germany) as described by (Mapesa, 2004). The durometers were calibrated using saturated solution of barium chloride and left to stand for 3 hours until water activity reading was at 0.900. Ten grams of bread was finely chopped into small pieces and placed in triplicates in the durometers and the water activity determined after 3 hours at a temperature of 20⁰C.

pH

The pH was measured using a pH meter. The bread samples in triplicates were subjected to pH analysis of the glass electrode (Hinga *et al.*, 1980). Ten grams of finely chopped bread samples was transferred into 100ml shaking bottle and 50ml of distilled water was added and shaken for 2 hours in reciprocal shaker. The pH was determined by a pH meter (PHS-3B) after a short but vigorous shaking. The pH meter was calibrated with buffers 4.0 and 7.0.

Moisture content

Moisture content was measured using oven drying AOAC Methods (2006). The bread samples were finely chopped and two grams of the sample weighed in moisture dishes then placed in an oven (Electrolux, Sweden) at (105⁰C for 4 hours). The % moisture content (MC) was calculated as follows:

$$MC\% = \frac{W_2 - W_3}{W_1} \times 100$$

Where: W₁ was the weight of the sample,

W₂ was the weight of sample plus dish;

W₃ was the weight of the dry sample plus dish.

3.7.2 Storage of bread and microbial analysis

The loaves of bread were packed in sterile plastic bags and stored in controlled oven (Sekonic, model SK-50P) set at 25⁰C to determine the storage time (in days) until mold growth on the surface of bread became visible (Lonner and PreveAkesson, 1989).

3.7.3 Microbial analysis

The total viable count (TVC), fungi (yeast and molds) and coliform counts were carried out in order to determine the shelf life of the product. These counts were carried out on day 0, day 2, day 4 and day 6 since bread spoils within 2-5 days (FAO, 2011).

3.7.4 Sample and media preparation

Samples were prepared by weighing 10 gm. of the sample and blending it with peptone water using a blender. Then serial dilutions were made up to 10⁻⁵. Plate count agar (PCA) was used for TVC, Potato dextrose agar (PDA) for yeast and molds while MacConkey agar (MCA) was used coliforms. The agar media were prepared by weighing the required amount of agar and dispensing into glass bottles and sterilizing in an autoclave at 121⁰C for 15 seconds. After sterilization the agar media were removed from the autoclave to allow cooling to about 48⁰C. This was done because, if the agar is too hot, the bacteria in the sample may be killed. If the agar is too cold, the medium may be lumpy once solidified.

3.7.5 Pour plate procedure

The diluents of 10⁻⁴ and 10⁻⁵ for each sample were used for pour plating. One milliliter of each diluent was transferred into sterilized petri dishes which were already labeled for the different tests using a micropipette. The glass bottle containing the agar media was opened and the rim passed through a Bunsen burner before pouring the media to the petri dishes containing the sample in order to ensure aseptic transfer. The petri dishes were then swirled gently to mix the sample with the agar. The agar was allowed to solidify then the petri dishes inverted and incubated at 30⁰C for 12 hours.

3.7.6 Isolation and enumeration of microorganism

After incubation the petri dishes were removed from the oven and the grown colonies counted and expressed as colony forming unit per gram (cfu/g) of the sample. After counting the distinct colonies were subculture on selective media to obtain pure cultures for identification.

3.8 Statistical analysis

Data obtained from physico-chemical, functional properties of cassava flour and proximate, alveograph, physical properties and sensory evaluation on wheat cassava bread was analyzed by SAS Version 9.1 for Analysis of Variance (ANOVA) using General Linear Model (GLM) procedure. Means separation was done using least significant difference (LSD) method (Gacula Jr, 2013) at $P \leq 0.05$. Principal component analysis (PCA) was performed on sensory attributes using PROC FACTOR procedure analysis data in order to reduce the set of attributes to a smaller set of variables called factors based on patterns of correlation among the original variables (Lawless and Heyman, 2010).

CHAPTER FOUR RESULTS

4.1 Cassava varieties cultivated in Migori County

Results from the cross-sectional survey show that fourteen different cassava varieties were grown in Migori County (Suna east and Rongo) (Table 1 and 2). Out of the fourteen varieties the following cassava varieties were more prevalent in Migori County compared to the others; *Selele*, *Merry go Round*, MH95/0183, MH95/0193, MH96/093, MH95/6484 and *Migyera*. *Migyera*, MH95/0183 and MH95/0193 cassava varieties were common in both Suna east sub-county and Rongo Sub-county. The improved varieties were more prevalent compared to the indigenous varieties.

Table 1: Types of cassava varieties grown in Migori County

| Suna east sub county | | | |
|---------------------------------|-----------------------|---------------------------|-----------------------|
| Indigenous varieties | Preference (%) | Improved varieties | Preference (%) |
| 1. <i>Ndege Olwaro</i> | 0.2 | 1. <i>Migyera</i> | 22.2 |
| 2. <i>Obaro dak</i> | 0.2 | 2. MH95/0183 | 22.2 |
| 3. <i>Adhiambo Lera</i> | 0.2 | 3. TR14 | 0.2 |
| 4. <i>Merry go round</i> | 22.2 | 4. MH95/0193 | 2.0 |
| | | 5. MH96/093 | 8.0 |
| Rongo sub county | | | |
| 1. <i>Selele</i> | 20 | 1. <i>Migyera</i> | 13.3 |
| 2. <i>Merry go round</i> | 11.1 | 2. MH95/0183 | 17.8 |
| 3. <i>Madam</i> | 4.4 | 3. MH95/0193 | 15.6 |
| 4. <i>Rawo onyoni</i> | 2.2 | 4. MH95/6484 | 13.3 |
| 5. <i>Oduogo</i> | 2.2 | | |

4.2 Physico-chemical properties of cassava flour

4.2.1 The effect of the cassava variety and stage of the maturity on the dry matter and cyanide content

The dry matter content, cyanide content of raw cassava tuber and cyanide content of dried cassava flour (HQCF) from different cassava cultivars harvested at different maturity periods are shown in (Table 2). Dry matter content varied from 25.33% for MH96/093 at 6 month to 47.21% for MH95/0183 at 15 month. The cyanide content of fresh cassava roots ranged between 2.937 mg/kg for *Migyera* 9 months to 9.093 mg/kg for MH95/0183 at 17 month. After processing the tubers to flour the level of cyanide content significantly reduced with percentage of 77.668% as shown in (Figure 4).

Table 2: Dry matter content, tuber cyanide content and flour cyanide content of different cassava cultivars and at different maturity stages

| Variety | Maturity (Months) | Dry matter (%) (fresh tubers) | Cyanide (mg/kg) (fresh tubers) | Cyanide (mg/kg) (processed flour) |
|-----------------------|----------------------|----------------------------------|------------------------------------|--------------------------------------|
| MH95/0183 | 6 | 40.920 ± 0.090 | 5.610 ± 0.322 | 1.103 ± 0.040 |
| MH95/0183 | 9 | 42.707 ± 1.235 | 7.153 ± 0.309 | 2.160 ± 0.000 |
| MH95/0183 | 12 | 47.210 ± 0.340 | 3.927 ± 0.176 | 1.080 ± 0.000 |
| MH95/0183 | 15 | 44.407 ± 0.050 | 3.937 ± 0.095 | 2.160 ± 0.000 |
| MH95/0183 | 17 | 39.167 ± 0.255 | 9.093 ± 0.117 | 1.080 ± 0.000 |
| MH95/0193 | 6 | 38.363 ± 0.074 | 4.973 ± 0.120 | 1.107 ± 0.046 |
| MH95/0193 | 9 | 44.427 ± 0.165 | 6.470 ± 0.000 | 1.113 ± 0.058 |
| MH95/0193 | 12 | 47.050 ± 0.170 | 6.430 ± 0.000 | 1.080 ± 0.000 |
| MH95/6484 | 9 | 41.777 ± 0.695 | 6.430 ± 0.000 | 1.080 ± 0.000 |
| MH95/6484 | 12 | 43.907 ± 0.444 | 8.620 ± 0.000 | 1.080 ± 0.000 |
| <i>Migyera</i> | 9 | 40.513 ± 0.051 | 2.937 ± 0.093 | 2.160 ± 0.000 |
| <i>Migyera</i> | 12 | 41.250 ± 0.530 | 5.350 ± 0.052 | 1.080 ± 0.000 |
| <i>Migyera</i> | 15 | 43.517 ± 0.153 | 7.523 ± 0.046 | 1.080 ± 0.000 |
| MH96/093 | 6 | 25.330 ± 0.370 | 5.410 ± 0.052 | 2.160 ± 0.000 |
| MH96/093 | 9 | 35.600 ± 0.060 | 6.473 ± 0.080 | 2.160 ± 0.000 |
| MH96/093 | 15 | 37.610 ± 0.495 | 6.470 ± 0.080 | 1.080 ± 0.000 |
| <i>Selele</i> | 9 | 38.230 ± 0.380 | 9.660 ± 0.000 | 1.103 ± 0.040 |
| <i>Selele</i> | 12 | 46.690 ± 0.145 | 6.420 ± 0.035 | 1.080 ± 0.000 |
| <i>Merry go round</i> | 12 | 44.710 ± 0.217 | 7.547 ± 0.046 | 1.080 ± 0.000 |
| <i>Merry go round</i> | 15 | 41.570 ± 0.080 | 9.710 ± 0.000 | 2.153 ± 0.011 |

Means ± standard deviation values are in triplicates

4.2.2 Effect of processing of cassava tubers on the cyanide content

The cyanide content of the cassava roots significantly reduced after processing the roots into high quality cassava flour. *Merry go round* and *Selele* had the highest level of cyanide for the fresh tubers of 8.629, 8.040 mg/kg while *Migyera* had the lowest cyanide content for the fresh cassava tubers of 5.270 mg/kg (Table 3). MH95/6884 and MH95/0193 lost the highest cyanide while MH96/093 and *Merry go round* lost the least.

