MICROBIAL AND CHEMICAL PROFILE OF FERMENTED CASSAVA LEAVES FROM SELECTED KENYAN COASTAL VARIETIES

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I Samuel Mumira Mwathi, hereby declare that this Dissertation is my original work and to the best of my knowledge, it has not been submitted for any award or examination in any other institution of Higher learning.

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DEDICATION

I dedicate this work to my mother Wamaitha Mwathi, my father Kenneth Mwathi, my Sister Ngina Mwathi, my brother Kahuria Mwathi, my uncle Prof. Kimani Waithaka and my entire family for their unending support and prayers during my masters study period.

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ABBREVIATION AND ACRONYMS

AOAC- Association of Official Analytical Chemists

CFU- Colony Forming Unit

FAO – Food and Agriculture Organization of the United Nations

ISO- International Organization for Standardization

Mg- Milligrams

Ml- Millilitres

Cfu/g - colony forming units per gram

Cfu/ml- colony forming units per millimetres

KNBS- Kenya National Bureau of Statistics

MPN- most probable number

ODK-open data kit

GENERAL ABSTRACT

Cassava (Manihot escutenta, Crantz), is one of the high yielding, disease and drought resistant crop that can be used as an alternative to maize in Kenya. The leaves are highly nutritious and serve as an alternative to green leafy vegetables. However, cassava leaves production, consumption and processing has been low due to the lack of a well-structured cassava value chain and standard postharvest handling practices. Additionally, the high content of anti-nutrients discourages consumption at household. This study sought to evaluate the harvesting and postharvest practices, microbial and chemical profile of fermented cassava leaves from selected Kenyan coastal varieties. A total of 247 respondents were nominated from the two counties 120 and 127 respondents in Kilifi and Taita Taveta respectively. A completely randomized experimental design was used for chemical and microbial analysis. Almost all respondents (99.6%) grew cassava for food and Kibanda meno was the most preferred variety. In both counties, farmers harvested few leaves or piecemeal by handpicking and most commonly in the morning hours. Additionally, cassava value addition was limited to drying (82.6%) and fermentation (4.1%). The respondents (65.2%) preserved cassava for a maximum of 15 days. Fermentation followed by oven-drying and sun-drying significantly ($p<0.001$) reduced the tannins, oxalates and cyanide to recommended levels. The sensory scores of the fermented leaves averaged at 5 points on a seven-point hedonic scale stating, that they were likeable in comparison to the non-fermented samples. The results also indicated that lactic acid bacteria (LAB) were the predominant microorganisms in cassava leaves fermentation. The mean log CFU of yeasts and molds, LAB and coliforms were 6.96, 7.99 and 8.70 respectively. Leaf position and cassava leaves variety significantly ($p<0.001$) influenced microbial load during fermentation. Since LAB is the predominant microorganism in cassava leaves fermentation there is need for isolation of its pure cultures. This study concludes

that fermentation reduces the anti-nutrient content in cassava leaves making it safe for consumption thus should be adopted for value addition of cassava leaves.

CHAPTER ONE: INTRODUCTION

1.1 Background Information

Micronutrient malnutrition prevalence is still rampant in developing countries despite the availability of nutritious food and high investment in agriculture (Underwood, 2000). Food security also remains an issue to be addressed as Kenya makes a step towards achieving vision 2030 (Mwang'ombe, 2013). Cassava (*Manihot escutenta, Crantz*), has been one of the crops promoted by Ministry of Agriculture, livestock, Fisheries and Irrigation through pillar two of The Big Four agenda on Food Security. Partly because it's high yielding, drought and disease resistant. Global production of cassava as at 2017 Food and Agriculture statistics was 277.1 million tonnes annually (FAO, 2017). The leading countries were Nigeria at 59.5 million tonnes annually, Democratic Republic of Congo 31.6 million tonnes and Thailand at 30.9 million tonnes. In Africa Nigeria leads the production followed by Democratic Republic of Congo which is also world number 5 biggest producer. Other major producers in Africa are Angola at 11.8 million tonnes annually. In East Africa region Tanzania is the leading producer at 5.01 million tonnes annually followed by Kenya at 1.1 million tonnes annually and Rwanda at 1.041 million tonnes annually (FAOSTAT, 2017).

Western and Coastal regions are the major cassava producing areas, producing over 80% of the recorded cassava output in the country. Cassava roots are low in protein and ash (minerals) but are 80 to 90% carbohydrates by dry weight basis with 80% been starch and low amounts of maltose, sucrose and glucose (Gil and Buitrago 2002). Consumers relying on cassava roots as their main diet are prone to nutrient malnutrition particularly protein and minerals. However, cassava leaves

are high in crude protein content and a well-balanced amino acid profile comparable to fresh egg although methionine, lysine and isoleucine are absent. They are also high in vitamins, minerals and fibre (Iglesias et al., 2015).

Cassava roots and leaves contain anti-nutrients that bind minerals and other nutrients causing them to be indigestible and unavailable for absorption in the body (Wobeto et al., 2006). They include cyanogenic glucosides, phytate, fibre, nitrate, polyphenols, oxalate and saponins (Siritunga and Sayre, 2003). Cyanogens occur in three forms: cyanogenic glucosides (95% linamarin and 5% lotaustratin), cyanohydrins and free cyanide. Cassava leaves contain 10 times cyanide content of roots (Bradbury and Denton, 2014). Acute poisoning associated with consumption of 50 to 100 mg of cyanide are rare however, prolonged consumption of small amounts of cyanide is associated with severe health problems including konzo (spastic paraparesis) , glucose intolerance, tropical neuropathy and goitre (Ernesto et al., 2002).

Different Processing methods are used to reduce cyanogenic compound and other anti-nutrients to allowable consumption levels. Their effectiveness depends on processing step, sequences utilised and are often labour intensive and time consuming. They include fermentation, boiling, drying, sun drying, and oven drying, shredding and soaking. Combination of two or more of these processes improves effectiveness and also improves nutrients retention.

Fermentation extends the shelf-life, safety, palatability and sensory quality of the raw product, reduces undesirable and toxic compounds, and may increase the availability of proteins and vitamins. Furthermore, some Lactic Acid Bacteria strains are well-known probiotics and it has been postulated that lactic fermented foods may also have positive effects on human gastrointestinal health (Mathara and Trierweiler, 2016).

Studies have reported reduction of specific anti-nutrients and retention of the desired nutrient. However, little information has been published on these processes usage with different storage methods to test their fitness for utilisation by local communities and industrial use both in cost, complexity of technology and time consumed. This study will assess the microbial and chemical content of lactic acid bacteria fermented leaves in combination with other processes from selected varieties.

1.2 Statement of the problem

Despite cassava being high yielding, drought and disease resistant crop and performing better than cereals under unconducive weather and climatic condition including poor soil conditions and erratic rainfall, its importance to help reduce food insecurity and micronutrients deficiency has not been fully utilised in Kenya. Cassava leaves are rarely utilised by local communities in Kenya due to the scare of high cyanide and other anti-nutrients, ineffective processing methods to reduce the cyanide and anti-nutrients levels and presence of other alternative vegetables. Fermentation is among the preferred methods that can reduce the cyanide and anti-nutrient content, increases the shelf-life of the leaves to last through drought period, improve safety, palatability and sensory quality of the raw leaves, and may increase the availability of proteins and vitamins. There is limited information on systematic studies of fermentation of cassava leaves in Kenyan context.

1.3 Justification

Climate change and increase in population necessitate use of drought resistant high yielding crops to improve food security. Local communities in the Coast of Kenya utilize cassava leaves and uses simple traditional methods such as fermentation, pounding and sun drying to reduce the cyanide levels. Standardization and optimization of the traditional methods will lead to production of cassava leaves products that are of uniform chemical and microbial quality. These products will

be more available and easy to use which would save labour and time households use in food preparation and adoption by other communities will be easy. Standardization of cassava fermentation, storage and packaging will unravel the problem of insufficient control characterised by traditional method of fermentation. This can be adopted by food industries and small scale producers to enhance cassava leaves production and food security.

This was envisioned in Agriculture sector development strategy 2010- 2020 and in pillar 2 of the Big 4 Agenda of the Kenya government under Ministry of Agriculture, Livestock and Fisheries (MOA, 2013). They plan on achieving 100% food and nutrition security, increase average daily farmer income by 34%, reduce malnutrition in children below 5 by 27% and create 1000 agro processing small scale enterprises (KNBS, 2010).

1.4 Study Objectives

1.4.1 Main Objective

To assess harvesting and postharvest handling practices, microbial and chemical profile of fermented cassava leaves from selected Kenyan Coastal varieties.

1.4.2 Specific Objectives

i. To assess the harvesting and postharvest handling practices and utilisation of cassava leaves in Taita Taveta and Kilifi County
ii. To determine chemical properties, anti-nutrient content and sensory characteristics of fermented cassava leaves
iii. To determine changes in microbial population during fermentation of cassava leaves

1.5 Research Questions

i. What are the harvesting and postharvest handling practices and utilisation of cassava leaves in Taita Taveta and Kilifi County

ii. What are the changes in nutrients (vitamin A, Vitamin C and minerals (calcium, zinc and iron) and anti-nutrients (cyanide, oxalates and tannins) during fermentation of cassava leaves?

iii. What are the changes in microbial (lactic acid bacteria, coliforms, mould and yeast), during fermentation of cassava leaves?

CHAPTER TWO: LITERATURE REVIEW

2.1 Production of cassava

There has been an outstanding upsurge in the global production of cassava in the 21st century. In the period 1980 and 2011, there was a 44% increase in the global harvested area from 13.6million ha to 19.6 million ha (Howeler, Lutaladio & Thomas, 2013). Furthermore, the production of the root crop almost doubled during this same period. Interestingly, the total production of Cassava in Africa, Southeast, and Eastern Asia is at the same rate as that of the staple food in the respective continents, which maize and rice. By 2017, The global production of cassava according to 277.1 million tonnes annually Figure 1. The three highest producing countries were Nigeria at 59.5 million tonnes annually, the Democratic Republic of the Congo at 31.6 million tonnes annually, and Thailand at 30.9 million tonnes.

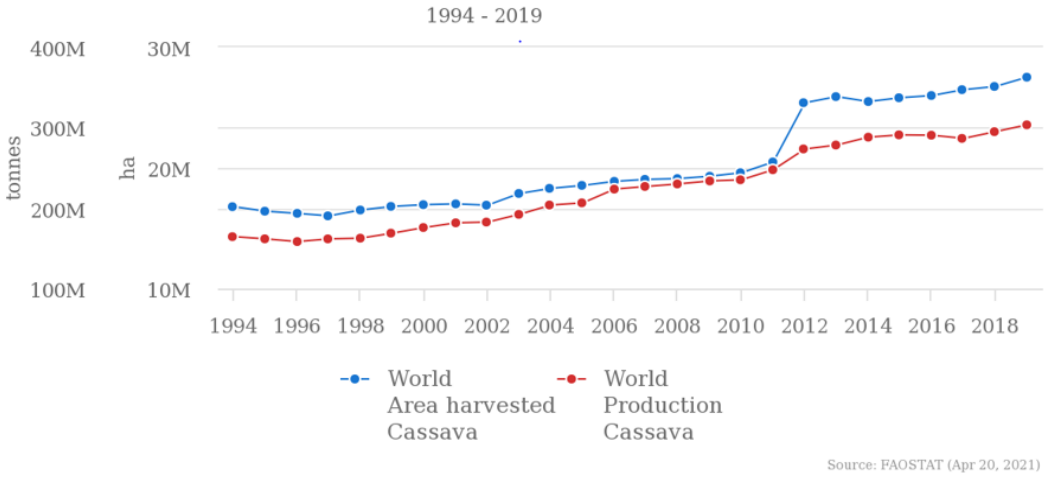

Figure 2.1 Global production of cassava between 1994 and 2018 (FAO, 2021)

Sub-Saharan Africa is the largest contributor to the current global production of cassava, contributing to 140.9 million tonnes. This region has witnessed a 56% increase in the total harvested area, 25% increase in the total yield between 1980 and 2011 (Akinpelu et al., 2011).

