DETERMINATION OF OPTIMUM HARVESTING TIME AND DEVELOPMENT OF CASSAVA ROOT- LEAF FLAKES WITH IMPROVED PROTEIN, MINERALS AND VITAMINS

BY

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DECLARATION

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DEDICATION

I dedicate this work to my late mother Naomi Nyevu Bulushi for the confidence she imparted in me to pursue the desires of my heart even from a tender age and to my children Joseph Kitole, Jolline Nyevu, Joan Nyevu and Josephine Umazi for their total support and patience, and the encouragement they accorded me during the time of studies.

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LIST OF ACRONYMS AND ABBREVIATIONS

ASAL - Arid and Semi-Arid Lands

AV - Acid Value

AOAC - Association of Official Analytical Chemists

DM - Dry matter content

Dwb - dry weight basis

FAO - Food and Agriculture Organization

HCN - Hydrogen Cyanide

IFDIC - International Fertilizer Development Center

KARI - Kenya Agricultural Research Institute

KALRO - Kenya Agricultural and Livestock Research Organization

SAS - Statistical Analysis System

PV- Peroxide Value

PDA - spread plate Agar

PCA - pour plate agar

SIFOR - Smallholder Innovation for Resilience

WHO - World Health organization

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GENERAL ABSTRACT

The current study intended to bridge a gap where coastal Kenya contributes up to 27% of the cassava produced nationally, yet remains highly insecure in terms of food and nutrition. This is at an interface of scientific efforts that led to development of improved cassava varieties suitable for the region. The improved varieties were developed by Kenya Agricultural and Agricultural Research Institute (KARI currently known as KALRO). This study targeted to develop flakes from cassava roots and leaves. Two improved varieties- Karembo and Tajirika against one local variety - Kibanda Meno were evaluated for suitability. Cassava roots were harvested at crop age 3, 6, 9 and 12 months, while leaves for nutrients profiling, were harvested at 3, 6 and 9 months after planting. A total of 18 blends of flakes were formulated from a varied cassava leaf combinedwith root material. Blend 20% fresh leaf material combined with fermented root that emerged thebest preferred by panelists was further evaluated for its nutritional value, shelf life, and suitable packaging material. The results on roots showed significant ($p \leq 0.05$) interaction effect of varieties and crop age on nutrients. Karembo and Tajirika had peak dry matter of 43.42% and 41.42% respectively at 12 months while the peak for Kibanda Meno was 44.99 % at 9 months. Mean starch content on dry weight varied with variety; Karembo (91.3%), Kibanda Meno (93.4%) and Tajirika (85.5%). Karembo and Tajirika had peak carbohydrates at 12 months while the peak for Kibanda Meno was at 9 months. Vitamin C was highest at 6 months for Karembo (6.4 mg /100 g) and Kibanda Meno (6.6 mg / 100g). Tajirika had highest vitamin C (7.0 mg / 100 g) at 9 months. Iron was peak at 3 months for Karembo (3.63 mg / 100 g) and Tajirika (5.33 mg / 100 g) but at 9 months for Kibanda Meno (7.12 mg / 100 g). Peak Zinc was 2.1 mg /100 g for Karembo at 6 months while for Kibanda Meno (1.94 mg / 100 g) and Tajirika was (1.75 mg / 100 g) at 12 months. Hydrogen cyanide increased from 8.6 to 9.3 mg / kg in Karembo between 9 and 12 months, it decreased from 7.8 to 5.2 mg / kg and from 5.1 to 4.5 mg / kg in Tajirika and Kibanda Meno, respectively. Leaves profiling showed significant ($p \leq 0.05$) effect of crop age on leaf nutrients content. Nutrients peaks were; moisture content (74%) at 3 months in Karembo and Tajirika, 75% at 9 months for Kibanda Meno; dry matter (50%), at 6 months, across varieties; fat (1.0 g / 100 g), was highest in Kibanda Meno at 3 months; protein (36 g/100 g), was highest in Kibanda Meno at 9 months. Vitamin C was highest in Karembo (1236 mg / 100 g) at 9 months. Vitamin A was 190 to 638 mg /100 g, at 6 months across the varieties. Iron and Zinc were 10 mg / 100 g, peak at different crop ages. Cyanide was 7.4 mg / kg, peak at 9 months. Formulation of cassava root - leaf flakes was best accepted at 20% leaf component added on to fermented root. Nutritional value analysis showed that the blend had vitamins A and C improved by 353% and 53%, minerals- iron and zinc by 5.6% and 85% respectively and protein by 430% compared to flakes processed from 100% root. Results on storage and packaging, showed moisture content to be significantly influenced by packaging material as it increased during storage, across the blends, with highest levels (10.75%) registered in kraft material on day 3. Paper insulated polythene had highest nutrients' levels;- protein at 27.68 g / 100g vitamins A (576.85 mg / 100 kg), Zinc (1.17 mg /100 g), iron 3.69 mg /100 g), fibre 6.12 g /100 g by day 5. Fat was highest at 9.71 g /100 g in the plastic material. Acid and peroxide values gradually increased in all the packages from 0 to 3.6 mg KOH / g and 0 to 6.3 mg / kg respectively. Microbial counts showed paper insulated polythene to be efficient in microbial growth in both spread plate and pour plate methods. The study concluded that roots of varieties - Karembo and Tajirika can be harvested as from 9 months while Kibanda Meno - can be harvested as from 6 months after planting. Levels of leaves micro nutrients are higher over the tender age. Cassava root - flakes is preferred at 20% fresh leaf blended with fermented root material that has improved levels of vitamins, minerals and protein, compared to pure cassava root products. It is recommended that harvesting of both roots and leaves be staggered.

CHAPTER ONE
GENERAL INTRODUCTION

1. 1 Background Information

Agriculture is the backbone of Kenya's economy, employing over 80% of rural communities. It is the major source of food and / or a sole source of livelihood for Kenya's coastal communities (Mwamachi *et al*., 2005). The coast region, experiences harsh climatic conditions and registers high poverty levels. Fortunately, cassava (*Manihot esculenta Crantz*) grows in sub-optimal agricultural conditions and hence grows in diverse agro - ecological zones in the region. Cassava not only serves as a food but also doubles up as a cash crop with potential for industrial uses (KARI, 2009; FAO 2005) and is of great economic importance worldwide (FAO, 2011). According to a report by Kariuki *et al*., (2002), cassava is increasingly becoming an important food and cash crop in Kenya. In Western and coastal Kenya, cassava is second to maize in importance as a food crop (Kiura, 2006). It is a crop that has the potential of reducing poverty, categorically in Kenya since it does well in diverse agro-ecological zones.

The coastal region of Kenya majorly covers Kwale and Kilifi counties that according to government report, implies 71% of its communities are people living below poverty line. Prevalence of malnutrition is also high, with most affected being children and antenatal women. (Rao *et al*., 2011). The two counties lie in the Arid and Semi-Arid (ASAL) with a common phenomenon of erratic and unreliable. Cassava is the most popular as it is drought tolerant and less costly in terms of inputs requirements making the region to contribute up to 27% national cassava production. It is a food crop that is grown for its roots witch are rich in carbohydrates, thus supplying much needed household calories to over 500 million households in the tropical countries (FAO, 2005). Cassava roots are, however, of low nutritional value compared to cereal staples, apart from being an energy source. Cassava roots have approximately 30% starch, 1.4% proteins, 3.8% soluble dietary fiber and 0.6 % ash (Infante *et al*., 2012; Dischen *et al*., 2013) that are low as compared to staples like maize, sorghum and millet. Maize has higher nutrient contents, averagely; 73% starch, 5.8% protein, 0.8 fiber and 1.2% ash (FAO, 2003; Jocelyne et al., 2020) while millets and sorghums constituents are approximated 60.3% starch, 7% protein, 4.13% dietary fiber and 2.8% ash (Singh et al., 2012; Elshazali *et al*., 2011; Saleh *et al*., 2013). Low nutritional value in cassava roots makes households that continuously use it as staple food to be exposed to nutrient malnourishment especially protein deficiency (Kevin *et al*., 2010). Vitamin content and other nutrients such as iron are also low in the roots (Lancaster and Brooks, 1983) compared to leaves that have 26.8% protein, 24% starch, 6.4% ash and 10.0% dietary fiber (Montagnac, 2009; Fasae, 2009) and can be used to fortify the cassava root based diets. The leaves are good sources of protein, calcium, iron and vitamins which can provide valuable supplements to predominantly starchy diets. Cassava leaves compare favorably with other green leafy vegetables generally regarded as good protein sources (Montagnac, 2009). The amino acid composition of cassava leaves shows that, except for methionine, the essential amino acid values exceed those of the FAO protein reference and RDA (Montagnac, 2009; Lancaster and Brooks, 1983). Studies have shown that the proteins embedded in cassava leaves are equal in quality to the protein in egg (Montagnac, 2009; Lancaster and Brooks, 1983). Cassava leaves and roots therefore, if properly processed and developed into end products for human consumption, can provide a balanced diet that could protect millions of African children against malnutrition (FAO, 2001; Babaleye, 1996; Montagnac *et al*., 2009). The current study undertook to develop cassava root - leaf flakes with improved nutrients, shelf stable and high potential for commercial production. The development of the product targeted school going children and pregnant women to easily access nutrients, for better health.

1. 2 Statement of the Problem

World Health Organization (WHO) recognizes three main deficiencies with global significance that include deficiencies of vitamin A, Iron and Iodine, and protein (WHO, 2014). Other deficiencies are those that can have serious health implications if they are localized: vitamin D (rickets); vitamin B1 (beri beri); zinc (stunting) and niacin (pellagra). The world population at risk is estimated at 190 million of which 40 million are already deficient, including 14 million with eye lesions (WHO, 2014 Rivera *et al*., 2003). Iron deficiency, the cause of anemia is the most common and widespread nutritional disorder in the world affecting over 30% of the world population (WHO, 2014). Protein deficiency is widespread and serious nutritional disorder, present in adults as well as in children (Henley *et al*., 2010; FAO, 2015; Carvalho, 2001). Critical protein deficiency in children occurs in form of kwashiorkor, while protein - calorie deficiency occurs in form of marasmus. In Kenya, under-nutrition is high in young children particularly in north Eastern and coastal regions (MI, 2009; CBS, 2014). Prevalence of deficiencies is approximated at 84% for vitamin A 36.8% for Iodine; 69% anemia among children of age 5 to 6-year-old and 5.5% (anemia) for women across the regions (Klaver and Mwadime 1998; MI, 2009). Prevalence of underweight, stunting and wasting was noted to have persisted for over a decade and women were seen to be affected by persistent chronic energy deficiency and under-nutrition in 2005 (FAO, 2005).

The coast region contributes up to 27% of the national cassava production and cassava remains an important food crop with roots commonly consumed in pure form as boiled, roasted, fried chunks and crisps (Kiura, 2006). Locals limitedly consume leaves that are a rich source of proteins, minerals and vitamins (Montagnac, 2009). Formulations involving both the roots and the leaves for human consumption are lacking though they could improve the nutrient density of cassava products for both the rural and urban populations.

Efforts to develop new cassava varieties by Kenya Agricultural Research Institute (KARI) led to release of new varieties among them Karembo and Tajirika in 2008 (KARI, 2009; Gethi, 2008). The varieties were, however, developed for high yields and pests and disease resistance especially cassava mosaic and cassava brown streak diseases. The new varieties along with the local varieties have not been evaluated for suitability for product development (KARI, 2009). Nutrients development over maturity stages and nutrients status in both roots and leaves in these varieties have limitedly been determined.

The presence of unsafe levels of cyanide in the roots and leaves of some varieties of cassava is a major problem as cyanide is known to be toxic to humans. The level of cyanide in the roots and leaves across maturity stages of popular varieties of cassava grown in the coastal region of Kenya has hardly been addressed that prompted breeders (Gethi, 2008) to place a recommendation for the same. There is limited or no data on cyanide content in relation to maturity stages of the improved and local cassava varieties' roots and leaves grown in the coast region of Kenya. This information is important in the selection of suitable varieties and optimum harvesting time with regard to manufacturing of value added products.

There has been increased promotion of high yielding cassava varieties for production as a means towards food security; but cassava is highly perishable (IFDC, 2010; Kiura *et al*., 2006; Abong' *et al*., 2016) and postharvest loss reduction has remained a challenge to actors along the value chain (IFDC, 2010). Perishability coupled with bulkiness exacerbate transport costs, thereby reducing profit margins (IFDC, 2010). High perishability state of roots has made raising cassava to a market competitive crop not possible and postharvest loses have remained high. Selling of fresh roots - the common practice of most cassava famers has limited cassava to fresh markets. Currently, there is limited diversity of shelf stable cassava based products that are rich in nutrients, and have potential to improve the nutritional status of the coast region with a potential for commercial production as well.

1. 3 Justification of the Study

Efforts to achieve food and nutrition security require increased production and utilization of the available food crops where cassava and maize remain the most important food crops in Africa (Eke *et al*. 2012). Increased production alone without reducing postharvest losses still makes food and nutrient security far from being achieved (Brimer *et al*., 2013). Postharvest loses remain high for most agricultural commodities but processing technologies have proved effective for reduction (Brimer *et al*., 2013). For the case of cassava, processing is important both for postharvest loss reduction and reduction of hydrogen cyanide to safe levels (Brimer *et al*., 2013). The current study undertook to develop cassava root - leaf flakes that is shelf - stable, rich in nutrients with potential for commercial production, targeted for income generation if adopted for processing by the cassava processing producer groups in the coast region of Kenya, a region known to have high poverty levels. The product with improved nutrients was primarily targeted to allow for school going children and pregnant women to easily access the imbedded nutrients. Where also, with improved incomes, food and nutrition insecurity may be reduced to some extent. Utility of cassava cannot be over emphasized and increased utilization has potential to trigger demand and consequently influence commercial cassava processing (Eke *et al*., 2012). Utilization can be increased when there is a wide diversity of products in the market.

Cassava roots are recommended to be harvested at 12 months after planting (Muli *et al*., 2008; Nhassico, 2008) but this only applies under ideal food security situations. Under food shortage, farmers start harvesting cassava as early as three or six months after planting when they notice cracks on the ground (FAO, 2005). The study undertook to determine the trend of nutrient development across the growing period (cassava field period) in order to highlight the effect of the early harvesting of cassava roots as well as identify the nutrients peaks suitable for harvesting. The work was carried out in the coast region of Kenya.

Most cassava products are processed from pure form of cassava roots processed into dried chips, flour, starch, crisps and animal feeds (Kariuki *et al*., 2006). Literature however shows that protein and fat content in roots is low at about 1- 2 % and 0.3%, respectively, but the leaves are, rich with crude protein surpassing that of legumes and leafy legumes. The crude protein content in cassava leaves also compares well to that of fresh eggs. The leaves are also rich in minerals at the rate of 2 to 5 times higher than that of the roots. The leaves have a greater concentration of calcium - 100 times higher than in roots and the phosphorus content is 2 to 3 times higher in roots than in the leaves (Montagnac, 2009). Vitamins and minerals are also high though with some variations in cassava leaf meal (CLM) on dry matter basis. Indeed, thiamin and niacin contents are 4 to 5 times higher in leaves than in roots, and riboflavin and vitamin C are 10 to 12 times higher in the leaves. Vitamin A in the form of pro-vitamin A carotenoid is of high quantity. The leaves from cassava varieties grown in the coast region were profiled in order to determine nutrients levels that can be documented to entice cassava leaves utilization in a region that is nutrient insecure.

Dry processed food products are prone to moisture absorbance that leads to spoilage over period of storage time (Chang *et al*., 2000). Storage temperatures together with type of packaging materials used help preserve processed products for prolonged shelf life. Cold temperatures are known to preserve commodities better however dry processed products have relatively long shelf life even when stored under room temperatures. It is also expensive and almost un-affordable for communities and or firms to establish and maintain cold rooms, as well as it is un-economical to store dry products under cold temperatures. The suitable packaging material, shelf-life of cassava roots - leaf flakes were therefore determined under room temperatures.

1.4 Objectives

1.4.1 Overall objective:

To determine the optimum harvesting time and to develop flakes from cassava roots and leaves with improved protein, minerals and vitamins contents for improved food and nutrition security.

1. 4. 2 Specific objectives:

(i). To determine peak nutrients and cyanide content in 4 maturity stage/s of cassava roots from 3 selected popular coastal cassava varieties

(ii). To profile nutrients from cassava leaves of 3 selected varieties in coastal Kenya

(iii). To develop cassava root - leaf flakes with improved protein, vitamin C and A and minerals Iron and Zinc

(iv). To determine shelf-life of cassava root - leaf flakes.

1. 5 Hypotheses

(i). There is no variation in nutrient and cyanide content of roots across maturity stages in cassava varieties grown in coastal region of Kenya.

(ii). Cassava leaves from the popular coastal varieties are of low quality due to the harsh climatic condition experienced in the region

(iii). Inclusion of cassava leaves in flakes has no effect on the quality of cassava flakes

(iv). Packaging materials have no effect on flakes' shelf-life

CHAPTER TWO

LITERATURE REVIEW

2. 1 Climatic Conditions in Arid and Semi-Arid (ASAL) Regions

Cassava is the most important food in terms of caloric intake (Rosenthal *et al.*, 2008). Rice, wheat, and maize account for only one third of total calories consumed in sub-Saharan Africa (FAO, 2011). Most people in this region derive about 1000 calories a day (about 50% of food intake) from cassava (Nweke, 1996; FAO, 2011). While cassava continues to be a vital subsistence crop for small scale farmers, it is also an increasingly important crop at regional and global scales for industrial uses (Rosenthal *et al.*, 2008). In the coast region of Kenya where agricultural climatic conditions are harsh and unfavourable for production of maize, cassava is grown in diverse agro ecological zones from zone CL 2 to zone CL5. This makes the region to contribute 30% of the national cassava production (Mwamachi *et al.*, 2005). Cassava leaves and shoots are also widely eaten as vegetable (Ogunniyi, 2011; Wobeto, 2006). Cassava is gradually being transformed from a famine reserve commodity and rural staple food to a cash crop for urban consumption (Philips *et al.*, 2004). This requires an intervention that will increase cassava based products availability and consumption, and or utilization within households and in the market as well. Making cassava products available in the market will be a means of response to the developing demand in the urban (FAO, 2006). The project undertook to develop a new product that has potential for nutritional improvement as well as commercial production.

2. 2 Cassava as a Crop

Cassava is a root food crop that thrives in sub-optimal climatic conditions (Nweke,1996). It is ranked fifth in importance in the world but ranked 3rd in Kenya after rice and maize (FAO, 2005). It is ranked second after maize in western and coast regions of Kenya (KARI, 2013). In recent years the importance of cassava has gone a notch higher as it is drought tolerant in the face of global warming effects (FAOSTAT, 2013). It has also been highly ranked on the basis of its potential in industrialization (Bechoff *et al*. 2018).

Due to harsh climatic conditions the region registers poverty levels that range from 13% to 90% at sub-county level, across all the 140 sub-counties (World Bank, 2016). Prevalence of malnutrition especially in children due to nutrients deficiencies is also high. Some of the symptomatic deficiencies found in the region include kwashiorkor - (protein deficiency), anemia - (iron deficiency) and growth retardation (zinc deficiencies) (FAO, 2005). Vitamin C also known as, ascorbic acid, is an antioxidant. It plays a vital role in protecting the body from infections and especially skin diseases like scurvy. It is a nutrient that is not synthesized by the human body and therefore it must be acquired from dietary sources. (Naidu, 2003).

Cassava remains an important food crop in the coastal region of Kenya, ranked second after maize (FAOSTAT, 2013). The crop has successfully been incorporated into many farming systems (FAO, 2005; FAO, 2005). This has made cassava to be very popular among smallholder farmers. It is one of the traditional crops, and it is significant in coastal dishes and menus (Githunguri, 1995). The coast region experiences unfavorable temperatures and rainfall but cassava thrives in sub optimal conditions. This makes it the only food crop of hope to farmers. It therefore has the potential to provide food to rural households during harsh weather conditions when other food crops have failed (Imungi *et al*., 1989; FAO, 2003).

Cassava breeders focus on agronomic traits Bechoff *et al*., (2018) that include disease and pest resistance and improvement of yields in selected varieties. KARI. (2009) report proves the same as the newly released varieties were reported to have been evaluated in the national performance trial on basis of the three parameters as mentioned above - (pest and disease resistance and high yield). Scientific attention is mostly drawn towards dry matter hence limited research has been conducted to evaluate the quality (in terms of nutrients content) of cassava roots harvested earlier than recommended, especially for the newly released varieties. The parameter that has been studied most in research on cassava root quality is dry matter content, whereas the roots also have other components that include carbohydrates, fiber, and ash among others as reported by (Motagnac, 2009).

A study by Hidayat. (2002) who studied ninety-nine varieties of cassava observed that there was significant correlation between plant age and cyanide potential of roots and leaves. He reported cyanide content as higher in younger leaves compared to older ones. This is an indicator that cyanide potential of the crop drops as plant ages. Chotineeranati (2006) also observed a similar trend in his studies on cassava age in months after planting and cyanide content. Gomez in (1985) found cassava cultivars harvested at 6, 8, 10 and 12 months to show lower leaf and rootparenchyma dry matter contents, lower crude fiber in the roots and the leaves at younger age. Crude protein levels in root peel, were lower but sugar content higher while there was lower cyanide concentration in root tissues (Gomez *et al*., 1985). Most of the parameters studied were affected by their cultivar as well as by plant age (FAO, 2003).

2. 3 Cassava Roots

Cassava roots are composed of flesh and peel. The edible fleshy portion makes up 80 - 90% of the cassava roots while the peel makes up 10 - 20% (Aro *et al*., 2010). Fresh roots are composed of about 30% starch giving the highest yield of starch per unit area of any crop known (Tonukari, 2004). The flesh of the tuber is composed of about 62% water and 35% carbohydrates (Motagnac *et al*., 2009). The roots are energy dense and twice rich in carbohydrate than leaves (Motagnac *et al*., 2009). Eighty percent of the carbohydrates in the roots are starch (Gil and Buitrago, 2002); 83

% is in the form of amylopectin and 17% is amylose (Rawel and Kroll, 2003). The glycolipids are mainly galactose - diglyceride (Gil and Buitrago, 2002). Cassava leaves also have carbohydrates but the content is lower measuring approximately 7 to 18g / 100 g and is comparable to that of green - snap beans (7.1 g /100 g), carrots (9.6 g /100 g), or green soybeans (11.1 g /100 g), and it is higher than those of leafy vegetables such as green leaf lettuce (2.8 g /100 g) and New Zealand spinach (2.5 g /100 g) (Gil and Buitrago, 2002). Protein and fat content in roots is low at about 1-2% and 0.3%, respectively (Tonukari, 2004).

Cassava roots also contain micronutrients that can be exploited to improve nutrient security for the cassava growing and/or consuming communities. In recent years, consumers and food processors have shown considerable interest in foods' nutritional quality, (Shobha *et al*., 2012). Exploitation of micronutrients especially in cassava roots can only be fully achieved if studies are carried out to determine their levels as well as identify the crop age at which they are at peak.

2. 4 Perishability of Cassava Roots

Cassava roots are highly perishable, which is a common characteristic of fresh agricultural commodities. Marketing of fresh cassava roots has proved to be a challenge due to bulkiness and high perishability (IFDC, 2010; USDA- NARCS, 2013). Processing the roots before marketing them is advantageous in terms of transportation and storability (FAO, 2010). In order to reduce post-harvest losses many different processing techniques have been employed to convert raw cassava tubers into various usable products that include; granules and flakes, flours, pastes and chips that are of a wide range. These products have different flavours and appearances for different markets (Brimer *et al*., 2013). The project undertook to develop a shelf stable ready to eat product as a means of diversifying cassava utilization, also providing means of nutrients access to urban families and cassava growing communities while targeting school going children and pregnant women. Other major beneficiaries in this study were to be women groups that can undertake cassava processing at a cottage industry level. The pregnant women and school going children would be able to accesses improved levels of protein, minerals iron and zinc and vitamins C and A in the formulated cassava root-leaf flakes.

2. 5 Cassava Leaves

The leaves are, rich with crude protein (26.8g./100) (Fasae *et al*., 2009; Arnieyantie, 2012) surpassing that of legumes and leafy legumes except for soybean that is approximately 13.0/ 100g (Montagnac, 2009). The crude protein content in cassava leaves is comparable to that of fresh egg (Westby, 1988; Okigbo, 1980). Leaves are also rich in minerals (Bradbury and Denton, 2011; Austin *et al*., 2009). Mineral content of cassava leaves is 2 to 5 times higher than that of the roots Gil and Buitrago. (2002). The roots typically have more phosphorus, but the leaves have a greater concentration of calcium (Gil and Buitrago, 2002). Phosphorus content is 2 to 3 times higher in roots than in the leaves at 27 mg /100 g and 211 mg / 100 g respectively (Montagnac, 2009; Madruga, 2000) and Chavez *et al*., (2007) reported cassava leaves to be more concentrated in vitamins and minerals than the roots, though with some variations in cassava leaf meal (CLM) on dry matter basis (mg / kg). Nutrients reported were; - iron; 61.5 to 270, potassium 8 to 16.9, magnesium 2.6 to 9.7, copper 6.2 to 50, zinc 30 to 63, and manganese 50.3 to 263. While thiamin and niacin contents were reported to be at 4 to 5 times higher in leaves than in roots, and riboflavin and vitamin C being 10 to 12 times higher in the leaves. Gil and Buitrago. (2002) reported cassava leaves to have a high quantity of vitamin A in the form of pro-vitamin A carotenoid. Vitamin E, however, is low in both the leaves and roots. Arginine in leaves is relatively lower than in roots (Wobeto *et al*., 2006) but compares well with that of eggs (Salcedo *et al*., 2010).

Cassava leaves are rich in a range of the symptomatic nutrients including protein but they are underutilized (Achidi *et al*., 2005). Underutilization is a consequent result of limited information on the nutritional and safety qualities of the leaves. Bechoff *et al*., (2018) indicates that knowledge of health benefits of food and food products is key in influencing consumer preference whereas consumer preference influences consumption and / or utilization of products. The limited availability of scientific information on the leaves' nutritional and safety qualities therefore hampers their popularization. Determination and consequent availability of this information would be used to promote utilization of cassava leaves. Studies indicate that lately consumers and food processors are interested in understanding nutritional quality of foods and food products (Shobha *et al*., 2012). Currently consumers are keen to know the nutritional value of what they eat, for "you are what you eat."

Safety in utilization of cassava leaves is dependent on the levels of hydrogen cyanide that has been found to be poisonous when ingested in large quantities (Bokanga *et al*., 1996; Bradbury 2014).