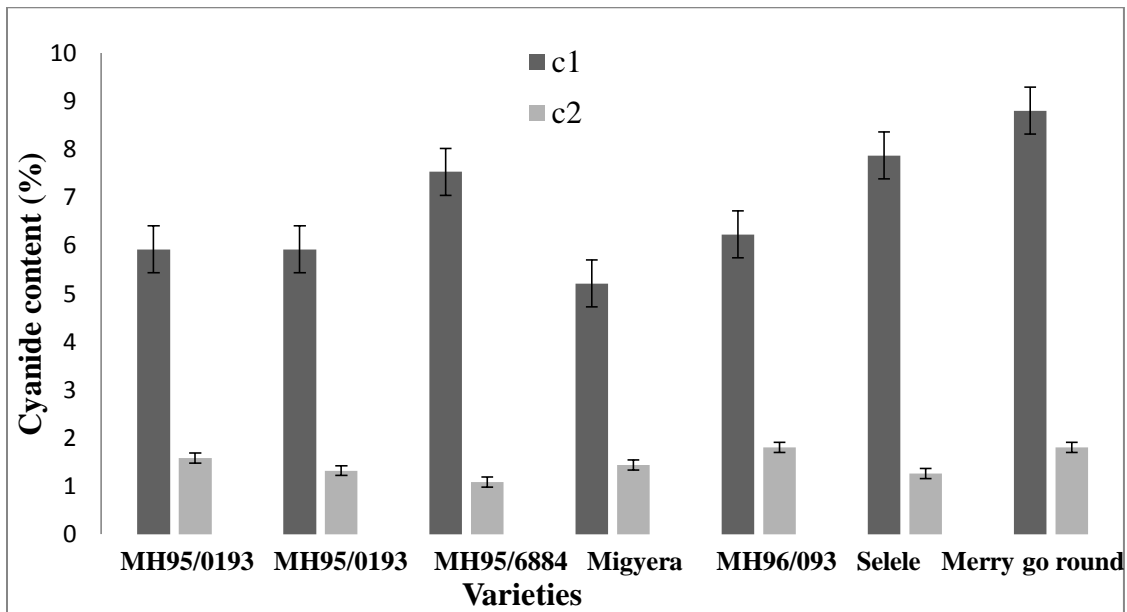


Figure 4: Effects of processing of cassava into HQCF on the cyanide content

C1- Cyanide content of fresh cassava, C2- Cyanide content of processed flour

4.2.3 Effects of maturity stage on the dry matter content

The dry matter content yield of cassava tubers increased with age of cassava from six months to twelve months then it started decreased to 17 months as shown in Figure 5.

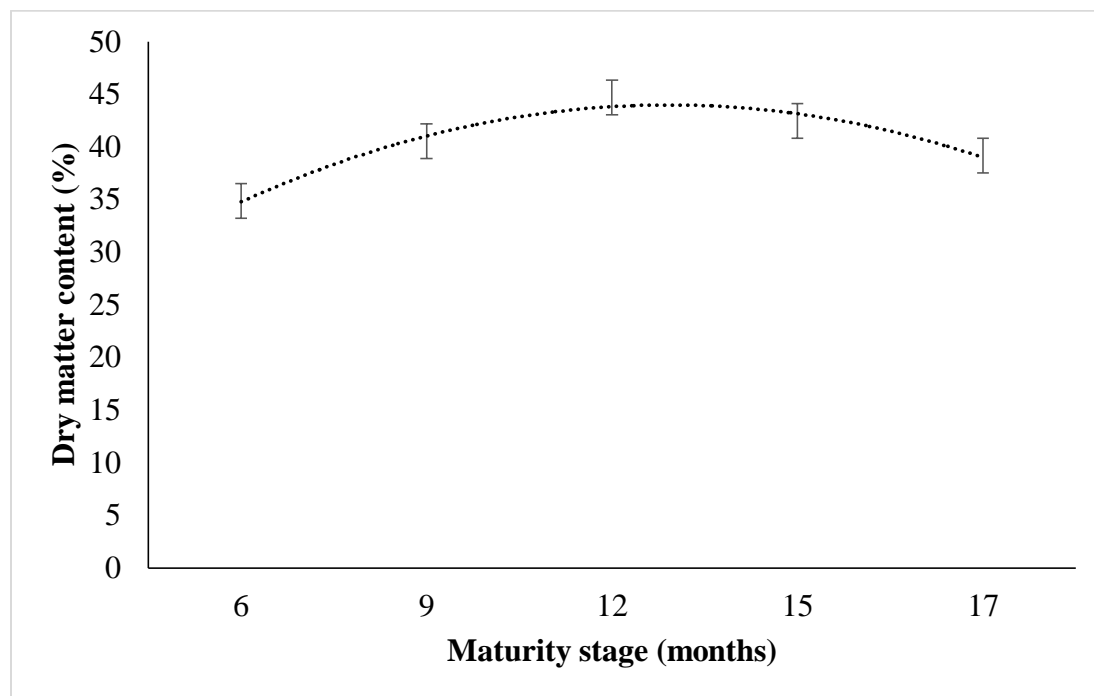


Figure 5: Effects of maturity stage (months) on dry matter content of fresh cassava tuber for all cassava varieties

4.3 Functional properties of cassava flour

4.3.1 The effect of the cassava variety and stage of the maturity on the functional properties of cassava flour

The functional properties of cassava flour (water binding capacity, swelling power, gelatinization temperature and peak viscosity) from different varieties and harvested at different maturity stage are shown in Table 3. The water binding index ranged between 0.253g/g for *Migyera* at 15 months to 2.967g/g for MH95/0183 at 9 months. The water binding capacity reduced with increase in maturity stage for most of the varieties.

Swelling power varied between 12.080g/g for MH96/093 at 15 months to 19.273 g/g for MH95/0183 at 17 months. For *Migyera* the swelling power increased in increase in maturity stage. The gelatinization temperatures ranged between 52°C for *Selele* at 12 months to 62°C for

MH95/0183 at 9 months. The peak viscosity varied between 580 BU to 586 BU for different varieties.

Table 3: Functional properties of cassava flour

| Maturity (months) | Variety | Water binding(g/g) | Swelling power(g/g) | Gelatinization temp(^oC) | Peak viscosity(BU) |
|--------------------------|-----------------------|---------------------------|----------------------------|---|---------------------------|
| 12 | MH95/0183 | 2.633 ± 0.070 | 18.110 ± 0.414 | 56.000 ± 0.000 | 580 |
| 15 | MH95/0183 | 1.643 ± 0.040 | 14.463 ± 0.450 | 58.000 ± 0.000 | 586 |
| 17 | MH95/0183 | 1.473 ± 0.078 | 19.273 ± 0.940 | 53.667 ± 0.000 | 580 |
| 6 | MH95/0183 | 2.967 ± 0.015 | 12.253 ± 0.096 | 55.667 ± 0.000 | 580 |
| 9 | MH95/0183 | 2.780 ± 0.026 | 13.653 ± 0.073 | 62.000 ± 0.000 | 580 |
| 12 | MH95/0193 | 2.443 ± 0.039 | 14.893 ± 0.135 | 55.667 ± 0.577 | 580 |
| 6 | MH95/0193 | 2.470 ± 0.056 | 13.513 ± 0.220 | 53.667 ± 0.577 | 580 |
| 9 | MH95/0193 | 2.470 ± 0.056 | 16.860 ± 0.177 | 58.000 ± 1.000 | 580 |
| 12 | MH95/6484 | 1.640 ± 0.036 | 15.573 ± 0.107 | 52.333 ± 0.577 | 585 |
| 9 | MH95/6484 | 2.780 ± 0.242 | 12.890 ± 0.098 | 56.000 ± 1.000 | 580 |
| 12 | <i>Migyera</i> | 1.627 ± 0.057 | 13.493 ± 0.250 | 61.667 ± 0.577 | 580 |
| 15 | <i>Migyera</i> | 0.253 ± 0.015 | 13.493 ± 0.250 | 56.000 ± 1.000 | 580 |
| 9 | <i>Migyera</i> | 2.763 ± 0.085 | 17.773 ± 0.067 | 57.667 ± 0.577 | 582 |
| 15 | MH96/093 | 1.540 ± 0.017 | 12.080 ± 0.293 | 61.667 ± 0.577 | 585 |
| 6 | MH96/093 | 2.780 ± 0.242 | 13.253 ± 0.611 | 58.000 ± 0.000 | 585 |
| 9 | MH96/093 | 2.733 ± 0.075 | 14.577 ± 0.091 | 55.667 ± 0.577 | 590 |
| 12 | <i>Selele</i> | 1.577 ± 0.021 | 16.547 ± 0.162 | 52.000 ± 2.000 | 580 |
| 9 | <i>Selele</i> | 2.547 ± 0.031 | 16.350 ± 0.142 | 57.667 ± 0.577 | 580 |
| 12 | <i>Merry go round</i> | 1.950 ± 0.046 | 14.640 ± 0.572 | 57.667 ± 0.577 | 585 |
| 15 | <i>Merry go round</i> | 1.243 ± 0.045 | 18.110 ± 0.413 | 58.110 ± 0.414 | 585 |

Means ± standard deviation values are in triplicates.