The root crop is primarily grown by small-scale farmers with limited resources, who do not incorporate any external inputs (Akinpelu et al., 2011). Ideally, the plant is grown with other crops, including maize, legumes, bananas, and rice. West African has witnessed the highest cassava productivity, where there was a 60% increase in the total production between 1980 and 2011, with particular strongholds in Ghana and Nigeria (Akinpelu et al., 2011). Even though the average yields of cassava in other regions of Africa remains comparatively low, Howeler et al. (2013) estimate the production of cassava is accelerating at a higher rate than any other crop, including maize. For the past three years, between 2016 and 2019, the production of cassava in Kenya has been increasing steadily.

Kenya produced 1.12 million tonnes in 2019, which was an increase from 0.9 million tonnes in 2018, as shown in Figure 2. Over 80% of recorded output is sourced from Western and coastal regions. About 60% of this quantity, produced in Western and Nyanza regions, while 30% is obtained from the eastern province. Cassava contributes to approximately 9% of the total caloric diet of Kenyans.

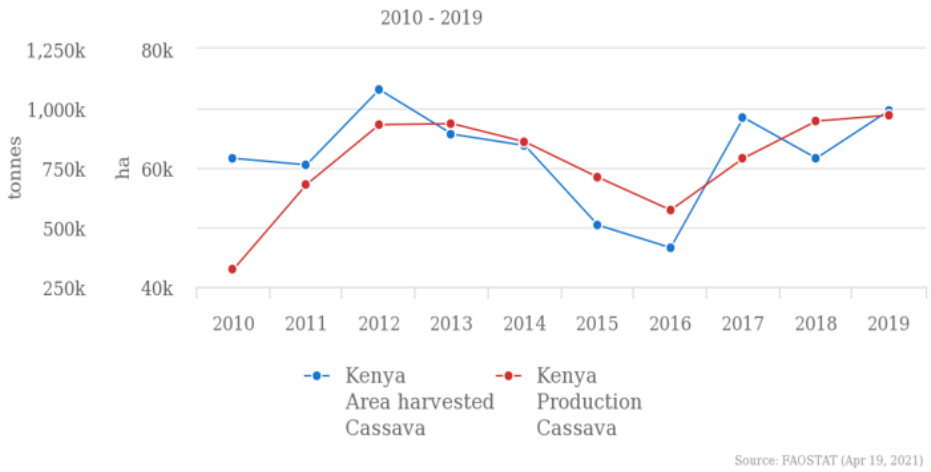

Figure 2.2 Production of Cassava in Kenya between 2010-2018 (FAO, 2021)

Cassava can be grown in areas with poor soils, where rainfall is inconsistent and unpredictable, making it highly attractive to small-scale farmers. The cassava plant also has a high tolerance to acid soils and has formed a symbiotic relationship with soil-borne fungi, which help it in the uptake of phosphorus and micronutrients (Howeler et al., 2013). Planting is through the propagation of stem cutting, meaning the plating materials are readily available, which has largely contributed to an increase in production. In Kenya, both abiotic and non-abiotic factors constraint the production of cassava. Specifically, the use of contaminated planted material, which leads to cassava mosaic disease, is the main challenge.

2.2 Consumption and utilization of cassava leaves

Cassava leaves are consumed as a vegetable in Africa, Asia, and South America. According to Achidi et al. (2005), a survey on cassava leaves utilization in Africa reported that the consumption varied from high (above 60% of the population consumed cassava leaves) to moderate (40% to 60%) of the population consumed cassava leaves) low (< 40% of the population consumed cassava leaves) and none for areas that didn't cultivate the crop or had no data on cassava leaves consumption. For instance, in DRC Congo, above 60% of the population consumed cassava leaves;

Tanzania Mozambique, Malawi, and Zambia cassava leaves were consumed by 40-60 percent of the population. In Kenya, the consumption was less than 40 percent, as indicated in Figure 3 (Achidi et al., 2005).

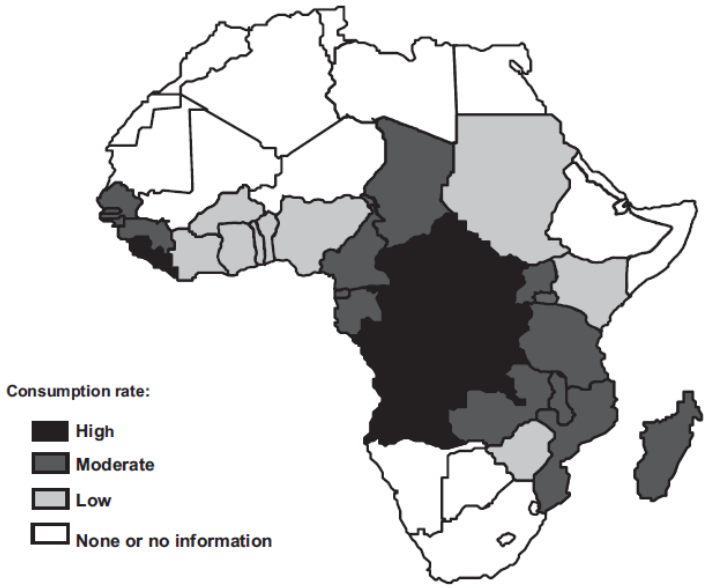

Figure 2.3: Cassava leaves utilization as food in Africa (Achidi et al., 2005)

Cassava leaves are available all year round and contain appreciable amounts of protein, minerals, and vitamins. Most consumers of cassava leaves prefer to consume the young and tender leaves, specifically the top 10 leaves (Achidi et al., 2005). The most common methods of preparation include shredding, boiling, followed by pounding or chopping. Spices are also added, and the meal is eaten as a sauce or a stew. On rare occasions, the leaves are sun-dried and pulverized into flour (Latif and Müller, 2015).

2.3 Nutritional Value of Cassava leaves

Cassava leaves are rich sources of protein, and vitamin C A, B. They also contain an appreciable amount of minerals, including Phosphorous, Magnesium, Potassium, and Calcium (Montagnac,

2009). Comparison of the nutrient level in cassava leaves with other vegetables is detailed in Table 2.1.

Table 2.1 Comparison of nutrients in (100g edible fresh portion) of cassava leaves and other African Leafy Vegetables

Scientific name	Crude protein (g)	β-carotene (mg)	Vitamin C (mg)	Calcium (mg)	Iron (mg)
Vigna unguiculata	4.2	7.4	70 -100	350	15
Solanum nigrum	3-6	9.6	40-140	250	17
Gynandropsis gynandra	10-13	9.4	130-180	434	15
Corchorus olitorus	8	7.5	170-210	270	8
Amaranthus spp	4-5	10	90-160	800	15
Clotalaria brevidens	4-5	7.2	110-130	270	4
Basella alba	5	4	100	250	4
Cucurbita	3-5	5.6	170-175	400	11
Brassica oleraceae var.Acephala	5	5.3	100	250	4
Lactuca sativa	1.4	0.6	15	35	1
Ipomea batatas	3.2	2.7	20	85	4.5
Manihot esculenta	28.1	8.8	90.2	1509.4	16.7

Caroline and Muchoki (2007)

The nutrient level in cassava leaves varies with specific factors, including the geographical location, variety, age of the plant, and environmental conditions. In a study conducted by Nekesa (2016), there was a significant difference among local cassava varieties. Nekesa (2016) reported that at three months, zinc, crude protein, and iron content were higher, while at nine months,

vitamin C and calcium content were higher. The crude protein content on cassava leaves was reported to reduce with an increase in plant maturity and levels between 20% and 31.6% (Nekesa, 2016). β carotene content in cassava leaves was also reported to decrease with an increase in maturity and levels. Ascorbic acid content in cassava leaves was reported to increase with plant maturity and varied significantly with cassava variety. Calcium content in cassava leaves was reported to increase with plant maturity, and ranges between 520 mg/100g and 153 mg/100g were reported (Nekesa, 2016). Iron content in cassava leaves was reported to decrease with plant maturity and range between 270 and 1780 mg/kg (Nekesa 2016).

Cassava leaves have appreciable amounts of beta carotene between 8.5mg and 15.0 mg. In comparison with other ALVs, Cassava leaves have a higher content of beta carotene than Sweet potato leaves (3.2mg) and Jute mallow (7.5mg). The Vitamin C content of cassava leaves is comparable to other leafy vegetables between 60mg to 370mg\100 (Montagnac, 2009). Comparison of ascorbic acid content between cassava leaves with other leafy vegetables, peanuts leaves, and sweet potatoes was 293.3 mg/100g, and 308 mg/100g, respectively (Wobeto *et al.*, 2006), and cowpea leaves 303 mg/100g (Muchoki, 2007). In a comparison of nutrients between cassava and other leafy vegetables, Nekesa (2016) reported that cassava leaves had ash content values between 4.0% and 7.7% that indicated lower mineral concentration (Kendall 2010). The mineral concentration was lower as compared to cowpeas 12.1 % (Muchoki, 2007), sweet potatoes 12.87% (Lwasai 2012), and amaranth 17.8% (Aduwesi 1999).In comparison to other leafy vegetables. Wobeto 2006 reported a similar value of 30.6% in cowpea leaves(Wobeto *et al.*, 2006), while sweet potatoes were reported to be at 24.85% (Antia 2006). Other studies reported higher values of 55.72 to 64.12mg/100g (Wobeto *et al.*, 2006) and 294.77 and 310.88 mg/100g (Simao et al., 2013). The iron content of cassava leaves ranges between 61.5 to 270mg/kg (Madruga and

Camara, 2000). In a comparison of cassava leaves and other leafy vegetables, a study by Yadav 2002 reported lower values in amaranth and iron 34.14 and 26.54 mg/100g, respectively (Yadav, 2002).

2.4 Anti-nutrients in cassava leaves

Anti-nutrients factors are synthetic and natural compounds that hinder the absorption of nutrients. The antinutrients occurring in cassava include cyanide, phenolic compounds, oxalates, nitrates, and nitrites (Aregheore, 2012), as shown in Table 2.2.

Table 2.2 Comparison of Anti-nutrient factors in African Leafy Vegetables.