2. 6 Hydrogen Cyanide

Both cassava roots and leaves contain cyanogenic glucosides that are presented in form of linamarin and lotaustralin. (Bradlbury *et al*., 1999). Linamarin is the toxic compound that produces toxic component in cassava - hydrogen cyanide (HCN) (Cardoso *et al*., 2005). Linamarinis the most representative glucoside accounting for about 80% of the total cassava glucoside (Cardoso *et al*., 2005). It is also the free form of cyanogenic glucosides (Cardoso *et al*., 2005). Linamarin is broken down by the enzyme linamarase also found in cassava tissues to release cyanohydrin, (Macrae *et al*., 1993; Montagnac, 2009). Cyanide in cassava is classified into two types: 1) bound cyanide; and 2) free cyanide present as the cyanohydrin which acts as free HCN gas above 26^{o}C (Bokanga *et al*., 1996). Studies on leaves nutrients and safety qualities in relation to cyanide levels are useful in the endeavor towards popularizing leaves consumption and /or utilization. The present study therefore undertook to profile moisture/dry matter, protein, fat, vitamins A and C, minerals - zinc and iron and hydrogen cyanide in cassava leaves across field periods of 3, 6, and 9 months after planting, for three popular cassava varieties grown in the coast region of Kenya.

2. 6. 1 Hydrogen cyanide levels in cassava

Cyanide is a toxic component found both in cassava roots and leaves (Kwok, 2008). Cyanogenic glucosides are the bound forms of hydrogen cyanide that occur as linamarin and lotaustralin (FAO, 2007). Cyanogens are widely distributed throughout the plant, with large amounts in the leaves and the root cortex (skin layer), and generally smaller amounts in the root parenchyma (Cardoso *et al*. 2005). Cassava varieties have been recognized under sweet and bitter categories: those which can be safely eaten without processing, and are referred to as sweet type and those that when eaten raw, boiled or roasted without prior processing, are dangerous to humans and animals on account of their cyanogenic potential. Their cyanide content is mostly more than 50 ppb and are referred to as bitter type (Eke *et al*., 2012; USP 2010). When cassava tissues are damaged linamarin gets exposed to enzyme linamarase, (released upon tissue damage, as in many forms of processing), acetone cyanohydrin and glucose are produced (Ferguson and Kawuki, 2006: Zidenge *et al*., 2017; Libot *et al*., 2017) thus reducing the cyanogenic potential. Cassava processing therefore reduces cyanogenic compounds to safe levels when properly carried out.

In as much as cassava is known to contain hydrogen cyanide that is poisonous to both humans and livestock, Bechoff *et al*., (2018), there is still limited information on this compound concerning its concentration in cassava roots at various stages of growth specifically for newly released KARI varieties. Determination of hydrogen cyanide levels in cassava will enhance consumers' safety especially those that consume the varieties grown in coastal Kenya. Consumption of unsafe levels of hydrogen cyanide for prolonged periods has been associated with the Konzo disease which mainly affects children Topor *et al*., (2008). This study undertook to evaluate cassava root dry matter, carbohydrate, energy, starch, fiber, ash, protein, fat, hydrogen cyanide and selected micronutrients contents in relation to crop age.

2. 7 Processing Methods

Methods of processing cassava range between chipping roots and drying, steeping and fermentation (Quaye *et al*., 2009; Nweke, 1996). Steeping is however not very common as it requires substantial amount of water for it to be undertaken, more so, steeping is traditionally carried out on rivers Oyetayo, (20010) to allow the moving water to rinse out any anti- nutrients present in the roots (Hahn et al., (1986); Fasuyi *et al*., (2005) indicated that traditional methods such like drying, pounding and long periods of boiling could remove anti- nutrients in cassava. The ultimate goal/s in processing include (a) elimination of anti- nutrients, (b) improving shelf life (Quaye, 2009), (c) improving quality (d) improving palatability and general acceptability (e) bulkiness reduction and (f) product differentiation, among others. However, with cassava being known to contain anti- nutrients, major goals remain to be; elimination of the anti- nutrients and improvement of palatability, general acceptability and utilization (Montagnac *et al*., 2009). Wheatley and Chuzel. (1993) documented the objectives of cassava processing as to; (a) diversify the food and its uses that will lead to market expansion, (b) reduce bulk that will relatively reduce transport costs, (c) improve storability by space and time, and (d) reduce cyanide levels for safe consumption. Cassava leaves on the other hand are commonly processed through pounding using pestle and motor (Bradbury, 2014), then cooked or fermented. The current study undertook to developed cassava root - leaf flakes using fresh and fermentation methods in order to generate different blends of cassava flakes. The major steps followed in formulating the flakes are illustrated in a flow chart (Figure 1)

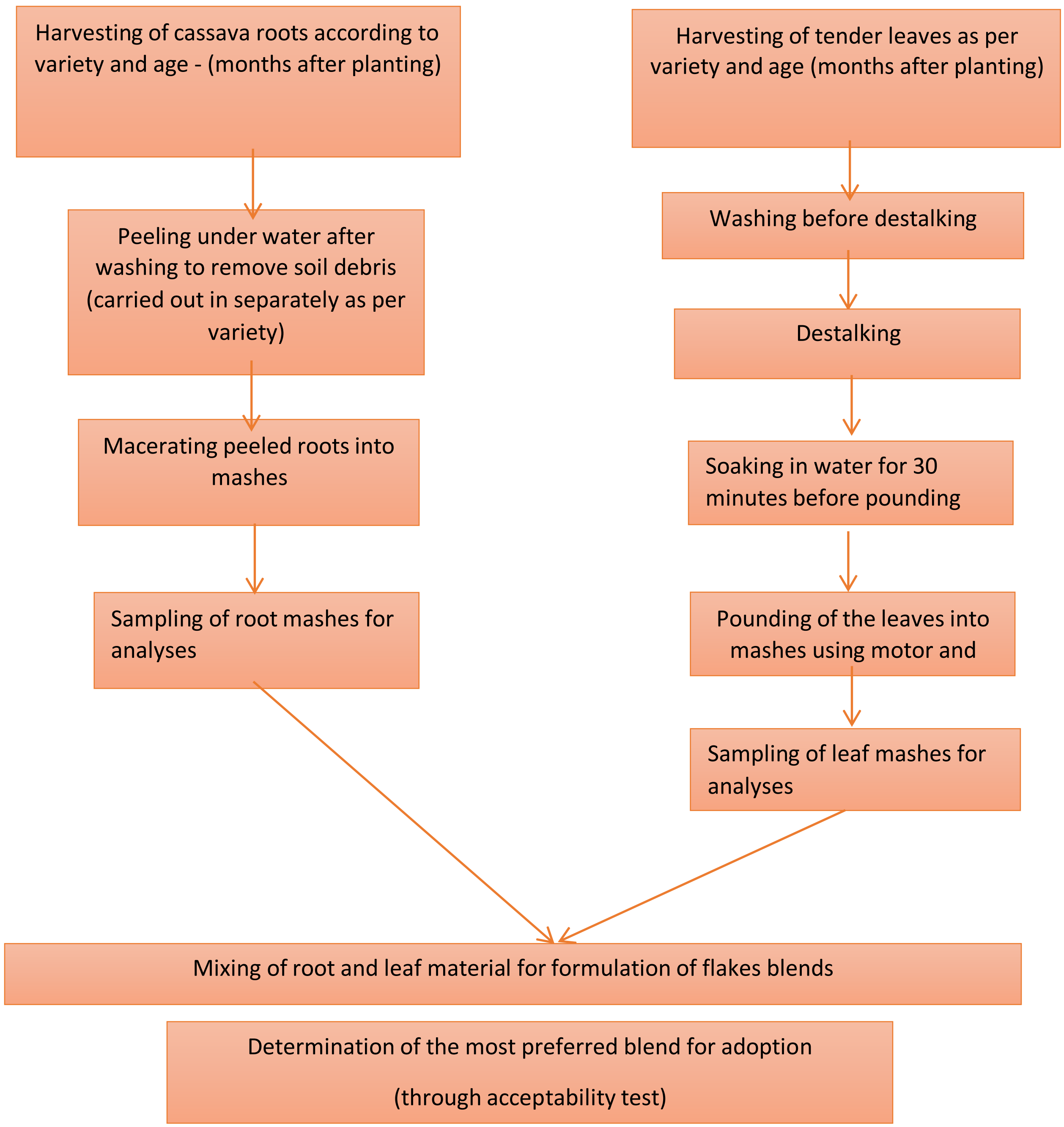

Figure 1: Major steps of cassava roots and leaves processing and, flakes formulation as described by Quaye *et al*., (2009) Bradbury, (2014) and Meilgaard, *et al*., (1999); respectively.

CHAPTER THREE

INFLUENCE OF CROP AGE ON NUTRITIONAL QUALITY AND HYDROGEN CYANIDE CONTENT OF ROOTS FROM POPULAR COASTAL KENYAN CASSAVA VARIETIES

Abstract

Cassava is an important crop in coastal Kenya commonly harvested at 12 months after planting but earlier during food shortages. There is limited information that guides on the peak period of nutrients that can guide on the suitable period of harvesting and / or addressing the effect of early harvesting on root quality especially for newly released varieties. Varieties Tajirika, Karembo and Kibanda Meno (roots) were harvested at different ages from coastal agricultural Research Centres, studied for nutritional quality and hydrogen cyanide levels for safe utilization. Results showed significant ($p \leq 0.05$) interaction effect of varieties and crop age on studied nutrients. Karembo and Tajirika had peak dry matter of 43.42 % and 41.42% respectively at 12 months while the peak for Kibanda Meno was 44.99% at 9 months. Mean starch content on dry weight varied with variety; Karembo (91.3%), Kibanda Meno (93.4%), and Tajirika (85.5%). Karembo and Tajirika had peak carbohydrates at 12 months while the peak for Kibanda Meno was at 9 months. Vitamin C was highest at 6 months for Karembo (6.4 mg /100 g) and Kibanda Meno (6.6 mg / 100 g). Tajirika had highest vitamin C (7.0 mg / 100 g) at 9 months. Iron was peak at 3 months for Karembo (3.63 mg / 100 g) and Tajirika (5.33 mg /100 g) but at 9 months for Kibanda Meno (7.12 mg /100 g). Peak Zinc was 2.1 mg /100 g for Karembo at 6 months while for Kibanda Meno (1.94 mg / 100 g) and Tajirika was (1.75 mg /100 g) at 12 months. Hydrogen cyanide increased from 8.6 to 9.3 mg / kg in Karembo between 9 and 12 months, it decreased from 7.8 to 5.2 mg / kg and from 5.1 to 4.5 mg / kg in Tajirika and Kibanda Meno, respectively. Harvesting of cassava roots should be

staggered across crop ages to maximize on peaks of different root nutrients and processing is important.

3. 1 Introduction

Cassava remains an important food crop in the coastal region of Kenya, ranked second after maize (FAOSTAT, 2013). The crop has successfully been incorporated into many farming systems (FAO, 2005; FAO, 2005). This has made cassava to be very popular among smallholder farmers. It is one of the traditional crops, and it is significant in coastal dishes and menus Githunguri. (1995). The coast region experiences unfavorable temperatures and rainfall but cassava thrives in sub optimal conditions. This makes it the only food crop of hope to farmers. It therefore has the potential to provide food to rural households during harsh weather conditions when other food crops have failed (Imungi *et al*., 1989).

In 2008, the Kenya Agricultural Research Institute released eight improved cassava varieties for production and consequent utilization by farmers in coastal Kenya KARI (2009). These varieties were bred for high yield, pest and disease resistance. Post-harvest issues were hardly considered. This is however a common phenomenon where breeders give more emphasis on agronomic traits for targeted varieties with limited studies on postharvest taken in to consideration Bechoff *et al*., (2018). This created information gaps that need to be addressed. For example, the new varieties still need to be studied to determine their nutrient build up trends- a recommendation documented by (Gethi *et al*., 2008).

Cassava roots are recommended to be harvested at 12 months after planting (Muli *et al*., (2009); Nhassico, 2008) but this only applies under ideal food security situations. Under food shortage, farmers start harvesting cassava as early as three or six months after planting when they notice cracks on the ground (FAO, 2005).

Cassava breeders focus on agronomic traits Bechoff *et al*., (2018) that include disease and pest resistance and improvement of yields in selected varieties KARI. (2009) report proves the same as the newly released varieties were reported to have been evaluated in the national performance trial on basis of the three parameters mentioned above - (pest and disease resistance and yield). Scientific attention is mostly drawn towards dry matter hence limited research has been conducted to evaluate the quality of cassava roots holistically and the effect of early harvesting than recommended, especially for the newly released varieties. The parameter that has been studied most in research on cassava root quality is dry matter content, whereas the roots also have other components that include carbohydrates, fiber, and ash among others as reported by (Motagnac, 2009).

Cassava roots also contain micronutrients that can be exploited to improve nutrient security for the cassava growing and / or consuming communities. In recent years, consumers and food processors have shown considerable interest in foods' nutritional quality, (Shobha *et al*., 2012). Exploitation of micronutrients especially in cassava roots can only be fully achieved if studies are carried out to determine their levels as well as identify the crop age at which they are at peak.

It is important to note that cassava is also known to contain hydrogen cyanide that is poisonous to both humans and livestock, Bechoff *et al*., (2018). There is still limited information on this compound concerning its concentration in cassava roots at various stages of growth specifically for newly released KARI varieties. Hydrogen cyanide is the bound form of glucoside produced by linamarin that is toxic to both human and animals (Cardoso *et al*., 2005). Determination of hydrogen cyanide levels will enhance consumers' safety especially the consumers of the cassava varieties grown in coastal Kenya. Consumption of unsafe levels of hydrogen cyanide for prolonged periods has been associated with the Konzo disease which mainly affects children Topor *et al*., (2008). This study evaluated cassava root dry matter, carbohydrate, energy, starch, fiber, ash, protein, fat, hydrogen cyanide and selected micronutrients contents in relation to crop age in months after planting.

3. 2 Materials and Methods

3. 2. 1 Acquisition of raw materials

Roots from three cassava varieties that are popular in the coastal region of Kenya were used for this study (plate 1). These three varieties were; Tajirika, Kibanda Meno and Karembo. Kibanda Meno is an indigenous variety that registers early maturity, high dry matter and sweetness that has led consumers to enjoy it when eating it raw, roasted or boiled (Mwamachi, 2005). Kibanda Meno is also known to be more resilient in drought resistance. It is the local variety that is commonly grown alongside the improved varieties as means of risk mitigation over crop loss (Wekesa *et al*., 2017). Though Kibanda Meno has low yield (2.02 t / acre) and susceptible to diseases as reported by Gethi *et al*., (2008), the coastal communities have remained persistent on their preference to grow Kibanda Meno (Mwamachi, 2005). Karembo and Tajirika are improved varieties that were bred and released by KARI in the year 2008. These varieties were bred for pest and disease resistance and also high yields. Karembo has a yield potential of up to 27 tons per hectare and Tajirika has yield potential of up to 25 tons per hectare (Muli *et al*., 2009).

The varieties: were obtained from mother blocks at three Kenya Agricultural and Livestock Research Organization (KALRO) Centres. The three Centers were; Masabaha, Matuga and Mtwapa. A total of 4 healthy plants were randomly selected and harvested at 3, 6, 9 and 12 months after planting. Harvesting of roots was carried out using a hoe. The hoe was used to carefully remove top soil in order to dig out the roots while ensuring minimal bruises. A total of 3 medium sized roots were harvested from each plant. The roots were then packaged in labeled polyethylene bags per variety and per age group. The labelled roots were then put in cooler boxes for overnight transportation to the University of Nairobi for analysis in the Food Science Laboratories. The roots were prepared by washing before and after peeling, and macerating using laboratory blender. Macerated root mashes were well mixed accordingly and sampled in replicates for analysis

3. 3 Laboratory analytical methods

3. 3.1 Determination of moisture /dry matter (DM) content

Moisture content determination was carried out using gravimetric method with a few modifications. About 5 g of macerated sample were weighed in crucibles and dried for 4 hours at 105 ^{0}C in an air oven as described by AOAC. (2001) method 925.10.

3. 3.2. Determination of starch content

Cassava starch content was determined by standard methods AOAC. (2012) method 985.29, with minimal modification where filtration of roots' mash to remove starch was done using a cheese cloth instead of a sieve with known mesh size. Before filtration, root mash had been mixed with distilled water at ratio of 2:1.

3. 3. 3 Determination of energy content

The energy content was obtained by multiplying the value of crude protein, fat and carbohydrate by factors of 4, 9 and 4 respectively as indicated by (Omosuli, 2014).

3. 3.4 Determination of crude fiber

Crude fiber was determined as described by AOAC. (2000) method 985.29. A duplicate 4 g sample of root mash was boiled for 30 minutes in 25 ml of 2.04 N H_2SO_4 diluted to 200 ml with boiling distilled water. The mixture was then filtered by use of a Buchner funnel slightly packed with glass wool. The collected residue was washed thrice with ethanol, followed with air oven drying at 105 ^{0}C for 2 hours then ashed at 550 ^{0}C in a muffle furnace.

3. 3. 5 Determination of ash content

Briefly 4 g sample of cassava root mash in duplicate was burnt in porcelain crucible using Bunsen burner (low flame) for 10 minutes then transferred to a Muffle furnace, ashed at 550 °C for 4 hours as guided in AOAC. (1995) method 923.03.

3. 3. 6 Determination of crude fat content

Briefly 5 g sample was extracted using soxhlet extractor for 8 hours using 200 ml petroleum ether (40 - 60 ^{0}C). Crude fat content was calculated after evaporating the solvent and the residue dried in an air oven at 105 ^{0}C for 1 hour as guided by (Mishra. (2017).

3. 3. 7 Determination of protein content

Protein content in cassava roots was determined as per AOAC. (2005) method 979.09.

3. 3. 8 Determination of hydrogen cyanide content

Hydrogen cyanide determination was carried out using distillation method. Cassava roots were macerated using a laboratory blender and samples of 10 g root mash was placed into distillation flask and allowed to stand for three hours before distillation. Distillation and consequent determination of hydrogen cyanide was carried out as guided in AOAC. (2016) method 915.03.

3. 3. 9 Determination of carbohydrates content

Carbohydrates were determined by difference. The total of moisture content, fat, ash, protein and fibre contents were subtracted from 100 as guided in FAO. (2003)

3. 3.9. 1 Determination of Vitamin C content

Approximately 15 ml (10%) TCA, was added into flat bottomed flask containing cassava roots mash and filtered. A total of 15 ml filtrate sample was collected. The filtrate sample was then mixed with 5 ml of 4% potassium iodide solution then titrated. The rest of procedure was followed as described in AOAC. (2013) method 967.21.

3. 3. 9.2 Determination of iron and zinc contents

Cassava root mash sample (4 g) for determining mineral Iron was ashed in a muffle furnace at 500^{o}C for 4 hours. Then digested by adding 10mls of 20% HCL to boiling, then filtered into 100 ml volumetric flask and topped to mark using distilled water. Using atomic absorption spectrophotometer (A.A.S) mineral iron and zinc was determined according to AOAC. (2016) method 99. 10.

3. 3 .9.2 Experimental design and data analysis

The statistical design used in the study was Complete Randomized Design, where varieties and crop growth stage were independent variables. Data were collected on the following parameters: Hydrogen cyanide levels, Carbohydrates in, Starch, Fiber and Dry matter, protein, fat and selected micronutrients. Results from laboratory analyses were subjected to the analysis of variance (ANOVA), using Statistical Analysis System (SAS version 9.1). Means were separated using Least Significant Difference (LSD), the differences being significant when $p \leq 0.05$.

3. 4 Results and Discussion

3. 4. 1 Dry matter and starch content

There was significant ($p \leq 0.05$) interaction between variety and crop age on dry matter, starch, carbohydrate, energy, fiber, ash and hydrogen cyanide Kibanda Meno contents. While varieties Karembo and Tajirika attained the highest root dry matter content (43.42% and 41.42% respectively) that significantly different ($p \leq 0.05$) at 12 months after planting, the local variety Kibanda Meno attained the highest dry matter content (44.99 %), 9 months after planting (Figure 2).

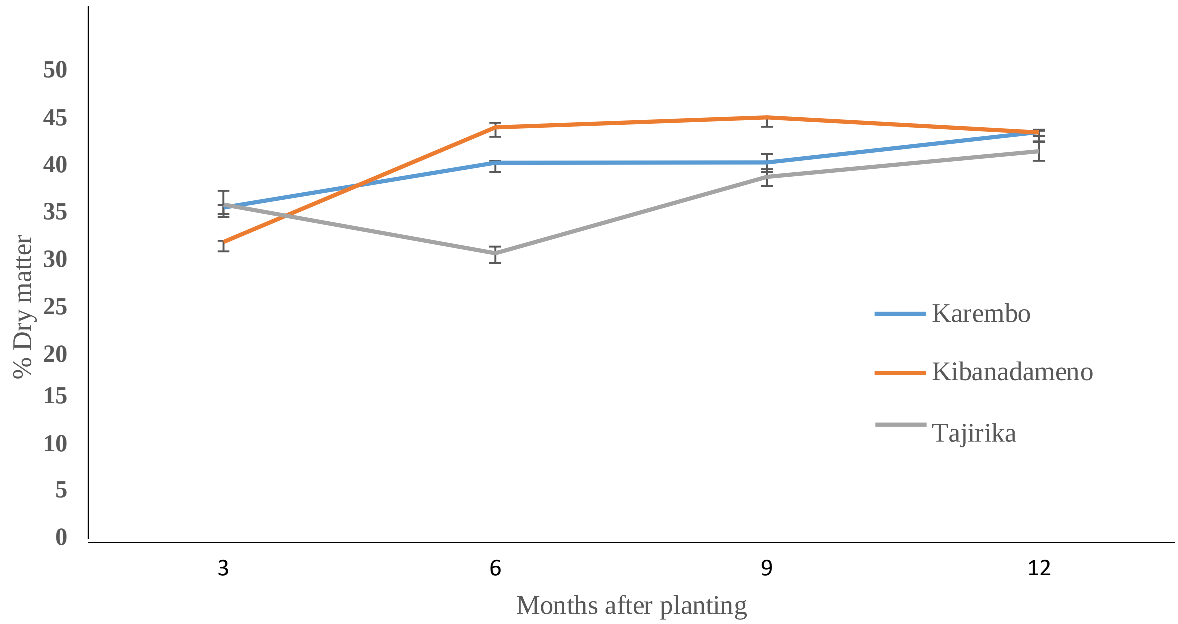

Figure 2: Dry matter content (%) of cassava roots as affected by crop age. The bars indicate standard error bars.

There was no significant difference ($p \leq 0.05$) in dry matter content for Karembo and Kibanda Meno harvested at either 6 or 9 months after planting (Figure 2). Tajirika harvested at 9 months after planting had 38.7% dry matter content as compared with 30.6% at 6 months. Between 9 and 12 months after planting, Karembo and Tajirika had accumulated 8.0% and 7% higher dry matter content, respectively. The dry matter content of Kibanda Meno dropped by 8% when left in the field beyond 9 months and harvested at 12 months after planting. Meanwhile Karembo has a stem that is characterized by short internodes (Muli *et al.*, 2009) and producers have adopted it for seed multiplication, a part from the high yields. Variety Tajirika has most of its traits almost similar to those found in the local variety Kibanda Meno, though its dry matter content is a bit lower. Tajirika however has long roots with medium thickness that make them preferred in the fresh market (Muli *et al.*, 2009).

These results indicate that farmers who use root dry matter content as the criterion for choice of cassava variety will still prefer the local variety, Kibanda Meno, since they would be able to harvest it as early as six months after planting especially at times of dire food shortages. However, the improved varieties Karembo and Tajirika give yields of up to 27 and 25 t $/ha^{-1}$ respectively compared to Kibanda Meno that gives 5 to 10 t $/ha^{-1}$ under similar conditions and cultural treatment. Therefore, farmers who adopt the improved varieties will be better off, even though they will have to wait a little longer to start harvesting their cassava. Dry matter content of the coastal varieties is higher compared to those reported by Montagnac (2009) and Benesi (2004) that ranged between 35% and 38%. It is important to note that dry matter in cassava plays a major role for a variety to be accepted for both consumption and production Bechoff et al., (2018). Dry matter is also the indicator component for higher starch and carbohydrates content that are sought for to supply the daily energy requirements for the cassava growing households and the cassava growing communities. The results in this study showed a 13% drop in dry matter at 6 months after planting of variety Tajirika (Figure 3). Agronomists have linked such scenario with growth pattern, especially when the crop is at its maiden stage of attaining crucial biomass to support its growth and or flowers. It is well demonstrated when cassava is cut back to allow for a ratoon crop, dry matter drops to non-palatable state as the new biomass establishes. Gethi. (2008) reported higher biomass at 6 months after planting of the improved varieties. However, this remains an information gap that could be established through research.

3. 4. 2 Starch content

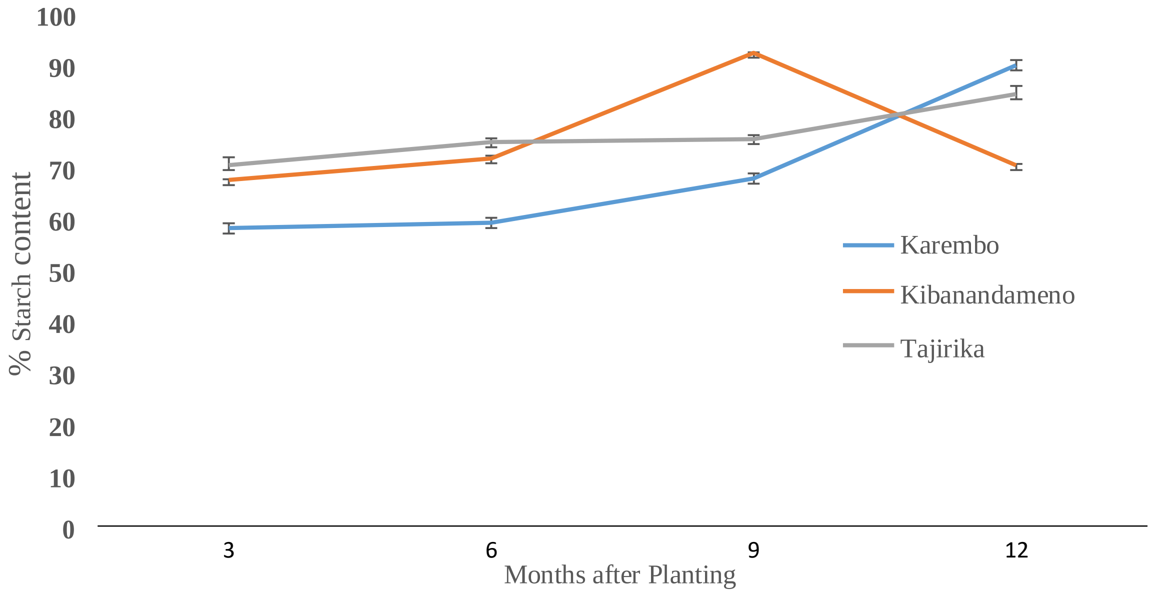

Figure 3: Variation of Starch content (% wet weight basis (wwb)) of cassava roots as affected by crop age. The bars indicate standard error of means.

There was significant ($p \leq 0.05$) effect of crop age on starch content in Kibanda Meno. The starch content of Kibanda Meno increased from 68.2% to 93% (on dry weight basis (wwb)) between 3 and 9 months after planting (Figure 3). Starch content in variety Kibanda Meno reduced by 3.3% when harvested at 12 months instead of 9 months after planting. On a wet weight basis (wwb), the results showed that the three varieties evaluated in this study had starch content ranging from 68.5% to 93% at 9 months after planting. These results compared well with those reported by Mouafi *et al*., (2018). Varieties studied by Montagnac *et al*., (2009) had starch content that correspond well with the results in the current study though they were reported on wet basis. Montagnac's varieties had starch content range of 32 - 35% - reported on wet weight basis.