4.4 Wheat-cassava flour

4.4.1 Proximate composition of wheat-cassava flour

The effect of different substitution levels of cassava flour with wheat flour on the protein content, gluten content, moisture content and water activity is shown in Table 4. It was found out that the protein content, gluten content, moisture content and water activity of wheat-cassava composite flour decreased significantly ($P < 0.05$) with increase in substitution of baker's flour with cassava flour. The control which is baker's flour had the highest values for protein and gluten of 13.2 % and 31.43%, respectively while there was no significant difference ($P > 0.05$) for the protein content and gluten content among the three cassava varieties at each substitution level. It was also found out that the highest moisture content was in the baker's flour (control) with 13.43% and the lowest was 12.67% in the MH95/0193 cassava variety at 30% substitution level. Similarly, the highest water activity was in the baker's flour (control) with 63.20% and the lowest was 55.17% in the MH95/0193 cassava variety at same substitution level as it's for moisture content.

Table 4: Proximate composition of wheat-cassava flour

| Variety | Ratio | Protein | Moisture content | Water activity | Gluten |
|----------------|-------|------------------------------|-------------------------------|--------------------------------|-------------------------------|
| Wheat(control) | 100:0 | 13.2±0.17^a | 13.43±0.12^a | 63.20± 0.26^a | 31.43±0.31^a |
| MH95/0183 | 95:5 | 12.80±0.23 ^b | 13.30±0.10 ^b | 62.33± 0.35 ^b | 29.70±0.17 ^b |
| | 90:10 | 12.20±0.17 ^c | 13.23±0.06 ^{bc} | 61.90± 0.26 ^c | 28.33±0.15 ^c |
| | 85:15 | 11.77±0.06 ^d | 13.27±0.06 ^c | 60.73± 0.15 ^d | 25.63±0.15 ^d |
| | 80:20 | 11.33±0.06 ^e | 12.97±0.06 ^d | 59.83± 0.35 ^e | 25.17±0.06 ^e |
| | 75:25 | 10.80±0.10 ^f | 12.90±0.10 ^d | 58.80± 0.10 ^f | 23.50±0.17 ^f |
| | 70:30 | 9.83±0.31 ^g | 12.77±0.12 ^e | 57.37± 0.20 ^g | 19.63±0.58 ^g |
| MH95/0193 | 95:5 | 12.70±0.10 ^b | 13.33±0.06 ^b | 62.50± 0.10 ^b | 29.37±0.15 ^b |
| | 90:10 | 12.37±0.05 ^c | 13.27±0.06 ^{bc} | 61.23± 0.20 ^c | 28.17±0.15 ^c |
| | 85:15 | 11.67±0.12 ^d | 13.30±0.10 ^c | 60.37± 0.32 ^d | 25.80±0.20 ^d |
| | 80:20 | 11.17±0.12 ^e | 13.06±0.06 ^d | 59.67± 0.12 ^e | 25.30±0.10 ^e |
| | 75:25 | 10.90±0.10 ^f | 13.07±0.06 ^d | 58.23± 0.32 ^f | 24.30±0.20 ^f |
| | 70:30 | 9.27±0.29 ^g | 12.67±0.06 ^e | 55.17± 0.28 ^g | 20.57±0.15 ^g |
| <i>Selele</i> | 95:5 | 12.80±0.10 ^b | 13.30±0.10 ^b | 62.60± 0.26 ^b | 29.27±0.15 ^b |
| | 90:10 | 12.03±0.06 ^c | 13.20±0.00 ^{bc} | 60.20± 0.20 ^c | 27.43±0.12 ^c |
| | 85:15 | 11.80±0.10 ^d | 13.27±0.00 ^c | 59.50± 0.36 ^d | 26.37±0.21 ^d |
| | 80:20 | 11.13±0.15 ^e | 13.13±0.15 ^d | 58.53± 0.21 ^e | 25.53±0.15 ^e |
| | 75:25 | 10.70±0.00 ^f | 13.10±0.17 ^d | 57.80± 0.17 ^f | 23.77±0.15 ^f |
| | 70:30 | 9.88±0.12 ^g | 12.87±0.06 ^e | 56.73± 0.32 ^g | 22.37± 0.15 ^g |

Values are means ± SD triplicate determinations. Means in the same column followed by the same letter are not significantly different ($p < 0.05$) from each other.

4.4.2 Rheological properties

The effect of different substitution levels of cassava flour with wheat flour on the rheological properties of the dough is shown in Table 5 as determined using an alveograph. It was found out that the dough extensibility, the dough strength and dough deformation energy reduced

significantly ($P<0.05$) with increase in cassava flour substitution levels. The type of the cassava variety also had a significant ($P<0.05$) effect on dough rheological properties with the dough made from MH95/0193 cassava variety having weaker rheological properties at each level of substitution while MH95/0183 variety having the strongest.

Table 5: Rheological properties of composite of the dough

| Variety | Ratio | Length-L(mm) | Height-P(mm) | W(Deformation energy-Joules) |
|------------------|------------------|-------------------------------|-------------------------------|--------------------------------|
| Wheat | 100:0 | 76.53±0.49^a | 90.30±1.04^a | 270.53±7.20^a |
| MH95/0183 | 95:5 | 72.20±0.30 ^c | 66.33±0.55 ^b | 215.16±5.59 ^b |
| | 90:10 | 71.63±0.57 ^c | 52.53±0.55 ^{cd} | 157.83±2.30 ^d |
| | 85:15 | 53.57±1.16 ^e | 37.57±0.61 ^f | 93.24±5.07 ^f |
| | 80:20 | 43.90±0.92 ^h | 42.23±0.70 ^h | 71.40±2.45 ^h |
| | 75:25 | 31.63±0.71 ^k | 29.60±1.37 ^j | 40.54±2.91 ^j |
| | 70:30 | 26.17±0.47 ^l | 27.83±0.31 ^k | 33.14±4.82 ^k |
| | MH95/0193 | 95:5 | 74.40±0.57 ^b | 53.07±0.67 ^c |
| 90:10 | | 46.97±0.91 ^g | 40.83±0.32 ^g | 70.85±6.19 ^h |
| 85:15 | | 26.00±0.75 ^l | 18.57±0.57 ^m | 45.56±5.45 ^j |
| 80:20 | | 16.50±1.34 ^m | 17.63±0.45 ^m | 18.31±2.62 ^l |
| 75:25 | | 11.37±0.61 ⁿ | 15.80±0.36 ⁿ | 13.73±1.31 ^{lm} |
| 70:30 | | 9.40±0.44 ^o | 11.70±0.60 ^o | 8.50±0.66 ^m |
| Selele | 95:5 | 71.80±0.98 ^c | 51.97±0.15 ^d | 155.65±1.96 ^d |
| | 90:10 | 57.93±0.31 ^d | 50.80±0.46 ^e | 109.44±5.64 ^e |
| | 85:15 | 49.17±0.30 ^f | 42.00±0.95 ^f | 79.57±4.60 ^g |
| | 80:20 | 41.87±0.76 ⁱ | 32.43±0.47 ⁱ | 57.33±5.45 ⁱ |
| | 75:15 | 32.83±0.85 ^j | 31.87±0.25 ⁱ | 41.20±2.99 ^j |
| | 70:20 | 16.73±0.78 ^m | 19.90±0.60 ^l | 11.77±0.66 ^{lm} |

Values are means ± SD triplicate determinations. Means in the same column followed by the same letter are not significantly different ($p<0.05$) from each other.

4.5 Wheat-cassava composite bread

4.5.1 Physical properties of wheat-cassava bread

The physical characteristics of wheat-cassava composite bread are presented in Table 6. The specific volume and form ratio of the composite bread decreased significantly ($P < 0.05$) with increased substitution levels with cassava flour. The control with 100% Baker' flour had the highest values of $4.77 \text{ cm}^3/\text{g}$ and 1.77 for specific volume and form ratio, respectively while the sample made from MH95/0183 cassava flour at 30% substitution level had the lowest values of $2.15 \text{ cm}^3/\text{g}$ and 0.86 for specific volume and form ratio, respectively. Different cassava varieties had significantly different ($P < 0.05$) effect on the specific volume and form ratio of the bread where *Selele* variety had the highest and MH95/0183 variety had the lowest specific volume and form ratio. The specific volume and form ratio for *Selele* variety was $3.07 \text{ cm}^3/\text{g}$ and 1.34 respectively while MH95/0183 variety had $2.78 \text{ cm}^3/\text{g}$ and 1.18 respectively.