Vegetable	Phytates	Oxalates	Tannins	Cyanide	Nitrates
Talinum triangulae	210.54	28.93	1.01	23.81	5.5
Amaranthus hydridus	155	47.35	0.67	24.36	4.99
Manihot esculenta	191.25	15.74	0.65	25.69	3.58
Telfariria. occiedetails	84.72	48.17	0.89	25.4	7.88
Solanum nigrum	97.21	2.99	0.16	24.18	4.08
Crassocephalum crepidiodes	249.16	13.2	9.58	24	3.93
Cindosculus aconitifolis	313.67	23.11	0.76	28.71	5.1

Source: Aregheore (2012)

2.5 Anti-nutrients in cassava leaves

2.5.1 Cyanide

Cyanide is a toxic compound occurring as a cyanogenic glucoside and is associated with adverse health effects in humans, thus restricting the consumption of cassava. Cassava is classified into three categories according to their levels of cyanhidric acid content; Non-toxic (Less than 50 mg HCN/kg), Moderately toxic (50-100 mg HCN/kg), and very toxic with more than 100mg HCN/kg) (Kobawila *et al.*, 2005). The

recommended maximum consumption level by FAO/WHO is 10 mg/kg dry weight. The levels of cyanide in cassava leave maybe six times more as compared to the cassava roots subject to change from one variety to another (Fasuyi, 2005). Despite the different processing techniques playing a part in reducing the levels of cyanogenic glucosides, other factors such as drought and famine can cause an increase in demand of the cassava leaves, causing a compromise in the processing methods (Fasuyi, 2005). Levels of cyanide in cassava are determined by various factors that include variety, soil condition, and weather (Aregheore, 2012). Nekesa 2016 reported that the levels varied among *Kibanda meno, Tajirika,* and *Karembo,* which are varieties grown in the Kenyan Coast. The levels were 409.3 to 633.3 mg/kg in *Kibanda Meno* 324.6 to 829.3 mg/kg in *Tajirika* and 843 to 1849 mg/kg in *Karembo* (Nekesa, 2016). Cyanide levels also increased with maturity, with the increase being significant at nine months as compared to both 3 and 6 months (Nekesa, 2016). Wobeto 2006 also reported an increase in cyanide levels at 12, 14, and 17 months (Wobeto *et al.*, 2006). In comparison with other leafy vegetable levels of cyanide in sweet potatoes was 30.24mg/100g which was lower than in cassava leaves as reported by Antia in 2014 (Antia, 2014).

2.5.2 Oxalates

Oxalates are di-carboxylic acids in the plant, such as cassava; they impact the bioavailability of magnesium and calcium negatively (Massey, 2007). Oxalates bind calcium, forming calcium oxalates, or are excreted through urine. Individuals with kidney stones are advised to reduce oxalate intake and increase calcium intake (Massey, 2007). Levels of oxalates in cassava vary from 1.35 to 2.88g/100g of total dry weight (Wobeto, 2006). In comparison with other African leafy vegetables, cowpeas leaves had a lower value of 18.89g/100g (Muchoki, 2010). Sweet potato leaves were also reported to have lower values at 33.16 g/100g (Laswai, 2011). Nevertheless,

oxalates contain antioxidant and anti-carcinogenic properties when consumed in appropriate amounts (Musa and Ogbadoyi, 2014).

2.5.3 Tannins

Plant tannins are polyphenols that have a high molecular weight and can bind with proteins as well as carbohydrates (Natesh et al., 2017). They are classified into hydrolyzable and non-hydrolyzable. The hydrolyzable form of tannins in vegetables can be easily broken down when suspended in solutions of acids, bases, and some other enzymes (Natesh et al., 2017). These polyphenols are antioxidants and can bind with minerals hence reduce their bioavailability (Nekesa, 2016). Research also has shown that high levels of polyphenols can cause infertility among women of childbearing age because it may affect reproductive hormones (Greenwell, 2000).

2.6 Traditional processing and preservation methods of Cassava in East Africa

There are different methods of preparation and processing of cassava leaves. The different methods of preparation vary from one community to another**.** The traditional processing methods differ with locality and preference of the consumers and include shredding, boiling, fermentation, pounding, drying, and soaking (Umuhozariho *et al.*, 2013).

2.6.1 Boiling and Pounding

Boiling is one of the most utilized processing methods for cassava leaves. In Rwanda, only the topmost leaves of the cassava plant are plucked and then grounded into fine form, using mortar and pestle (Nekesa, 2016). The cooking process involves boiling for about 30 minutes and eventually consumed with milk.

Bradbury & Denton (2011) reported a rapid loss (96%) in the total cyanide content after 40 minutes of boiling cassava leaves at 55°C. Boiling reduces cyanide content due to the increased evaporation

of hydrogen cyanide and cyanohydrins caused by heat. Notably, increasing the period of boiling also increases the rate of cyanide removal. (Bradbury & Denton ,2011). The aqueous solution obtained after boiling must be discarded as it contains poisonous cyanogen. It has been reported that increasing the volume of water during boiling and reducing the size of cassava leaves by shredding improve the efficiency of boiling to remove cyanide (Aura, 2013). Research by (Lola 2009) showed that all nutrition contents in cassava leaves reduced after boiling while anti-nutritional components of tannins and oxalates were also significantly reduced by 39.7% and 20%, respectively.

However, as a single process, boiling is not an effective process to remove cyanide as high temperatures involved lead to the breakdown of vitamins and proteins (Bradbury and Denton, 2014). The project was education. Pounding the cassava leaves prior to boiling increases cyanide removal while preserving the nutrients. Bradbury & Denton (2011) reported that pounding cassava leaves for 15 minutes reduce the cyanide content by 8%. Boiling the pounded leaves for 10 minutes resulted in a 98% reduction in cyanide. Generally, pounding reduces the need to extend the boiling time for the efficient removal of cyanide. Ojiambo (2017) reported that pounding, soaking, and boiling the sweet cassava leaves resulted in a 95% reduction in cyanide after 20 minutes in the different varieties grown in Kakamega county. Pounding ruptures the cell structure in cassava leaves by promoting the rapid breakdown of cyanogenic glycosides and increasing contact between linamarase enzyme and Linamarin, causing hydrolytic breakdown (Ojiambo, 2017).

2.6.2 Steeping

Steeping is soaking the cassava leaves in water (5 to 10 times its unit weight) and leaving it for a unit time (Fasuyi, 2005). As reported by Fasuyi, cassava leaves were steeped in 5 times their weight for 24 hours; the leaves retained 69.1 % of cyanide (hydrocyanic acid) and 99.1% of tannins

(Fasuyi, 2005). Steeping reduces cyanogenic glycosides through solubilization (Nebiyu & Getachew, 2011). For storage purposes, farmers have also turned to blanching or steeping with preservatives (Singh, 2019). The method is simple and can be utilized during peak seasons. Optimization of the steeping methods as well as quality assessment of the vegetables is the biggest point of concern as far as steeping is concerned. For effective removal of cyanide, steeping must be combined with other methods, including boiling, pounding, or drying.

2.6.3 Drying

Drying helps in reducing water content in vegetables. This subsequently inhibits the growth of microorganisms (Nekesa, 2016). Before drying, enzyme inactivation needs to be put into consideration, and this can be achieved through blanching (Nekesa, 2016). In the drying process, the endogenous linamarase enzyme catalyzes the removal of cyanogenic glucoside hence determine the accumulation of free cyanide in dried cassava leaves and roots (Kehinde AT, 2013b). According to Fasuyi, two main methods of drying are used, sun drying and oven drying (Fasuyi, 2005). In the sun drying, the cassava leaves are dried for 2-3 days in the open sun while continuously turning to prevent fungal growth. Fasuyi reported that the method achieved retention of 4.1 hydrocyanic acid and 63.9% of tannins (Fasuyi, 2005). In oven drying at 80-90 ºC for 24 hours, Fasuyi reported a retention of 61.6% of hydrocyanic acid and 48% of tannins (Fasuyi, 2005) .

During oven drying of cassava leaves, cyanide removal is more effective at higher temperatures of drying. Modesto et al. (2019) reported that oven drying cassava leaves for 30 minutes at 70° C led to a 75% reduction in HCN, as compared to 94.11% at 80°. Wobeto et al., (2004). Also reported a 62-to 80% reduction in total HCN after 90 minutes of oven drying at 30°. Therefore,

higher temperatures should be used for shorter periods of time, while lower temperatures may require more time for effective cyanide removal.

The drying of cassava leaves causes changes in the nutritional content. Vitamin C was also reported to decrease during solar drying as it is thermo-labile. Calcium levels were reported to increase by 12.1% during solar drying of cassava leaves (Nekesa, 2016). The ash content of cassava leaves also increased by 4% during solar drying.

2.6.4 Combination of processing methods

To improve the effectiveness of cyanide, phytate, and tannin removal, several techniques are combined (Fasuyi, 2005). Shredding and sun-drying leaves retained 3.7% cyanide, while steeping and oven drying retained 69.1% cyanide. Oven-dried leaves retained the least amount of tannin at 48%. Shredding and sun-drying had the same effect as sun drying and steaming as they retained 42% of phytate (Fasuyi, 2005). Combination of three mild methods

i.e., pounding followed by steeping for 2 hours in the sun and finally three washes in water was reported to remove cyanide content to 28%, 12%, and 1%. The cassava leaves also retained their bright green color and texture (Bradbury and Denton, 2011). Pounding and boiling for ten minutes was reported to reduce cyanide to zero but greatly reduced water-soluble Vitamin B and Vitamin C proteins and methionine (Bradbury and Denton, 2011). Pounding cassava leaves for ten minutes in a pestle and mortar followed by washing in water twice their weight was reported to reduce cyanide levels to 8% (Bradbury and Denton,2011). Immersing the cassava leaves in water ten times their weight and changing the water followed by immersion in water for 2 hours at 50^0 c was reported to reduce cyanide by 7% (Bradbury and Denton, 2014). Fermentation and oven drying was reported to remove 85.6% of phytates and 52% polyphenols (Montagnac, Davis & Tanumihardjo, 2008).

2.6.5 Fermentation

Fermentation among the oldest processing methods that improve the sensory profile of a food. Fermentation is also used to increase shelf-life, sensory and nutritional properties (Ochieng, Owaga and Njoroge, 2018). For cassava, the aim of fermentation is to increase the nutritional value by increasing the protein availability and reducing the level of toxic cyanogenic to levels safe for human consumption (Hawashi, 2019). Fermentation also prolongs shelf-life and improves sensory qualities (Kehinde, 2013). Fermentation can be grouped into spontaneous and control fermentation (Hawashi et al., 2019).

Spontaneous fermentation provides favorable conditions on suitable microorganisms for fermentation while killing all other microorganisms. Waluchio (2016) demonstrated successful spontaneous fermentation through the addition of 3% salt and 3% sugar to the raw cassava leaves for 16 days at ambient temperature between 22-25°C.

Controlled fermentation is done when natural fermentation might be unstable, or the bacteria growth is minimal. In controlled fermentation, starter cultures are isolated, characterized, and used as single or combined cultures. The quality of products and sensory characteristics can therefore be controlled (Hawashi et al., 2019). Controlled fermentation has been successfully demonstrated by Barati et al. (2019) by using starter culture *Bifidobacterium, Lactobacillus acidophilus,* and *Streptococcus thermophilus* for 21 days at 37°C. Furthermore, Hawashi et al. (2019) reported a reduction of cyanide content in cassava leaves less than 10ppm after 60 hours of solid-state fermentation using *Saccharomyces cerevisiae* as a starter culture, in addition to 1% sucrose concentration, 60% moisture content, and 0.5% of urea.