Kibanda Meno had the highest starch content of 93% (wwb) compared to Karembo and Tajirika that had 76% and 69% (wwb) respectively when harvested at 9 months after planting. This scenario could be due to varieties' genetic characteristics and also as influenced by other factors including climatic conditions as postulated by Salvador *et al*., (2014); Bechoff *et al.*, (2018); Burns *et al.*, (2012). These values are relatively high in comparison to those reported by Eke *et al*., (2007) where 39 cassava varieties showed percent starch content that ranged between 20.2% and 31.8%. Inasmuch as the coastal region is known for poor soil fertility and harsh climatic conditions the varieties are competing well in starch build up with varieties grown under favourable conditions elsewhere. The trend in the current study also shows a consistent accumulation of starch content in roots through to 12 months after planting.

3. 4. 3 Proximate composition

There was significant ($p \leq 0.05$) difference in carbohydrate content accumulation for the improved varieties and Kibanda Meno - the local variety. The improved varieties had peak accumulation of carbohydrates at 12 months while Kibanda Meno had its peak at 9 months after planting. Crop age 6 to 9 months had significant ($p \leq 0.05$) effect on carbohydrate content of Kibanda Meno and Tajirika. Tajirika had the highest increase in this age range at 31.8%. The carbohydrate content of Karembo was 37.6% at 9 months and 40.5% (wwb) at 12 months after planting (Table 1). These results are within the range of carbohydrates contents that was reported by Richardson. (2013) who tested six mature improved varieties harvested at between 12 and 13 months after planting. The six varieties evaluated by Richardson. (2013) had carbohydrates content of between 26.3% and 39.6% (wwb). However, the nutritional content of cassava depends on geographic location, variety, age of the plant and the specific plant part (root or leaves) and environmental conditions Salvador *et al*., (2014); Bechoff *et al*., (2018); Burns *et al*., (2012).

Crop age had significant ($p \leq 0.05$) effect that resulted to change in energy content of cassava roots between 3 and 12 months after planting. There was 17.9%, 41.3% and 40.1% increase in energy (kcal / 100 g dry weight) content, for Karembo, Kibanda Meno and Tajirika, respectively during this period. Variety Karembo had 11% increase in energy content from 3 to 6 months after planting while Kibanda Meno and Tajirika had 38% and 17.1% increase respectively over the same period. Energy content in Kibanda Meno increased by 3% while energy content in Tajirikaroots increased by 7% between 6 months after planting and 9 - 12 months after planting, while variety Karembo added 9.4% energy content over the same period of time. These results show thatenergy content of coastal varieties is averagely higher compared to those varieties reported by Montagnac. (2009). At 12 months after planting energy contents of the coastal varieties were as follows: Karembo had 169.8 kcal / 100 g dry weight, Kibanda Meno had 155.7 kcal / 100 g dry weight and Tajirika had 161.2 kcal / 100 g wet weight. The varieties tested by Montagnac had energy content of between 110 and 149 kcal per 100 g dry weight. This is a positive indicator thatpopularizing cassava varieties in the coast region can help households acquire a bigger percentageof the daily required calories. This makes them more suitable for processing whole root product.

The change in fibre content was statistically significant ($p \leq 0.05$) across the three varieties. The three varieties showed to have built more fibre at 6 that continued to decline at 9 months after planting and reached to non-significance ($p \leq 0.05$) at 12 months after planting especially for Tajirika. At 6 months after planting, the fibre content of Karembo, Kibanda Meno and Tajirika increased by 53.5%, 41% and 49% respectively (Table 1). However, there was a decline in fiber content by 18%, 17% and 31% between 6 and 9 months for Karembo, Kibanda Meno, and Tajirika, respectively. The fiber content of Kibanda Meno increased by 30% between 9 and 12 months after planting. Tajirika had its fiber content decrease by 9% as it matured between 9 and 12 months after planting. The results indicate that the improved varieties (Karembo and Tajirika) become less fibrous when left longer in the field to be harvested at 12 months but Kibanda Meno accumulates more fiber when left to reach 12 months after planting. The fibre content in the coastal cassava varieties is rather low at 0. 9% to 1.3% (wwb) across the 3 varieties, as compared to results reported by Enidiok *et al*., (2008) who tested 7 cassava varieties from Ethiopian Agricultural Centre and found that fibre content in all of them ranged between 1.7% and 1.8%   However, Aileyegun *et al.*, (2017) reported fiber content in cassava ranging between 0.48 and 2.59% (wwb) in their studieson *gari*. The less fibrous cassava roots are the more palatable and well accepted by producers, consumers and processors Burns et al., (2012); Bechoff *et al*., (2018). The coastal varieties therefore would compete well with other varieties at farm gate, fresh or processing markets.

Ash content in the three varieties was between 0.67 to 0.98 in variety Karembo, 0.84 to 1.29 in variety Kibanda Meno and 0.65 to 1.03 in variety Tajirika. (Table 1). The results showed significant ($p \leq 0.05$) interaction between crop age and ash content. The change in ash content between 3 and 6 months after planting for variety Karembo was a 23.4% decline but crop age 6 to 9 months after planting showed a 2.7% increase in ash content. This is an indicator that variety Karembo's roots left in the field beyond 9 months after planting does not guarantee increase of nutrients. Variety Kibanda Meno had a significant ($p \leq 0.05$) change that was characterized with consistent increase in ash content of up 53.4%, from 3 to 12 months. Variety Tajirika showed a similar trend with Karembo where there was a significant ($p \leq 0.05$) decline of 36. 9% from 3 to 6 months after planting followed by 18.5% increase in ash content from 6 to 9 months after planting. From 9 to 12 months after planting Tajirika, however, showed 27.3% increased ash content (Table 1). However, the change in ash content for Tajirika was not statistically significant ($p \leq 0.05$). An increase in age from 3 to 6 months decreased the ash content by 23.5% and 36.9% in the roots of

Karembo and Tajirika, respectively, while the same increase in age increased the root ash content of Kibanda Meno by 12%. Variety Kibanda Meno had the highest ash content in comparison with the improved varieties. The results on improved varieties are high compared to those reported by Kalawole and Akigbala, (2010) where ash content was 0.54% (wwb) in roots harvested at 12 months and above after planting. However, Montagnac, (2009) reported ash content of 0.4 to 1.7% (wwb) from roots of assorted cassava varieties.

Plate 1: From left to right - Cassava Roots harvested Kibanda Meno; Karembo: Tajirika

Table 1: Proximate composition (%wwb) of three coastal cassava varieties as affected by crop age

Cassava root Nutrients	Cassava Variety	3 Months after planting	6 Months after planting	9 Months after planting	12 Months after planting
Carbohydrates %	Karembo	39.00 ± 0.01^{c}	37.28 ± 0.28^{de}	37.58 ± 0.37^{d}	40.48 ± 0.26^{ab}
	Kibanda Meno	30.01 ± 0.01^{h}	39.86 ± 0. 2^{b}	40.81 ± 0.24^{a}	37.12 ± 0.16^{de}
	Tajirika	35.84 ± 0.17^{f}	26.65 ± 0.06^{i}	35.15 ± 0.14^{g}	36.86 ± 0.69 ^{e}
Energy (kcal/100g)	Karembo	139.49± 0.63^{f}	155.0 ± 0.15^{d}	155.15± 1.40^{d}	169.81± 0.74^{ab}
	Kibanda Meno	122.81 ± 4.04^{h}	169.04 ± 1.77^{b}	173.52 ± 0.10^{a}	155.65 ± 0.68^{d}
	Tajirika	134.79 ± 0.63^{g}	115.12 ± 0.77^{i}	150.69± 1.08^{e}	161.23± 3.72^{c}
Fibre %	Karembo	0.71± 0.00^{i}	1.08± 0.03^{d}	0.93± 0.01^{g}	0.92± 0.01^{g}
	Kibanda Meno	0.83 ± 0.01^{h}	1.17 ± 0.01^{c}	1.03 ± 0.01^{ef}	1. 29 ± 0.01^{b}
	Tajirika	1.04± 0.01^{f}	1.55± 0.06^{a}	1.06 ± 0.01^{e}	1.01 ± 0.01^{f}
Ash %	Karembo	0.98 ± 0.02^{b}	0.75 ± 0.07^{e}	0.77 ± 0.04^{d}	0.67 ± 0.02^{f}
	Kibanda Meno	0.84 ± 0.01^{d}	0.94 ± 0.02^{c}	0.96 ± 0.01^{bc}	1.29 ± 0.01^{a}
	Tajirika	1.03 ± 0.01^{b}	0.65 ± 0.00^{g}	0.77± 0.06^{d}	0.98 ± 0.04^{b}

*Means with different superscripts in the same row are significantly different at 5 % level of significance

3. 4. 4 Fat content

Fat content was shown to be low ranging between 0.2 and 0.8% wwb (Table 2). The results of the current study showed significant ($p \leq 0.05$) interaction effect on cassava roots fat content. The variation on the coastal varieties is wider compared to 6 varieties studied by Richardson. (2013), whose range of variation was between 0.2 and 0.4% (wwb) fat content. The results conform to those reported by Ifeabunike, *et al*., (2017) who reported fat content of 0.78% for the bitter varieties and 0.27% for sweet varieties.

3. 4. 5 Protein content

Change in protein content for variety Karembo, from 3 to 6 months after planting was 74.7% decrease, 4.5% decrease from 6 to 9, however protein content increased from 0.21 % to 0.50% (wwb) from 9 to 12 months after planting (Table 2), this culminates to 138% increase. Variety Kibanda Meno showed a decrease of 37.8% from 3 to 6 months after planting, 7.14% increase from 6 to 9 months after planting and a decrease of protein content of 3.33% from 9 to 12 months after planting. Karembo had the highest protein content at 3 months after planting followed by Tajirika whose highest protein content was at 12 months after planting. Variety Kibanda Meno had its peak protein at month 6 while Karembo's peak was at month 3. This conforms to the study carried out by Richardson and Benesi where six varieties evaluated for quality showed a range of 1.20 to 2.10% protein at 12 and 13 months after planting Richardson, (2013); Benesi, *et al*., (2004). Chavez also observed that protein content is quite low in cassava roots Chavez *et al*., (2005), with Salcedo showing that protein content in cassava roots ranging between 1% and 3% Salcedo *et al.*, (2010). This shows that the popular varieties grown in coastal Kenya have lower protein content than other varieties elsewhere. It is advisable therefore that consumers supplement cassava roots dishes with protein source. Also in order to maximize on the little protein contained in cassava roots Tajirika has peak protein levels at 12 months, Kibanda Meno at 6 months and Karembo at three months after planting therefore it is advisable that harvesting of roots to be staggered accordingly

3. 4. 6 Hydrogen cyanide levels

The change in root hydrogen cyanide content between 3 and 12 months after planting was 0.12% to 9.34%, 0.12% to 5.24% and 0.13% to 4.47% for Karembo, Kibanda Meno and Tajirika respectively (Table 2). While longer period in the field from 6 to 9 months after planting had no effect on root hydrogen cyanide content in Karembo and Kibanda Meno Tajirika accumulation was 22% and 37% times more cyanide over the same period of time. Longer period in the field from 9 to 12 months showed accumulation of hydrogen cyanide content in the roots of Karembo by 8%, while the same period caused 33% and 12%, reduction of hydrogen cyanide content in the roots of Kibanda Meno and Tajirika by respectively. Results in this study show that the coastal varieties had hydrogen cyanide content that range between 0.12 mg / kg to 13.00 mg / kg dry weight. The results are higher compared to results reported by Onwuka and Ogbogu. (2007) whose two varieties (one improved and a local) had hydrogen cyanide levels of 3.1 mg / kg and 3.6 mg / kg respectively in roots harvested at full maturity, i.e. over 12 months after planting. The results are, however, comparing well with those reported by Ubwa *et al*., (2015). The accepted hydrogen cyanide levels by FAO/WHO. (2011) - codex standards are up to 10 mg / kg that is equivalent to 10ppm Endmemo. (2017) On the other hand hydrogen cyanide levels that were reported to be endemic with reference to Konzo disease, were between 47 and 614 mg / kg Rijssen *et al*., (2013); Bolhuis, (1966) as cited in Lukuyu *et al*., (2014) observed that Hydrogen cyanide content that is below 50 mg / kg is innocuous. The coastal varieties have hydrogen cyanide range of between

9.3 mg / kg and 12 mg / kg - conversion done using Endmemo. (2017) convertor table. Variety Karembo however has high cyanide content across the studied crop ages. Studies by Topor *et al*., (2008) showed storage of fresh roots caused hydrogen cyanide levels to decline. However, moisture stress on cassava roots in the field causes hydrogen cyanide level to rise- as reported by Srihawong *et al*., (2015). The trend shown in this study on hydrogen cyanide build up indicates a moisture stress on the roots as hydrogen cyanide was shown to rise as the roots were left longer in the field. Moisture stress is a common phenomenon in the coastal region of Kenya.

Table 2: Proximate composition (wwb) of three coastal Kenya cassava varieties as affected by crop age

Cassava root nutrients	Cassava varieties	3 months after planting	6 months after planting	9 months after planting	12 months after planting
Fat %	Karembo	0.87 ± 0.4^{a}	0.22 ± 0.01^{f}	0.21 ± 0.01^{g}	0.50 ± 0.06^{d}
	Kibanda Meno	0.45 ± 0.02^{d}	0.28 ± 0.01^{efg}	0.30 ± 0.01^{e}	0.29 ± 0.00^{ef}
	Tajirika	0.50 ± 0.01^{d}	0.29 ± 0.08^{f}	0.63 ± 0.04^{c}	0.77 ± 0.02^{b}
Protein %	Karembo	1.13 ± 0.03^{d}	0.77 ± 0.01^{fg}	0.74 ± 0.06^{h}	0.88 ± 0.05^{ef}
	Kibanda Meno	0.41 ± 0.01^{h}	1.54 ± 0.11^{ab}	0.94 ± 0.00^{e}	1.15 ± 0.00^{d}
	Tajirika	1.33 ± 0.08^{c}	1.49 ± 0.04^{b}	1.12 ± 0.05^{d}	1.63 ± 0.06^{a}
HCNmg/kg	Karembo	0.12 ± 0.00^{c}	8.5 ± 0.01^{b}	8.62 ± 0.76^{b}	19.34 ± 0.45^{a}
	Kibanda Meno	0.12 ± 0.01^{f}	2.62 ± 0.12^{e}	57.80 ± 0.38^{a}	4.47 ± 0.54^{d}
	Tajirika	13.00 ± 0.01^{f}	4.80 ± 0.30^{d}	$10 \pm 0.01^{c\,d}$	5.24 ± 0.03^{d}

*Means with the same superscript in the same row are not significantly different at 5% level of significance

3. 4. 7 Variation in vitamins C and A contents

The results of the current study showed significant ($p \leq 0.05$) interaction effect on cassava roots vitamin C content (Table 3.). The three varieties registered vitamin C content that ranged from 7 mg / 100g to approximately 70 mg / 100g across the 4 crop ages studied. After determining the% change per variety Karembo showed that the change in vitamin C content in was up to 585.89% increase from 3 to 6 months after planting, after which there was a 31 % reduction from 6 to 9 month after planting and then a further 26.79% reduction from 9 to 12 months after planting Variety Kibanda Meno had persistent decline in vitamin C content in the following trend from 3 to 6 months (796.21%), 6 to 9 months (7.97%) and 22.94% after planting, from 9 to 12 months after planting. Variety Tajirika had a vitamin C content increase of 445.38% from 3 to 6 months after planting. Then an increase of 23% from 6 to 9 months after planting and a decrease of 13.76% from 9 to 12 months after planting. Therefore, varieties Karembo and Kibanda Meno showed a decreasing trend in vitamin C content when left in the field for a period beyond 6 months after planting, while Tajirika's vitamin C content started decreasing when left in the field beyond 9 months after planting (Table 3). There was therefore significant ($p \leq 0.05$) interaction effect between vitamin C content (mg/100 g dry weight) and crop age.

The results showed substantial high content of vitamin C in all the three varieties, Karembo 64.2 mg /100 g, Kibanda Meno 66 and mg Tajirika 69.6 mg / 100 g. These results are higher than those reported by Montagnac. (2009) whose results were 14.9 to 50 mg /100 g. They however compare well with those reported by Davidson *et al*., (2017). Salvador *et al*., (2014) postulates that chemical content of cassava varies depending on the plant part, climatic and environmental condition and agro - ecological location of production. Peak vitamin C content also significantly varied, Tajirika. Karembo and Kibanda Meno attained their highest vitamin C content at 6 months after planting.

Tajirika had its highest level of vitamin C at 9 months after planting. The results indicate that cassava roots for Karembo and Kibanda Meno loose vitamin C when left in the field longer than 6 months after planting. The percent drop in vitamin C content from 6 to 9 months after planting was as follows; Karembo 30.9% and Kibanda Meno 7.9%when harvested at 9 months after planting instead of harvesting them at 6 months after planting. Tajirika's vitamin C content however drops when the variety is left in the field beyond 9 months after planting (Table 3).

3. 4. 8 Variation in mineral contents

The change in variety Karembo for mineral iron content was a persistent accumulation from 3 to 6 months after planting (41.05%) and 25.23% from 6 to 9 months after planting. At 9 to 12 months after planting the results showed 12.62% decline in mineral iron content (Table 3). Variety Kibanda Meno had 28.9% decline in mineral iron content from 3 to 6 months, then had an increase of 134.98% from 6 to 9 months after planting, then showed a decrease of 26.97% from 9 to 12 months. Variety Tajirika showed a decrease of 46.15% from 3 to 6 months after planting, then another decrease of 17.42% from 6 to 9 months after planting. There was further decrease of 5.48% mineral iron content from 9 to 12 months after planting. The results therefore showed significant ($p \leq 0.05$) interaction effect on cassava roots in relation to iron content (Table 3). Kibanda Meno had significantly ($p \leq 0.05$) higher iron content in all the crop ages compared to the improved varieties. At 3 months after planting, Kibanda Meno had 4.3 mg /100 g dry weight iron content that indicated to be higher 14.7% than Karembo's iron content, that stood at 3.6 mg /100 g dry weight. Peak iron content was found to be at the tender crop age of 3 months after planting for the improved varieties. Karembo (3.6 mg/ 100g wet weight) and Tajirika (5.3 mg /100 g wet weight), while variety Kibanda Meno had its peak iron content (7.12 mg /100 g wet weight) at 9 months after planting. These results are within the range reported by Ifeabunike *et al.*, (2017) and Davidson *et al*., (2017), who reported iron content of 2.25 mg /100 g wet weight for bitter varieties and 4.0 mg /100 g wet weight for the sweet varieties. Studies carried out by Chavez et al. (2000) showed a mean iron content of 9.6 mg /100 g dry weight in cassava roots, while Montagnac's results were at 0.3 mg /100 g wet weight to 14.0 mg /100 g wet weight Montagnac. (2009). This is however expected since cassava nutrients and / or physical characteristics are influenced by several factors as stated by (Salvador *et al*., 2014).

Change in mineral zinc content showed of 176.32% accumulation in variety Karembo when left in the field from 3 to 6 months after planting, then there was a 60% reduction when the crop was left in the field from 6 to 9 months after planting, and a further 73.81% accumulation of mineral zinc from 9 to 12 months after planting. Variety Kibanda Meno showed 54.72% increase in mineral zinc content from 3 to 6 month after planting. There was 18.28 % reduction of mineral zinc from 6 to 9 months after planting. There was 190% accumulation of mineral zinc content from 9 to 12 months after planting. Variety Tajirika showed 96.83% change from 3 to 6 months after planting. The period between 6 to 9 months after planting caused a 20.97% decline in mineral zinc content but there was 16.67% accumulation at 9 to 12 months after planting. The results of the current study also showed significant ($p \leq 0.05$) interaction effect on cassava roots in relation to zinc content (Table 3.). Variety Karembo had the highest Zinc (2.1 mg /100g wet weight) content at 6 months after planting. Tajirika and Kibanda Meno had their peak zinc content at 12 months that were 1.75 mg /1 00 g wet weight and 1.94 mg / 100 g wet weight respectively. Tajirika's Zinc content at the peak was 1.75 mg / 100 g while Kibanda Meno was 1.46 mg/100g wet weight. These results are within the range that was reported by Charles *et al*., (2005). His five cassava varieties had zinc content range of between 1.4 and 4.25 mg /100 g wet weight.

Table 3: Variation of vitamin C, Zinc and Iron contents (Mcg/100 g, wwb) in three coastal Kenya cassava roots as affected by crop age

Root Nutrients	Cassava Varieties	3 Months after planting	6 Months after planting	9 Months after planting	12 Months after planting
	Karembo	9.36 ± 0.721^{g}	$64.2 \pm .35^{abc}$	44.3 ± 0.06^{e}	32.43 ± 0.78^{f}
Vit' C	Kibanda Meno	7.39 ± 0.61^{g}	66.23 ± 3.7^{a}	60.95 ± 0.96^{bcd}	46.97 ± 7.75^{e}
	Tajirika	10.38 ± 0.08^{g}	56.61 ± 0.78^{d}	69.63 ± 0.74^{a}	60.05 ± 1.0^{cd}
Iron	Karembo	3.63 ± 0.03^{bed}	2.14 ± 1.6^{d}	2.68 ± 0.12^{c}	2.41 ± 0.18^{d}
	Kibanda Meno	4.26 ± 0.03^{bc}	3.03 ± 1.31^{c}	$7.12 \pm 2.2\ 3^{a}$	5.20 ± 0.08^{b}
	Tajirika	5.33 ± 0.00^{b}	2.87 ± 1.72^{c}	2.37 ± 0.24^{d}	2.24 ± 0.18^{d}
Zinc	Karembo	0.76 ± 0.01^{e}	2.10 ± 0.28^{a}	0.84 ± 0.21^{de}	1.46 ± 0.29^{bc}
	Kibanda Meno	0.53 ± 0.03^{e}	0.82 ± 0.05^{d}	0.67 ± 0.5^{e}	1.94 ± 0.25^{a}
	Tajirika	0.63 ± 0.05^{e}	1.24 ± 0.00^{cd}	1.50 ± 0.07^{bc}	1.75 ± 0.14^{ab}

* Means with the same superscript in the same row are not significantly different at 5% level of significance

3. 5 Conclusion and Recommendation

The current study concludes that the crop age has influence on cassava roots' quality but leaving the crop in the field for longer period after planting does not warrant maximum root quality in terms of nutrient levels. At 9 months after planting, several nutrients evaluated in this study are at higher levels but the micronutrients are at higher levels between 3 and 6 months after planting.The study therefore recommends staggered harvesting of cassava roots at different crop ages particularly as from 6 months but harvesting at around 9months is best based on dry matter and starch content.

CHAPTER FOUR

PROFILING OF CASSAVA LEAF NUTRIENTS FOR QUALITY AND SAFETY FOR HUMAN CONSUMPTION IN COASTAL KENYA

Abstract

Coastal Kenya is food and nutrients insecure with harsh climatic conditions, but contributes 27% of the national cassava production. Cassava leaves are nutritionally rich but moderately consumed. Enhanced leaves' utilization could reduce nutrients' insecurity. Nutritional and safety information availability would popularize them. This study determined nutrients in varieties Tajirika, Karembo and Kibanda Meno, at 3, 6, and 9 months after planting. Leaves harvested from the localities, were analyzed at the University of Nairobi. Results showed significant ($p \leq 0.05$) effect of crop age on nutrients content. Nutrients peaks were; moisture content (74%) at 3 months in Karembo and Tajirika, 75% at 9 months for Kibanda Meno; dry matter (50%) at 6 months, across varieties; fat (1.0 g / 100 g) was highest in Kibanda Meno at 3 months; protein (36 g / 100 g) was highest in Kibanda Meno at 9 months. Vitamin C was highest in Karembo (1236 mg / 100 g) at 9 months. Vitamin A was 190 to 638 mg /100 g, at 6 months across the varieties. Iron and Zinc were 10 mg / 100 g, peak at different crop ages. Cyanide was 7.4 mg / kg, peak at 9 months. Cassava leaves micronutrients and dry matter, are peak at 3 and 6 months, while cyanide progressively builds up. The young leaves would be recommendation for consumption based on relative cyanide safety.

4. 1 Introduction

Cassava is a root food crop that thrives in sub-optimal climatic conditions (Nweke, 1996). It is ranked fifth in importance in the world but ranked 3rd in Kenya after rice and maize (FAO, 2005). In recent years the importance of cassava has gone a notch higher as it is drought tolerant in the face of global warming effects (FAOSTAT, 2013). It has also been highly ranked on the basis of its potential in industrialization (Bechoff *et al*., 2018). Due to cassava being drought tolerant, it has been possible for the coast region of Kenya to produce up to 30 % of the national cassava production (Opondo *et al.*, 2020), amidst harsh climatic conditions. Cassava is therefore a popular food crop, and positioned among the top in the list of coastal traditional dishes and menus FAO. (2005). It is ranked second most important food crop after (Githunguri *et al*., 2017). Cassava production is majorly for human consumption. It is commonly produced as a subsistence crop.in most of the tropical countries. Over 93% of its production is for human consumption (Sagaf and Saha 2019). It is evidenced that cassava can enhance food base and mitigate poverty (FAOSTAT, 2018) especially for coastal Kenya where poverty levels are high.

Due to harsh climatic conditions in the coastal region of Kenya, the region registers poverty levels that range from 13% to 90% at sub-county level, across all the 140 sub-counties (World Bank, 2016). Prevalence of malnutrition is also high especially in children due to nutrients deficiencies (KNBS, 2015). Figures by UNICEF show that the region and the rest of ASAL counties and urban informal settlements, had a total number of children (6 - 59 months that required treatment for acute malnutrition by April 2019, in Kenya. The estimated figures show that out of 541,309; 113, 941 suffered severe acute malnutrition and 427,368 had moderate acute malnutrition (UNICEF, 2019). The symptomatic presentation of the deficiencies found in the region include kwashiorkor - (protein deficiency), anemia - (iron deficiency) and growth retardation (zinc deficiencies) FAO/WHO. (2005). Vitamin C also known as, ascorbic acid is an antioxidant. It plays a vital role in protecting the body from infection and especially skin diseases like scurvy. It is a nutrient that is not synthesized by the human body and therefore it must be acquired from dietary sources Canterbury. (2013).

Cassava leaves are rich in a range of the symptomatic nutrients including protein but they are underutilized (Achidi *et al*., 2005). Underutilization is a consequent result of limited information on the nutritional and safety qualities of the leaves, amongst other factors (Walucho, 2019). Bechoff *et al*., (2018) indicated that knowledge of health benefits of food and food products is key in influencing consumer preference whereas consumer preference influences consumption and / or utilization of products. The limited availability of scientific information on the leaves' nutritional and safety qualities therefore hampers their popularization. Determination and consequent availability of this information could be used to promote utilization of cassava leaves. Studies indicate that lately consumers and food processors are interested in understanding nutritional quality of foods and food products (Shobba *et al*., 2012). Currently consumers are keento know the nutritional value of what they eat, for "you are what you eat."