Table 6: Physical properties of wheat-cassava bread

| Sample | Ratio | Specific volume(cm³/g) | Form ratio |
|-------------------------|--------------|--|--------------------------------|
| Baker's flour(A) | 100:0 | 4.77 ± 0.06^a | 1.77 ± 0.03^a |
| MH95/0183 | 95:5 | 3.56 ± 0.05 ^d | 1.54 ± 0.01 ^b |
| | 90:10 | 3.04 ± 0.05 ^g | 1.38 ± 0.02 ^e |
| | 85:15 | 2.81 ± 0.01 ^j | 1.22 ± 0.02 ^h |
| | 80:20 | 2.74 ± 0.02 ^l | 1.09 ± 0.02 ^e |
| | 75:25 | 2.35 ± 0.04 ^q | 0.93 ± 0.02 ^o |
| | 70:80 | 2.15 ± 0.04 ^s | 0.86 ± 0.01 ^p |
| Mean | | 2.78 ± 0.47^c | 1.18 ± 0.25^c |
| MH95/0193 | 95:5 | 3.63 ± 0.06 ^c | 1.37 ± 0.01 ^h |
| | 90:10 | 3.10 ± 0.06 ^f | 1.33 ± 0.00 ^g |
| | 85:15 | 2.95 ± 0.03 ⁱ | 1.20 ± 0.02 ⁱ |
| | 80:20 | 2.76 ± 0.05 ^k | 1.16 ± 0.00 ^j |
| | 75:25 | 2.69 ± 0.05 ⁿ | 1.13 ± 0.00 ^k |
| | 70:80 | 2.37 ± 0.04 ^p | 1.01 ± 0.01 ^m |
| Mean | | 2.95 ± 0.40^b | 1.20 ± 0.13^c |
| Selele | 95:5 | 4.19 ± 0.04 ^b | 1.75 ± 0.02 ^b |
| | 90:10 | 3.45 ± 0.02 ^d | 1.54 ± 0.01 ^b |
| | 85:15 | 3.19 ± 0.04 ^e | 1.42 ± 0.01 ^c |
| | 80:20 | 2.99 ± 0.02 ^h | 1.35 ± 0.02 ^f |
| | 75:25 | 2.39 ± 0.01 ^o | 1.02 ± 0.02 ^l |
| | 70:80 | 2.19 ± 0.01 ^r | 0.94 ± 0.03 ⁿ |
| Mean | | 3.07 ± 0.69^b | 1.34 ± 0.29^b |

Values are means ± SD triplicate determinations. Means in the same column followed by the same letter are not significantly different (p<0.05) from each other.

4.5.2 Effects of substitution level of the wheat cassava bread

The cross-sections of bread made from the different wheat- cassava ratios are shown in Figure 6. Substitution of baker's flour with cassava flour had effects on the shape of the bread and crumb appearance. The shape of the bread changed from oval to flat at the top with increased substitution of baker's flour with cassava flour. Substitutions of cassava up to 15% produced bread with a good shape. The crumb firmness of the bread increased with increase in substitution of baker's flour with cassava flour which caused formation of more spaces between the crumb particles.

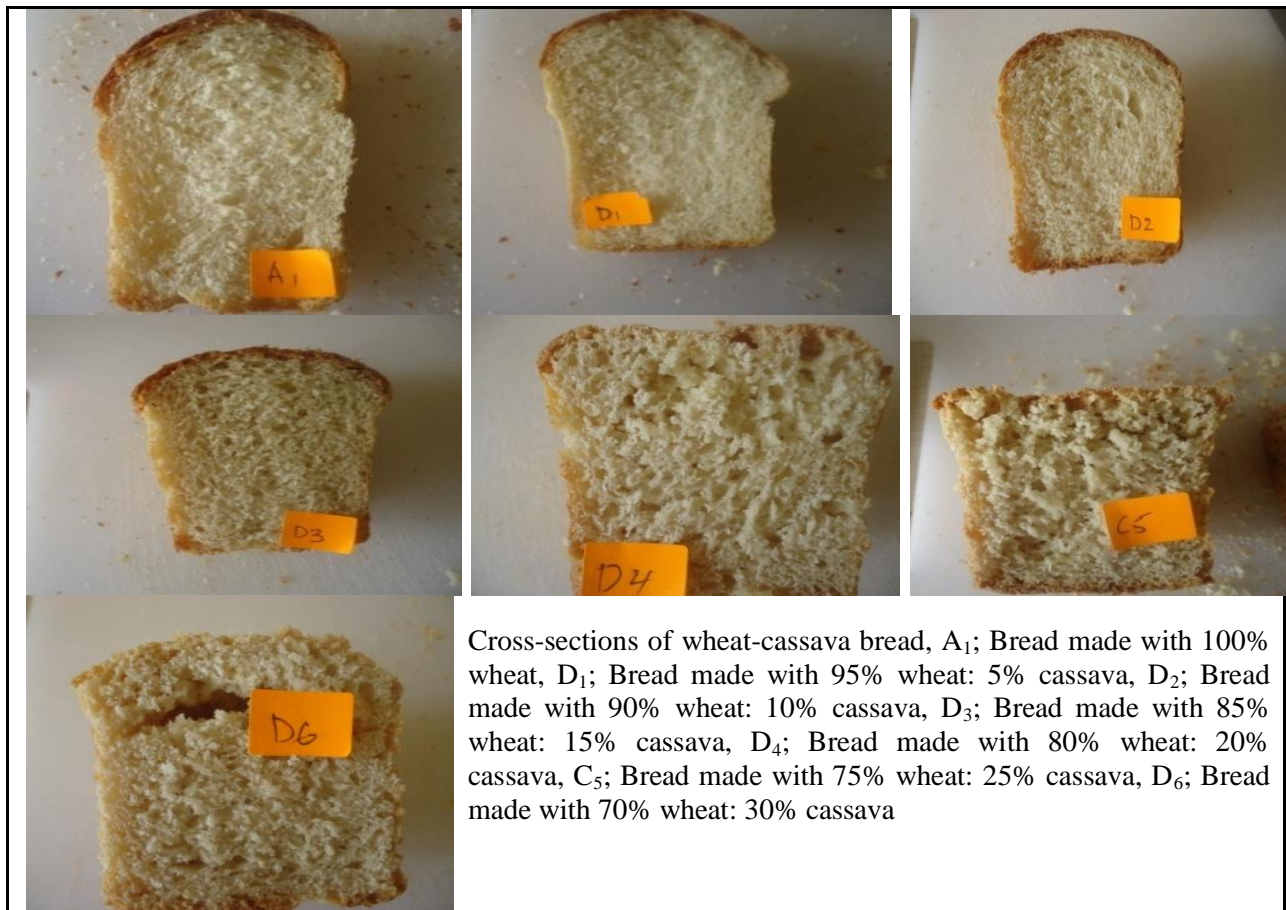


Figure 6: Cross-sections of wheat-cassava composite bread

4.5.3 External sensory loaf characteristics of wheat-cassava composite bread

The results of external loaf characteristics are shown in Table 7. The likeability of loaf shape, texture, crust color and aroma reduced with increase in substitution of wheat with cassava flour for all varieties. Bread made from 100% wheat flour didn't have significant difference from

bread made up to 15% substitution with cassava flour for loaf shape, texture, crust color and aroma for MH95/0183 and *Selele* variety. There was significant difference ($P < 0.05$) in the liking of bread made from 100% wheat and bread made with substitution of cassava at 20%, 25% and 30% for the loaf shape, texture, crust colour and aroma.

Table 7: External loaf characteristics of wheat-cassava composite bread

| Sample Code | Ratio | Loaf shape | Texture | Crust color | Aroma |
|----------------------|-------|---------------------------------|---------------------------------|--------------------------------|--------------------------------|
| Control | 100:0 | 3.91±0.13^{ab} | 3.95±0.11^{ab} | 3.66±0.13^{bc} | 3.95±0.13^a |
| MH95/0183 | 95:5 | 4.25±0.12^a | 3.89±0.19^{ab} | 3.61±0.21^c | 3.54±0.20 ^b |
| | 90:10 | 3.82±0.15^{ab} | 3.71±0.13^b | 3.54±0.15^c | 3.25±0.20 ^{bc} |
| | 85:15 | 3.75±0.18^{ab} | 3.68±0.17^{bc} | 3.82±0.15^a | 3.61±0.19^{ab} |
| | 80:20 | 3.61±0.15^b | 3.50±0.20 ^c | 3.71±0.17^b | 3.43±0.20 ^{bc} |
| | 75:25 | 3.39±0.20 ^c | 3.25±0.22 ^d | 3.36±0.19 ^d | 3.00±0.17 ^{bc} |
| | 70:30 | 3.36±0.20 ^c | 3.14±0.22 ^e | 3.11±0.22 ^e | 2.93±0.16 ^c |
| Mean | | 3.70 ± 0.95^{ab} | 3.53 ± 1.03^c | 3.53 ± 0.98^c | 3.30 ± 0.96^b |
| MH95/0193 | 95:5 | 4.25±0.20^a | 4.07±0.19^a | 4.32±0.19^a | 3.90±0.17^a |
| | 90:10 | 3.71±0.19^{ab} | 3.86±0.20^{ab} | 3.71±0.20^b | 3.75±0.18^a |
| | 85:15 | 3.61±0.17^b | 3.36±0.19 ^d | 3.54±0.16 ^c | 3.36±0.19 ^{bc} |
| | 80:20 | 3.57±0.16^b | 3.17±0.17 ^e | 3.47±0.15 ^{cd} | 3.27±0.16 ^c |
| | 75:25 | 3.60±0.16^b | 3.07±0.17 ^e | 3.37±0.19 ^d | 3.03±0.17 ^c |
| | 70:30 | 3.23±0.20 ^c | 3.27±0.20 ^d | 3.20±0.19 ^{de} | 3.20±0.18 ^c |
| Mean | | 3.66 ± 1.00^b | 3.47 ± 1.06^c | 3.60 ± 0.89^c | 3.42 ± 0.98^b |
| <i>Selele</i> | 95:5 | 4.13±0.18^{ab} | 4.03±0.13^a | 3.93±0.16^a | 3.70±0.15^a |
| | 90:10 | 4.27±0.16^a | 4.20±0.15^a | 3.87±0.16^b | 3.67±0.16^a |
| | 85:15 | 4.00±0.17^{ab} | 3.87±0.15^{ab} | 4.00±0.14^a | 3.73±0.14^a |
| | 80:20 | 3.87±0.13^b | 3.53±0.14 ^c | 3.67±0.14^{bc} | 3.43±0.16 ^b |
| | 75:25 | 3.50±0.18 ^c | 3.10±0.12 ^e | 3.30±0.19 ^d | 3.57±0.18 ^b |
| | 70:30 | 3.13±0.20 ^c | 3.03±0.20 ^e | 3.07±0.17 ^e | 2.90±0.19 ^c |
| Mean | | 3.81 ± 1.01^{ab} | 3.63 ± 0.93^{bc} | 3.64 ± 0.94^c | 3.50 ± 0.93^b |