2.6.5.1 Microbial changes after fermentation

Various bacteria and fungi are associated with the fermentation of cassava leaves. The bacteria include *Bacillus Lactobacillus* and *Leuconostoc,* while the fungi include *Penicillin, Fusarium,* and *Saccharomyces* (Sobowale and Oyewale, 2008). During submerged fermentation using lactic acid bacteria as reported by Anyogu, dominant isolates were *Lactobacillus plantarum*, *Weissela confuse, Lactobacillus rhamnosus* and *Lactobacillus paracasei* (Anyogu *et al.*, 2014). Anyogu further reported antimicrobial against indicator bacteria which included *E. coli*, *Salmonella enterica Typhimirium, Bacillus cereus,* and *Staphylococcus* within 48 hours. This was attributed to an increase in acid production (Anyogu et al., 2014).

During a combination of submerged and solid-state fermentation as reported by Indrastuti & Estiasih (2018), the first process was submerged fermentation for three days followed by submerged fermentation for three days (Indrastuti & Estiasih, 2018) reported a reduction in PH from 6 to 4.5 and the dominant bacteria was lactic acid bacteria. He also reported that after the fermentation there was a significantly high level of yeast than molds. He also noted that different cassava varieties had different microbial growth patterns (Indrastuti and Estiasih, 2018).

2.6.5.2 Sensory qualities of dehydrated fermented cassava leaves

Traditional and indigenous cassava roots and leaves are characterized by a bitter taste which sometimes leads to the undesirable quality of their end products (Kehinde , 2013). Fermentation is among the first step that is utilized to develop flavor in cassava leaves, and roots. Dehydration process helps to stabilize the product after fermentation and improve its shelf life (Kehinde, 2013). Fermented cassava leaves were reported to be liked and preferred as compared to non-fermented cassava leaves by Sanni and Jaji. Fermented samples were likable as compared to non-fermented

samples and had an average sensory score of 5 points on a seven-point hedonic scale(Sanni & JaJi, 2003) .

2.7 Research gaps

There is need for more research to be done on cassava varieties to identify varieties that have low levels of anti-nutrients for both leaves and roots. Also, identify the most effective methods for elimination of anti-nutrients from cassava leaves.

CHAPTER THREE

HARVESTING AND POSTHARVEST HANDLING PRACTICES AND UTILISATION OF CASSAVA LEAVES IN TAITA TAVETA AND KILIFI COUNTY

3.0 Abstract

Cassava (Manihot escutenta, Crantz), is one of the high yielding, disease and drought resistant crop that be used as an alternative for maize in Kenya. The leaves are highly nutritious and serve as an alternative to green leafy vegetables. However, cassava leaves production, consumption and processing has been low due to the lack of a well-structured cassava value chain and standard postharvest handling practices. Additionally, the high content of anti-nutrients discourages consumption at household. This study evaluated the harvesting, postharvest handling practices, utilization and value addition of cassava leaves in Kilifi and Taita Taveta counties.A total of 247 respondents were selected from the two counties 120 and 127 respondents in Kilifi and Taita Taveta respectively. The results indicated that majority of the cassava farmers were female (56.3%). The mean age of respondents was 48.82±15.08 years with more than three in every ten respondents (32.0%) being middle aged (36-50 years). Most of respondents had attained primary education (55.1%) and education was significantly ($\chi 2=27.433a$, $p<0.001$) associated with gender with males being more educated as compared to females. Almost all respondents (99.6%) grew cassava for food and Kibanda meno was the most preferred variety. In both counties, farmers harvested few leaves or piecemeal by handpicking and most commonly in the morning hours. Half of the respondents (50%) sorted cassava leaves after harvesting based on damage, size and color. Additionally, cassava value addition was limited to drying (82.6%) and fermentation (4.1%). The respondents (65.2%) preserved cassava for a maximum of 15 days. This study concludes that poor

postharvest handling and low value addition of cassava leaves contributes significantly to the low production and consumption.

3.1 Introduction

Cassava (*Manihot escutenta, Crantz*), has been one of the crops promoted by Ministry of Agriculture, livestock, Fisheries and Irrigation through pillar two of The Big Four agenda on Food Security partly because it's high yielding, drought and disease resistant (Ouma, 2019). Global production of cassava as at 2017 Food and Agriculture statistics was 277.1 million tonnes annually (FAO, 2017). The leading cassava producing countries are Nigeria at 59.5 million tonnes annually, Democratic Republic of Congo 31.6 million tonnes and Thailand at 30.9 million tonnes. In Africa Nigeria leads the production followed by Democratic Republic of Congo which is also world number 5 biggest producer. Other major producers in Africa are Angola at 11.8 million tonnes annually. In East Africa region Tanzania is the leading producer at 5.01 million tonnes annually followed by Kenya at 1.1 million tonnes annually and Rwanda at 1.041 million tonnes annually (FAOSTAT, 2017). Western and Coastal regions are the main cassava producing areas, producing over 80% of the recorded cassava output in the country (Nekesa, 2016).

Cassava is the third most vital food as a source of carbohydrate around the world and in precisely in sub-Sahara Africa. Both the roots and leaves are equally important source of nutrition, however, they have anti-nutrient cyanogenic glucosides that are harmful to consumers (Van and Wredle, 2012). The age and climatic conditions influence the level of anti-nutrients in cassava leaves (Nawiri et al., 2017). Nutritionally, cassava leaves are rich in proteins, vitamins and iron and this is dependent on variety with leaves from yellow flesh root varieties having more (Wargiono, 2002). The leaves contain 7-10 percent protein which is high as compared to other vegetables (FAO, 1999). The cyanide content of the leaves is reduced by processing through fermentation,

drying, pounding, frying, shredding and maceration processes (Nawiri et al., 2017). Cassava leaves processing is not a common practice in Kenya due to low production and consumption. In the Western, Coastal and part of the Eastern regions of the country, there is high production of both the leaves and roots. The leaves are consumed as a vegetable and is sometimes fermented or dried to reduce anti-nutrients and extent shelf-life.

Cassava leaves are highly perishable and susceptible to physiological changes that set in immediately after harvesting. These changes have been attributing to the huge postharvest losses of the leaves and roots (Djabou et al., 2017). Without proper harvesting and postharvest handling practices, the losses could go beyond 50%. Cassava leaves are harvested mainly by handpicking by picking a few leaves or uprooting the whole plant. Postharvest handling practices for vegetables include sorting or grading, packaging, storage and transportation apply to cassava leaves (Womdim et al., 2012). In Kenya, there are no standard postharvest handling practices for cassava leaves leading to poor handling and increased postharvest losses. Little credible information available to quantify these losses (Naziri et al., 2014). There is need to develop standard postharvest handling practices for cassava leaves aimed at reducing the losses and extending shelf life.

Cassava root is consumed as a snack in most parts of Sub-Saharan Africa mostly as a breakfast accompaniment for tea. There is increased utilization and value addition of the roots to various products including *gari*, cassava flour, boiled cassava, cassava paste, fried cassava and fermented cassava flour (Karuri et al., 2001). On the other hand, processing of cassava leaves is minimal and only fermentation, sun drying and pounding have remained the most common practice which are mainly aimed at reducing the cyanide content in the leaves (Nawiri et al., 2017). This calls for more involvement of food processors and researchers on cassava leaves processing into shelf-

stable products. This will increase cassava leaves consumption. In Kenya, cassava is produced largely in the coastal and Western region hence the choice of Kili and Taita Taveta as the study areas was based on their high cassava lead production and consumption. This study intended to assess the harvesting and postharvest handling practices, and utilization of cassava leaves in both counties.

3.2 Materials and Methods

3.2.1 Study Design

The study was cross-sectional baseline survey in Kilifi and Taita Taveta Counties. Survey was conducted January 2020 and total of 247 farmers including 120 from Kilifi and 127 from Taita Taveta. The data were collected using semi-structured questionnaires by use of digital Open Data Kit application. The data pertained to the harvesting, postharvest handling practices and utilization of cassava leaves in the two Counties.

3.2.2 Study area

The study was undertaken in Kilifi and Taita Taveta (Figure 1). Kilifi County is one of five counties that comprise the Kenyan coast line. It's about 507 kilometers from Nairobi and the Port town of Mombasa is 65 kilometers away. It's among the smaller counties by land mass in Kenya with an area of 12,245.90km2 of which 109km2 is the Indian Ocean water mass. Kilifi experiences two seasons of rain with an average rainfall of 900mm per annum while temperatures range between 21–35^{o}C (Ouma, 2019). The main economic activities are fishing, agriculture, mining manufacturing and tourism. Main subsistence crops grown in the County are maize and cassava while cash crops include coconuts, cashew nuts, sisal and citrus fruits such as mangoes and

Horticulture (Flowers, Fruits, and Vegetables) and pineapples (Tirra et al., 2019). Half of the land in the county is arable.

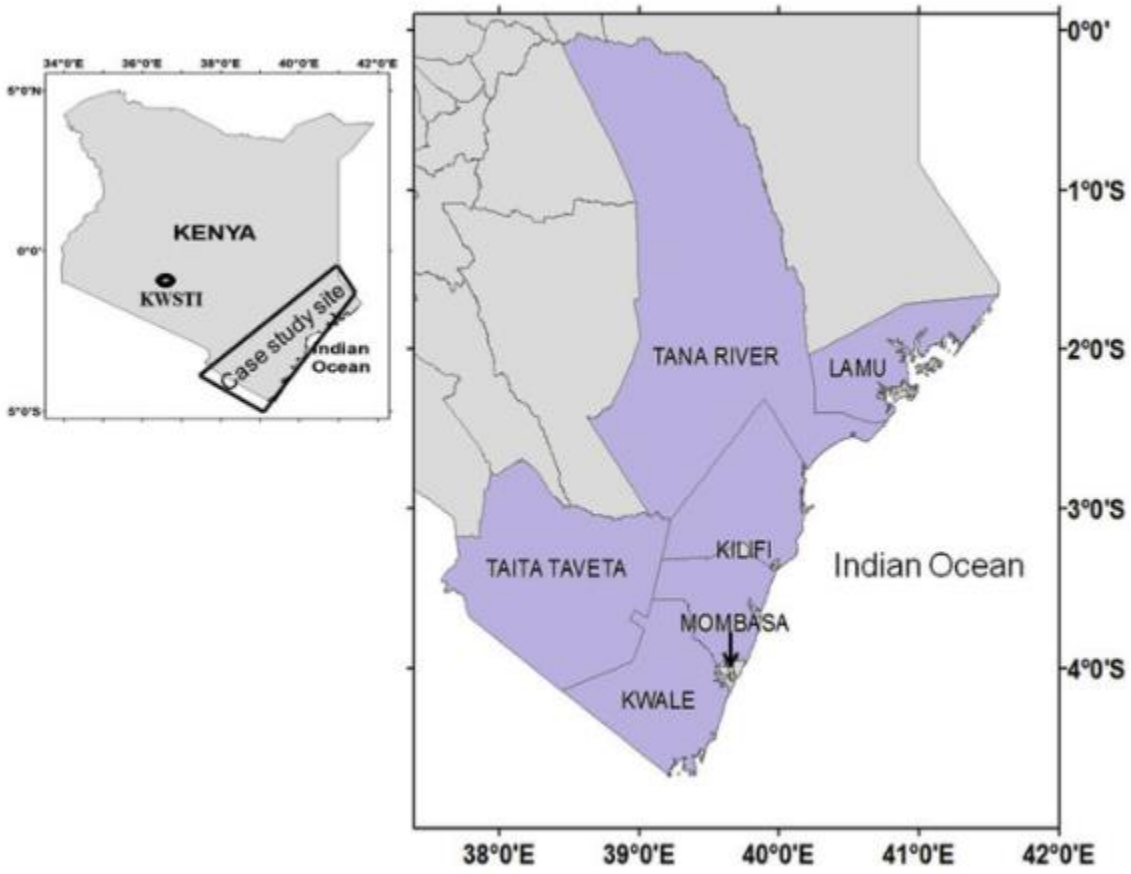

Figure 3.1: Map of Kilifi and Taita Taveta Counties (Mulanda et al., 2018)