Safety in utilization of cassava leaves is dependent on the levels of hydrogen cyanide that has been found poisonous when ingested in large quantities (Bokanga, 1996; Bradbury, 2014). Both cassava roots and leaves contain cyanogenic glucosides that are presented in form of linamarin and lotaustralin (Bradbury, 2014). Linamarin is the toxic compound that produces toxic compound hydrogen cyanide (HCN) (Cardoso *et al*., 2005; Jackson et al., 2020)). Linamarin is the most representative glucoside accounting for about 80% of the total cassava glucoside. It is also the free form of cyanogenic glucosides. Linamarin is broken down by the enzyme linamarase alsofound in cassava tissues to release cyanohydrin (McRae *et al*., 1993). Cyanide in cassava is classified into two types: (1) bound cyanide; and (2) free cyanide present as the cyanohydrin whichacts as free HCN gas above 26 ^{o}C (Bokanga, 1996). Determination of leaves nutrients and safety qualities in relation to cyanide levels could assist in the endeavor towards popularizing leaves consumption and /or utilization.

Major cassava products processed in coastal Kenya region are fried cassava chips (cassava French fries (21 %) and cassava flour (11%) (Githunguri *et al*., 2017). There are currently limited reports on processing and value addition and utilization of cassava leaves. This is because the leaves are mostly categorized as a byproduct (Latif and Muller, 2015).

The present study therefore undertook to profile moisture/dry matter, protein, fat, vitamins A and C, minerals - zinc and iron and hydrogen cyanide in cassava leaves across field periods of 3, 6, and 9 months after planting. Profiling of nutrients was done on three popular cassava varieties grown in the coast region of Kenya, with an aim to determine the effect of crop age on the leaves nutrients content.

4. 2 Materials and Methods

4. 2. 1 Cassava leaves

The cassava leaves that were used in the present study were from popular varieties Tajirika, Karembo and Kibanda Meno. The varieties were selected from the coastal counties from rain fed farms and mother blocks. Tajirika and Karembo are improved varieties released by KARI in 2009 (KARI, 2009: Gethi *et al*., 2008). Kibanda Meno is an indigenous variety that has roots which mature early, known for high dry matter and sweetness making it very popular (Mwamachi, 2005). This indigenous variety is commonly grown alongside the improved varieties as means of risk mitigation over crop loss (Wekesa *et al*., (2017). The coastal communities have remained persistent on their preference to grow it (Mwamachi, 2005; Lewa *et al*., 2019). The variety Karembo has a stem that is characterized by short internodes (Muli et al., 2009) and producers have adopted it for seed multiplication (Sagaf and Saha 2019). Variety Tajirika has most of its traits that are moderately similar to those found in the local variety Kibanda Meno (Muli *et al*., 2009). The varieties/ plants for harvesting leaves were identified by age in months after planting - 3, 6 and 9 months. The selection was carried out from mother blocks that had been planted over the long rains of year 2015. Long rains in the coastal region starts from March and subsides by August (as shown in the coastal Kenya rainfall calendar - (Metrology department, 2014).

The coastal Kenya is mainly an ASAL region with pockets of fertile areas that are less than 1/8 of the region. It lies in the climatic zones 2 and 5 (CL2 and CL5) that fall in sub-humid to ASAL. The soil types range from white loose sand to black clay soils with average rainfall of 1,300 mm (mean of between 350 to700 mm per annum) and mean annual temperature of 24ºC to 35ºC (Dicosta and Wamicha, 2016)

The harvesting of leaves was carried out from mother blocks planted at Kenya Agricultural Research Centres; Masabaha, Matuga and Mtwapa. Plants for harvesting were randomly selected and labeled. Cassava leaves were harvested from the labeled plants, targeting the top tender leaves from leaf one to five - counting from the plant top as done by (Bradbury, 2014). Harvested leaves were packed in polyethene bags and labeled as per the variety and crop age. Labeled bags were put in cool boxes and transported overnight to University of Nairobi food laboratories for physico -chemical analysis.

4. 3. Laboratory analyses

4. 3. 1 Cassava leaves preparation.

At the laboratory the leaves were destalked and the leafy portions were pounded using pestle and motor as guided by (Bradbury, 2014).

4.3. 2 Determination of moisture / dry matter (DM) content

Moisture content determination was carried out using gravimetric method with a few modifications. About 5 g of pounded sample were weighed in crucibles and dried for 4 hours at 105 ^{o}C in an air oven as guided in AOAC. (2001) method 925.10.

4.3. 3 Crude fat determination

Approximately 5 g sample was extracted using soxhlet extractor for 8 hours using 200 ml petroleum ether (40-60 ^{o}C). Crude fat content was calculated after evaporating the solvent and the residue dried in an air oven at 105 ^{o}C for 1 hour as guided in (AAFCO, 2014).

4. 3. 4 Protein determination

Protein content in cassava roots was determined as per AOAC. (2005) method. 979.09.

4. 3. 5 Determination of hydrogen cyanide

Hydrogen cyanide determination was carried out using distillation method. Cassava roots were macerated using a laboratory blender and samples of 10 g root mash was placed into distillation flask and allowed to stand for three hours before distillation. Distillation and consequent determination of hydrogen cyanide was carried out as in AOAC. (2016) method 915.03

4. 3. 6 Determination of Vitamin C

Approximately 15 ml (10%) TCA, was added into flat bottomed flask containing cassava roots mash and filtered. A total of 15 ml filtrate sample was collected. The filtrate sample was then mixed with 5 ml of 4 % potassium iodide solution then titrated with N- bromisumccinimide solution. The rest of procedure was followed as described in AOAC. (2012) method 967.21.

4. 3. **7 Determination of vitamin A**

Approximately 2g sample of crushed leaf was weighed, 25 ml of acetone were added to extract colour, and the rest of steps followed were as guided in AOAC (2006) method 98.25.

4. 3. 8 Determination of iron and zinc Content

Cassava root mash sample (4 g) for determining mineral Iron was ashed in a muffle furnace at 500ºC for 4 hours. Then digested by adding 10ml of 20% HCL and heated to boiling, then filtered into 100 ml volumetric flask and topped to mark using distilled water. Using atomic absorption spectrophotometer (A.A.S) mineral iron and zinc were determined according to AOAC. (2016) method 99. 10.

4. 3. 9 Experimental design and data analysis

The statistical design used in the study was Complete Randomized Design, where varieties and crop growth stage were independent variables. Data were collected on the following parameters: Hydrogen cyanide levels, moisture and dry matter, protein, fat and micronutrients vitamins C and A and minerals zinc and iron. Results from laboratory analyses were subjected to the analysis of variance (ANOVA), using the General Linear Model (GLM) of the Statistical Analysis System (SAS version 9.1). Means were separated using Least Significant Difference (LSD), the differences being significant when $p \leq 0.05$.

4. 4 Results and Discussion

4.4. 1 Moisture contents

The results showed that there was significant ($p \leq 0.05$) effect in the leaf moisture content as affected by crop age. Moisture content in variety Karembo showed a significant ($p < 0.05$) drop of 6.7% from 3 months after planting to 6 months after planting. There was 16% extra accumulation of moisture as the crop was left longer in the field from 6 months after planting to 9 months after planting. Variety Kibanda Meno showed approximately 20% drop in moisture content from 3 months after planting to 6 months after planting. But the variety accumulated more moisture of approximately 40% when it was left to stand in field from 6 months after planting to 9 months after planting. Variety Tajirika had up to 74% moisture content at 3 months that later showed a 16% drop at 6 months after planting. At 9 months after planting moisture content of Tajirika leaves increased by 16% from the level reached at 6 months after planting. All the three varieties had the lowest leaf moisture content at 6 months after planting.

Moisture content compared across the three varieties (Table 4), the results showed a significant ($p \leq 0.05$) difference within the varieties. Variety Tajirika had significant ($p \leq 0.05$) highest moisture content when compared with varieties Karembo and Kibanda Meno at the initial period of 3 months after planting. At 6 months after planting variety Karembo was shown to have significant ($p \leq 0.05$) lowest moisture content although all the three varieties had registered a declining trend. At nine months after planting variety Kibanda Meno had significantly ($p \leq 0.05$) higher moisture content when compared with Karembo and Tajirika.

The phenomenon of the coastal varieties showing lower moisture content at 6 months after planting was unique. However, it may have probably been caused by erratic rainfall that is commonly experienced in the coast region of Kenya, where the present study was conducted. Metrology department housed at KALRO Mtwapa recorded 870 mm of rain over the month of September 2015 (KALRO 2016). This was a clear demonstration of the erratic rain preterm that has lately been observed globally, being caused by global warming. This is because under normal circumstances moisture level is expected to reduce as the crop age advances. This is because it is expected that there will be more buildup of fibre and other nutrients. The leaf moisture content values found in this study are slightly higher than those reported by Kuobala (Kuobala *et al.,* 2015). Kuobala's results for leaves from five genotypes showed moisture content of approximately 66% though he harvested the leaves at 12 months after planting. Oresegun's results showed his varieties to contain moisture in a range of 63% to 96% (Oresegun *et al.*, 2016). Oresegun however did not specify the time of leaf harvesting. These results therefore are only indicative results showing that cassava leaves have the capacity to contain up to 96% moisture with no specific crop age.

4.4. 2 Dry matter content

The dry matter contents were between 34.84% - 43.24% in Karembo, 35.41 - 32. 60% in Kibanda Meno and 34.95 - 41.24% in Tajirika leaves respectively (Table 4). There was significant ($p \leq 0.05$) effect of crop age on leaf dry matter that was indicated through change in the leaf dry matter content. Variety Karembo had accumulated extra 6.7% dry matter when it was left to stand in the field from 3 months after planting to 6 months after planting. There was no significant ($p \geq 0.05$) change in the leaf dry matter content of variety Karembo between 6 and 9 months after planting. Variety Kibanda Meno accumulated 4.9% more dry matter in its leaves when left in the field up to 6 months after planting. Variety Tajirika left in the field between 3 months after planting and 6 months after planting accumulated 6.6% extra dry matter. The leaf dry matter content of variety Tajirika however decreased by 3.3% between 6 and 9 months after planting. The present study showed that peak dry matter content in the leaves is at 6 months after planting for all the three varieties studied. At 3 months after planting, the three varieties registered a common dry matter content of approximately 35%. At nine 9 months after planting Kibanda Meno had the lowest dry matter content at 32.60% while Karembo and Tajirika had approximately 40% dry matter.

Dry matter shown to be high at 6 months after planting is an indicator that the three cassava varieties grown in the coast region are most vegetative at 6 months after planting. Dry matter content was shown to vary significantly ($p \leq 0.05$) within the varieties, especially at 9 months after planting (Table 4). In as much as the three varieties had significant ($p \leq 0.05$) differences in their dry matter contents at this crop age of 9 months after planting, variety Kibanda Meno had the significantly ($p \leq 0.05$) lowest dry matter content during this period. The dry matter contents reported in the current study are in agreement with those reported by Kuobala (Kuobala *et al.*, 2015). Kuobala's results showed dry matter content of cassava leaves that ranged from26% to 35%. Wobeto's results were however at 29.54%. The varieties studied by (Salvador, 2014) had leaves' dry matter ranging between 19 to 20.3% (Wobeto *et al.*, 2006). Those studied by Montagnac also had values within the range of the coastal varieties at between 25 - 35% (Montagnac *et al.*, 2009). The results reported by Ravindran *et al.*, (1998) had dry matter that ranged from 11% to 21%. The results of the current study also indicate that even under harsh climatic conditions experienced in coastal Kenya, the popular cassava varieties are substantially vegetative and are comparing well with other varieties. Shirpley and Thi-Tam. (2002) in their study on dry matter content against dry matter concentration postulated that dry matter is associated with many critical aspects of plant growth and survival. The scientific explanation of this fact is that dry matter content that is also referred to as the mass fraction of dry matter in the SI system is the ratio of dry mass to fresh mass of the plant part, making dry matter content to be direct pointer of the fresh mass (Garnier *et al.*, 2001). The leaf fresh mass is influenced by leaf variation architecture. When the venation architecture is ideal and well-structured it becomes an efficient irrigation system in the plant leaves (Tabassum *et al.*, 2016); thus leaf vein density(LVD) and vein thickness are the two major properties of the plant survival.

4. 4. 3 Fat content

The results showed significant ($p \leq 0.05$) effect of plant age on cassava leaves' fat content. Fat content was shown to be low ranging from 0.28% to 1.28% on a dry weight basis (Table 4). Fat content decreased as the crop stayed longer in field, a phenomenon registered in all the three studied varieties. The drop was however significant ($p \leq 0.05$) in varieties Kibanda Meno and

Tajirika only. Variety Kibanda Meno had 1.28% fat content at 3 months after planting but dropped to 0.72% at 6 months after planting and further dropped to 0.28% at 9 months after planting. The total loss of fat content in Kibanda Meno between 6 months after planting and 9 months after planting is approximately 37.6%. Variety Tajirika's fat content was registered at 0.83% at 3 months and stayed stable up to 6 months after planting. But when the variety was left longer in the field to reach 9 months after planting, it showed a significant ($p < 0.05$) drop of fat content of approximately 62%. (Table 4), Variety Kibanda Meno had the significantly ($p \leq 0.05$) highest fat content at 3 months after planting. Kibanda Meno had significantly ($p \leq 0.05$) the lowest fat content when it reached 9 months after planting. The fat content of the three varieties had highest fat content at 3 months after among the three varieties (Table 4). However, Kibanda Meno showed to higher levels than the rest of the varieties at 3 months and also had lowest fat content on reaching 9 months after planting. This indicates that the leaves of variety Kibanda Meno are more susceptible to losing fat as the crop is left to stand longer in the field. The results in this study are lower than those reported by Oresegun (Oresegun *et al*., 2016). Oresegun's results showed fat content that ranged between 5.6 mg / 100 g to 13.3 mg /100 g. The coastal varieties therefore are of lower quality in terms of fat content. A fact that calls for consumers to be encouraged to add a bit of fat during processing of the leaves for consumption, in relation to RDA. Total fat RDA is about 44 to 77g of fat per day if one eats 2,000 calories a day; – culminating to about 20 to 35% of total calories (ODPHP, 2020).

4. 4. 4 Protein content

The protein contents leaves were; 16.1 - 23.2% in Karembo, 13.8 -21.8% in Kibanda Meno and 13.8% -21.8% in Tajirika respectively (on a dry weight basis) (Table 4). The results showed significant ($p \leq 0.05$) influence of age of the crop (months after planting) on protein content of

Protein content in Kibanda Meno was 13.82% at 3 months after planting, 24.42% at 6 months after planting and 36.1% at 9 months after planting. Protein content significantly ($p \leq 0.05$) increased as variety Kibanda Meno was left to stay longer in the field. Kibanda Meno had a total change in protein content that was up to 161.2%. There was also significant ($p \leq 0.05$) variation of protein content within the varieties especially between 6 to 9 months after planting (Table 1). Variety Kibanda Meno had significantly ($p \leq 0.05$) the highest protein content at 9 months after planting as compared to the improved varieties Karembo and Tajirika.

Protein contents in the current study are low compared with those reported by Oresegun. (2016). They are however much higher than the range reported by Montagnac (Montagnac *et al*., 2009)**.** Oresegun reported protein content range between 40.2 mg / 100 g to 48.9 mg / 100 g. Wobeto's varieties had protein content that ranged between 25 mg / 100g to 137 mg /100 g (Wobeto *et al*., 2006). Ngundi went an extra mile to profile amino acid in cassava leaves (Ngundi et al., 2003). Amino acid is the precursor of protein and his results indicate that the main amino acid found in the cassava leaves are glutamate, alamine, asparartate and leucine (Ngundi *et al*., 2003). Montagnac *et al*., (2009) and Oresegun *et al*., (2016) pointed out that cassava leaves are a potential rich source of protein. A report by FAO/ WHO. (2005) indicated that amino acid profile in cassava leaves is well balanced and even exceeds that found in fish, soybean, and eggs. However, the most important (essential) is leucine but also present in cassava leaves are; isoleucine, tryptophan, histide and valine FAO/ WHO. (2005).

4. 4. 6 Hydrogen cyanide content

The hydrogen cyanide content in Kibanda Meno was 0.2 - 7.1 mg / kg, in Tajirika, leaves contained 0.2 - 5.9 mg / kg in Karembo the level was 0.2 - 7.4 mg/kg on dry weight basis respectively (Table 1). The results showed significant ($p \leq 0.05$) influence of crop age on hydrogen cyanide content in cassava leaves (Table 4). All the three varieties Karembo, Kibanda Meno and Tajirika had highest hydrogen cyanide levels at 9 months after planting. Variety Kibanda Meno however had significantly ($p \leq 0.05$) lower hydrogen cyanide levels at all the crop ages of 3, 6 and 9 months after planting. Kibanda Meno values were; 0.15 mg / kg at 3 months after planting, 5.29 mg / kg at 6 months after planting and 5.86 mg / kg at 9 months after planting. On the other hand, Hydrogen cyanide levels in Karembo were; 0.17 mg / kg at 3 months after planting but rose to 7.07 mg / kg at 9 months after planting. Variety Tajirika had 0.22 mg / kg at 3 months after planting and 7.39 mg / kg at 9 months after planting. A comparison within varieties there was shown to be a significant ($p \leq 0.05$) variation especially when the varieties reached 6 and 9 nine months after planting (Table 4). The levels of Hydrogen cyanide in all the three varieties are within acceptable range in reference to Codex standard - (accepted 10ppm that is equivalent to 10 mg / kg) (Codex 1989). The results of the current study are also lower than those reported by (Ifeabunike *et al*., 2017; Salvador *et al*., 2014). Topor *et al*., (2008) studied Hydrogen cyanide from cassava leaves that were harvested from 14 farms in her region and registered results that reached up to 50 mg / kg, although there was no clarification whether among the varieties, there were bitter and or wild varieties or all were the domesticated varieties. However, (Kuobala *et al*., 2015; Burns *et al*., 2012; Salvador *et al*., 2014) and Bechoff *et al*., (2018), all link variations found in cassava leaves' nutrients to be as a result of varietal, climatic and environmental influences.

Table 4: Proximate nutrients and Hydrogen Cyanide (HCN) content in leaves of three coastal cassava varieties, across four crop ages - months after planting

Cassava leaf content	Cassava varieties	3 months	6 months	9 months
Moisture content (%)	Karembo	74.16 ± 0.57^{ab}	59.93 ± 4.20^{d}	69.82 ± 2.00^{b}
	Kibanda Meno	73.85 ± 0.75^{ab}	68.99 ± 0.70^{c}	75.42 ± 4.89^{a}
	Tajirika	74.11 ± 0.191^{a}	67.10 ± 0.83^{c}	70.81 ± 2.44^{abc}
Dry matter (%)	Karembo	34.84 ± 0.57^{bc}	54.31 ± 2.59^{a}	43.24 ± 2.01^{a}
	Kibanda Meno	35.41 ± 0.75^{bc}	44.98 ± 0.70^{a}	32.60 ± 4.89^{c}
	Tajirika	$34..95 \pm 0.20^{bc}$	48.44 ± 1.39^{a}	41.24 ± 2.44^{ab}
Fat (mg/100g wwb)	Karembo	0.85 ± 0.01^{b}	0.73 ± 0.11^{bc}	0.70 ± 0.02^{bc}
	Kibanda Meno	1.28 ± 0.01^{a}	0.73 ± 0.19^{b}	0.28 ± 0.40^{d}
	Tajirika	0.83 ± 0.021^{b}	$0.69 \pm 0,78^{b}$	0.43 ± 0.5^{c}
Protein(mg/100gwwb)	Karembo	16.11 ± 0.08^{b}	20.41 ± 0.38^{b}	23.20 ± 0.83^{b}
	Kibanda Meno	13.82 ± 0.29^{b}	24.42 ± 2.16^{ab}	36.10 ± 16.84^{a}
	Tajirika	13.76 ± 0.03^{b}	$21\ .84 \pm 0.81^{b}$	21.79 ± 2.35^{b}
	Karembo	0.17 ± 0.01^{d}	5.47 ± 1.29^{b}	7.07 ± 0.77^{ab}
HCN (mg/kg)	Kibanda Meno	0.15 ± 0.01^{d}	5.29 ± 0.04^{bc}	5.86 ± 0.11^{b}
	Tajirika	0.22 ± 0.01^{d}	4.13 ± 0.62^{c}	7.39 ± 0.52^{a}

* Means with different superscripts in a row are significantly different at 5 % level of significance

4. 4. 7 Vitamin C

The vitamin C content in Karembo was 27.1 - 1236.5 mg / 100 g, 36.6 - 281.6 mg / 100 g in Kibanda Meno and 151.2 mg / 100 g - 761.7 mg /100 g in Tajirika leaves respectively, the content being per 100 g dry matter (DM) (Table 5). There was significant ($p \leq 0.05$) effect of age of the crop on vitamin C content. All the varieties showed a progressive change with a consistent accumulation of vitamin C content from the tender crop age of 3 months after planting up to 9 months after planting. Variety Karembo showed the highest content of vitamin C at 1226.54 mg / 100 g, followed by variety Tajirika at 761.66 mg / 100g. The local variety Kibanda Meno showed the least vitamin C content. The increase in vitamin C content in variety Karembo was 904 % from 3 months after planting to 9 months after planting. For variety Kibandameno, the increase in vitamin C content was 356% from 3 months after planting to 6 months after planting and 68 % from 6 months after planting to 9 months after planting. For variety Tajirika, increase in vitamin C content was 54% from 3 months after planting to 6 months after planting and 226.6% from 6 months to 9 months after planting. The results of the current study also depict a significant ($p \leq 0.05$) variation of vitamin C within the varieties (Table 5). The variation is persistent from the tender field period of 3 months after planting through to 9 months after planting (Table 5). The results also show that the coastal varieties contain higher vitamin C than those reported by Kuobala *et al*., (2015). Vitamin C content in Kuobala's varieties ranged from 6 mg / 100g to 362.65 mg / 100g. This therefore shows that the coastal varieties are superior in accumulating vitamin C. The change pattern reported in these results however conforms to the pattern reported by Wobeto *et al*., (2006). His results of leaf meal nutrients from five cassava varieties showed peak vitamin C at 17 months after planting when compared with vitamin C content at 15 months after planting and12 months after planting. Vitamin C content in varieties studied by Kuobala *et al*., (2015) was upto 41µg/g that is equivalent to 0.41 mg /100 g. Vitamin C content reported by Simao et al., (2013)had levels that reached between 171 mg /100 g and 569 mg / 10 0g. Simao's 4 varieties had however been harvested at crop age of 10, 12, and 14 months after planting.

4. 4. 8 Vitamin A

The vitamin A contents and were 164.9 - 301.5 mg / 100 g in Karembo, 161.1 - 253.5 mg / 100 g in Kibandameno and 177.2 - 226.1 mg /100g in Tajirika leaves respectively, the content being per 100g dry matter (DM) (Table 5). The results showed significant ($p \leq 0.05$) influence of crop age on leaf content of vitamin A. Variety Karembo had an almost 50% increase of vitamin A between 3 and 6 months after planting. The age of 6 months after planting marked the peak of vitamin C content in variety Karembo. The trend of change shown in vitamin A content in variety Karembo was a sharp drop of up to 473% when the variety was left in the field to reach 9 months after planting. Variety Kibanda Meno had peak vitamin A content at 3 months after planting with a consistent drop of 4.60 % between 3 and 6 months after planting and 33.41% between 6 and 9 months after planting respectively. Variety Tajirika also showed a decreasing trend of vitamin A content that was similar to that of variety Kibanda Meno when left longer in the field. The peak vitamin A content in variety Tajirika was at 3 months after planting that was followed by a consistent drop of 21% from 6 months after planting to 9 months after planting. The results also depict a significant ($p \leq 0.05$) variation among varieties across all the ages of 3, 6 and 9 months after planting (Table 5). The results of the current study on vitamin A content of the coastal varieties conform to those reported by Montagnac *et al*., (2009) and (Chavez *et al*., 2000) who studied cassava leaves nutrient levels of 5 varieties at crop age -; 12, 15 and 17 (months after planting). His varieties showed a decreasing trend where all the varieties had peak vitamin A at 12 months after planting. They registered least levels at 17 months after planting. For example, at: 12 months after planting the range was-; 113 mg / 100 g to 131 mg / 100 g; at 15 months after planting the range was-; 50.4 mg / 100 g to 71mg / 100 g and 60 mg / 100 g to 92 mg / 100 g 17 months after planting. The current study also had similar trend where vitamin A had decreased in all the three varieties on reaching 9 months after planting (Table 5). Kuobala's varieties ranged between 22 mg /100 g to 1817 mg /100 g Kuobala *et al.*, (2015). Oresgun had varieties that contained vitamin A of up to 816µ/g - that is equivalent to 0.81 mg / 100 g Oresegun *et al.*, (2016). Simao *et al.*, (2013) reported vitamin A ranging from 50 mg / 100 g to 73 mg / 100 g. At crop age 14 months, Simao's varieties registered a declining trend in the levels of vitamin A.

4. 4. 9 Mineral iron

The iron content in Karembo was 672.5 - 960.8 mg / 100g, 100.8 - 752.6 mg / kg in Kibandameno and 303.6 - 515.0 mg / kg in Tajirika leaves respectively (Table 5). Results showed significant ($p \leq 0.05$) effect of crop age on iron content. Karembo had higher iron content at all crop ages compared to Kibanda Meno and Tajirika varieties. Peak iron content was shown to be at the tender age of 3 months after planting for the improved varieties (Karembo and Tajirika). There was a significant ($p \leq 0.05$) decrease of 30% in Karembo leaves at 6 months after planting when compared with iron content at 3 months after planting. Similar trend was depicted in variety Tajirika. Variety Kibanda Meno showed persistent accumulation of leaf mineral iron content from 3 months after planting to 9 months after planting. Kibanda Meno had peak iron content at 9 months after planting. There was significant ($p \leq 0.05$) variation of iron content among the varieties at all the three crop ages (Table 2). The results in this study are high in iron content compared to those reported by Kuobala *et al*., (2015). Kuobala reported Iron content that ranged between 57 mg / 100 g to 76 mg / 100 g. Wobeto's varieties showed peak iron content at 2 months after planting that ranged from 134 mg /100 g to 18177 mg /100 g (Wobeto *et al*., 2006). Chavez reported a mean iron content of 9.4 mg / 100 kg. The results of the current study are however within the range reported by Montagnac and Wobeto. The varieties that were reported by Wobeto had iron content that was accumulated in a trend similar to the trend in the current study -; that iron content decreases with an increase in crop age. At 12 months after planting - 202 mg / 100g to 225 mg / 100g; at month 15 - 133 to 203.51mg / 100g; 17 months after planting 120 mg / 100g to 148mg / 100g (Wobeto *et al*., 2006). This trend was also observed in the current study (Table 5). Varieties Tajirika however showed to increase its iron content that peaked at 9 months after planting. This is however expected since cassava nutrients and or physico characteristics are influenced by several factors as stated by Salvador *et al*., (2014) and Montagnac et al., (2009). Salvador's iron content was between 0.4 - 8.3 mg / kg (Salvador et al. 2014). Iron content in varieties studied by Oresegun had results that ranged from 321 mg / kg to 460 mg / kg when harvested at maturity (Oresegun *et al*., 2016; Awubyika *et al*., 1993) reported results in similar range of the current study. Chavez et al., (2000) reported content of 9.4 mg /100g.