Values are means ± SD triplicate determinations. Means in the same column followed by the same letter are not significantly different ($p < 0.05$) from each other.

4.5.4 Internal sensory loaf characteristics of wheat-cassava composite bread

There were significant differences ($P < 0.05$) in the internal loaf characteristics of bread made from the baker's flour (control) and the wheat-cassava composite bread at different substitution levels as shown in (Table 8). However, the composite bread with MH95/0193 and *Selele* varieties with 5% level of substitution and MH95/0183 variety with 10% level of substitution had a higher rating on internal loaf characteristics than the control. The liking of the crumb softness decreased with increase in cassava substitution. There was no significant difference ($P > 0.05$) in the taste of bread made from unblended wheat flour (control) and bread made up to 15% cassava flour. There was also no significant difference in the overall acceptability of bread made from baker's flour (control) and that made of cassava flour up to 15% substitution. *Selele* variety still had the best liking for crumb color, crumb softness, taste and overall acceptability compared to MH95/0183 and MH95/0193 varieties.

Table 8: Internal loaf sensory characteristics of wheat-cassava composite bread

| Sample Code | Ratio | Crumb color | Crumb softness | Taste | Overall acceptability |
|------------------|-------------|--------------------------------|---------------------------------|---------------------------------|---------------------------------|
| Control | 100:0 | 3.93±0.12^a | 4.16 ± 0.91^a | 3.97 ± 0.13^{ab} | 4.16 ± 0.11^a |
| MH95/0183 | 95:5 | 3.68±0.15 ^b | 4.04 ± 0.84^a | 3.64 ± 0.16^{ab} | 3.89± 0.16^{ab} |
| | 90:10 | 4.00±0.17^a | 3.39 ± 0.83 ^b | 2.89 ± 0.18 ^c | 3.61±0.16^{ab} |
| | 85:15 | 3.96±0.15^a | 3.11 ± 0.81 ^{bc} | 3.36 ± 0.19 ^{bc} | 3.86±0.16^{ab} |
| | 80:20 | 3.57±0.21 ^b | 3.07 ± 1.09 ^{bc} | 3.54 ± 0.20 ^{bc} | 3.57±0.20 ^b |
| | 75:25 | 3.50±0.20 ^b | 2.96 ± 0.96 ^c | 2.89 ± 0.19 ^c | 3.29±0.16 ^c |
| | 70:30 | 3.29±0.20 ^c | 2.96 ± 0.92 ^c | 3.03 ± 0.22 ^{bc} | 3.21±0.18 ^c |
| | Mean | | 3.67 ± 0.99^b | 3.26 ± 0.99^{bc} | 3.23 ± 1.04^{bc} |
| MH95/0193 | 95:5 | 4.14±0.18^a | 4.18 ± 0.90^a | 3.86 ± 0.18^{ab} | 4.29±0.17^a |
| | 90:10 | 3.86±0.18^a | 3.82 ± 0.96^{ab} | 3.71 ± 0.18^{ab} | 3.89±0.15^{ab} |
| | 85:15 | 3.68±0.16 ^b | 3.43 ± 0.96 ^c | 3.61 ± 0.18 ^b | 3.68±0.14 ^b |
| | 80:20 | 3.53±0.14 ^b | 3.07 ± 0.94 ^c | 3.43 ± 0.18 ^{bc} | 3.60±0.13 ^b |
| | 75:25 | 3.40±0.14 ^{bc} | 3.17 ± 0.91 ^c | 3.17 ± 0.16 ^{bc} | 3.60±0.16 ^b |
| | 70:30 | 3.37±0.16 ^c | 2.83 ± 1.05 ^d | 2.77 ± 0.16 ^c | 3.13±0.15 ^c |
| | Mean | | 3.66 ± 0.89^b | 3.41 ± 1.04^b | 3.43 ± 0.98^b |
| Selele | 95:5 | 4.00±0.15^a | 4.00 ± 0.95^a | 4.00 ± 0.16^a | 4.10±0.15^a |
| | 90:10 | 3.90±0.15^a | 3.80 ± 0.85^{ab} | 3.77 ± 0.16^{ab} | 4.20±0.15^a |
| | 85:15 | 3.93±0.14^a | 3.80 ± 0.8^{ab} | 3.67 ± 0.15^b | 3.93±0.14^{ab} |
| | 80:20 | 3.67±0.15 ^b | 3.30 ± 0.99 ^{bc} | 3.37 ± 0.15 ^c | 3.47±0.17 ^d |
| | 75:25 | 3.37±0.15 ^c | 2.97 ± 0.35 ^c | 3.10 ± 0.15 ^c | 3.20±0.15 ^d |
| | 70:30 | 3.27±0.17 ^c | 2.57 ± 1.17 ^c | 2.70 ± 0.22 ^c | 3.07±0.17 ^d |
| Mean | | 3.69 ± 0.87^b | 3.41 ± 1.07^c | 3.44 ± 1.00^c | 3.67 ± 0.96^b |

Values are means ± SD triplicate determinations. Means in the same column followed by the same letter are not significantly different (p<0.05) from each other.

4.5.5 Principal component analysis (PCA)

The results from PCA show the existence of two principle components (factors) for the seven sensory attributes of the bread. The first factor accounted for 54.5% while the second factor

accounted for 11.08% of total variation (Table 9). The first factor consists of attributes such as loaf shape, texture and crust colour. These are the most influential sensory characteristics which the customer will use to judge the bread.

Table 9: Principal component factor loadings for wheat-cassava bread attributes

| Sensory attribute | Principle component scores | |
|---|-----------------------------------|-----------------|
| | Factor 1 | Factor 2 |
| Shape | 0.72582 | -0.27769 |
| Texture | 0.76142 | -0.27372 |
| Crust colour | 0.82319 | -0.22191 |
| Crumb colour | 0.60180 | 0.43848 |
| Crumb softness | -0.38595 | 0.70152 |
| Aroma | -0.24988 | 0.81716 |
| Taste | -0.24683 | 0.80378 |
| Proportion of the total variance | 54.5% | 11.08% |

4.6 Shelf life determination of wheat-cassava composite bread

4.6.1 Biochemical composition of wheat-cassava bread.

The water activity of the bread ranged between 0.96 for bread baked with 100% wheat to 0.92 for bread made with 30% cassava flour as shown in Table 10. The results show that the water activity reduced with increase in the level of cassava substitution.

The pH value ranged between 6.68 for 100% wheat to 6.47 for 30% cassava bread. The acidity of the bread slightly increased with increase in cassava flour.

The moisture content of the bread ranged between 34.93 % for bread from 5% cassava flour to 31.35 % for bread made from 15 % cassava flour. There was no significant trend in the moisture content of the bread made from different ratios of wheat and cassava flour.

Table 10: Biochemical properties of fresh baked loaves of bread

| Sample and level of substitution | Water activity (a_w) | P_H | Moisture content (%) |
|---|--|-------------------------|-----------------------------|
| A₀ (Control)-100:0 | 0.96 | 6.68 | 32.23 |
| A₁ - 95:5 | 0.96 | 6.64 | 34.93 |
| A₂ -90:10 | 0.95 | 6.65 | 33.82 |
| A₃ -85:15 | 0.94 | 6.58 | 31.35 |
| A₄ -80:20 | 0.93 | 6.56 | 33.79 |
| A₅ -75:25 | 0.93 | 6.52 | 32.32 |
| A₆ -70:30 | 0.92 | 6.47 | 31.56 |

Ratio of (wheat: Cassava) A₀ – (100 %:0%), A₁-(95%:5%), A₂-(90%:10%), A₃ – (85%:15%), A₄ (80%:20%), A₅-(75%-25%) and A₆– (70%- 30%)

4.6.2 Total viable counts (TVC)

The bread made from 100% wheat had the highest TVC while the bread with 30% cassava had the lowest TVC for the three consecutive times that the analysis was done as shown in (Table 11). After the second day the TVC increased to high numbers and there was noticeable smell of spoiled bread. The growth of microorganism was highest on the sample (100% wheat flour) that had higher water activity and pH than samples (30% cassava flour) that had lower water activity pH values.