Taita Taveta County **(Figure 3.1)** is located in the Coastal region of Kenya bordering Tana River, Kitui Makueni, Kwale and Kilifi, Kajiado and the Republic of Tanzania on the Southern side. The county covers an estimated area of 17,084.1km^2 and has an estimated population of 340,671 persons according to 2019 census (KNBS, 2019). The county lies between longitude 37^0 36$^{"}$east and 30^0 14$^{"}$ east and latitude 2 ^{0} 46$^{"}$ south and 4^0 10$^{"}$ south. Altitudes range from 500 metres above sea level to almost 2300 m at the highest point in the county Vuria Peak. Taita Taveta is mainly

dry, with the exception of Taita Hills which are considerably wet (Katumbi et al., 2021). Rainfall distribution is usually uneven, with higher rainfall amounts being recorded in highland areas as compared to the lowlands. Annually, mean rainfall is 650 mm (County Government of Taita Taveta, 2018). The average temperature in Taita Taveta County is 23^0C, with lows of 18^0C in the hilly areas and rises to about 25^0C in the lower zones (Tirra et al., 2019).

3.2.3 Study population

The study population included cassava farmers in Kilifi and Taita Taveta County. A structured questionnaire was used to determine the farmers' demographics, social economic status, methods of preparation and methods of utilization of cassava leaves.

3.2.4 Sample size determination

The sample size for the laboratory analysis was determined as per the Fisher's formula (Fischer et al., 1998).

$$N= Z^2pq \div d^2$$

Where;

n - Quantity of sample size desired

P- Ratio in the selected population who cultivate cassava (20%)

q- (1-p) - the ratio in the selected population expected not cultivate cassava (80%)

d- level of statistical significance set (0.05)

Z- Normal standard variation at the required confidence level, a 95% confidence level will be used.

Therefore;

n= (1.962* 0.2*0.8) ÷ (0.052) = 247 respondents

3.2.5 Sampling criteria

The two counties were purposively selected as among the highest producers of cassava in Kenya. Two Sub-Counties were purposively selected in each County based on high production quantities. Wards that were producers of cassava from the two Sub-Counties were all included in the study. Villages and farmers were randomly selected in these wards to be interviewed.

3.2.6 Inclusion and Exclusion criteria

The study included farmers of cassava in both Kilifi and Taita Taveta counties of ages above 18 years who have been growing cassava for a minimum of 3 years.

3.2.7 Research tools and instruments

The study involved personal interview and focused group discussions and structured questionnaires. Each questionnaire contained two sections one for the demographics and the utilization and preparation sections.

3.2.8 Data collection procedures

The data collection was done using ODK (open Data Kit) tool. Forms (XLS forms) were built and uploaded on a mobile device and used to collect data.

3.2.9 Data quality control/validation

The pre-coded questionnaires were pretested in a different setting with the same factors of interest as the study area. Relevant corrections were done followed by pre-coding.

3.2.10 Data analysis

Data analysis was done using statistical package for Social Sciences Software (IBM SPSS Statistics for Windows, version 25 (IBM Corp., Armonk, N.Y., USA). Means were used to analyse age of respondents. Chi square was used to analyse association of income levels, academic qualifications and age groups with preparation and utilization methods.

3.2.11 Ethical clearance

Clearance to conduct research was applied and issued by County Government of Kilifi and County Government Taita Taveta. Informed oral consent was obtained from all respondents participating in the study.

3.3. Results

3.3.1 Social Demographics characteristics of cassava producing households

Majority of cassava producers largely consisted of females (56.3%) who took part in farming activities. The mean age of respondents was 48.82±15.08 years with more than three in every ten respondents (32.0%) being middle aged (36-50 years). Most of the cassavas producing households (64.8%) were headed by males and about 14.2% of them had a size of 6 members. More than half of the respondents (68.0%) owned less than 2 acres of land even though majority (80.2%) practiced farming as their main occupation. About 10.5% of the respondents were from low income households with an annual income of approximately 600 US Dollars. Moreover, more than half of the respondents (55.1%) had attained basic primary education, however, a few (5.7%) had attained tertiary education with 20.6% being illiterate (did not attend school). Other socio-demographic characteristics of the respondents are represented in table 3.1. There was a significant (χ^2=27.433[a], p<0.001) association between gender and respondent's level of education with males being more

educated as compared to females. The county of origin of respondents significantly associated (χ^2=47.627[a], p<0.001) with their level of education with Taita Taveta having more educated respondents as compared to Kilifi county.

Table 3.1: Socio-demographic characteristics of Cassava producing households

Social demographic characteristic		%N N=247
Sex	Male	43.7
	Female	56.3
Age group	Youth <35 years	22.3
	Middle aged (36-50) years	32.0
	Upper middle aged(51-60) years	20.2
	Retiree	25.5
Household Head	Male	64.8
	Female	35.2
Education level	None(did not attend school)	20.6
	Primary	55.1
	Secondary	18.6
	Tertiary	5.7
Marital status	Married	78.1
	Separated	2.8
	Widowed	14.2
	Single	4.0
	Divorced	0.8
Religion	Christianity	88.3
	Muslim	11.3
	traditionalists	4.5

3.3.2 Cassava production in Kilifi andTaita Taveta Counties

Almost all respondents (98.0%) grew cassava in their farms and the main reason of growing cassava for majority of them (99.6%) was for food (Figure 3.2). There were no significant differences ($p>0.05$) between Kilifi and Taita Taveta counties based on cassava production. Majority of the respondents (67.6%) were growing the Kibanda meno variety. There was a significant association ($\chi^2=94.528^a$, $p<0.001$) between the county and cassava varieties grown

(Figure 3). More than six in every ten respondents (69.6%) consumed cassava leaves. The county of origin was significantly associated (χ^2=28.96[a], p<0.001) with cassava consumption with more consumers coming from Kilifi (59.9%) as compared with Taita Taveta (22.7%)(Figure 3.4). The reason for cassava consumption for more than eight in every ten respondents (87.8%) was the availability of the cassava leaves while 58.1% of the respondents considered consuming the leaves due to their nutritional value however, the reasons for cassava consumption differed significantly (χ^2=86.98[a], p<0.001) with county of respondent (Figure 3.5).

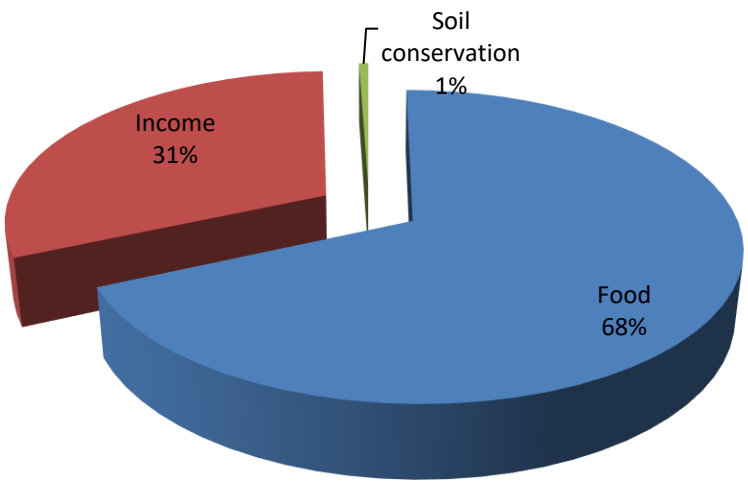

Figure 3.2: Reasons for growing cassava in both Kilifi and Taita Taveta Counties

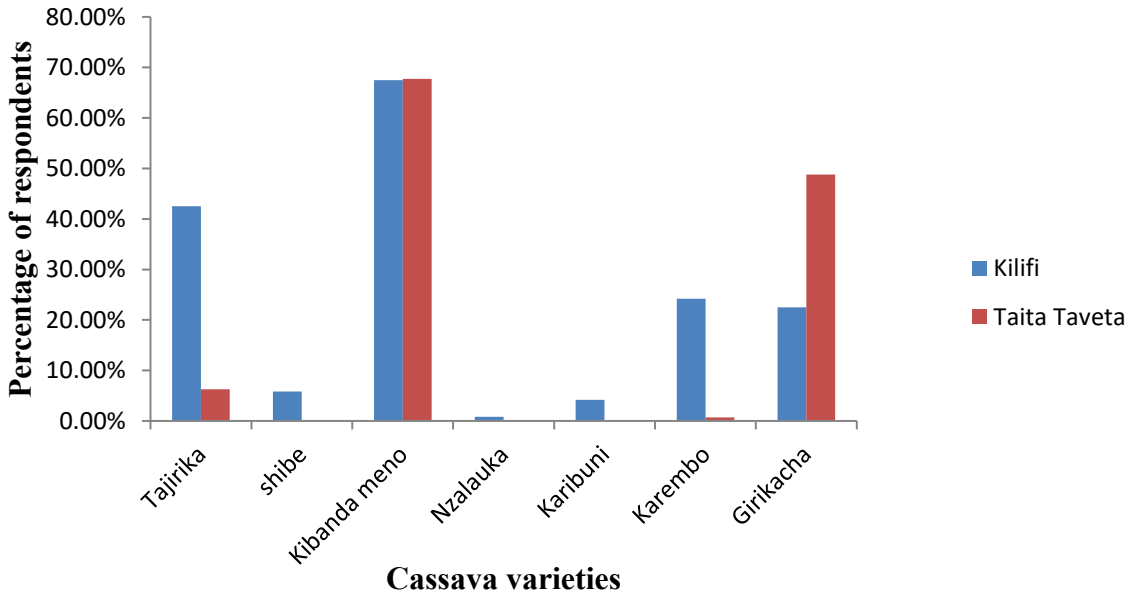

Figure 3.3: Cassava varieties grown in Kilifi and Taita Taveta Counties, Kenya (p<0.001)