4. 4. 9.1Mineral zinc

The mineral zinc content in Karembo was 3.66 - 7.18 mg / 100 g, 3.60 -5.68 mg/100g in Kibanda Meno and 3.22 - 6.43 mg/ 100 g in Tajirika leaves respectively (Table 5). There was significant ($p \leq 0.05$) effect of crop age on zinc content of cassava leaves. Variety Karembo had the highest zinc content at 3 months after planting followed by variety Tajirika. The change in mineral zinc content showed a persistent drop from 3 months after planting to 9 months after planting for both the improved varieties. Lowest zinc content was registered in variety Karembo. The decrease in zinc content in Karembo from 3 months after planting to 9 months, was 49% while it was 36.7% in variety Kibanda meno. Karembo and Kibanda Meno had peak content of mineral zinc at the initial age of 3 months after planting. Variety Tajirika had its peak zinc content at 9 months after planting. There was significant ($p \leq 0.05$) variation of mineral zinc content among the varieties (Table 5). At 3 months after planting Karembo had the highest zinc content but had declined levels that went lower than the levels in Tajirika, on reaching 9 months after planting. The zinc content at 9 months after planting in Tajirika was still lower compared with Karembo zinc content at 3 months after planting. Ravindran *et al*., (1998) observed that age of cassava leaves influenced the levels of micronutrients. These results are high compared to those reported by Kuobala *et al*., (2015) but within the range that was reported by (Wobeto *et al*., 2006). Wobeto also noted that zinc contend decreased as the crop age increased. At 12 months after planting his results were -; 42.8 mg / 100 g to 52.3 mg / 100g; at 15 months they were 35.8 to 67. 1 mg / 100 g; and at 17 months after planting they were, 37.6 mg / 100 g to 64.1 / 100g. Kuobala's varieties had zinc content that ranged between 13 mg / 100 g to 30 mg /100 g (Kuobala *et al*., 2015). Wobeto's varieties had substantially high zinc content that ranged between 3 mg /100 g to 882 mg / 100g (Wobeto *et al*.,2006). Oresegun's varieties had zinc content that ranged between 36 mg /100 g to 70 mg /100 (Oresegun et al. 2016). For those results reported by Salvador *et al*., (2014) had zinc content that was equivalent to 0.25 mg /100 g. Chavez' varieties had mean zinc content of 5.2 mg / 100 g (Chavez *et al*., 2000). Ravindran and Ravindran, (1998) reported on zinc content that ranged from 89 to 230 / kg. Burns' *et al*., (2012) varieties had leaves that had zinc content between 50 mg / 100 g to 120 mg /100 g. However higher zinc content was reported by Fasuyi. (2005). who had results that were within the range reported by Achidi. (2005). Achidi's results were up to 448.76 mg / 100 kg. Kuobala *et al*., (2015) expounded on the importance of zinc to include the synthesis of protein and nucleic acid in the plants. Oresegun's *et al*., (2016) varieties had leaves that had zinc content between 36.3 mg / 100 g to 69 mg / 100 g (Oresegun's *et al*., 2016).

Table 5: Means of cassava leaf micronutrients content mg/100g (wwb) leaf for three coastal varieties across three crop ages – (months after planting)

Cassava leaf contents	Cassava varieties	3 months	6 months	9 months
Vitamin C	Karembo	27.08 ± 0.50^{f}	272 ± 13.08^{c}	1236.5±11.9^{a}
(mg/100 g)	Kibanda meno	36.6 ± 22.42^{f}	7.19± 4.12d^{e}	281.6 ± 84.7^{c}
	Tajirika	151.19 ± 4.87^{e}	233± 61.00^{cd}	761.66±62.90^{b}
Vitamin A	Karembo	301.50 ± 12.9^{b}	638.17±64.83^{a}	164.9± 31.4^{f}
(mg/100 g)	Kibanda meno	253.5 ± 10.83^{bc}	241.82± 11.46^{cd}	161.0 ±32.2^{f}
	Tajirika	226.12 ± 25.1^{cde}	192± 30.33d^{ef}	177.2 ± 3. 6^{ef}
Iron	Karembo	9.61± 3.93^{a}	6.73±29.80^{bc}	8.19 ± 5.6^{ab}
(mg/100g)	Kibanda meno	10.08 ± 3.32^{f}	3. 83 ±26.91^{e}	7.53 ± 7.6^{b}
	Tajirika	5.15 ± 217.1^{cd}	3.04± 5.8^{e}	3.59± 5.9^{de}
Zinc	Karembo	7.1 8 ± 26.9^{a}	5.12 ± 2.27^{abcd}	3.6 ± 3.51^{b}
(mg/100g)	Kibanda meno	5.68 ± 1.21^{abcd}	3.76 ±6.19^{b}	3.6 ± 0.98^{c}
	Tajirika	6.06 ± 26.96^{abc}	3.22 ±5.28^{d}	6.43 ± 1.55^{ab}

* Means with the different superscripts in a row are significantly different at 5% level of significance

4. 5 Conclusion and Recommendations

The study concludes that crop age has effect on nutrients content in the leaves of coastal Kenya varieties that are also of high quality between 3 and 6 months after planting, with substantial protein content but low in fat content. The coastal varieties are also superior in micro nutrients contents (vitamin A and C, mineral iron and zinc) compared to those varieties reported by other researchers, while hydrogen cyanide levels are within acceptable range though the improved varieties tend to accumulate more cyanide towards reaching 9 months after planting thus proper processing is important. The cassava leaves from coastal varieties are therefore safe for human consumption and are recommended to be picked as early as three months.

Plate 2: Destalked tender leaves of cassava

CHAPTER FIVE

FORMULATION OF CASSAVA ROOT - LEAF FLAKES AND EVALUATION OF ITS ACCEPTABILITY AND NUTRITIONAL VALUE

Abstract

Cassava is an important food crop grown for its roots to supply daily needed calories to households in the cassava growing communities. In Kenya, the coastal region contributes up to 30% of the national cassava production though it remains food insecure with high prevalence of malnutrition. Cassava roots that are commonly consumed are deficient in most nutrients except carbohydrates while the leaves that are rich in a range of nutrients including protein, vitamins and minerals are moderately consumed as vegetables. This study sought to formulate flakes from cassava roots and leaves, a snack with improved nutrients' content. Formulation of flakes was done through mixing roots and leaves in varied levels ranging from 0% to 50%. Fermented and unfermented flakes were developed. A total of 18 formulations were developed before consumer acceptability and nutritional contents were determined in the most preferred blends. The results showed cassava root - leaf flakes were best accepted when fermented root material is blended with 20% leaf component. Percent leaf content above 40 % was unacceptable as such blends exuded unpleasant aroma. The nutritional value showed that cassava root-leaf flakes has vitamins A and C improved by 353% and 53%, minerals- iron and zinc by 5.6% and 85% respectively and protein by 430% when compared with flakes processed from 100% cassava root. It is recommended that more studies be carried out to determine bioavailability and nutritional effect of consumption of the flakes on children and pregnant women.

5. 1 Introduction

Cassava is a food crop that has always remained a crop of hope, providing livelihood to up to 500 million small holder households in the tropical regions, Kenya included (FAO, 2011). It provides up to 500 calories per day to over 70 million households (Chavez *et al*., 2005; Chavez *et al*., 2000). It is the third most important source of calories in the tropics, after rice and corn according to FAO. (2011). It grows and produces well under suboptimal conditions (Bechoff, 2018; Burrell, 2003; Cock, 1982). It is commonly grown for its roots that are rich in carbohydrates and therefore have potential to supply much needed calories to the communities that grow it (FAO, 2011). The leaves are moderately utilized (Abok *et al*., 2016; Tonukari *et al*., 2004) although they are rich in a range of nutrients including protein (Montagnac *et al*., 2009). The tropical regions have harsh climatic conditions with Kenya having 88% of its area being Arid and Semi-Arid (ASAL) (Njoka *et al*., 2016). Coastal Kenya is one of the regions that fall in the ASAL agro ecological zones (Njoka *et al*., 2016). The harsh climatic conditions have exacerbated poverty levels in this region.

World Bank. (2016) reported poverty in coastal region to range from 13% to 90% at sub-county level, across the 140 sub counties. Poverty is a vicious circle that rolls from low incomes, to food and nutrients, and health insecurities. However inasmuch as the region registers high poverty levels, it contributes up to 27% of the national cassava production in Kenya. It is unfortunate that cassava leaves that are rich in a range of nutrients, are still underutilized even in the face of high levels of malnourishment in children and pregnant mothers. Studies have shown that protein content in cassava leaves compares well with protein content in eggs (Montagnac *et al*., 2009; FAO/ WHO, 2005). The underutilization of cassava leaves is most likely due to the fact that they are only used as a vegetable. A vegetable is mostly eaten as accompaniment to basal diets. This means vegetables are hardly utilized in the absence of the basal diets. A worse scenario is expected in a region that is food insecure. The present study undertook to blend cassava roots and leaves to come up with cassava root - leaf flakes with improved nutrients. Flakes is, an all-time snack that will serve as a means of diversification of cassava based products and utilization of cassava leaves. Methods of processing cassava include chipping roots and drying, steeping and fermentation (Quaye *et al*., 2009; Nweke, 1996; Montagnac, 2009). Steeping is however not very common as it requires substantial amount of water for it to be undertaken, more so, steeping is traditionally carried out in rivers (Oyetayo, 2006). This method allows the moving water to rinse out any anti-nutrients present in the roots (Hahn *et al*., 1986). Fasuyi *et al*., (2005) indicated that traditional methods such as drying, pounding and long periods of boiling could remove anti- nutrients in cassava. The ultimate goals in processing include elimination of anti- nutrients, improving shelf life (Quaye, 2009; Cardoso *et al*., 2005), improving quality, palatability and general acceptability, reducing bulkiness as well as product differentiation. It is important to note that fermentation method is the most commonly practiced in cassava processing since it is not only efficient in removing anti- nutrients but also improves palatability of the end product/s (Quaye *et al*., 2009). Fermented cassava products include- *gari*, - in East and West Africa, *chikwangue* or *fufu* in Central Africa, and sour starches in Latin America (Cardoso *et al*., 2005: Rainbault *et al*., 1996). Fermentation bio conserves cassava through acidification by lactic acid bacteria. The process has as well been known to be responsible for product stability, flavor improvement and cyanide elimination (Rainbault, *et al*., 1996). The current study undertook to develop cassava root - leaf flakes using fresh processing and fermentation methods to generate different blends of cassava flakes.

5. 2 Materials and Methods

5. 2. 1 Harvesting and preparation of cassava roots and leaves

Cassava roots were harvested from plants that were at the age of 6 months up to 12 months after planting while the leaves were harvested at an early crop age - 3 months after planting up to 9 months after planting. Both the roots and the leaves were randomly harvested from three cassava varieties; Karembo, Tajirika and Kibanda Meno. Before undertaking major processing, the roots were chipped using a manual chipper with chipping plate that has mesh size of 10 mm as guided by Dziedzoave *et al*., (2003). The leaves were pounded to mash using a motor and pestle as recommended by other researchers (Bokanga, 1994: Bradbury, 2014).

5. 2. 2 Processing methods

Cassava roots and leaves were processed differently, using fresh and fermentation methods.

5. 2. 3 Processing steps and procedures

The fresh method (method 1) involved harvesting of roots, washing before and after peeling (roots) as guided by Dziedzoave *et al*., (2003), chipping of roots using manual chipper sun drying root chips on raised beds in open sun before milling and grinding using a hammer mill. Processing of leaves in the fresh method involved harvesting of leaves which were then washed and destalked as guided Bradbury. (2014). Pounding of leaves was done using mortar and pestle before the leaf mash was dried in drying mesh under shade according to Bokanga. (1994) and Bradbury. (2014). Drying of both the root and leaf materials was up to 13% moisture content.

All steps followed in fresh method (plate 3) were also undertaken in the fermentation method (method 2) but in this case, roots and leaves mashes were subjected to spontaneous fermentation for 3 days as described by Bokanga *et al*., (1994) and Quaye. (2009) before drying and milling.

5. 2. 4 Formulation of cassava roots and leaves flakes

The mixing of leaf cassava root material with leaf meal was cried out using varied percentages in order to come up with different blends.

Plate 3: Processed cassava root

Plate 4: Processed cassava leaf meal

Table 6: Levels of leaf composition (%) in cassava root leaf flakes and the method of processing

Blends of Flakes	Processing Method		Percent Leaf	Name of Blend
Root flakes	**Fresh - root**	**No leaf**	**0**	**Control 1** (X0)
Root -leaf flakes	Fresh - root	Fresh - leaf	20	X14
Root - leaf flakes	Fresh - root	Fresh - leaf	30	X 15
Root - leaf flakes	Fresh - root	Fresh - leaf	40	X16
Root - leaf flakes	Fresh - root	Fresh - leaf	50	X17
Root -leaf flakes	Fresh - root	Fermented leaf	20	X8
Root - leaf flakes	Fresh - root	Fermented leaf	30	X 1
Root - leaf flakes	Fresh - root	Fermented leaf	40	X6
Root - leaf flakes	Fresh - root	Fermented leaf	50	X11
Root flakes	**Fermented- root**	**No leaf**	**0**	**Control 2** (X13)
Root -leaf flakes	Fermented- root	Fresh - leaf	20	X2
Root - leaf flakes	Fermented- root	Fresh - leaf	30	X 5
Root - leaf flakes	Fermented- root	Fresh - leaf	40	X9
Root - leaf flakes	Fermented- root	Fresh - leaf	50	X7
Root -leaf flakes	Fermented- root	Fermented leaf	20	X4
Root - leaf flakes	Fermented- root	Fermented leaf	30	X 12
Root - leaf flakes	Fermented- root	Fermented leaf	40	X3
Root - leaf flakes	Fermented- root	Fermented leaf	50	X10

Cassava roots and leaves were mixed in different ratios using a linear model for food formulation model ($C_T = C_r.W + C_l.W + E$) Where C_T = Total nutrient content; Cr. W= cassava roots by weight;

C_l.W = cassava leaves by weight and E = error tag that would allow for additives and consequential product improvement as guided (FAO / WHO, 2016: Stuart et al. 2015). The linear model was however modified where by cost factor was exempted. The mixing of roots and leaf majorly targeted protein RDA for mothers. The mixing of roots with leaves both differently processed resulted to different blends of root-leaf flakes.

5. 2. 5 Processing of cassava root - leaf flakes formulation

The blends were put in separate labeled jars. Each blend at a time was mixed with vegetable oil at the ratio of 5: 2 (blend: Oil). After mixing, the mixtures were separately molded using a double sleeve molding mat to extra thin sheets. The thin sheets were then transferred into baking trays for baking. Baking was done at 250^{o}C for 12 to 17 minutes, with continuous monitoring to ensure uniform browning of the flakes.

5. 2. 5. 1 Steps and procedures

The flakes were produced as follows:

Step 1; Mixing of cassava root material with leaf meal at different ratios

Step 2; Each mixture put in to separate jar

Step 3; 200 g of each mixture weighed and put in a bowl and added with 40g (equivalent to 4 table spoons) vegetable oil

Step 4; Kneading and Molding

Step 5; Transferring of molded thin sheets into baking trays

Step 6; Baking at 250^{o}C for 12 t0 17 minutes

5. 2. 6 Pairwise ranking of formulated flakes

Sensory evaluation of flakes was undertaken in order to test acceptability in terms of colour, taste, aroma and texture (Meilgaard *et al*., 1999). However, non-professional tasters have a tendency to object sensory evaluation when the list of samples to be evaluated is long. In this study the list of blends of cassava flakes totaled 18. Therefore, a criterion was participatory developed that would allow for elimination of some of the blends with outright low acceptability qualities in order to reduce the list to a manageable number of below 10 samples. A total of 30 panelists were asked to participatory identify one parameter among the four (colour, taste, aroma and texture) that would be used as a "knock out" criterion. The exercise was carried out according to participatory methods by Guijt. (2014). In this case the panelists were also required to indicate reason/s for their choice of the parameter they felt was more powerful. Each panelist was given a questionnaire that had the list of four parameters and they were instructed to choose and tick one parameter they felt was most powerful in product acceptability. They were also asked to give one or two reason/s to justify their choice. The outcome results (selected parameter) was used to carry out pairwise ranking.
Pairwise ranking was conducted according to; Runsell. (2001) and Liu. (2009). Each panelist had a chance to compare one blend against the rest of the blends and indicate the blend they preferred on the basis of aroma that had been adopted as the "knock out" criterion.

5. 2. 7 Sensory evaluation

A total of 30 adult panelists were randomly selected and recruited within KALRO Mtwapa and its environs. The panelists were invited to KALRO Mtwapa as a central place, where they were first briefed on what was expected of them as they evaluated the flakes. A brief explanation was given on how to evaluate each parameter. The blends of flakes were labeled using random codes and randomly placed in plates. The plates were placed in well-lit panel booths. Each panelist was provided with clean drinking water to use for mouth rinsing every after tasting a sample according to Meilgaard *et al*., (1999). Every panelist evaluated each blend and translated their preference in terms of scores as guided in Bechoff *et al*., (2016) using a 7-point hedonic scale. The parameters that were evaluated were color, taste, aroma, texture and overall acceptability. Acceptability and preference level was measured by use of scores as translated in 7 point hedonic scale preference scores: .i.e., 7 = (like extremely), 6 = (like most), 5 = (like), 4 = (neutral), 3 = (dislike) 2 = (dislike most), 1 = (dislike extremely). Sensory evaluation of flakes was undertaken in order to test acceptability in terms of colour, taste, aroma and texture

5. 3. Laboratory analyses

5. 3.1. 1 Determination of nutritional quality of the selected blend

The most preferred blend alongside the controls were taken to the University of Nairobi food laboratories to determine their nutritive values.

5. 3. 2 Determination of moisture content

Moisture content determination was carried out using gravimetric method with a few modifications. About 5 g of pounded sample were weighed in crucibles and dried for 4 hours at 105 ^{o}C in an air oven as guided in AOAC. (2001) method 925.10.

5. 3. 3 Crude fat determination

Approximately 2 g sample was extracted using soxhlet extractor for 8 hours using 200 ml petroleum ether (40 - 60 ^{o}C). Crude fat content was calculated after evaporating the solvent and the residue dried in an air oven at 105 ^{o}C for 1 hour as guided in AAFCO. (2014).

5. 3. 4 Protein determination

Protein content in the flakes samples was determined as per AOAC. (2005) method. 979.09.

5. 3. 5 Determination of crude fiber

A duplicate of approximately 2.5g sample of flakes samples were weighed and transferred to soxhlet extractor and extracted using petroleum ether, one after the other. The rest of the procedure was then followed according to AOAC (2000) method 985.29.

5. 3. 6 Determination of ash content

Briefly 4 g sample of cassava flakes (per blend), weighed in duplicate was burnt in porcelain crucible using Bunsen burner (low flame) for 10 minutes then transferred to a Muffle furnace, ashed at 550°C for 4 hours as guided in AOAC (1995) method 923.03.

5. 3. 7 Determination of carbohydrates

Carbohydrates were determined by difference. The total of moisture content, fat, ash, protein and fibre contents were subtracted from 100 as guided in FAO (2003).

5. 3. 8 Determination of hydrogen cyanide

Hydrogen cyanide determination was carried out using distillation method. Cassava flakes were crashed using motor and pestle, and samples of 10 g per blend were placed into distillation flask and allowed to stand for three hours before distillation. Distillation and consequent determination of hydrogen cyanide was carried out as in AOAC (2016) method 915.03.

5. 3. 9 Determination of Vitamin **C**

Approximately 15 ml (10%) TCA, was added into flat bottomed flask containing cassava flakes samples and filtered. A total of 15 ml filtrate sample was collected. The filtrate sample was then mixed with 5 ml of 4% potassium iodide solution then titrated with N- bromisumccinimide solution. The rest of procedure was followed as described in AOAC. (2012) method 967.21.

5. 3. 9.1 Determination of vitamin A

Approximately 2 g sample of crushed flakes was weighed, 25 ml of acetone were added to extract colour, and the rest of steps followed were as guided in AOAC (2006) method 98.25.

5. 3. 9. 2 Determination of iron and zinc content

Cassava flakes sample (4g) for determining mineral Iron was ashed in a muffle furnace at 500 ^oC for 4 hours. This was then digested by adding 10ml of 20 % HCL and heated to boiling, then filtered into 100 ml volumetric flask and topped to mark using distilled water. Using atomic absorption spectrophotometer (A.A.S) mineral iron and zinc were determined according to AOAC (2016) method 99. 10.

5. 3. 9. 3 Experimental Design

The experimental design that was used for setting up experiment on evaluation of the blend of flakes was Randomized Block Design (RBD). Panelists were treated as blocks and different blends of flakes were studied as treatments against sensory attributes - color, taste, aroma, and texture. The scores given by panelist were subjected to analysis of variance (ANOVA), using Statistical Analysis System (SAS version 9.1). Means were separated using Least Significant Difference (LSD), the differences being significant at $p \leq 0.05$.

5. 4 Results and Discussion

5. 4. 1 Participatory development of "knock out" criterion

The results from the 30 panelists showed that 76% indicated aroma to be the most powerful parameter to be used as "knock out" criterion (Figure 4). One of the reasons indicated by most of the panelists as means of justifying their choice on aroma as the most powerful parameter rather than important was; "because aroma can draw the attention of a consumer to look for and find a product even when the product is hidden out of sight". It was further justified as "It is after finding the product that a consumer can appreciate its colour, then taste and final feel its texture". Upon discussion however panelists agreed synonymously that the most important parameter in acceptability is taste. Additional reasons that were indicated by panelists as a way of justifying their choice are as follows:

(i) Aroma is part of the flavor that influences the taste of a product

(ii) A consumer first smells a product before they taste it especially when it is not a common one.

(iii) An offensive aroma leads to a product being rejected before it is tasted

(iv) Aroma ignites appetite

(v) Aroma is one of the major factors commonly used for product differentiation i.e. yogurt tastes the same but different blends have different aroma that influences the flavours.

Aroma was therefore adopted as the knock out criterion. Inasmuch aroma was adopted as the parameter for knock out criterion as justified by panelists. However, according to Ross et al. (2011) texture is also one of the most important parameter towards sensory acceptability by end users. The results in this study contradicted the postulation by Ross because, texture was rated lowest. However, Ross' argument was in reference to potato tubers, it may have been ranked differently when considering processed products such like flakes. Mcdougall *et al*., (2007) went further to indicate that texture is influenced by several factors that include genetics, environment and processing. According to Ross et al. (2011), these factors have not been fully investigated, especially where cassava is concerned. Beleia *et al*., (2006) argue that a large number of factors influence texture of cassava roots. These factors, as the researcher indicates, include starch swelling pressure, cell size, cell wall structure and composition and its breakdown during cooking. This is however an argument that addresses the importance of texture in relation to processing. From the sensory and acceptability perspective, (Chen and Rosenthal, 2015) described texture as a food characteristic that is internally linked to the food structure hence it is a collective term of sensory experiences arising from visual, audio and tactile stimuli. It is also important to realize that the arguments drawn by researchers cannot be categorized as feedback from consumers rather than derived from the point of literature and scientific reasoning. The panelists also had a chance to debate within themselves and gave reasons that rationally justified their choice. It can as well not be refuted that aroma can initiate consumers to put effort to find a product that is out of sight and/ or hidden from them. Whereas an offensive aroma can lead to an outright rejection of a product before it is tasted. The panelists went further not to underrate the role that taste plays in influencing acceptability of a product. They argued out within themselves that taste remains to be important but not as powerful when compared with aroma in relation to acceptability.

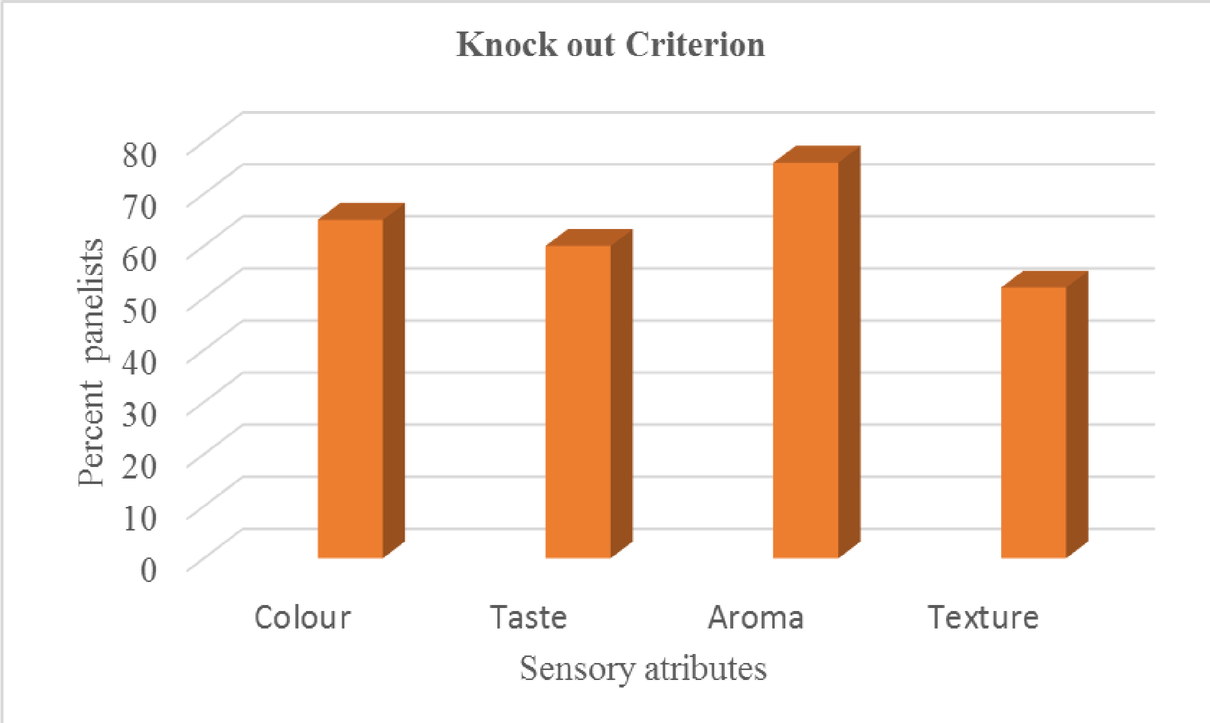

Figure 4: Knock out criterion as decided by sensory evaluation panelists

Formulation of flakes using fresh and fermented methods allowed the current study to develop a wide range of blends - up to 18. It was observed that after panelists adopted aroma as the knock out criterion, the blends that contained fermented root material were scored highly. This was a clear indication that fermentation of cassava roots positively influences the aroma- thus more acceptable. The observation is also in agreement with an argument projected by Westby (1994) that fermentation when carried out correctly develops improved flavours. This can as well be aligned with the scenario where fresh milk aroma and / or flavour is improved through fermentation.

5. 4. 2 Pairwise ranking

The results from pair wise ranking (Appendix 3), showed that the blends with fermented material either root or leaf were scored highly. It was also shown that the less the leaf component, the more the blend exuded appealing aroma hence preferred by panelists. Also, 30% fresh leaf in fermented root was better than (fresh root blended with 30% fresh leaf) and (fermented root blended with 30% fermented leaf). Since the leaf content in these three blends was at 30% yet they were rated differently, these results indicate that the processing method also has effect on the acceptability of cassava flakes. The blend that had most preferred aroma was the blend containing 20% fresh leaf combined with fermented root material (Appendix 3).