Table 11: Total Viable Counts at 25⁰C

| Bread sample | DAY 1(CFU/g Log₁₀) | DAY 2 (CFU/g Log₁₀) | DAY 4 (CFU/g Log₁₀) | DAY 6 (CFU/g Log₁₀) |
|---------------------|--------------------------------------|---------------------------------------|---------------------------------------|---------------------------------------|
| A0 | 5.40 | 5.54 | 6.48 | 7.40 |
| A1 | 5.29 | 5.46 | 6.48 | 7.29 |
| A2 | 5.23 | 5.31 | 6.42 | 7.23 |
| A3 | 5.00 | 5.23 | 6.30 | 7.00 |
| A4 | 4.90 | 5.02 | 6.18 | 6.90 |
| A5 | 4.51 | 5.00 | 5.60 | 6.50 |
| A6 | 4.30 | 4.99 | 5.64 | 6.30 |

Ratio of (wheat: Cassava) A – (100 %:0%), A₁-(95%:5%), A₂-(90%:10%), A₃ – (85%:15%), A₄ (80%:20%), A₅-(75%-25%) and A₆ – (70%- 30%).

4.6.3 Yeasts and moulds

The yeast and moulds were also highest on the 100% wheat bread (Table 12) and there were no coli-forms that were found on the samples. The growth of bacteria and fungi were directly related to the water activity and the pH of the product. High water activity and higher pH led to faster spoilage of the bread. Visible moulds were seen on the surface of the bread after the fourth day. Therefore from the results it is clear that the bread can take a maximum of three days before spoilage, however, if preservatives were used it could have taken a longer time before spoilage starts. The different moulds that were found in the bread samples are presented in (Figure 7). The types of moulds found include *Aspergillus* species, *Penicillium* species and *Rhizopus* species.

Table 12: Yeast and moulds count at 25⁰C

| Bread sample | DAY 1(CFU/g Log ₁₀) | DAY 2 (CFU/g Log ₁₀) | DAY 4 (CFU/g Log ₁₀) | DAY 6 (CFU/g Log ₁₀) |
|--------------|---------------------------------|----------------------------------|----------------------------------|----------------------------------|
| A0 | 4.30 | 5.53 | 5.70 | 5.90 |
| A1 | 4.00 | 5.35 | 5.51 | 5.66 |
| A2 | 4.00 | 5.15 | 5.40 | 5.60 |
| A3 | 0 | 5.00 | 5.32 | 5.54 |
| A4 | 4.00 | 4.95 | 5.31 | 5.48 |
| A5 | 0 | 4.78 | 5.27 | 5.40 |
| A6 | 0 | 4.48 | 5.18 | 5.33 |

Ratio of (wheat: Cassava) A – (100 %:0%), A₁-(95%:5%), A₂-(90%:10%), A₃ – (85%:15%), A₄ (80%:20%), A₅-(75%-25%) and A₆– (70%- 30%).

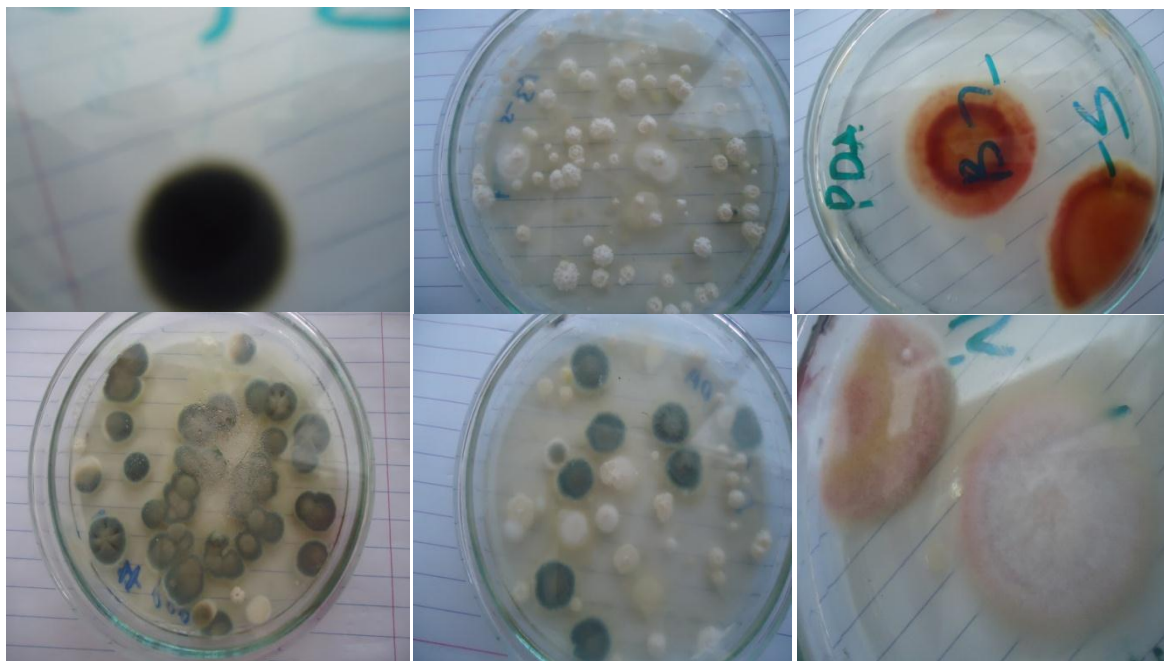


Figure 7: Moulds found in wheat cassava bread

CHAPTER FIVE DISCUSSION

5.1 Cassava varieties cultivated in Migori County

The results show that the improved varieties were more prevalent compared to the indigenous variety and this could be attributed to the fact that the improved varieties are more resistant to diseases, high yielding and mature very fast compared to the indigenous varieties thus more farmers preferred the improved varieties (Table 1). The improved varieties are resistance to mainly cassava mosaic disease (CMD) and cassava bacterial blight (CBB) (Moses *et al.*, 2008). *Migyera*, MH95/0183 and MH95/0193 were found in both sub-counties since they are the most preferred improved varieties due to their high resistance to diseases and have high yields. The indigenous varieties were not more prevalent since they were prone to diseases, low yielding and took more than 15 months to mature.

5.2 Physico-chemical properties of cassava flour

5.2.1 The effect of the cassava variety and stage of the maturity on the dry matter and cyanide content

The results in Table 2 show that different varieties had different dry matter content which is an important factor to consider when choosing a variety that will give the highest flour yields since dry matter is an indicator of flour yields (Nuwamanya *et al.*, 2009). MH95/0183, MH95/0193 and *Selele* had the highest dry matter content at 12 months compared to the other varieties at the same age which makes the three varieties to be more suitable for flour production. The cyanide content also differed for all varieties with *Selele* and *merry go round* having the highest cyanide content and *Migyera* having the least. This shows that the indigenous varieties had the highest cyanide compared to the improved maybe because they have not been improved to yield less cyanide.

The results also shows that dry matter content increased from 6 months up to 12 months then it started decreasing for MH95/0183, *Migyera* and MH96/093 as shown in Table 3. The increase in dry matter content from 6 months to 12 months shows that flour content was highest at 12 months since dry matter content is directly related to the flour yields (Nuwamanya *et al.*, 2009). The decrease in flour content after 12 months is an indicator that starch which is the main constituent of cassava flour (Stupak *et al.*, 2006) had started being degraded into simple sugars

(Sriroth et al., 1999) thus making harvesting at 12 months to be the best for most of the cassava varieties. The cyanide content also increased with time, like for *Migyera* the cyanide content increased from 2.94 mg/kg at 6 months to 7.52 mg/kg at 15 months. This might be attributed to the change in climatic condition. However, this study differed from another study by Cooke *et al.*, (1982) who reported that cyanide content of cassava roots was not affected by age that cyanide content of cassava tubers harvested between 6 and 14 months was not significantly different.

5.2.2 Effect of processing of cassava tubers on the cyanide content

Processing of cassava roots into high quality cassava flour reduced the levels of cyanide significantly for each cassava variety as shown in Figure 4. The processing was done through peeling the roots, washing, grating, sun-drying and milling it into flour. The highest reduction of cyanide was in MH95/6884 while the lowest was in *Merry go round* and MH96/093. This could be due to differences in the composition of different cassava varieties. The processing of the cassava roots reduced the cyanide content to safe levels according to the recommended levels by World Health Organization of 10 mg/kg to prevent cyanide poisoning. The reduction in cyanide content according to Essers *et al.*, (1996) was due to cell disruption during grating which enhances the contact between linamarase enzyme and cyanogenic glucosides that are hydrolyzed to glucose and cyanohydrins which are further decomposed into ketones and hydrogen cyanide.