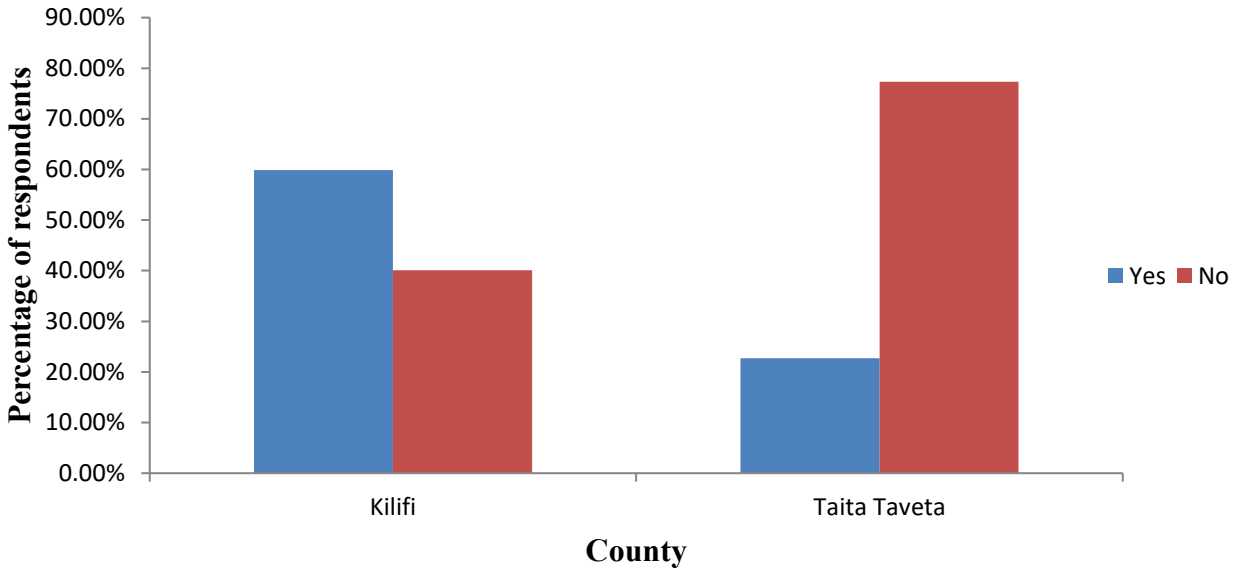

Figure 3.4: Consumption of cassava leaves in Kilifi and Taita Taveta Counties

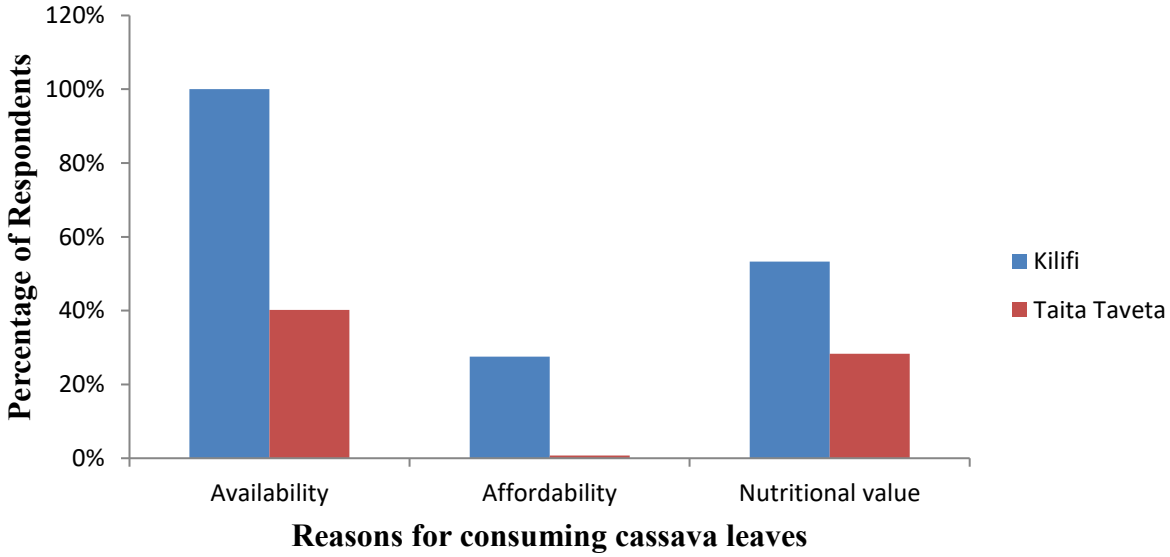

Figure 3.5: Reasons for cassava consumption in Kilifi and Taita Taveta

3.3.3 Cassava harvesting practices

Kilifi and Taita Taveta counties differed significantly (χ^2=71.80[a], p<0.001) in methods of harvesting cassava leaves .Those from Kilifi preferred harvesting (70.83%) few leaves while in

Taita Taveta piecemeal harvesting was the most common (Figure 3.6). The number of cassava leaves picked differed significantly (χ^2=71.70[a], p<0.001) among the two counties (Table 3.2). All respondents (100%) harvested few leaves or piecemeal because of the nutritional value while 22.2% of them did so due to the levels of anti-nutrient content in the leaves. Handpicking was the most preferred harvesting tool in both counties (Table 3.2). Most respondents (44.8%) reported that they harvested cassava leaves on need basis and in the morning hours (9am-12pm) and their main reason for this was temperature (54.1%).

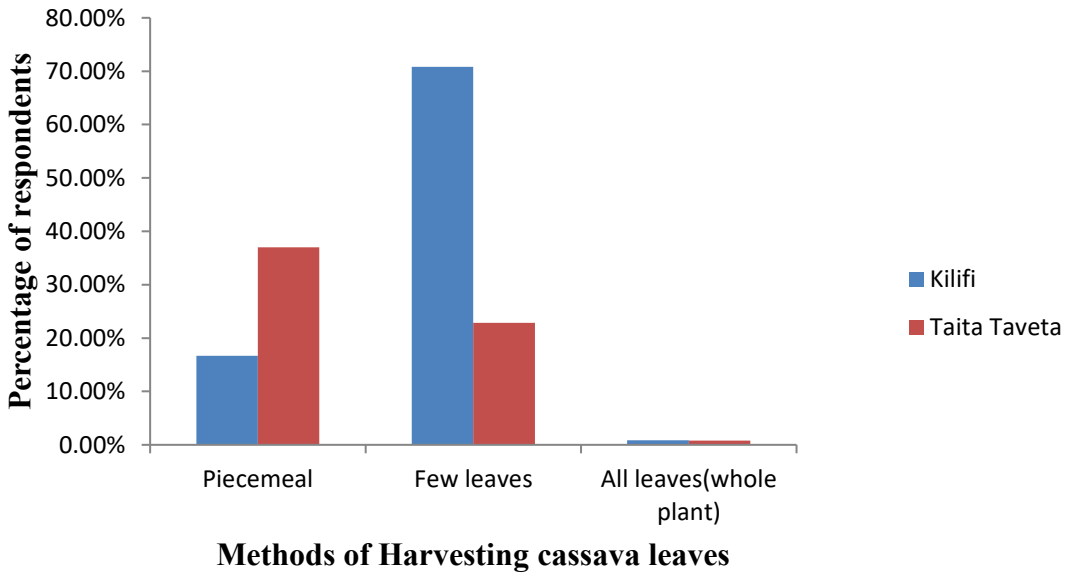

Figure 3.6: Methods of harvesting cassava leaves in Kilifi and Taita Taveta

Table 3.2: Harvesting Practices in Kilifi and Taita Taveta

Harvesting practice		**Kilifi %N N=120**	**Taita Taveta %N N=127**	**Significance**
Number of top leaves harvested	Top 2	3.33	9.44	**
	Top 3-4	41.67	18.11	**
	Top 5 and Above	32.5	3.94	**
Harvesting tools	Uprooting	14.17	45.67	**
	Jembe	14.17	45.67	**
	Panga	0	0.7	
	Handpicking	85.8	53.5	**
Time of harvesting	5am-9am	5.0	11.02	*
	9am-12pm	23.33	20.47	*
	12pm-4pm	1.67	1.57	
	After 4pm	10.0	3.94	*
	As needed	45.83	17.32	*

*Correlation is significant at the 0.05 level, **. Correlation is significant at the 0.001 level (Chi-square tests).

3.3.4 Postharvest Handling Practices

Half of the respondents (50%) sorted cassava leaves after harvesting however, this was not significantly different ($p>0.05$) in the two counties. The criteria for sorting the leaves differed significantly ($\chi^2=40.10^a$, $p<0.001$) between the two counties with Taita Taveta being poorest in sorting (Figure 3.7). The main reason for sorting / grading for more than six in every ten respondents (67.6%) was freshness, quality and removal of both damaged and diseased leaves. Pest damage was reported by 58.7% of respondents as the most common type of damage to cassava leaves observed during harvesting in both counties.

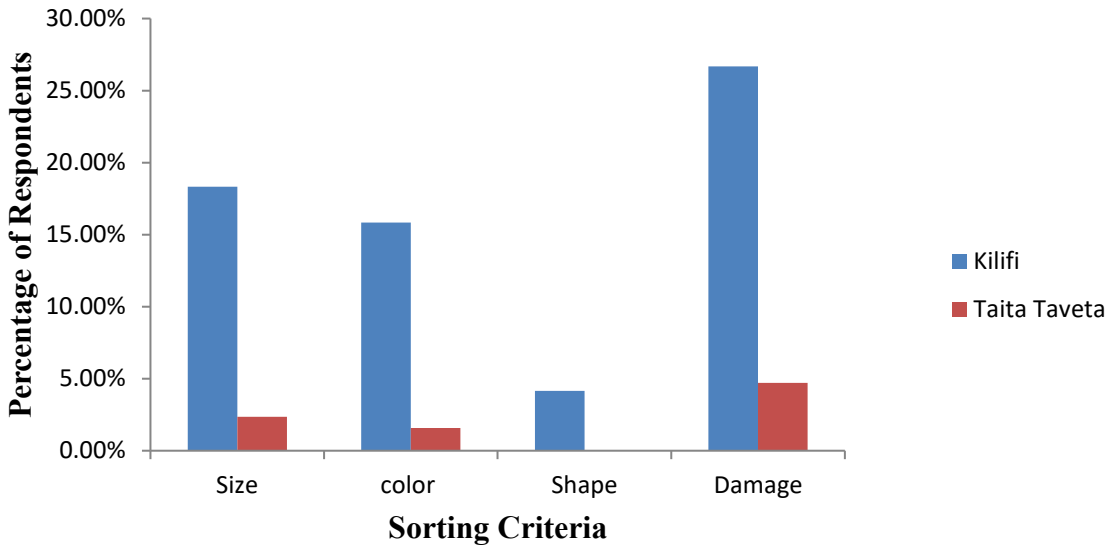

Figure 3.7: Cassava sorting criteria in Kilifi and Taita Taveta

3.3.5 Cassava Value addition and preservation practices

Preservation of cassava leaves was not a common practice in both Kilifi and Taita Taveta counties with only 19.17% and 2.36 respondents in Kilifi and Taita Taveta respectively preserving cassava leaves. More than eight in every ten respondents (82.6%) preserved cassava leaves by drying and only a few (4.1%) used fermentation as a preservation technique. Additionally, respondents (34.3%) reported that they learned about cassava leaves preservation from fellow farmers. About 65.2% of the respondents indicated that preservation of cassava leaves of up to 15 days. Processing of cassava leaves was not in the two counties and only 2.8% of the respondents did it. The few who processed the leaves did it for subsistence (71.4%). The most common cassava processed products were dried leaves (71.4%) and boiled and pounded leaves (42.9%). More than half of the respondents (60.0%) reported that drying was done to detoxify the cassava leaves and drying took place in the open yard. Color and texture was used by most of the respondents (60%) to check

quality of dried cassava leaves. All respondents (100%) preferred dried cassava leaves because they are easy to process.