Fermented root blended with 20% fresh leaf, was ranked second after 100% fermented root (control 2) it was, however ranked higher than 100% fresh root (control 1) The blends that had scored 10 points and above (Appendix 3) were picked for four parameters sensory evaluation - i.e. (color, taste, aroma and texture). These blends were; a) Fermented root blended with 30% fresh leaf, b) Fermented root blended with 20% fresh leaf, c) Fermented root blended with 20% fermented leaf, d) Fermented root blended with 30% Fermented leaf, e) Fresh root blended with 20% fermented leaf, f) Fermented root blended with 30% fermented leaf, g) 100% fresh root; h) Control 1- Fresh root 0% leaf; i) 100% fermented root - Control 2, as shown in (Table 6). From the perspective of using aroma as the knock out criterion, it was observed that the blends that contained fermented root material were scored higher than those that had fresh root material. There was inconsistence in the way the preference was presented in relation to blends that contained fresh leaf material. Some blends with fresh leaf were scored higher than others. However, it was well demonstrated that leaf material blended with root material was preferred when at lower percentage, whether in fresh or fermented form (Appendix 3).

5. 4. 3 Sensory evaluation using 7-point hedonic scale

The results on 4 parameters (color, taste, aroma, and texture) sensory evaluation of the blends showed significant ($p \leq 0.05$) difference in the way the panelists scored and consequently ranked them (Table 7).

5. 4. 3. 1 Colour

The blend/s of flakes that had most preferred colour were 100% fresh root (Plate 7) and 100% fermented root (Plate 6). The two blends however did not contain any leaf material and they were used as controls in the trial. The two blends had mean scores of 6.3 and 6.6 respectively. The fact that the two blends contained no leaf material could have led to their high scoring. This because it is common to find pure root cassava products such like flour, crisps, fried cassava root chunks etc. Whereas blending with cassava leaves is yet to be fully adopted especially in the coastal region of Kenya. It was therefore literal that consumers were going to find the poor root blends more likable than those blended with leaf material. The colour of the blends was scored significantly ($p< 0.05$) different. The was significant ($p< 0.05$) difference in colour of the Fresh root blended with 20% fresh leaf, Fermented root +20% fresh leaf (Plate 6) and Fermented root blended with 20% fermented leaf. In as much as the blends contained 20% leaf material, having been processed differently was observed to create variation on the colour that result to different preference by the panelists. The colour of Fermented root blended with 30% fermented leaf, Fermented root blended with 30% fermented leaf and Fermented root blended with 30% fresh leaf was also significantly ($p \leq 0.05$) different in the way the blends were preferred by panelists. The blend that was most preferred in terms of colour was Fermented root blended with 30% fermented leaf. Inasmuch as blends with less leaf material were more preferred, colour of Fermented root blended with 30%

Fermented leaf was not significantly ($p< 0.05$) different from that of Fermented root blended with 20% fresh leaf that contained fresh leaf.

5. 4. 3. 2 Taste

The results showed significant ($p \leq 0.05$) difference in the way panelists preferred their taste (Table 7). Fermented root + 30% fresh leaf, Fermented root blended with 30% fermented leaf and Fermented root + 30% Fermented leaf were significantly ($p < 0.05$) different in taste though they all contained 30% leaf material. Fermented root blended with 30% fresh leaf had a mean score of 3.77. Fermented root blended with 30% fermented leaf had a mean score of 4.83. Fermented root blended with 20% fresh leaf, Fermented root blended with 20 % fermented leaf and Fresh root blended with 20% fresh leaf were significantly ($p< 0.05$) different in taste though they all contained 20% leaf material. Fermented root blended with 20% fresh leaf had a mean score of 5.30 as rated on taste, was not significantly different to the taste in Fermented root blended with 30% fermented leaf inasmuch the latter contained higher leaf material at 30%.

5. 4. 3. 3 Aroma

The results showed significant ($p \leq 0.05$) difference in the panelists' preference of the aroma of most blends. The panelists however could not pick the difference in aroma of blends 30% fermented leaf blended with fermented root and the aroma of the blend with 20% fresh leaf blended with fermented root (Table 7). Bechoff *et al.*, (2018) postulated that fermentation of cassava is a way to develop specific and appreciated product flavours. It is important to note that panelists in the present study also associated aroma with flavours of a product. Aroma in Fermented root +30% fresh leaf was significantly ($p \leq 0.05$) different from aroma in Fermented root blended with 30%

fermented leaf and Fermented root blended with 30% fresh leaf, though they contained similar quantity of leaf material. Also Fermented root blended with 20% fresh leaf, Fermented root blended with 20% fermented leaf and Fresh root blended with 20% fresh leaf were significantly ($p< 0.05$) different in the way panelists preferred their aroma though all had 20% leaf material. The most preferred aroma was in the blend; Fermented root blended with 30% fermented leaf and Fermented root blended with 20% fresh leaf with scores of 2.93 and 5.37, and 5.33 respectively.

5. 4. 3. 4 Texture

The results showed significant ($p\leq 0.05$) difference on basis of panelists preference on the texture of the blends (Table 7). Fermented root blended with 20% fresh leaf, Fermented root blended with 20% fermented leaf and Fresh root blended with 20% fermented leaf had 20 % leaf material but were significantly ($p \leq 0.05$) different according to preference by panelists. A similar trend was also shown in blends Fermented root blended with 30% fresh leaf, Fermented root blended with 30% fermented leaf and Fresh root blended with 30% fermented leaf. That were shown to be significantly ($p< 0.05$) different according to preference by panelists. The texture of cassava blends was shown to be preferable when blends are fermented. This was depicted by the fact that the blend that had fermented root blended with 30% fresh leaf, was rated higher than control 1 that contained 100% fresh root despite that fact that it contained higher percent leaf material. The mostpreferred texture was found to be in blend - Fermented root blended with 30% fermented leaf witha mean score of 5.63 but not significantly different from 100 % fermented root, the control 2 withmean score of 6.17.

Sensory evaluation undertaken to evaluate colour in cassava root- leaf flakes indicated that the two blends that had 100% root with no leaf added had the most preferred colour. However, Bechoff *et al.*, (2018) recognizes food as culture. And culture is defined in oxford dictionary, as “the ideas, customs, and social behavior of a particular people or society". From this argument therefore, end users are bound to always prefer what they are used to eating against that which is new to them. The two blends had mean scores of 6.3 and 6.6, respectively. They were probably highly scored due to the fact that they contained no leaf material. It is common to find pure root cassava products such as flour, crisps, fried cassava root chunks etc. Blending is yet to be fully adopted, in the coast region. It was therefore literal that consumers were going to find pure root blends more likable than those blends that contained leaf material. Taste and aroma of blend 20% fresh leaf blended with fermented root could not be differentiated from the blend that contained 30% fresh leaf in fermented root material. This is an indicator that the consumers accept flakes that contains leaf material up to 30%, beyond which they will reject the product. And this was well demonstrated where all the blends the contained 40% leaf were rated low and or disliked during the sensory evaluation.

5. 4. 3. 5 Overall acceptability:

The results showed significant ($p \leq 0.05$) difference in the panelists' preference of the four parameters evaluated in each of the different cassava blends (Table 7). The blend with Fermented root blended with 20% fresh leaf (plate 5) had its attributes significantly ($p \leq 0.05$) preferred differently by panelists. Its overall acceptability score was 5.30. Fermented root blended with 20% fermented leaf had an overall acceptability at mean score of 4.97. Blend Fermented root blended with 30% fermented leaf was shown to have attributes that were significantly ($p \leq 0.05$) different as preferred by panelists. Its best rated attribute was texture in its overall acceptability. Blend Fermented root containing 30% Fermented leaf had its parameters' mean scores ranging from 5.20 to 5.63. However, its overall acceptability falls at mean score of 4.77. Blend Fresh root containing 20% fermented leaf had its attributes scored at mean score of 4.10 of its overall acceptability. The overall acceptability also confirmed that the blend with 30% fresh leaf material combined with fermented root, though rated lower than the 20% fresh leaf material combined with fermented root was at acceptable. Sensory data is however subjective and its subjectivity of is well explained by Babicz-Zielińska. (2006) who argues that food acceptance is influenced by Psychological state of individuals. According to Cardello. (2012), acceptance of food relies on physicochemical characteristics of the food interacting with human senses.

Plate 5: Flakes containing 20% leaf combined with root material

Plate 6: 100% fermented root flakes

Plate 7: 100% fresh root flakes

Table 7: Mean scores for color, taste, aroma, texture and overall acceptability of cassava flakes

Id	Blends of cassava flakes	Color	Taste	Aroma	Texture	Overall Acceptability
X1	30% fermented leaf + fresh root	3.50 ± 0.39^{d}	3.77 ± 0.409^{d}	2.93 ± 0.448^{e}	3.6 ± 0.554^{e}	3.50 ± 0.251^{e}
X2	20% fresh leaf + fermented root	6.07 ± 0.392^{b}	$5.30 \pm 0.\ 409^{bc}$	5.33 ± 0.447^{bc}	5.40 ± 0.554^{bc}	5.30 ± 0.251^{b}
X4	20% fermented leaf + fermented root	4.77 ± 0.393^{bc}	4.73 ± 0.409^{c}	4.97 ± 0.448^{c}	4.70 ± 0.554^{dc}	4.97 ± 0.251^{bc}
X5	30% fresh leaf + fermented root	4.90 ± 0.393^{bc}	4.83 ± 0.409^{c}	4.70 ± 0.448^{cd}	5.20 ± 0.554^{b}	5.00 ± 0.251^{bc}
X12	30% fermented leaf + fermented root	5.20 ± 0.393^{b}	5.57 ± 0.409^{b}	5.37 ± 0.447^{bc}	5.63 ± 0.554^{ab}	4.77 ± 0.251^{c}
X8	20% fermented leaf + fresh root	4.43 ± 0.393^{c}	3.63 ± 0.409^{d}	4.00 ± 0.448^{d}	4.20 ± 0.554^{de}	4.10 ± 0.251^{d}
X0 Control 1	Fresh root + (0% leaf)	6.30 ± 0.392^{a}	5.93 ± 0.409^{b}	6.00 ± 0.447^{ab}	2.30 ± 0.554^{e}	5.03 ± 0.25^{bc}
X13 Control 2	Fermented root+ (0% leaf)	6.63 ± 0.392^{a}	6.83 ± 0.408^{a}	6.50 ± 0.447^{a}	6.17 ± 0.554^{a}	6.37 ± 0.250^{a}

*Means with different superscripts along a column are significantly different at 5 % level of significance

5. 4. 4 Nutritive value of preferred cassava root-leaf flakes

The results of the current study showed that there was significant ($p \leq 0.05$) difference in the nutritive value of the most preferred blend of cassava root - leaf flakes; Fermented root +20% fresh leaf and the two controls that did not have any leaf material but 100% fermented and fresh root material. The two played representative role of the current status of available cassava products i.e crisps, cassava flour, fried chunks that are processed purely from cassava root. Fermented root +20% fresh leaf had significantly ($p \leq 0.05$) higher content of protein, carbohydrates, vitamin A, minerals iron and zinc, fibre and ash. It had significantly ($p \leq 0.05$) lower vitamin C than the controls. In comparison of cassava root - leaf flakes with flakes made from pure roots, most of the nutrients measured were significantly ($p \leq 0.05$) higher in the root - leaf (Table 8). The pure root flakes processed through fresh and fermented methods give an indicator of the common cassava products that are currently available to consumers. Cassava products commonly found in the market such as flour, crisps, roasted and fried chunks and fresh cassava root (Mwamachi *et al*., 2005). Comparing the results found in this study on vitamin c content with other food products, it was shown that the cassava root - leaf flakes had much higher vitamin C than that found in guava and or green pepper as reported by (Kareem, 2010; Essam *et al*., 2019). Determination of nutritive value of the Fermented root blended with 20% fresh root showed reduced vitamin C when compared with the controls. This could have been due to the fact that vitamin C is water soluble and heat labile hence could have been lost during processing (Montagnac *et al*., 2009). The roots that were processed through fermentation method may have resulted in a major loss of the vitamin. Cassava root - flakes, however, had higher protein content than wheat bread and other 4 types of cassava bread that were formulated by Maria *et al*., (2013). While the pure wheat bread contained 10 g / 100 g, the cassava-based types of bread ranged from 1.3 g / 100 g to 5.4 g /100 g protein.

Montagnac *et al*., (2009) reported on leaf meal that contained 34 g/100 g protein that is a little bit higher than the protein content in cassava root - leaf flakes. Vitamin A content of the root - leaf flakes was much higher than vitamin A content reported by Motagnac *et al*., (2009) on raw cassava. The researchers, however, indicated that processing increases vitamin A content in cassava products. Abok *et al*., (2016) reported vitamin C content in his cassava crisps ranging from 73 mg / 100 g to 136 mg / 100 g that is far much lower than the level of vitamin C contained in the cassava root - leaf flakes. The present study proves that cassava root - leaf flakes is an improved cassava product that is much superior in nutrients than most common pure cassava roots productscurrently available.

The Recommended Dietary Allowance (RDA) guides that males, depending on their weight, should averagely take protein amounting to between 45 to 63 g per day, vitamin A, averagely 1000µg, vitamin C 50 - 60mg, Iron 10 - 12mg, zinc- averagely 15 µg; Females, Children of age 4 - 9 years require 275 µg vitamin A or half of the stipulated quantities also depending on the body weight (FAO/WHO, 2001). The formulated cassava root - leaf flakes meet about a half ($^{1}/_{2}$) to three quarters (¾) of the RDA in most of the nutrients. This calls for further fortification of the product as way of up scaling to fully meet the RDA.

Table 8: Nutritive values (mg / 100 g wet weight basis) of cassava root – leaf of flakes

Nutrients	Blends of cassava flakes		
	X2	**X13**	**X0**
MC	11.17± 0.45[a]	10.34±0.44[a]	10.34± 0.44[a]
Carb's(g/100 g)	40.76±0.45[a]	37.18±0.45a	28.54±0.57[b]
Protein (g/100 g)	26.0±0.99[a]	4.9± 0.44[b]	6.51±0.45[b]
Fibre (g/100g)	8.24±0.44[a]	6.27±0.449[b]	6.88±0.438[b]
Ash (g/100g)	6.22±0.44[a]	6.68±0.45[b]	7.96±0.44[b]
Fat	8.26±0.47[a]	8.67±0.48[a]	8.22±0.44[a]
Vit A	488.4±7.61[a]	107.9±2.16[c]	269± 1.879[b]
Vit C	746.58± 5.55[a]	485.32± 27.58[b]	403.05± 1.52[b]
Iron	8.91±0.44[a]	4.8±0.438[b]	5.54±0.45[b]
Zinc	3.61±0.45[a]	3.42±0.438[a]	4.0±0.46[a]
Hydrogen Cyanide	2.7± 0.44[a]	3.5± 0.45[a]	3.7± 0.44[a]

*Means with similar superscripts in the same row are not significantly ($p \leq 0.05$) different

*Blends X2 = 20% fresh leaf material mixed with fermented root, X13 = 100% fermented root and X0 = 100% fresh root material.

5. 5 Conclusions and Recommendation

The study concludes that cassava root - leaf flakes is most acceptable when it contains leaf material at the level ranging from 20% to 30%, the lower the leaf composition the more the product is liked. Cassava root - leaf flakes has improved nutritional value in terms of vitamins, minerals and protein, in comparison to the common pure cassava root products. The study recommends more research to determine the shelf-life of the formulated flakes. Nutritionists may also determine the nutritional contribution of cassava root- leaf flakes to children and pregnant mothers - (targeted beneficiaries)

CHAPTER SIX

EFFECT OF PACKAGING MATERIALS ON KEEPING QUALITY OF CASSAVA ROOT - LEAF FLAKES

Abstract

Processing and value addition is necessary for fresh agricultural commodities in order to reduce perishability and prolong shelf life. The fresh commodities are also seasonal and bulky, however, processing and value addition not only make it possible to prolong shelf-life but also for product diversification. Shelf life is enhanced with proper packaging because packaging materials also influence the storage period through the preservation nutrients and sensory quality of stored food products. Three blends of cassava flakes identified by panelists as the most preferred (20% leaf, 100% fresh root, 100% fermented roots were developed and studied on accelerated shelf life trial. Storage period and packaging material were determined. The studied packaging materials were Kraft, insulated polythene and plastic, stored in an incubator at 55^0C and 75% relative humidity for 5 days. The results showed moisture content to be significantly influenced by packaging material whereby it increased over the storage period, across the blends, with highest levels (10.75-%) registered in kraft material on day 3. Insulated polythene was shown to contain highest nutrients' levels by day 5 with protein at 27.68 mg /100g vitamins A (576.85 mg/100 kg), Zinc (1.17 mg /100 g), iron 3.69 mg /100g), fibre 6.12 g /100g. Fat was highest at 9.71 g/100g in the plastic material. Acid and peroxide values gradually increased in all the packages from 0 to 3.6 mg KOH / g and 0 to 6.3 mg /kg, respectively. Microbial counts showed insulated polythene to be more restrictive in microbial growth in both spread plate and pour plate methods. The study therefore concluded that insulated polythene is the best packaging material for cassava flakes and the product's shelf life is up to 3 months.

6. 1 Introduction

Agricultural commodities and especially fresh commodities are known to have common characteristics that include seasonality, bulkiness, and high perishability (FAO, 2001). Cassava in its fresh form deteriorates to spoilage in a span of 72 hours (Abong *et al*., 2016). Perishability is however mitigated through processing that equally enables value addition, product diversification, leading to enhanced utilization and marketing and, market segmentation (Nweke, 2001). Processing also reduces commodity bulkiness and improves shelf life. Shelf life is mostly rated the most important factor that necessitates processing. However, in order to effectively improve product shelf life, the product storage has to be carried out under suitable temperatures as well as packaging materials. Packaging and packaging materials are most appropriate for storage and portability of a product besides the preservation (Kulcu, 2018). It is important to note that the packaging is an act of value addition as well.

Cassava roots and leaves are agricultural commodities whose common characteristics pose a challenge to producers. Cassava roots are consumed to supply much needed calories while leaves are moderately consumed but are a rich source of a range of nutrients including protein, (Achidi *et al*., 2005; FAO, 2000). Both the roots and leaves are highly perishable. This therefore necessitates processing, packaging and storage as a means to improve shelf life.

Shelf life has been defined by the consumer to mean a number of connotations that include; the time limit, that a product retains its sensory, intrinsic and physical qualities (Manzocco, and Nicoli, 2011: Hammond *et al*., 2015). Shelf life represents the length of time before the food is considered to be unsuitable for human consumption (Manzocco, and Nicoli, 2011; Conte, *et al*., 2013). Suitability and safety of a product are factors embedded in the quality of the product. Quality of a product includes the level of nutrients in a particular product (nutritive value of a product). A product that passes its shelf life date does not immediately become dangerous for human consumption, but rather no longer conforms to a set of given quality parameters (Moschopoulou *et al*., 2019). Other factors that affect shelf- life besides the product itself include the micro environment in which the food or product is packaged (Higgs, 2019). Shelf life is termed to have expired once the micro- environment within a packaged product begins to be conducive for microbial growth. Therefore, storage temperatures and packaging are some of the other factors that affect shelf life.

The efficiency of packaging in extending shelf life of foods and products depends on packaging material that are majorly; metals, glass, paper, polythene and plastics (Manzocco, and Nicoli 2007). Packaging materials are known to have influence on chemical reactions within the packaged food / products. These reactions can be monitored through parameters such as peroxide value, acid value and microbial count (Nychas *et al*., 2016). A definition of peroxide value is given as; the amount of oxygen consumed in the reaction that reduces all the unsaturated carbon bonds (C=C) in a given amount (mass) of a lipid mixture during autoxidation Bandyo, (2017). The oxygen ultimately forms peroxides. By use of peroxide method one can measure how unsaturated the fat/oil is (Bandyo, 2017). Another parameter measured in shelf life is acid value that simply means the number of milligrams of potassium hydroxide (KOH) necessary to neutralize the fatty acids in 1 gram of sample (Zailer, 2019).

Chemical reactions and microbial growth on food and food products bring about spoilage that end up compromising the products quality. Spoilage and its extent is normally evaluated through, the use of microbial count, among other methods. Key microorganisms depend on the nature of the product in question.

The current study therefore sought to determine the effect of packaging materials (Kraft paper, insulated polythene packets and plastic jars). The study under took to determine; (a) the amount of oxygen spent - unsaturated fat reduction (b) free fatty acids and (c) microbial count in the packaged blends of cassava flakes under ambient temperature.

6. 2 Materials and Methods

6. 2. 1 Sample preparation, packaging and storage

Blends of cassava flakes were formulated from 100% fresh roots, 100% fermented roots and 20% leaf + fermented root. Formulation of the flakes was carried out at KALRO Mtwapa food laboratory and transported to the University of Nairobi for shelf life trial. The choice of 20-% leaf material to be incorporated in the root was guided by an acceptability test that was carried out by 30 panelists on a variety of blends that contained varied ratios of cassava leaf added on to root material. The three blends of cassava flakes were differently packaged in duplicates in 200g standard packages made from different materials namely: Kraft paper bags, insulated polythene - packets and plastic jars. Packages were put in a laboratory incubator at 55^{o}C / 75% rh (relative humidity) for commencement of accelerated shelf life trial. Please note that the term accelerated shelf life, every span period of 24hours (one day) translates to a one month. This activity was carried out in the University of Nairobi food laboratories.

Nutrients in the stored blends were monitored using laboratory procedures in order to determine their levels during the storage period. Nutrient analyses were carried out on alternative days, 1, 3 and 5.

6. 3 Laboratory analyses

6. 3. 1 Determination of moisture content

Moisture content determination was carried out using gravimetric method with a few modifications. About 5 g of pounded sample were weighed in crucibles and dried for 4 hours at 105 ^{o}C in an air oven as guided in AOAC. (2001) method 925.10.

6. ***3. 2 Crude fat determination***

Approximately 2 g sample was extracted using soxhlet extractor for 8 hours using 200 ml petroleum ether (40 - 60 ^{o}C). Crude fat content was calculated after evaporating the solvent and the residue dried in an air oven at 105 ^{o}C for 1 hour as guided in AAFCO (2014).

6. 3. 3 Protein determination

Protein content in the flakes samples was determined as per AOAC (2005) method number 979.09.

6. 3. 4 Determination of ash content

Briefly 4 g sample of cassava flakes (per blend), weighed in duplicate was burnt in porcelain crucible using Bunsen burner (low flame) for 10 minutes then transferred to a Muffle furnace, ashed at 550°C for 4 hours as guided in AOAC (1995) method 923.03.

6. 3. 5 Determination of carbohydrates

Carbohydrates were determined by difference. The total of moisture content, fat, ash, protein and fibre contents were subtracted from 100 as guided in FAO (2003).

6. 3. 6 Determination of hydrogen cyanide

Hydrogen cyanide determination was carried out using distillation method. Cassava flakes were crashed using motor and pestle, and samples of 10 g per blend were placed into distillation flask and allowed to stand for three hours before distillation. Distillation and consequent determination of hydrogen cyanide was carried out as in AOAC (2016) method number 915.03.

6. ***3. 7 Determination of Vitamin C***

Approximately 15 ml (10%) TCA, was added into flat bottomed flask containing cassava flakes samples and filtered. A total of 15 ml filtrate sample was collected. The filtrate sample was then mixed with 5 ml of 4% potassium iodide solution then titrated with N- bromisumccinimide solution. The rest of procedure was followed as described in AOAC (2012) method number 967.21.

6. 3. 8. Determination of beta carotene

Approximately 2 g sample of crushed flakes was weighed, 25 ml added to extract colour, and the rest of steps followed were as guided in AOAC (2006) method number 98.25.

6. ***3. 9 Determination of iron and zinc content***

Cassava flakes sample (4g) for determining mineral Iron was ashed in a muffle furnace at 500 ^oC for 4 hours. This was then digested by adding 10ml of 20% HCL and heated to boiling, then filtered into 100 ml volumetric flask and topped to mark using distilled water. Using atomic absorption spectrophotometer (A.A.S) mineral iron and zinc were determined according to AOAC (2016) method 99. 10.

6. 3. 9. 1 Determination of peroxide value and acid value

A total of 18 packages were removed on alternative days 1, 3, and 5 to determine Peroxide value as guided in AOCS (1997) Cd 8b - 90 and acid value was done according to AOCS (1997) Ca 5a - 40.

6. 3. 9. 1. Microbial Enumeration

Microbial enumeration was carried out by first making decimal dilution to the power of 10^{-10} with steps observed as guided by (Sanders, 2012). Pour plate method was used to determine total viable count and yeasts and molds. Homogenate was prepared by mixing 225ml of diluent to 25g of food sample. Duplicate samples of 1 ml from 0.85-% total of 25 g per flakes' type and mixed well with the diluent. MRS agar was used in order to allow enumeration of lactobacilli bacteria, using spread plate method that was reported as log CFU / g. Smart Count Agar (SMA) was used for pour plate method in order to monitor total viable microbial growth. Smart count was used in order to monitor general microbial growth since it is a non-selective agar as opposed to MRS that selectively allows lactobacilli growth. Smart count agar was reported as log CFU / ml (Plate Count Agar). Sampling was carried out from each packaging material according to the packaged blend of flakes.

6. 3 Experimental Design and Data Analysis

The statistical design used in the study was Complete Randomized Design, where cassava flakes blends and packages (by material) were independent variables. Data were collected on the following parameters: peroxide value and acid value and, total viable count (TVC) and yeast and molds (Y&M). Results from laboratory analyses were subjected to the analysis of variance (ANOVA), using the General Linear Model (GLM) of the Statistical Analysis System (SAS version 9.1). Means were separated using Least Significant Difference (LSD), the differences being significant when $p \leq 0.05$.

6. 4 Results and Discussion

The three packaging materials Kraft (Kraft paper bag), insulated polythene packets and plastic jars had significant effect ($P \leq 0.05$) on moisture content of all the three blends of flakes; 20% cassava leaf blended with cassava root material, 100% fresh cassava root and 100% fermented cassava root

(Table 9). The effect of packaging materials on moisture content was registered across the storage period from day 1 to day 5. Please note that the term accelerated shelf life, every spurn period of 24hours (one day) translates to a one month. The blend with 20% cassava leaf material had a fluctuating trend in its moisture content. It showed a moisture decrease on day 3 but there was an increase of moisture content on day 5, in the insulated plastic packets. A similar trend was shown in the plastic package. There was, however, a consistent increase of moisture content in the Kraft package across the entire storage period. This scenario could have been caused by the fact that Kraft material is porous and probably was imbibing moisture from within the incubator. A comparison across the three packaging materials by day 5 showed that the paper insulated polythene packets had the lowest moisture content in all the three blends of cassava flakes across the storage period. Akingbala *et al*., (2005) however reported a decrease in moisture content of *gari* stored in polybags at ambient temperatures. On the other hand, the results of the current results agree with those reported by Shibby *et al*., (2017) who recorded an increase in moisture content in stored pineapple lassi with a variation in the different packaging materials. The variationof the results reported by the two different researchers Akingbala and Shibby could have risen from the difference in the nature of the stored products. Abong'*et al*., (2011); Ajayi *et al*., (2015)reported an increase in moisture content in their research on potato crisps and mushrooms respectively, packaged in different packaging material. Zeeman *et al*., (2007); Pillai, (2012) on the other hand reported that an increase in thickness of polymers (polythene) material increased the barrier properties of the film material making it more efficient on inhibiting moisture permeation. This confirms the characteristics shown in the insulated polythene packets.