5.2.3 Effects of maturity stage on the dry matter content

The increase in dry matter content from 6 months to 12 can be attributed to increase in the starch content and decrease in the water content of the tubers while the decrease in dry matter content from 12 months to 17 months can be attributed to breakdown of starch into simple sugars and increase fibrousness. The results are comparable to a similar study done by Apea-Bah *et al.*, (2011) who reported that the age at which cassava tubers were harvested significantly affected the moisture content of cassava which is directly related to its dry matter content. Another study as reported by Erikson, (2013) found that the age of cassava at harvesting affected bread making potential of cassava flour more than the cultivar or the genotype.

5.3 Functional properties of cassava flour

5.3.1 The effect of the cassava variety and stage of the maturity on the functional properties

Water binding capacity was lowest for Migyera at 15 months (0.25g/g) and merry go round (1.24g/g) at 15 months as shown in Table 3. These results shows that the water binding capacity decreased with increase in maturity stage for most of the varieties this is because break down of starch is a measure of the degree of paste stability (Tsakama *et al.*, 2010) which is related to water binding capacity. A low break down value suggests that the paste formed is more stable (Olufunmilola *et al.*, 2009). This is because there is stronger cross-linking within the starch granules of the flour, therefore flours with low water binding index will have a more stable paste compared to flours with high water binding index. Water binding capacity is an important functional property of starch that determine the viscosity, consistency and the ability of the starch to resist break down in various products.. The results were similar to another study by Aberi *et al.*, (2012) who reported that the values for water binding index ranged from 1.933g/g - 2.010g/g.

Swelling power was highest for MH95/0183 (19.273g/g) at 17 months and Merry go round (18.110g/g) at 15 months. These results show that swelling power increased with increase in maturity stage for most of the varieties. High swelling power results into high digestibility and ability to be used in different starch solution. Therefore, *Selele* and Merry go round had the best swelling power properties compared to the other varieties. Swelling power is an important functional property that is used in characterization of starches for bakers and manufacturers and is depended on the botanical origin of the starch (Dufor *et al.*, 1996). The different starches display different swelling powers at a given temperature and therefore affect the eating quality of cassava tubers and the use of starch in several industrial applications (Moorthy, 2002).

Different cassava varieties had different gelatinization temperatures at different maturity stages. MH95/0183 had the highest gelatinization temperature of 62 °C at 9 months while *Selele* had the lowest gelatinization temperature of 52 °C at 12 months. Gelatinization temperature is an important parameter that provides an indication of the minimum temperatures required for cooking a sample and energy costs. *Selele* at 12 months had the lowest pasting temperatures of 52°C which indicates that it can easily form paste hence more suitable in most food and non-food industrial processes because of reduced energy cost and time during processing. These results

were similar to a study done by Aberi *et al.*, (2012) who reported gelatinization temperatures of between 58.5°C- 65°C in cassava flour.

Most of the cassava varieties had peak viscosity at 580 BU at different maturity stage while MH96/093 had the highest peak viscosity of 570 BU. Peak viscosity is the maximum viscosity developed soon after heating. The variation in peak viscosity among the varieties might be as a result of minor differences in the amylose content among the varieties (Nuwamanya *et al.*, 2010b).

5.4 Wheat-cassava flour

5.4.1 Proximate composition of wheat-cassava flour

The protein and gluten content of the wheat cassava composite flour decreased with increase in cassava flour substitution as shown in Table 4. Cassava has no gluten and therefore its progressive increase at each substitution level was the reason for the decrease in the gluten content of the wheat-cassava composite flour. Similarly, cassava has very low protein content of 3- 4 % (Apea-Bah *et al.*, 2011) when compared to wheat flour which has protein content of between 9-13% (KEBS, 2009) and therefore the increase of cassava in the composite flour results in decrease of the overall protein content. However, from the results, substitution with cassava flour up to 20% would give good quality bread that meets the Kenyan standards that requires protein content of above 11% in flour used for baking bread (KEBS, 2009).

The moisture content and water activity also decreased with increase in substitution of wheat with cassava flour. This can be attributed to the differences in composition of wheat and cassava flour whereby wheat has more proteins and fats compared to cassava flour thus able to retain more moisture. The quantity of moisture and water activity plays a significant role in the spoilage of the bread because most of the spoilage microorganism will grow rapidly at higher moisture content and higher water activity (Banwart, 1989).

5.4.2 Rheological properties

The rheological properties of the dough; the length, height and deformation energy of wheat-cassava dough decreased with increase in substitution of wheat with cassava flour as shown in Table 5. The length represented the extensibility of the dough, the height represented the strength of the dough while deformation energy represented the energy required to bread a bubble of the dough. The decrease in the rheological properties of the dough with increase in substitution

levels of wheat with cassava flour indicate that baking quality of the dough was decreasing which resulted into weaker dough because the gluten levels were diluted Ribotta *et al.*, (2005). Wheat flour contains more gluten which contributes to elasticity of the dough by trapping carbon dioxide produced by yeast during fermentation Mepba *et al.*, (2007) and this is the reason why the bakers' flour had the highest values for dough extensibility, dough strength and deformation energy when compared to the composite dough.

5.5 Wheat- cassava composite bread

5.5.1 Physical properties of wheat-cassava bread

The specific volume and form ratio reduced significantly with increase in substitution of wheat with cassava flour as shown in Table 6. This reduction of specific volume and form ratio with increase in cassava flour can be attributed to dilution of gluten protein which is present only in wheat flour and is responsible for rising the dough during proofing and thus disruption of its rheological and mechanical properties (Schoenlechner *et al.*, 2013). The specific volume and form ratio are important quality parameters that influence consumer acceptability of the bread (Onyango *et al.*, 2015). *Selele* variety produced bread with better specific volume and form ratio compared to MH95/0183 and MH95/0193. Therefore the variety of cassava used had significant effects on the physical properties of the bread.

5.5.2 Effects of substitution level of the wheat cassava bread

Substitution of wheat with cassava flour significantly affected the loaf volume, shape and colour as shown in Figure 6. The change in volume and shape can be attributed to the decrease in gluten content in the flour which is responsible for increase in volume of the dough during fermentation due to accumulation of carbon dioxide gas. Substitution of wheat with non-wheat flour reduces and disrupts the formation of visco-elastic network that hinders entrapment of carbon dioxide within the particles thus leading to increased crumb firmness (Onyango *et al.*, 2015) which breaks easily. Substitutions of cassava up to 15% also produced bread with a better crumb firmness. Crumb firmness is also an important factor to consider when slicing the bread. When the firmness is increased it becomes difficult to slice the bread because it breaks easily. Increasing cassava flour causes significant decrease in the volume of the bread which is an important feature that the customer looks at before buying the product. The colour of the bread

changed from dark brown to light brown, this was due to decrease in protein content which is responsible for maillard reactions that lead to formation of a brown colour (Stadler *et al.*, 2002).

5.5.3 External sensory loaf characteristics of wheat-cassava composite bread

The results on external loaf characteristics (Table 7); loaf shape, texture, crust color and aroma showed that they were significantly affected by the rate of substitution of wheat with cassava flour and the variety. This can be attributed to the fact that increase in cassava flour in the composite flours reduced the protein and gluten content that are responsible for browning of bread through maillard (Stadler *et al.*, 2002) reaction and also bread volume which affects the external loaf characteristics. These results were similar to a study done by Masamba and Jinazali, (2014) who found out that the bread colour and texture decreased significantly with increase in substitution of wheat flour with cassava flour.

5.5.4 Internal sensory loaf characteristics of wheat-cassava composite bread

Significant differences were observed in the internal loaf characteristics; crumb colour, crumb softness and taste (Table 8) of bread produced from 100% wheat flour and bread made from the different cassava substitutions. The variety of cassava also affected the internal loaf characteristics with *Selele* variety having the highest liking of up to 15% followed by MH95/0193 at 10% and MH95/0183 at 5% substitution. This can be attributed to the composition of cassava flour which is less in proteins and fat that is useful in baking. These results were similar to another study that concluded that there was a general decrease in sensory scores such as aroma and taste with increase in the substitution level with cassava flour (Oluwamukoni *et al.*, 2011).

5.5.5 Overall acceptability of wheat-cassava composite bread

From the results the overall acceptability of the bread was significantly affected by the rate of substitution of wheat with cassava flour and variety. The bread made from 100% wheat flour was more accepted than bread made from the different cassava substitutions. However, there were no significant differences in the acceptability of 100% wheat bread and bread made from up to 20% substitution with flour from *Selele* variety and 15% from MH95/0193 and MH95/0183. The decreased overall acceptability of the wheat-cassava composite bread can be attributed to the fact that external and internal characteristics were affected by increased cassava flour in the bread.

These results were similar to a study done by Masamba and Jinazali, (2014) who reported that the acceptability of bread decreased with increase in wheat substitution with cassava flour.

5.5.6 Principal component analysis (PCA)

From the PCA results shape, texture and crust colour of the bread were the major factors the panelist used to score the bread as shown in Table 9. The results shows that external loaf characteristics are major factors that determine consumer acceptability of the bread and that substitution of cassava up to 15% will produce good quality wheat-cassava bread that is acceptable to the consumers. The second principle component include attributes like crumb softness, aroma and taste which are the internal loaf characteristics. Therefore this study shows that the external loaf characteristics are the most important attributes the bakers should put in mind while baking bread so that the consumers can accept the product.