3.4 Discussion

3.4.1 Social Demographics characteristics of cassava producing households

Cassava farming in Kilifi and Taita Taveta was dominated by women and this is linked to the fact that women play major roles in households and farm activities (Katumbi et al., 2021). Additionally, men dominate farming of cash crops where farm returns are high (Ogunlela and Mukhtar, 2009). Scarcity of arable land for farming was a common phenomenon in Kilifi and Taita Taveta as farmers owned less than 2 acres linked to the growing population and degradation of agricultural land (Peprah, Amoah and Akongbangre, 2014 and Mpozi *et al.*, 2020). Most cassava farmers had only attained basic primary education with low education level which correlates with other studies by Rahiel, Zenebe and Leake, (2018) and Katumbi *et al.*, (2021) where farmers were reported to have low level of education. The current study indicated that men are more educated than women which could be linked to societal beliefs where women are considered inferior to men even in education. Additionally, most women have low interest in education (Mareng, 2010; Nyaga and Ph, 2015).

3.4.2 Cassava production in Kilifi andTaita Taveta Counties

Cassava production is high in coastal, Western and Eastern regions of Kenya including Kilifi and Taita Taveta. The high production in these regions is linked to the favourable climatic conditions in the regions for cassava farming (Githunguri and Gatheru, 2017). These regions also have improved cassava varieties that do very well hence increased yield and productivity with slow but steady increase (Ouma, 2019). Consumption of cassava leaves was high in these regions linked to

high production of cassava in the area and the nutritional quality of cassava leaves as compared to other vegetables. The challenge with cassava leaves consumption and utilization is the presence of cyanogenic glucosides that are harmful to consumers. This makes consumers to be reluctant to consume cassava leaves as an alternative vegetable (Nekesa, 2016). More cassava leaves consumers were in Kilifi and this is attribute to the increased production and value addition of cassava in Kilifi with increased availability, awareness on nutrition and potential of cassava as both a vegetable and root (Karuri et al., 2001). Farming cassava mainly as a source of food in Kilifi and Taita Taveta was linked to the fact that cassava is a drought resistant crop and is considered a food security crop in the Sub-Saharan Africa. Additionally, cassava has a high production potential and highly nutritious with high protein content (Githunguri and Gatheru, 2017). Kibanda meno was the most occurring variety of cassava grown in Kilifi and Taita Taveta, this findings are similar to those of Nekesa ,(2016) where Kibanda meno variety was preferred due to high levels of nutrients and low anti-nutrient levels.

3.4.3 Cassava harvesting and Postharvest Handling Practices

Piecemeal and harvesting of few leaves was the most preferred harvesting technique in Kilifi and Taita Taveta counties mainly because the leaves were harvested for household consumption with minimal commercialization. Manual harvesting also gives better quality of the leaves because it minimizes physical damages (Id et al., 2019; Legg et al., 2007). Harvesting few leaves is linked to nutritional quality and low levels of anti-nutrient content as it is dependent on leaf maturity with the tender leaves being more nutritious compared to mature leaves (Munyahali et al., 2017; Van and Wredle, 2012). Cassava leaves harvesting at morning hours with the aim of avoiding high temperature that accelerates the rate of deterioration by lowering the field heat. This practice ensures that the leaves have low amounts of field heat to enhance the shelf life (Wargiono, 2002;

Nekesa , 2016). Increased temperature has been associated with increased enzymatic activity and cellular metabolism which hastens the rate of postharvest deterioration increasing postharvest losses (Mazumder, 2017; Sagrilo et al., 2003). Sorting or grading of cassava leaves was practiced in Kilifi and Taita Taveta mainly for quality especially leaf freshness, removal of both damaged and diseased leaves. The sorting criteria for the leaves were size, color and leaf damage (Paltrinieri, 2014). Pest damage has been a major cause of postharvest losses experienced by cassava farmers in Kilifi and Taita Taveta. Cassava postharvest losses are attributable to the high perishability and susceptibility to pests and diseases (Legg, 2017; Naziri et al., 2014).

3.4.4 Cassava Value addition and preservation practices

Cassava leaves consumption is very low in Kenya which is attributed to the presence of cyanogenic glucosides that are harmful to human hence discouraging consumers (Ouma, 2019). Kibanda Meno and Tajirika cassava varieties have been reported to have lower levels of cyanide (Nekesa, 2016) and were preferred by farmers in Kilifi and Taita Taveta. These anti-nutrients can be removed through processing and value addition of the leaves (Fasuyi, 2005). Several methods have been reported to reduce cyanide in cassava, fermentation, drying, baking, shredding, steaming (blanching) and frying (Nawiri et al., 2017). There was limited cassava leaves value addition in Kilifi and Taita Taveta with sun-drying being the most common method of value addition. Drying the cassava leaves is a key method of reducing cyanogenic glucosides in the leaves and extending the storage life (Nekesa, 2016; Ekpo and Baridia, 2020). Lactic acid fermentation of cassava leaves has been a common practice to reduce cyanide before consumption and the leaves are shredded or macerated before fermentation to expose the cyanogenic glucosides (Montagnac et al., 2009). Pounding of cassava leaves has also been reported to reduce cyanide content (Nawiri et al., 2017). Sun drying is preferred by farmers because it is affordable, however, during the rainy season which

is the season of glut, sun drying becomes a challenge due to low sun intensity (Nekesa, 2016). Cassava farmers should adopt fermentation of cassava leaves which also an affordable method of reducing cyanide and preserving the leaves.

3.5 Conclusion

Cassava leaves production in Kilifi and Taita Taveta is largely for household consumption with limited commercialization. Farmers produce only enough for household consumption. This is attributed to the existence of anti-nutrients in the leaves; methods such as sun drying and fermentation are being used in the two counties to reduce cyanide content in the leaves. Providing the farmers with effective storage facilities and training on cassava leaves value addition would provide an avenue for its improved utilization and appropriate postharvest handling. Improved value chain for cassava from leaves to the roots will increase production, consumption and marketability of cassava in the region.

CHAPTER FOUR

CHEMICAL PROPERTIES, ANTI-NUTRIENT CONTENT AND SENSORY CHARACTERISTICS OF FERMENTED CASSAVA LEAVES

4.0 Abstract

Cassava leaves are a nutritious delicacy in some parts of Africa. The leaves are used as vegetables and are rich in vitamins, minerals, iron and proteins. Consumption of cassava leaves is low due to the presence of cyanogenic glucosides, tannins, phyatates and oxalates in some varieties that cause acute and chronic poisoning. This study aimed at determining changes in chemical properties and anti-nutrient content of cassava leaves during fermentation. The cassava leaves had an average of 11.84 mg/100g of beta carotene and 21.70 mg/100g of vitamin C. Additionally, the leaves contained minerals including iron, zinc and calcium with an average of 19.37mg/100g, 16.33mg/100g and 741mg/100g respectively. The leaves contained an average of 2.44mg/100g oxalates, 0.303mg/100g tannins and 64.88mg/100g cyanide. Fermentation followed by oven-drying and sun-drying significantly ($p<0.001$) reduced the tannins, oxalates and cyanide to recommended levels. The sensory scores of the fermented leaves averaged at 5 points on a seven-point hedonic scale stating, that they were likeable in comparison to the non-fermented samples. This study concludes that fermentation reduces the anti-nutrient content in cassava leaves making it safe for consumption thus should be adopted for value addition of cassava leaves.

4.1 Introduction

Cassava (*Manihot esculenta crantz*) is the staple food majority of people in the developing world. It can survive in many soil types and is one of the most drought and disease resistant crops (Tefera, Ameha and Biruhtesfa, 2014). Nutritionally, cassava is rich in starch with high energy content but

has low protein content. Cassava leaves are source of vitamin C, B1, B2, and carotenoids (Wobeto, 2006). Cassava roots and leaves contains potentially toxic compounds, cyanogenic glucosides which cause acute cyanide poisoning, chronic poisoning and even death in man and animals when consumed (Perera et al., 2018). The amount of these toxic compounds varies according to cultivars and growing conditions (Tefera, Ameha and Biruhtesfa, 2014). Cassava leaves have higher proportions of cyanide compared to roots. The content can be as high as ten times (Bradbury and Denton, 2014). Cyanide is associated with iodine deficiency which might lead to goitre and cretinism. The level of cyanide in a cassava is determined by various factors such as variety, soil condition and weather.

Phytates have been known to bind proteins and minerals in the gastrointestinal tract, which makes them unavailable for absorption in the body (Aregheore, 2012). Similarly, Tannins and phenolics, also bind minerals and hinder action by digestive systems. In cassava leaves, this quantity is measured by non-specific assay (Vasconcelos *et al.*, 2007). Oxalates are known to bind the calcium and magnesium, forming calcium oxalate, a compound that is excreted through urine. On the other hand, oxalates contain anti- oxidants and anti-carcinogenic properties while consumed in the right amounts (Musa and Ogbadoyi, 2014).

The anti-nutrient content in cassava leaves can be reduced to acceptable levels using various traditional processing methods. The methods include shredding, boiling, fermentation, pounding, drying, and soaking (Umuhozariho *et al.*, 2011). The various traditional processing methods have various effects on anti-nutrients reduction and nutrients retention (Mathara and Trierweiler, 2016). One of the oldest food processing methods is fermentation is widely used for the purposes of increasing shelf-life, sensory and nutritional properties (Ochieng, Owaga and Njoroge, 2018). Specifically, for cassava leaves fermentation, the aim is to increase the nutritional value by

increasing the protein content availability, reduce the levels of toxic cyanogenic and increase the shelf life of the leaves. During the fermentation, there are significant changes in chemical and sensory properties of the leaves. This study aimed at assessing the chemical and sensory changes in cassava leaves during fermentation. The sensory scores of the fermented leaves averaged at 5 points on a seven-point hedonic scale (Sanni and JaJi, 2003) stating, that they were likeable in comparison to the non-fermented samples.

4.2 Materials and Methods

4.2.1 Study Design

Analytical evaluation was done to determine changes in nutrients (vitamin A, Vitamin C and minerals (calcium, zinc and iron) and anti-nutrients (cyanide, oxalates and tannins) during fermentation experiment of cassava leaves.

4.2.2 Experimental design

The experiment was arranged in a completely randomized design with 2 main fermentation treatments. Sub-treatments were 3 popular varieties, 2 top leaves groups and 8 chemical tests (Vitamin A, Vitamin C, Calcium, Zinc, Iron, Cyanide, Oxalate and Tannins the experiment was replicated twice.

4.2.3 Sample size determination

The sample size was 2 main fermentation treatments x 3 varieties x 2 top leaves groups x 8 chemical tests x 2 replicates = 192 samples.

4.2.4 Preparation of cassava leaves samples

The fresh cassava leaves were sorted, de-stemmed, washed in clean water and divided in 4 batches

4.2.5 Optimization of cassava leaves fermentation conditions

4.2.5.1 Determination of optimal concentration of added sugar and salt

The sorted cassava leaves were divided into equal 4 portions and fermented in lots of 250g, each lot was mixed thoroughly with 0,1, 2, 3 % concentration respectively of table salt (Kensalt, Kenya) followed by tight packing in 2-litre plastic beakers. They were allowed to stand for 20 minutes after which a polythene bag full of water was placed inside each container as a weight to press down the leaves with sugar and ensure that the experiment was air tight during fermentation. Fermentation was carried out at ambient temperatures (22º-26º C). During fermentation, samples of the fermenting liquor were withdrawn at regular intervals of 1, 4, 8 and 16 days for PH and total titratable acidity (TTA) determination. Fermentation lasted 16 days. The preliminary experiment was replicated twice. The sugar concentration that gave the highest total titratable acidity (TTA) and lowest PH was used for the other treatments in the experiment.