The packaging materials also had significant effect ($P \leq 0.05$) on protein content of in all the blends across the packaging materials over the storage period. Protein content was shown to reduce as the blends were stored longer. All the packaging materials registered declined levels of protein in the blend containing 20% cassava leaf and the blend containing 100% roots flakes. The blend containing 100% fermented root showed to be stable in all the three packaging materials. There was shown to be a decrease in protein content in the blend containing 20% leaf material blended with root materials packaged in paper insulated polythene packets. The results of the current study however differ from the results reported by Haruna *et al*., (2015) who reported an increase in protein in *gari* stored in specialized ware house. On the other hand, Shahidi (2019); Ozdal *et al*., (2013), argued that proteins in foods commonly form complexes with other food components. The presence or the absence of such chemical reactions could be the source of variations in the results reported in different studies, including the current study. By day 5 20 % cassava leaf blended with cassava root material packaged in insulated polythene was shown to contain higher levels of protein (on a decreasing rate) compared to other packaging materials, the blend containing 100% fresh root was shown to contain more protein in Kraft and the blend containing 100% fermented roots had no significant ($P \leq 0.05$) difference across the packaging materials. Tunick *et al*., (2016) also reported protein drop after twelve months in whey stored for 20 months. Moneim *et al*., (2007) however reported an increase in protein and ash levels in white cheese stored in different packages. These results of the current study show that packaging materials have effect on nutrientlevels of stored products, a fact that was reported by Willige. (2002), Hao *et al*., (2015) and Moneim *et al*., (2007).

Carbohydrates content in all the three blends were significantly ($P \leq 0.05$) affected by the packaging materials over the storage period. The blend containing 20% cassava leaf material blended with cassava root material had significant ($P \leq 0.05$) difference in carbohydrates content in day 3 compared to day 5. The significant ($P \leq 0.05$) effect was demonstrated by a drastic drop of carbohydrates levels in this particular blend across the three packaging materials. The blend containing 100% fresh root material showed a fluctuation trend with carbohydrates contained in kraft registering an increase. The scenario where carbohydrates were shown to register an increase in the levels could have been caused by the fact that kraft material is porous, a fact that could have caused a decrease or an increase in moisture content that consequently influence the carbohydrates levels. There was decrease in carbohydrates in the laminated polythene and plastic packages. The blend containing 100% fermented root material showed a significant ($P \leq 0.05$) drop by day 5 especially in kraft and paper insulated polythene packets, but there was an increase in the plastic material package. These variations could have been caused by the nature of the products or the nature of the packaging materials. In a scenario where there is a moisture decrease, carbohydrates tend to show increased levels. The results of the current study agree with results reported by Haruna *et al*., (2015) and Ajayi *et al*., (2015), the two researchers reported similar trends in their results where there were variations in carbohydrates levels of products packaged in different packaging materials. Haruna *et al*., (2015) had his *gari* showing fluctuating trends, but registereda peak increase of carbohydrates levels after 1year storage up. Ajayi's study showed carbohydrates packaged in aluminium laminated packages, to be higher than levels in other packages.

There was significant effect ($P \leq 0.05$) of packaging material on fat content across the three blends. Significant ($P \leq 0.05$) difference was also shown within one blend packaged in the different packaging material. Equally significant difference in fat content was registered across the storage period from day 1 to day 5. 20% cassava leaf blended with cassava root material was showed to significantly ($P \leq 0.05$) higher levels of fat content in plastic than in kraft and gunny packages. Fat content in plastic was higher at 21% than in Kraft and 20.7% than in gunny. However, this difference was not significant ($P \leq 0.05$). It was found that X0 had higher fat content in the plastic

Ash content was significantly ($P \leq 0.05$) affected by packaging materials. Variation was registered within one blend packaged in different packages in the blend containing 100% fresh root material and the blend containing 100% fermented root material. The variation was however registered in day 1 and 5. The blend containing 100% fresh roots packaged in gunny had significantly ($P \leq 0.05$) higher ash levels than in kraft and plastic. A similar trend was depicted in the blend containing 100% fermented root material. The blend containing 20% cassava leaf blended with cassava root material was least affected by packaging material across the storage period. This scenario could be indicating that 20% cassava leaf blended with cassava root material is more shelf stable product than the blend containing 100% fresh root material and the blend containing 100% fermented root material. Itwarnge and Achimba. (2009 reported an increase in ash content during fermentation of cassava root mashes for *gari* processing. While Moneim confirms that packaging materials have effect on ash in the packaged food and food products (Moneim *et al*., 2007), The variation in the effect of the packaging material across the blends could be arising from the difference in composition of the different blends and the difference in the method of processing.

package than in Kraft and gunny. Similarly, the blend containing 100% fermented root had significantly ($P \leq 0.05$) higher fat content in gunny than in kraft and plastic packages. Hao *et al*., (2015), studied effect of packaging films on canola oil under photo oxidation conditions. Hao reported packaging films to have high effect on the canola oil. Hao's study confirmed that packaging films have effect on oils, a fact that is also confirmed in the current study. Wellige (2002) on his study argued that rancidity of food is mostly minimized when the packaging material ensures good barrier to oxygen and also protects the food product from light.

Table.9: Effect of packaging materials on macro nutrients content (g/100 g dwb) of cassava flakes during 5 days' storage

S - life /	Nutrients	20% cassava leaf flakes			100% fresh cassava root flakes			100% fermented cassava root flakes		
		Kraft	Ins' polythene	Plastic	Kraft	Ins' polythene	Plastic	Kraft	Ins' polythene	Plastic
Day 1	MC	9.00 ± 1.138^{bcdefg}	9.28 ± 0.00^{bcdefg}	9.77 ± 0.02^{abcd}	10.07 ± 0.22^{abc}	7.13 ± 0.48^{h}	8 ± 0.70^{fgh}	9.89 ± 0.63^{abcd}	6.80 ± 0.69^{h}	$8..94\pm0.06^{bcdefg}$
	Pro'tn	25.26 ± 0.43^{d}	27.68 ± 0.141^{a}	25.72 ± 0.05^{c}	6.66 ± 0.30^{h}	6.47 ± 0.61^{hi}	6.83 ± 0.09^{h}	5.39 ± 0.13^{kl}	5.76 ± 0.26^{jk}	4.56 ± 0.01^{m}
	Carb	40.39 ± 0.52^{a}	40.28 ± 0.57^{a}	39.80 ± 2.72^{a}	28.78 ± 3.81^{h}	25.92 ± 0.19^{kl}	26.32 ± 0.82^{k}	25.23 ± 0.82^{mn}	26.18 ± 0.04^{kl}	28.81 ± 3.76^{h}
	Fat	5.73 ± 0.20^{m}	5.75 ± 0.14^{m}	7.25 ± 0.28^{h}	5.57 ± 0.13^{mn}	6.35 ± 0.00^{kl}	7.81 ± 0.09^{f}	5.38 ± 0.23^{no}	6.28 ± 0.00^{kl}	6.93 ± 0.08^{ij}
	Ash	2.24 ± 0.35^{c}	2.23 ± 1.04^{c}	2.69 ± 0.32^{c}	2.13 ± 0.27^{c}	3.62 ± 0.24^{b}	6.99 ± 3.17^{a}	2.85 ± 0.13^{a}	2.38 ± 0.71^{c}	3.14 ± 0.42^{b}
Day 3	MC	9.70 ± 0.42^{abcd}	8.20 ± 1.09^{defgh}	9.48 ± 1.39^{abcd}	7.50 ± 2.12^{gh}	7.16 ± 0.25^{h}	8.45 ± 0.07^{cdefgh}	8.47 ± 0.75^{cdefgh}	7.23 ± 0.39^{gh}	8.23 ± 1.74^{defgh}
	Pro'tn	22.94 ± 0.01^{f}	25.46 ± 0.78^{cd}	24.27 ± 0.00^{e}	6.65 ± 0.32^{h}	6.48 ± 0.61^{hi}	6.85 ± 0.09^{h}	4.40 ± 0.120^{m}	4.66 ± 0.26^{m}	4.44 ± 0.12^{m}
	Carb	24.76 ± 1.29^{n}	$29.94\pm020f^{g}$	30.93 ± 1.14^{de}	30.57 ± 1.46^{ef}	26.14 ± 594^{kl}	$25.65\pm0.93l^{m}$	29.31 ± 1.61^{gh}	27.65 ± 0.74^{ij}	30.79 ± 2.85^{de}
	Fat	7.09 ± 0.08^{hi}	8.28 ± 0.9^{d}	7.88 ± 0.03^{ef}	8.08 ± 0.06^{de}	10.31 ± 0.042^{a}	9.13 ± 0.04^{c}	7.21 ± 0.01^{h}	7.91±0.03ef	7.53±0.07g
	Ash	3.12 ± 0.22^{a}	2.99 ± 0.35^{a}	3.00 ± 9.56^{a}	2.48 ± 0.33^{a}	3.32 ± 0.20^{a}	3.38 ± 0.18^{a}	2.50 ± 0.01^{a}	2.97 ± 0.33^{a}	2.67 ± 0.21^{a}
Day 5	MC	10.21 ± 0.20^{ab}	9.70 ± 2.97^{abcd}	10.17 ± 0.35^{abc}	10.75 ± 0.35^{a}	8.47 ± 0.71^{cdefgh}	9.44 ± 1.39^{abcde}	9.96 ± 0.71^{abc}	7.71 ± 0.41^{efgh}	7.72 ± 0.35^{efgh}
	Protein	8.49 ± 0.27^{g}	27.25 ± 0.27^{c}	24.15 ± 0.06^{e}	2.8 ± 0.24^{l}	5.56 ± 0.01^{kl}	$5.32\pm .24^{l}$	4.74 ± 0.12^{m}	4.52 ± 0.035^{m}	4.41 ± 0.13^{m}
	Carb	27.94 ± 1.22^{i}	33.36 ± 1.40^{b}	29.20 ± 1.30^{h}	31.30 ± 0.42^{d}	27.15 ± 0.55^{j}	25.65 ± 0.93^{lm}	26.42 ± 0.26^{k}	24.88 ± 0.18^{n}	32.27 ± 0.76^{c}
	Fat	5.10 ± 0.03^{p}	6.47 ± 0.05^{k}	7.78 ± 0.05^{f}	4.34 ± 9.02^{q}	6.40 ± 0.04^{kl}	9.71 ± 0.04^{b}	5.18 ± 0.03^{op}	6.79 ± 0.06^{j}	6.21 ± 0.35^{l}
	Ash	3.53 ± 0.67^{a}	3.10 ± 0.32^{b}	2.64 ± 0.22^{c}	3.14 ± 0.34^{b}	25 ± 0.14^{a}	1.87 ± 0.67^{c}	1.44 ± 0.44^{c}	3.31 ± 0.76^{a}	2.31 ± 0.04^{b}

*Means with the same superscripts in a row are not significantly ($p\leq 0.05$) different

The three blends showed significant ($P \leq 0.05$) variation in their vitamin C content, especially in day 1 and 3 across the three packaging materials. 20% cassava leaf blended with cassava root material showed significant deference in vitamin C packaged in gunny. Gunny package showed higher levels of vitamin C than in Kraft and plastic. The blend with 100% fresh roots showed significant ($P \leq 0.05$) difference in vitamin C packaged in gunny. However, this blend had the lowest levels of vitamin C content gunny. The blend containing 100% fermented root material packaged had in the plastic jars had significant ($P \leq 0.05$) deference in the levels of vitamin C. The levels of vitamin C in this package was the lowest in levels. This scenario where each product is affected differently by the type of packaging material could be due to the product structural difference resulting from their composition and the method of processing, as postulated by Tunick *et al*., (2016). By virtue of structural variations in the different blends, consequently each of the blends had different texture, thus different capacities of moisture permissibility whereby vitamin C is water soluble. Moisture in the packages originates from different sources that include, the air that is normally trapped at the top of each packaging in the absence of vacuum sealing, moisture contained in the packaged product, and moisture laden in the air that may seep through the packaging materials or seals as argued by Tunick *et al*., (2016). Fluctuations in vitamin C levels could be an indirect indication of moisture permeability in the different packages resulting to the effect that different packaging material have on packaged food. Abong *et al*., (2011); Burgos et al. (2009) and Galani *et al*., (2017) reported fluctuation of vitamin C that was caused by high storage temperatures -above 25^{o}C. There was however wide variation of vitamin C content across the blends, registered by day 5 that showed vitamin C to be significantly ($P \leq 0.05$) low. Islam *et al*., (2015) also reported a similar trend in his studies on effect of packaging material as affecting carrot stored under different conditions. Kaleen *et al*., 2015 went further to explain that 2% of vitamin

C is lost every time a storage container is opened, this was in relation to orange juice stored in different containers.

There was significant ($P \leq 0.05$) effect of packaging material on vitamin A content in all the three blends of cassava flakes. However, 20% cassava leaf blended with cassava root material was shown to retain more vitamin A content when packaged in gunny than when stored in plastic and Kraft. A major difference was however registered between day 1 and day 3. The blend containing 100% fresh cassava roots and 100% fermented roots were least affected by packaging material as they showed non-significant difference in the levels of vitamin A across the different packages. This an indicator that pure root products are more stable during storage, inasmuch as they initially contained low levels of vitamin A. All the blends however had extremely low vitamin A across the different packages by day 5. The variation in the levels of vitamin A shown within the blends could have been caused by the nature and composition and, the method of processing the products. The results also showed paper laminated polythene packets to contain higher levels of vitamin A therefore demonstrating to be a more suitable package than the other packages. The results of the current study agree to results reported by Ayowale *et al*., (2016). Ayowale reported vitamin A content to be well retained in high density polythene However, Oluwalana *et al*., (2015) reported drastic drop of vitamin A levels stored under different temperatures. This probably could mean that even if vitamin A is packaged well in a suitable packaging material it is still mandatory that the storage is done under predetermined suitable temperatures too.

There was significant effect ($P \leq 0.05$) of packaging material on mineral zinc content that was shown in blend 20% cassava leaf blended with cassava root material. Zinc levels in 20% cassava leaf blended with cassava root material stored in gunny remained significantly ($P \leq 0.05$) higher across the storage period when compared with levels of the same blend stored in Kraft and plastic materials. A significant effect ($P \leq 0.05$) was also shown in the blend containing 100% fresh root material when packaged and stored in paper laminated polythene packets. Zinc content in the blend containing 100% fermented root material was however shown to be higher in the plastic material. The nature and composition and, the processing method of the products could have beenthe source of the variation since factors cause physical and biodegradation difference thus affecting the retention of the mineral zinc. All the three blends had zinc content that significantly ($P \leq 0.05$) dropped by day 5 in all the packages. Olayiwola *et al.*, (2012) reported similar trend on studies carried out on vegetables packaged in different packaging materials.

There was significant effect ($P \leq 0.05$) of packaging material on mineral iron content across the three blends over the storage period of five days. The results however showed that each blend was affected differently by each packaging material. 20% cassava leaf blended with cassava root material packaged in gunny had the highest iron content while the lowest iron content in 20% cassava leaf blended with cassava root material was shown to be in plastic package. A similar trend was shown in the blend containing 100% fresh root material whose iron levels were also found to be higher in the gunny package than in plastic and Kraft. However, iron content in the blend containing 100% fermented root material was found to be lowest in the paper laminated polythene package though not significantly different from the levels found in the Kraft package. Significant variations were however registered between day 1 and 3. By day 5 all the packages showed non-significant ($P \leq 0.05$) difference in each of the blends across the different packages. The results conform to results reported by Huma *et al.*, (2007). The iron levels in fortified whole meal wheat flour, packaged in tin boxes and polypropylene bags showed variations. Huma reported variation of iron content as affected by days of storage and type of packaging material.

Table 10: Effect of packaging materials on micro nutrients content (mg/100 g dwb) of cassava flakes during 5 days' storage

S - life / Nutrients		20% cassava leaf flakes			100% fresh cassava root flakes			100% fermented cassava root flakes		
		Kraft	ns' polythene	Plastic	Kraft	Ins' polythene	Plastic	Kraft	Ins'-polythene	Plastic
Day 1	Vit A	315.03±49.94^{b}	476.54±11.40^{a}	323.09±22.85^{b}	387.71±91.38^{a}	298.86±11.42^{ab}	306.93±0.00^{b}	290.80±45.67^{b}	355.40±31.39^{b}	460.40±54.12^{a}
		4852.13±178.43^{b}	6640.58±58.69^{a}	4886.34±105.58^{b}	70.00±9.11^{hi}	269.00±7.07^{f}	249.95±7.16^{fg}	96.24±1.91^{hi}	107.50±7.78^{hi}	152.63±6.52^{gh}
	Zinc	0.39±0.21f	1.10±0.40^{def}	0.28±0.05^{f}	7.92±0.10^{a}	7.77±0.05^{ab}	8.86±0.24^{a}	0.69±0.67^{f}	0.20±0.04^{f}	0.19±0.02^{f}
	Iron	7.12±1.35^{ab}	7.85±0.72^{a}	5.93±0.79^{bc}	2.49±0.96^{fg}	2.18±0.59^{gh}	2.28±0.84^{gh}	0.99±0.02^{hij}	1.34±0.58^{ghij}	1.80±0.13^{gh}
Day 3	Vit C	323.09±45.69^{b}	395.78±39.88^{a}	322.68±45.11^{b}	242.32±0.00^{b}	177.70±91.39^{c}	249.82±94.31^{b}	242.32±0.00^{b}	210.02±68.56^{b}	137.31±34.27^{c}
	Vit A	361.97±16.96^{f}	807.11±22.83^{c}	582.40±0.00^{d}	70.00±29.11^{hi}	65.75±23.24^{hi}	55.65±32.18^{hi}	14.37±0.820^{i}	44.74±28.76^{i}	49.48±23.32^{i}
	Zinc	0.96±0.38^{def}	2.39±0.39^{cd}	1.49±0.18^{def}	3.32±0.76^{c}	1.30±0.03^{def}	6.38±3.00^{b}	1.52±0.78^{def}	1.23±0.18^{def}	2.21±0.30^{cde}
	Iron	6.12±0.21^{bc}	6.24±0.27^{bc}	6.95±1.27^{ab}	2.48±0.63^{fg}	2.54±1.00^{fg}	2.08±0.25^{gh}	1.52±0.79^{ghij}	1.08±0.28^{hij}	1.80±0.03^{gh}
Day 5	Vit C	161.56±22.85^{c}	145.39±22.84^{c}	177.70±28.53^{c}	193.85±22.84^{c}	127.62±22.29^{c}	129.24±22.85^{c}	177.695±22.85^{c}	113.08±0.00^{c}	161.55±22.85^{c}
	Vit A	457.85±82.38^{e}	576.85±5.87^{d}	278.58±33.76^{f}	0.00±000^{j}	81.61±8.77^{hi}	67.88±9.21^{hi}	0.00±0.00^{j}	94.40±13.81^{hi}	24.59±11.59^{i}
	Zinc	0.08±0.04^{f}	0.18±0.09^{f}	0.15±0.00^{f}	0.80±0.95^{ef}	1.17±1.32d^{ef}	1.11±0.23 ^{def}	0.34±0.24^{f}	0.20±0.14^{f}	0.28±0.11^{f}
	Iron	0.40±0.21^{j}	0.99 ±0.33^{hij}	0.46±0.271^{j}	2.28±0.35^{gh}	3.69±0.35^{ef}	3.64±0.16^{ef}	1.80±0.69^{gh}	1.34±0.41^{ghij}	0.395±1.71ghi

*Means with the same superscripts in a row are not significantly ($p \leq 0.05$) different

Reactions taking place in the packages were captured in AV & PV (acid value and or peroxide value), values during the storage period (Figure 5 showing Acid values - AV). AV values in 20% cassava leaf blended with cassava root material (X2) were shown to be significantly ($P \leq 0.05$) lower in gunny packages (section (a) figure 5) compared with the values found in Kraft (section b) and plastic (section c). The trend showed acid value that had a persistent increase but slowed down after day 3 of storage. This is an indicator that reactions were slowing down either because of the product going beyond its peak deterioration or due to oxygen exhaustion in the packages. Blend 100% fresh root material (X0) mirrored the trend where the opposite was depicted.

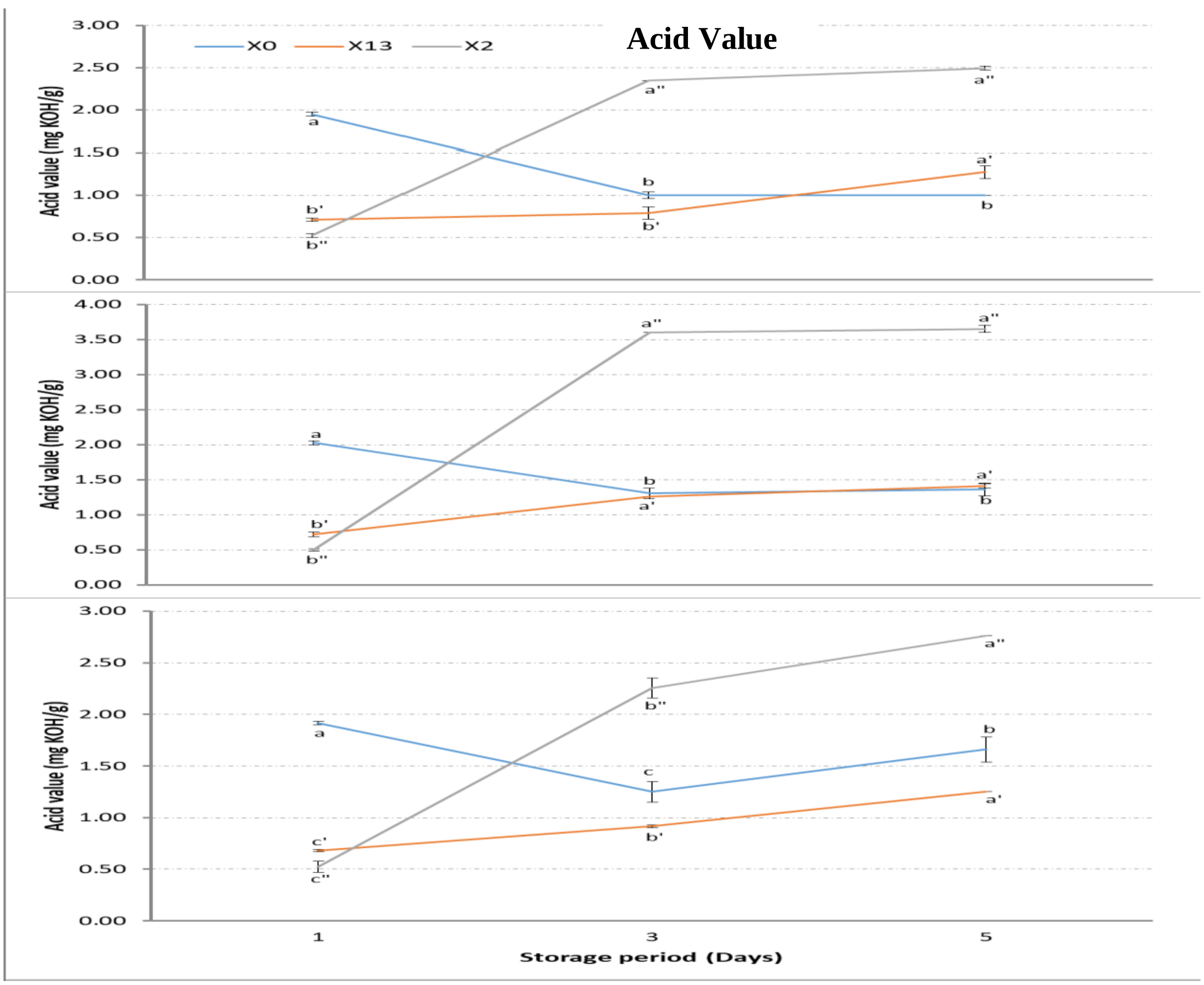

Figure 5. Variation of Acid value as affected by packaging material (a; gunny, b; Kraft c; plastic) during storage of cassava flakes

The blend showed a downward trend where AV drastically dropped from day 1 but stated to rise after day 3 of storage. The scenario was peculiar since it is expected for oxygen trapped in any package to cause chemical reactions immediately after the inception of storage. However, the nature of a product also plays a role over such reactions. Probably the blend X0- 100% fresh root was too compact to allow significant reactions during the early stages of storage. Blend 100% fermented root showed a more stable trend of AV generation whose variation was probably due to the method of processing. This is because the method by which a product is processed influences quality including the structural makeup of the product. A comparison of AV in the three blends showed that gunny package (paper laminated polythene) had the lowest values across the storage period. These results of the current study are different from results reported by Bukola *et al.*, (2015) where the researcher reported AV and PV increase alongside storage period. The researcher however studied different oils stored differently on shelf, cupboard and refrigerator. The uniformity was expected since it was one product (vegetable oil) packaged in different packaging/ materials, while the current study evaluated different products in different packaging material.

PV in 20% cassava leaf blended with cassava root material (X2) was significant ($P \leq 0.05$) different on day 5 in kraft and plastic packages, gunny showed no significance ($P \leq 0.05$) across the storage period. (Figure 6). The plastic material registered the highest PV on reaching day 5 with a trend that showed persistent increase. PV in the kraft packages showed a fluctuating trend. PV in the blend containing 100% fresh root material showed a significant difference in all the packages across the storage period. Kraft packages showed a persistent increase over the storage period with the highest values registered on day 5. PV value in the gunny packages dropped over the storage period and registered lowest value on day 5.

within the packages. Mathaus. (2010) relates steep increase in PV to drastic deterioration of lipids.