5.6 Shelf life determination of wheat-cassava composite bread

5.6.1 Biochemical composition of wheat-cassava bread

The water activity of the wheat-cassava bread decreased with increase in substitution of wheat with cassava flour as shown in Table 10. This can be attributed to the fact that wheat flour has high content of protein than cassava flour thus making it trap more water. Water activity is an important factor that influences the growth of microorganism in food products therefore influencing the shelf life of the food product. Most microorganisms will grow at higher levels of water activity. The pH also decreased with increase in substitution of wheat with cassava flour. pH is also an important factor that affects the growth of microorganism. Different microorganism will grow at different pH values. The moisture content was not affected by the level of substitution of wheat with cassava flour. However there were differences in the moisture content of the different bread samples. This can be due to different evaporation. of water during baking

5.6.2 Total viable counts (TVC)

The rate of growth of microorganism (TVC) reduced with increase in substitution of wheat flour with cassava flour as shown in Table 10. This can be attributed to the fact that the water activity also had the same trend thus favouring growth of more microorganisms at high water activity. Microorganisms respond differently to water activity, pH and moisture content depending on microbial growth and the production of microbial metabolite. Microorganisms generally have optimum and minimum levels of a_w for growth depending on other growth factors in their

environments. For example, gram negative bacteria are generally more sensitive to low aw than gram positive bacteria (Banwart, 1989). *Bacillus subtilis* caused ropiness in bread which was characterized by color change from brown black, a rotten fruit like odor and stringy bread crumb

5.6.3 Yeasts and moulds

The growth of yeasts and moulds also decreased with increase in substitution of wheat with cassava flour as shown in Table 12. This can also be attributed to the decrease in the growth factors; water activity and pH. Normal cooking temperatures usually destroys fungal spores however, if the post handling of the baked product is not done in the right way it thus contributes to contamination of the bread thus needs to be controlled (Saranraj, 2012).

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1 CONCLUSIONS

1. Out of fourteen varieties that were grown in Migori County the following were more common; *Selele*, *Mary go round*, MH95/0183, MH95/0193. *Mabul*, *Arama* /and *Migyera*.
2. Variety and maturity stage during harvesting had significant effects on physico-chemical and functional properties of cassava flour. *Selele*, MH95/0183 and MH95/0193 varieties had the best physico-chemical and functional properties compared to the other varieties.
3. Cassava flour obtained at 12 months after harvesting on average had better flour yields and functional properties and processing of cassava tubers into high quality cassava flour (HQCF) significantly reduces hydrogen cyanide to safe levels.
4. Substitution of baker's wheat flour with cassava flour decreased the protein and gluten content of the flour with increase in substitution. Cassava varieties also affected the rheological properties and physical properties of the bread. External loaf characteristics played a major role in the acceptability of wheat-cassava bread compared to the internal loaf characteristics.

6.2 RECOMMENDATIONS

The study recommends the following;

1. More research on the different cassava varieties grown at different locations needs to be done in order to characterize all the varieties in Kenya
2. Development of other products like cakes, crisps and buns from wheat - cassava composite flour from these cassava varieties should be done.
3. Use of bread preservatives and bread improvers can be studied in order to increase the shelf life and the quality of wheat-cassava composite bread.
4. Further research to enrich cassava flour with protein can also be tried to improve the quality of bread and also maybe increase the substitution level of cassava flour.

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APPENDICES

Appendix 1: Questionnaire of varieties of cassava grown in Migori County

Serial no.

1.0 GENERAL INFORMATION

- a. Name of the farmer.....
- b. Gender Male..... Female.....
- c. Sub County..... Location..... Village.....

2.0 which variety(s) do you grow on your farm?

.....
.....

Reason(s) for growing these varieties.....

.....

3.0 At what maturity stage do you harvest your cassava?

.....

Reason(s).....

.....

4.0 How do you utilize your cassava after harvesting?

.....

.....

Reason(s).....

.....

Appendix 2: Sample preparation table

| SAMPLE | MH95/0183 | MH95/0193 | SELELE | WHEAT |
|---------------|------------------|------------------|---------------|--------------|
| | % | % | % | % |
| Control(A1) | 0 | 0 | 0 | 100 |
| B1 | 5 | | | 95 |
| B2 | 10 | | | 90 |
| B3 | 15 | | | 85 |
| B4 | 20 | | | 80 |
| B5 | 30 | | | 70 |
| C1 | | 5 | | 95 |
| C2 | | 10 | | 90 |
| C3 | | 15 | | 85 |
| C4 | | 20 | | 80 |
| C5 | | 30 | | 70 |
| D1 | | | 5 | 95 |
| D2 | | | 10 | 90 |
| D3 | | | 15 | 85 |
| D4 | | | 20 | 80 |
| D5 | | | 30 | 70 |

Appendix 3: Bread sensory evaluation score card

Panelist name

Date.....

Using the score card below, please examine the bread samples in terms of loaf symmetry, texture, crust color, crumb color, taste, aroma and overall acceptability. The numbers in brackets represent the maximum scores that can be awarded for each attribute.

| Sample Code | External Loaf Characteristics | | | Internal Loaf Characteristics | | | | |
|-------------|-------------------------------|-------------|-----------------|-------------------------------|--------------------|-----------|-----------|---------------------------|
| | Loaf shape (5) | Texture (5) | Crust Color (5) | Crumb Color (5) | Crumb Softness (5) | Aroma (5) | Taste (5) | Overall Acceptability (5) |
| 1. | | | | | | | | |
| 2. | | | | | | | | |
| 3. | | | | | | | | |
| 4. | | | | | | | | |
| 5. | | | | | | | | |
| 6. | | | | | | | | |
| 7. | | | | | | | | |
| 8. | | | | | | | | |
| 9. | | | | | | | | |
| 10. | | | | | | | | |

Key: 5-Like extremely, 4-Like, 3- Neither Like nor Dislike, 2-Dislike, 1-Dislike Extremely

Comments.....

Appendix 4: Physical and functional properties of cassava

| Source | DF | DM | C1 | C2 | SP | WBC | GT | PV |
|--------------------|----|-----------------------|----------------------|---------------------|----------------------|---------------------|----------------------|----------------------|
| Variety | 6 | 105.40 ^{***} | 11.65 ^{***} | 0.66 ^{***} | 9.60 ^{***} | 1.20 ^{***} | 21.70 ^{***} | 55.95 ^{***} |
| Maturity | 4 | 122.19 ^{***} | 11.88 ^{***} | 0.83 ^{***} | 20.34 ^{***} | 4.80 ^{***} | 26.34 ^{***} | 5.47 ^{***} |
| Variety(Maturity) | 9 | 16.73 ^{***} | 8.82 ^{***} | 0.80 ^{***} | 13.19 ^{***} | 0.35 ^{***} | 24.08 ^{***} | 17.78 ^{***} |
| Standard Deviation | | 0.41 | 0.12 | 0.02 | 0.36 | 0.091 | 0.76 | 0 |
| R ² | | 0.99 | 0.99 | 0.99 | 0.98 | 0.98 | 0.95 | 1 |
| C.V | | 0.99 | 1.88 | 1.49 | 2.36 | 4.27 | 1.34 | 0 |

^{***}; Significant at P<0.0001, DF; Degree of freedom, DM; Dry matter content, C1; Cyanide content of fresh cassava tubers, C2; Cyanide content of cassava flour, SP; Swelling power of cassava flour, WBC; Water binding capacity of cassava flour, GT; Gelatinization temperature of cassava flour, Peak viscosity of cassava flour,

Appendix 5: Publication



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Effect of different cassava varieties (*Manihot esculenta*) and substitution levels in baking of wheat-cassava composite bread on physical properties and sensory characteristics

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ABSTRACT

The ever increasing worldwide cost of wheat flour used in the baking and confectionery industries has necessitated the search for alternative cheaper flour from the locally available crops. Cassava crop (*Manihot esculenta*) is one of such crops that have been identified because its flour has great potential to be utilized as a partial substitution of wheat flour. This study investigated the effect of different varieties and flour substitution levels of cassava with wheat flour in baking of wheat-cassava composite bread and benchmarked with attributes of common wheat bread on the market. Three cassava varieties; MH95/0183, MH95/0193 and *Selele* were used in the wheat-cassava flour blending at different ratios; 95:5, 90:10, 85:15, 80:20, 75:25 and 70:30 whereas the bread from baker's flour was used as the control. Baking was done using Pup –loaf method. The proximate composition of the flour blends and alveograph properties; length (dough extensibility), height (dough strength) and W (Deformation energy) of the dough blends were determined. The specific volume and form ratio of the breads was calculated whereas the sensory evaluation of bread was carried out using 25 semi-trained panelists. The study found out that the proximate components of the blended breads reduced with increase in cassava substitution for all the cassava varieties. Composite flour with MH95/0183 were found to have better alveograph properties while composite bread with *Selele* had the highest specific volume and form ratio and sensory properties. Bread made from 5%, 10% and 15% cassava flour didn't have significantly ($P < 0.05$) different sensory properties from the control. The external loaf characteristics were the major factors the panelist used to rate the acceptability of the bread. Results of this study show that cassava flour can be used in the reconstitution of bread so as to reduce costs.

Key words: Cassava, Bread, Varieties, Substitution level, Sensory evaluation