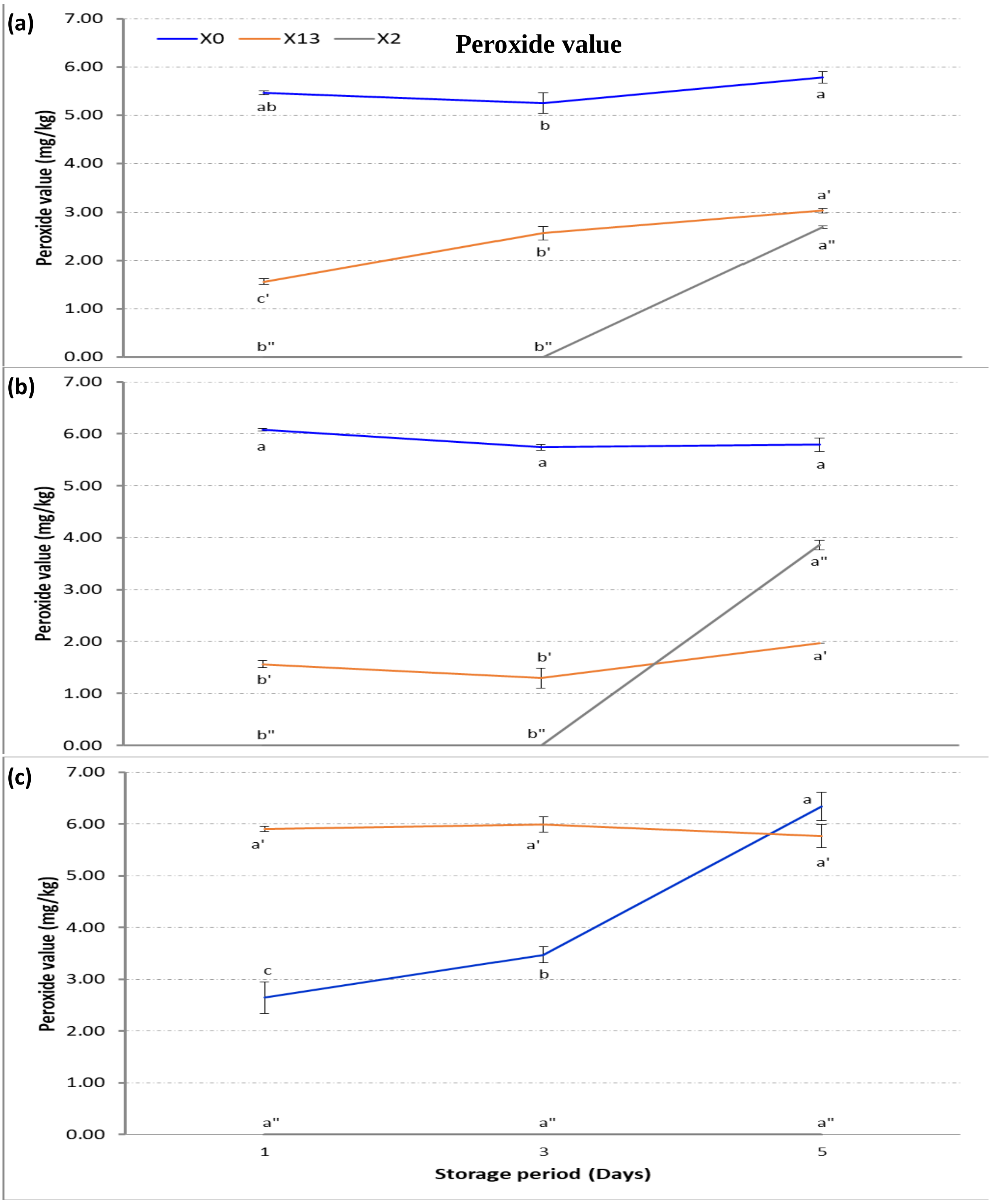

Figure 6. Variation of peroxide value as affected by packaging material (a; Kraft, b; plastic c; gunny) during storage of cassava flakes

A similar trend was shown in the plastic packages for the blend containing 100% fresh root material. Lower values were recorded in day 5 in both gunny and plastic packages. The blend containing 100% fresh root material (X0) showed significant ($P \leq 0.05$) across day 1 to 5. PV in kraft showed an increasing trend and registered highest values on day 5. A similar trend was shown in gunny and plastic packages. The trend shown in 20% cassava leaf blended with cassava root material packaged in gunny where PV values dropped along the storage period was peculiar but probably chemical reactions stopped after all the oxygen trapped in the packages had been expended thus forming a vacuum.

The case in the current study would therefore mean oils contained in 20% cassava leaf blended with cassava root material were well preserved in the gunny package as indicated by the none significance ($P \leq 0.05$) trend across the storage in PV. This scenario therefore depicts that there were minimal reactions taking place in the gunny package. This however does not only depict efficiency of the packaging material alone but possibly the ambient temperatures and duration of storage too. It is also a clear indicator that blend 20% leaf combined with fermented root is a shelf stable product. On the other hand, Jacobsen et al., (2008) reiterated that a combination of trace metals with hydro peroxides elevated levels of PV resulting to rapid deteriorations. Elevated PV was registered in kraft and plastic packages for all the three blends; - 20% cassava leaf blended with cassava root material, 100% fresh root flakes, and 100% fermented root flakes. Gong *et al.*, (2018) argued that hydro peroxides are formed not only in storage but also during processing procedures this would mean that chemical reactions take place even before packaging and storage of products.

A microbial count on the stored blends of cassava flakes targeting lactobacilli through spread plate method done from day 1 to day 5 (Figure 7) showed significant ($P \leq 0.05$) effect of packaging material on the lactobacilli growth over the storage period. The results expressed in log CFU/g showed higher level of bacterial growth in 20% cassava leaf blended with cassava root material packaged in plastic. A similar trend was found in the blend containing 100% fermented root material, but no growth was found in the blend containing 100% fresh root material in all the three packaging material.

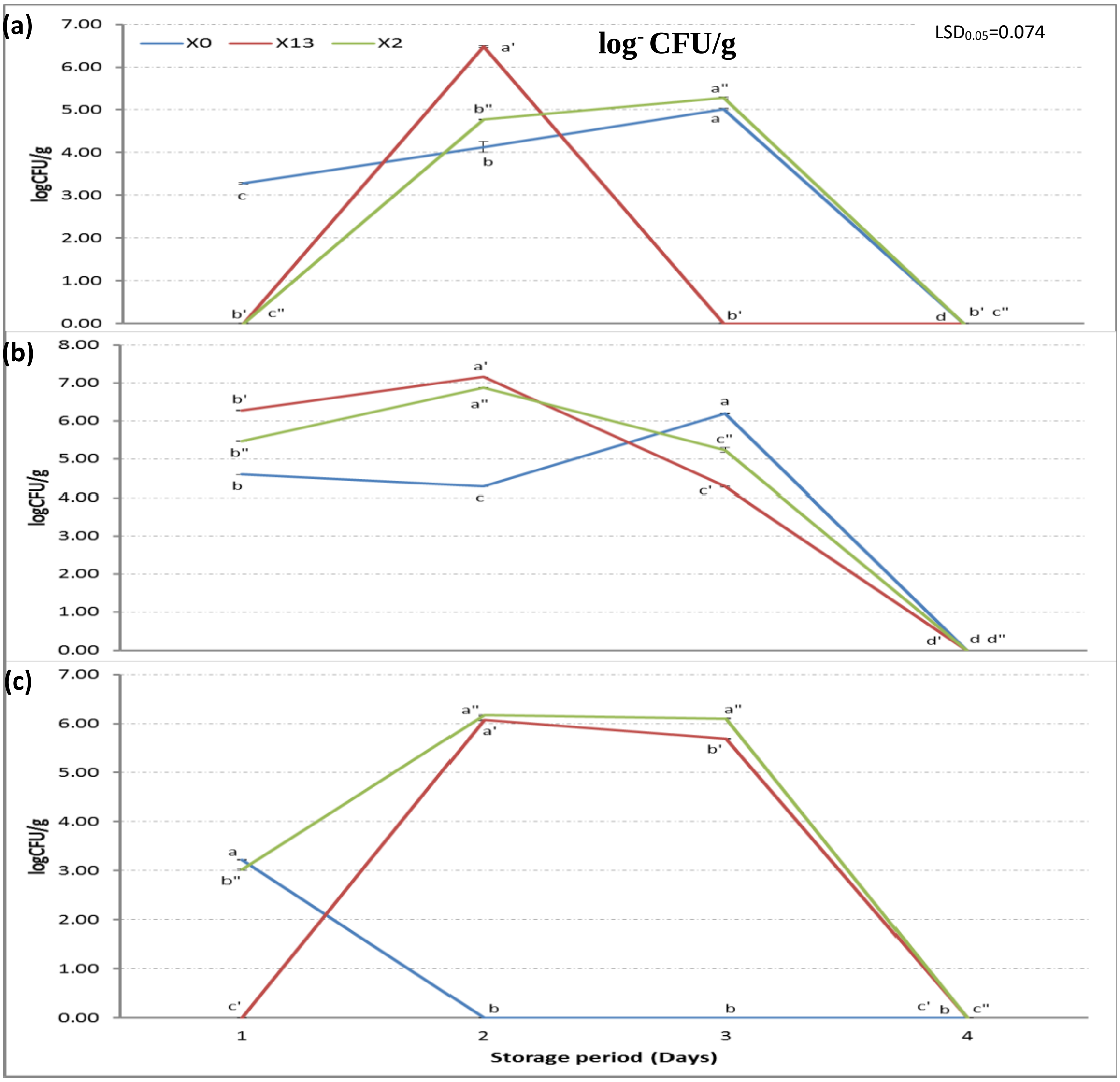

Figure 7. Effect of package material, on microbial count (lactobacillus) through spread plate method (log^{-} CFU/g) during storage of flakes.

This however confirms the fact that the product was processed from fresh cassava roots. The counts found in different packages are as a result of the difference in the nature of the packaging materials. Kraft paper is porous and would allow adequate aeration on the packaged products. On the other hand, lactobacilli are anaerobic bacteria that thrive well in less aerated environment. The higher count in the plastic is an indicator of a conducive environment of for lactobacilli, showing that the plastic jars were not efficiently airtight. The lower count found in gunny package also expresses the nature of the gunny that is polythene reinforced with white paper material thus creating a none-porous characteristic of the package. There is likelihood that the higher microbial counts registered in 20-% cassava leaf blended with cassava root material and 100% fermented root material emanated from the fermentation process that the blends underwent during the formulation stage. The laminated polythene packages were sealed using a sealing machine as opposed to the plastic jars that have place-on cover tops without screwing threads. The sealing of the laminated polythene packages could have as well created a near vacuum environment within the packets thus inhibiting most growth of the lactobacilli. Terpou *et al*., (2019) argued that several factors influence the viability of probiotics found in food products at the stage of processing and even storage. These factors include the intrinsic nature of the product such like oxygen, pH, titratable acidity, etc. also processing parameters such like fermentation conditions would affect the viability of these probiotics. Stanton *et al*., (2001) stated that temperatures up to 30^{o}C allow growth of lactic acid bacteria. The storage environment (temperature and humidity) in a laboratory incubator are set to mimic ambient shelf storage. This is demonstrated by the varied values of lactobacilli in the different cassava blends. Zahra *et al*., (2016) drew emphasis on the success in processing of foods to be greatly dependent on protective antimicrobial processing that call for optimizing of factors including oxygen, sunlight exposure, moisture and water activity and microbial contaminants. This means packaging and packaging materials require proper selection for effective preservation and storage of foods. Better and carefully selected packaging material with the use of latest technologies can save tons of food (Zahra *et al*., 2016)

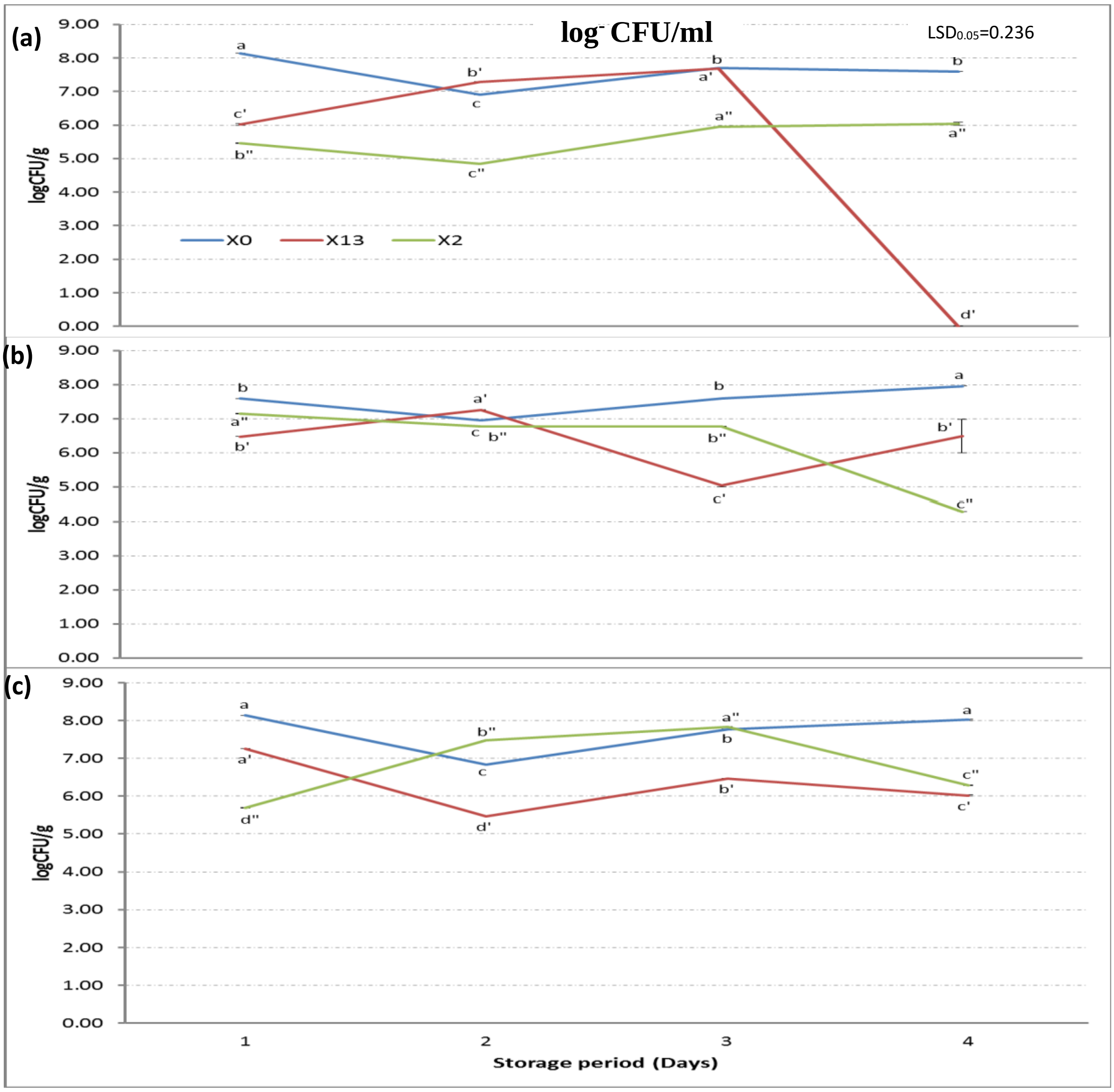

Figure 8. Effect of package material, on microbial (yeast and molds) count through pour plate method storage (log^{-} CFU/ml) during storage of flakes.

A microbial count done on the stored blends of cassava flakes through pour plate method showed significant ($P \leq 0.05$) effect of packaging material on the blends across storage period (Figure 8). The results were expressed in log PCA. There was a variation on how the blends were affected by the packaging material during the storage period; – On day one, the blend containing 20% cassava leaf material and 100% fermented were shown to have significantly ($P \leq 0.05$) higher microbial counts in the Kraft packages than in plastic and insulated polythene (plate 8). The higher growth in the Kraft could likely be due to the porosity of the package allowing general growth of spoilage microbes. However, the blend with 100% fresh root material had the highest counts across all the packages. This is an indicator that controlled fermentation is a means of food preservation that restricts microbial growth and allows only the probiotics i.e. the lactobacilli. The higher growth in count shown in the blend containing 100% fresh root material was probably due to the fact this particular blend did not undergo fermentation thus allowing invasion of all kinds of microbes. Terpou *et al*., (2019) expressed clearly in the statement; processing parameters such like fermentation conditions would affect the viability of these probiotics. This would also mean when fermentation is carried out well, it ensures optimum viability of probiotics. On this basis, the less growth of spoilage microbes in 20 % cassava leaf blended with cassava root material and 100% ferment root flakes is an indicator that probiotics were optimum viable hence counteracted growth of the spoilage microbes. Siroli *et al*., (2017) stated that plastic material for packaging allows transmission of storage microbes. However, this was in relation to fruits and vegetables packaged in plastic containers and cardboards.

6. 5 Conclusion and Recommendations

The three blends of cassava flakes are of good quality up to 3 months, where most nutrients are at average levels, while packaging materials have significant effect on nutrients' levels in the packaged cassava based flakes, packaging materials also have effect on the length of storage period, the study hereby concludes that best time to store cassava flakes is up to 3 months, and the best packaging material is insulated polythene. The study recommends further studies to be carried to identify storage pests for cassava root-leaf flakes that were not covered in the current study.

Plate 8: Packages and packaging materials

CHAPTER SEVEN

GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

7. 1 General Discussion

Coastal cassava varieties as studied in the current study demonstrated to be more resilient towards harsh climatic conditions since the nutrients levels in the roots compared well with nutrients levels determined in other varieties grown in other regions that probably were grown under conducive climate for agricultural production. The assumption that the latter varieties were grown under conducive climatic conditions arises from the fact that researchers such as Quaye *et al*., (2009); Salcedo *et al*., (2010) and Bechoff *et al*., (2018), never highlighted harsh climate factors in their study reports. The results of the current study showed that the coastal varieties are superior in vitamin C content compared to the latter varieties referenced to in this study. Nutrients buildup determined at crop age 3 months demystified the fact that cassava growing communities harvest cassava roots as early as 3 months after planting especially at times of food shortage. This is because roots at this tender stage were shown to have substantial nutrients levels i.e. carbohydrates and energy. Inasmuch, dry matter was shown to be low in the roots at this particular stage of growth period. It was also established that other nutrients were even higher during this tender stage. Protein in the tender roots was also substantial especially in variety Karembo. Tajirika and KibandaMeno protein build up rose in levels alongside the crop age. Hydrogen cyanide in the coastal varieties is fairly low, a fact that justifies the reason why the coastal communities mostly consumeraw cassava roots Mwamachi *et al*., (2005) The current study also indicate that the coastal varietiesare early maturing. This however did not warrant a conclusion for the roots to be harvested at thistender age, rather it would be prudent that harvesting is staggered. This is because some researcherslike Wobeto and Nweke had to harvest their study varieties as from 12 months after planting to 17months after planting (Wobeto *et al*., 2006; Nweke, 1996). This is a pointer that the studied crop ages were a common practice in the country/ region of study in reference.

It was established in this study that aroma was the most powerful parameter in the process towards a product acceptability (Figure 4). The reasons given to justify the decision were authentic and convincing, thus aroma was used to knock out other blends. Taste was synonymously proved to be very important too. Blending cassava root with leaves was however accepted at lower ratios of below 20%, though, 30% leaf composition in cassava flakes was equally accepted since there was no significance in most of the evaluated sensory parameters. Physical qualities like crispiness during storage was not determined in the current study. However, crispiness of flakes is not very mandatory for acceptability since cassava flakes can be reconstituted using hot water or milk before consumption. Cassava root - leaf flakes is a snack food of convenience that can be consumed dry or reconstituted at any time of the day. It was observed that the three blends of cassava could be reconstituted to a smooth consistence after being soaked in water or milk for a period of 4 to 7 minutes. The nutritive value of cassava flakes blended at 20% leaf in root materialwas determined to cover up to half ($\frac{1}{2}$ - $\frac{1}{3}$) the RDA nutrients requirement per a serving. It is therefore important that the product - cassava root - leaf flakes is studied for nutrient bioavailability on the targeted beneficiaries- school going children and pregnant women.

Evaluation of packaging materials suitable for packaging and storage of cassava flakes indicated that paper laminated polythene (gunny) was suitable for preserving quality of cassava flakes especially. Paper laminated polythene preserved cassava flakes against both chemical and microbial reactions. Storage of cassava flakes up to 3months was shown to be most appropriate since most nutrients showed drastic drop in their levels when stored beyond the 3 months.

7. 2 Conclusions

The most suitable plant age for harvesting roots is at 9 months after planting. Harvesting of cassava leaves should start at 3 months after planting and be staggered from varied crop ages. The tender leaves are the most suitable for human consumption. The leaves of the coastal varieties are of superior in quality compared to other varieties. Cassava root-leaf consumption and or utilization has potential to mitigate nutrient insecurity. The most acceptable level of leaf component for formulating cassava root - leaf flakes is at 20%. Cassava root- leaf flakes is best packaged in paper insulated material. Flakes are suitably stored at room temperature and best before the elapse of 3 months' storage period.

7. 3 Recommendations

The current study recommends staggered harvesting of both the roots and leaves since each crop age has some substantial nutrients at varying levels. Cassava root - leaf flakes are recommended leaf material not to exceed 30%. The study also recommends more nutritionally oriented research to carried out in order to determine uptake flakes nutrients by human body especially the targeted school going children and pregnant women. The national and county governments should develop policies that promote production of cassava crop and utilization of its roots and leaves for manufacture of not only cassava root - leaf flakes, but also other nutritious food products.

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Appendices

Appendix 1; Sensory evaluation participants

Sensory Evalution Participants for cassava root- leaf flakes

S/N	NAME	STATUS	ID NUMBER	SIGN
1	BEJA PASCAL ZUMA	YOUTH	12902166	[illegible]
2	KOMBO MWAMUNGA	YOUTH	[illegible]	[illegible]
3	JULIET MWAKA CHAKA	WOMAN	[illegible]	
4	JUDITH M. MWITHANJA	WOMAN	[illegible]	[illegible]
5	STEPHEN M. KIHARA	MAN	[illegible]	[illegible]
6	I HASSAN . M . YAWA	MAN	[illegible]	[illegible]
7	MESALIMU DZOMBO	WOMAN	[illegible]	[illegible]
8	MJENI CHAKA MDUNE	WOMAN		
9	REBECCA .N. KUTSELA	YOUTH	[illegible]	[illegible]
10	TERESIA .M. KILONZI	PLWD		
11	MDACHI BEDZINE	YOUTH		
12	CHINYAVU BENGOME	WOMAN	[illegible]	[illegible]
13	UMAZI CHITI NYALE	WOMAN	[illegible]	[illegible]
14	MNYAZI TSUMA MRISA	YOUTH	[illegible]	[illegible]
15	YUSUF MVURYA	YOUTH	[illegible]	[illegible]
16	REHEMA MARY JULIUS	WOMAN	[illegible]	[illegible]
17	JABU MWANGOME	MAN	25375107	[illegible]
18	NYAWA CHITSANIA	MAN	21369462	[illegible]
19	TSUMA NGAO	YOUTH		
20	DORCUS WATIRI	WOMAN	[illegible]	[illegible]
21	UMAZI BAKARI	WOMAN	[illegible]	[illegible]

S/N	NAME	STATUS	ID NUMBER	SIGN
24	DZOMBO MANGALE	[illegible]	14595088	[illegible]
25	SALMAN CHAKA	YOUTH	[illegible]	[illegible]
26	SAIDI MZUNGU	MAN	[illegible]	[illegible]
27	MWATSIMBA SIMBA MBEGA	MAN	[illegible]	[illegible]
28	SAMSON ROCHA NDEGWA	MAN		
29	NYAWA KALIMBO MWERO	MAN	[illegible]	[illegible]
30	ZACHARIA NIWERESA [illegible]	MAN	3582852	[illegible]
31	STEPHEN NDORO CHAKA	MAN	8397447	[illegible]
32	MJENI CHAKA	YOUTH		
33	VICTOR MZINGA	MAN	2162316	[illegible]
34	ESTHER UMAZI NDEGWA	YOUTH	39356336	[illegible]
35	NDORO CHAKA TSUMA	YOUTH	29916971	
36	GINDO NYAWA			
37	AMIN SUYA		8413887	[illegible]
38	SAMUEL MVURYA		14599966	[illegible]
39	JACKSON MULINGE		10314609	[illegible]
40	REBECCA NDEGWA		27684245	[illegible]
41	JANE WANJA CHEGE		10220838	[illegible]
42				

Appendix 2; Sensory evaluation questionnaire

Sensory evaluation questionnaire

Sensory evaluation for Cassava root- leaf flakes: Date ………………………

Name of the panellist………………………………… **Sex ……………………….**

Please score your preference of the parameters- (colour, taste, aroma and texture as listed below using the following 7-point hedonic scale preference scores: i.e.,
7 = (like extremely), 6 = (like most), 5 = (like), 4 = (neutral), 3 = (dislike) 2 = (dislike most), 1 = (dislike extremely).

Parameters	**Put your score here**
Colour	
Taste	
Aroma	
Texture	

Remarks……………………………………………………………………………………..

……………………………………………………………………………………………….

……………………………………………………………………………………………….

………………………………………………………………………………………………

………………………………………………………………………………………………

Appendix 3

Pairwise ranking of cassava blends according to preference as evaluated on Aroma

Blend of cassava flakes	Codes	X0	X14	X15	X16	X 17	X8	X1	X6	X11	X13	X4	X5	X9	X7	X2	X12	X3	X10	Score	Rank
Fresh root + 0% fresh leaf	**X0**		X0	X0	X0	X0	X8	X1	X0	X0	X13	X0	X0	X0	X0	X2	X12	X0	X0	**12**	**6**
Fresh root + 20% fresh leaf	**X14**			X14	X14	X14	X8	X1	X14	X1	X13	X4	X5	X14	X14	X2	X12	X14	X14	**9**	**9**
Fresh root + 30% fresh leaf	**X15**				X15	X15	X8	X1	X15	X15	X13	X4	X5	X15	X15	X2	X12	X15	X15	**8**	**10**
Fresh root 40% + fresh leaf	**X16**					X16	X8	X1	X6	X16	X13	X4	X5	X9	X16	X2	X12	X3	X16	**4**	**14**
Fresh root 50% + fresh leaf	**X17**						X8	X1	X6	X11	X13	X4	X5	X9	X7	X2	X12	X3	X10	**0**	**18**
Fresh root + 20% fermented leaf	**X8**							X8	X8	X8	X13	X8	X8	X8	X8	X2	X12	X8	X8	**14**	**3**
Ferment' root + 30% fresh leaf	**X1**								X1	X1	X13	X1	X1	X1	X1	X2	X12	X1	X1	**13**	**5**
Fresh root + 40% fermented leaf	**X6**									X6	X13	X4	X5	X9	X6	X2	X12	X3	X6	**5**	**13**
Fresh root 50% + 50%fermented leaf	**X 11**										X13	X4	X5	X9	X11	X2	X12	X3	X10	**1**	**17**
Fermented root + 0% leaf	**X13**											X13	X13	X13	X13	X13	X13	X13	X13	**17**	**1**
Fermented root + 20% fermented leaf	**X4**												X4	X4	X4	X2	X12	X4	X4	**11**	**7**
Fermented root + 30% fermented leaf	**X5**													X5	X5	X2	X12	X5	X5	**10**	**8**

Pairwise ranking of cassava blends according to preference as evaluated on Aroma

Blend of cassava flakes	Codes	X0	X14	X15	X16	X 17	X8	X1	X6	X11	X13	X4	X5	X9	X7	X2	X12	X3	X10	Score	Rank
Fermented root + 40% fresh leaf	**X9**														X9	X2	X12	X3	X9	**6**	**12**
Fermented root + 50% fresh leaf	**X7**															X2	X12	X3	X10	**2**	**16**
Fermented root +20% fresh leaf	**X2**																X2	X2	X2	**16**	**2**
Fermented root + 30% Fermented leaf	**X12**																	X12	X12	**14**	**3**
Fermented root +40% Fermented leaf	**X3**																		X3	**7**	**11**
Fermented root+ 50% fermented leaf	**X10**																			**3**	**14**