4.2.5.2 Determination of starter culture levels

The sorted cassava leaves were divided into 3 equal portions and fermented in lots of 250g as in the first preliminary above. Each portion contained the different cassava leaves varieties. Isolation and enumeration of most dominant Lactobacillus species (*Lactobacillus plantarum, Lactobacillus Fermentum or Lactobacillus brevis*) was done by plate count on Lactobacilli de Man, Rogosa, and Sharpe (MRS) agar. Colonies were counted as viable numbers of microorganisms (cfu/g) per gram. The dominant microorganisms were cultured and isolated and were used as starter culture for the other fermentation treatments. The amount of starter culture determined was varied to determine the percentage of starter culture that gives highest total titratable acidity (TTA) and lowest PH. This level was used for the other treatments in the experiment. Different treatments as indicated in Table 3.

Table 4.1: Treatment of cassava leaves for fermentation

	Treatments	T1	T 2	T 3	T 4
1	Sugar (% concentration)	0	1	2	3
2	Salt (% concentration)	0	1	2	3
3	Sugar + salt (% concentration)	0 + 3	1+ 2	2 + 1	3+ 0
4	Starter culture (% concentration)g/l	0.05	0.1	0.15	0.2

4.2.6 Fermentation of cassava leaves

The cleaned leaves were cut into 5 millimetres thickness and weighed. 1 kilogram of each of the 3 popular varieties top 4 leaves and 1 kilogram of top 5-8 leaves was weighed. Batch 1 was emptied into an air tight bucket and the bucket and covered with a sheet of polyethylene paper (fermentation vessel). The bucket was kept at ambient temperatures of 22º- 25º for 16 days (spontaneous fermentation). For Batch 2, one kilogram of each of the 3 popular varieties top 4 leaves and 1 kilogram of top 5-8 leaves was weighed. The percentage concentration of sugar and the percentage concentration of salt by weight determined to give the lowest PH and highest total titratable acidity (TTA) in 3.2.2.2 was added to the mixture. The mixture was gently mixed and the bucket covered with a sheet of polyethylene paper. The bucket was kept at ambient temperatures of 22º- 25º for 16 days. Batch 3, one kilogram of each of the 3 popular varieties top 4 leaves and 1 kilogram of top 5-8 leaves was weighed. Selected starter culture from 3.2.2.4 above (*Lactobacillus plantarum, Lactobacillus Fermentum or Lactobacillus brevis*) at the rate of 2mg/Kg was added. The mixture was gently mixed and the bucket covered with a sheet of polyethylene paper. The bucket was kept at ambient temperatures of 22º- 25º for 16days. Batch 4, one kilogram of each of the 3 popular varieties top 4 leaves and 1 kilogram of top 5-8 leaves was weighed. Selected starter culture from 4.2.2.4 above (*Lactobacillus plantarum, Lactobacillus Fermentum or Lactobacillus brevis*) at the

rate of 2mg/Kg and optimal percentage concentration of sugar concentration and salt optimal percentage concentration by weight from 4.2.2.4 above were added. The mixture was gently mixed and the bucket covered with a sheet of polythene paper. The bucket was kept at ambient temperatures of 22º- 25º for 16 days.

4.2.7 Analytical methods

4.2.7.1 Determination of Vitamin A (Beta Carotene)

Vitamin A was determined as beta-carotene using the method of Astrup et al. (1971) as modified by Imungi and Wabule (1990).

4.2.7.2 Determination of Vitamin C (Ascorbic acid)

The samples were analyzed for ascorbic acid content by indophenols methods No. 985.01 (AOAC, 1990) and titration with 2, 6 dichlorophenoliindophenol dye.

4.2.7.3 Determination of minerals: Calcium, zinc and Iron

Calcium, zinc and iron were determined according to the method of Ezeonu et al., (2002) using an Atomic Absorption Spectrophotometer 500(AAS 500) PG Instruments Limited, Alma Park Wibtoft Leicestershire, England LE175BE. The amount of elements was calculated against their standards as indicated: Absorbance (ppm)/sample weight x 100 = ppm

4.2.7.4 Determination of cyanide

The samples were analyzed for cyanide content with method number 915.03B. (AOAC, 1990).

4.2.7.5 Determination of Oxalates

The samples were analyzed for soluble and total oxalates content with titrimetric method as described in (AOAC, 1999).

4.2.7.6 Determination of tannins (total phenolic compounds)

The samples (250mg in 10ml of 70% aqueous acetone) were extracted for 2hr at 30 C using Gallenkamp orbital shaker (Survey, UK). Pigments and fats were first removed from the leaves by extracting with diethyl ether containing 1% acetic acid. Thereafter, the total polyphenols (as tannic equivalent) were determined in 0.05, 0.2 or 0.5ml aliquot using Folin Cocalteu (Sigma) and standard tannic acid (0.5mg/ml) as described by Makkar & Goodchild (1996).

4.2.8 Data analysis method

The data gathered was analysed using the analysis of variance (ANOVA) and means were separated using High Significant Difference (HSD) Test calculated at 95% confidence interval using R programming.

4.3 Results

4.3.1 Chemical profile of cassava leaves

The cassava leaves contained an average of 11.84 mg/100g of beta carotene and 21.70 mg/100g of vitamin C (Table 4.2). Additionally, the leaves contained minerals including iron, zinc and calcium with an average of 19.37mg/100g, 16.33mg/100g and 741mg/100g, respectively. The leaves contained an average of 2.44mg/100g oxalates, 0.303mg/100g tannins and 64.88mg/100g cyanide.

Table 4.2: Chemical profile of cassava leaves

Parameter	Beta carotene mg/100g	Vitamin C mg/100g	Tannins g/100g	Oxalate Mg/100g	Cyanide Mg/kg	Iron mg/100g	Zinc mg/100g	Calcium mg/100g	Moisture%

Min	3.99	10.34	0.1030	1.280	33.99	15.00	12.00	563.0	65.00
Mean	11.835	21.70	0.3034	2.441	64.88	19.37	16.33	741.8	76.39
Max	25.410	42.86	0.7810	2.880	90.12	27.00	21.00	960.0	89.00

Table 4.3: Effect of cassava variety on chemical changes during fermentation

Variety	Fermentation	Leaf position	Beta Carotene(mg/100g) DM	Vitamin C mg/100g DM	Tannins(g/100g) DM	DM Oxalates (mg/100g) DM	DM Cyanide (mg/kg) DM	Iron mg/100g DM	Zinc mg/100g DM	Calcium mg/100g DM	Moisture %
Kaleso	Raw	Bottom	7.30±0.28^{a}	16.69±1.13^{a}	.51±0.00^{abc}	2.70±0.03^{ab}	73.94±9.21^{abcd}	22.00±2.83^{a}	16.00±1.41^{abc}	789.50±0.71^{abc}	74.50±4.95^{a}
		Top	10.13±0.01^{a}	14.80±0.74^{a}	.37±0.01^{abc}	2.82±0.08^{ab}	75.66±0.61^{abcd}	22.50±6.36^{a}	17.00±1.41^{abc}	782.50±3.54^{abc}	83.50±2.12^{a}
	Spontaneous	Bottom	7.45±0.64^{a}	17.75±0.01^{a}	.21±0.00^{bc}	2.83±0.06^{ab}	41.17±10.15^{e}	19.50±0.71^{a}	16.50±2.12^{abc}	799.00±1.41^{abc}	69.00±5.66^{a}
		Top	11.15±0.23^{a}	17.46±1.80^{a}	.21±0.00^{bc}	2.80±0.01^{ab}	48.76±2.23^{de}	16.50±2.12^{a}	17.00±1.41^{abc}	789.50±0.71^{abc}	78.00±15.56^{a}
	Salt and sugar	Bottom	13.23±0.00^{a}	15.89±0.00^{a}	.19±0.00^{bc}	1.35±0.00^{d}	47.15±0.00^{de}	15.00±0.00^{a}	18.00±0.00^{abc}	630.00±0.00^{c}	71.00±0.00^{a}
		Top	15.95±0.08^{a}	16.25±2.78^{a}	.20±0.00^{bc}	1.69±0.47^{bcd}	49.95±0.87^{de}	17.00±1.41^{a}	16.50±0.71^{abc}	772.50±17.68^{bc}	75.50±9.19^{a}
	Salt-sugar-starter culture	Bottom	12.80±1.27^{a}	23.38±3.67^{a}	.31±0.23^{abc}	2.62±0.26^{ab}	72.77±4.48^{abcd}	17.50±2.65^{a}	17.75±1.26^{abc}	688.00±12.65^{c}	74.75±8.66^{a}
		Top	13.03±3.36^{a}	35.18±5.55^{a}	.31±0.23^{abc}	2.35±0.23^{abc}	66.03±5.65^{abcd}	18.00±1.83^{a}	15.00±1.41^{bc}	744.25±75.37^{bc}	76.50±8.19^{a}
KMP	Raw	Bottom	6.65±0.01^{a}	34.01±11.07^{a}	.27±0.01^{bc}	2.87±0.01^{a}	82.82±4.01^{ab}	20.50±0.71$_{a}$	12.50±0.71^{c}	952.50±10.61^{a}	75.00±5.66$_{a}$
		Top	7.64±0.04^{a}	19.72±4.88^{a}	.33±0.00^{abc}	2.25±0.09^{abcd}	86.66±4.90^{a}	20.00±1.41^{a}	14.50±0.71^{bc}	857.00±4.24^{ab}	77.50±10.61^{a}
	Spontaneous	Bottom	17.18±0.10^{a}	11.37±1.39^{a}	.22±0.00^{bc}	2.62±0.08^{ab}	49.73±0.56^{de}	18.50±0.71^{a}	15.50±0.71^{bc}	799.00±1.41^{abc}	77.00±1.41^{a}
		Top	15.43±0.62^{a}	14.54±1.05^{a}	.20±0.00^{bc}	2.16±0.25^{abcd}	47.77±3.95^{de}	16.50±0.71^{a}	18.50±0.71^{ab}	750.00±2.83^{bc}	76.50±4.95^{a}
	Salt and sugar	Bottom	18.10±0.01^{a}	14.55±1.04^{a}	.19±0.01^{bc}	1.39±0.09^{cd}	49.29±3.03^{de}	18.00±4.24^{a}	15.00±1.41^{bc}	849.50±2.12^{ab}	73.00±5.66^{a}
		Top	17.91±0.03^{a}	13.32±4.21^{a}	.19±0.00^{bc}	1.36±0.11^{d}	53.24±2.58^{cde}	21.00±2.83^{a}	14.50±2.12^{bc}	782.50±3.54^{abc}	82.00±5.66^{a}
	Salt-sugar-starter culture	Bottom	17.17±8.79^{a}	30.07±11.13^{a}	.14±0.02^{bc}	2.27±0.14^{abcd}	72.77±4.48^{abcd}	19.25±3.30^{a}	16.25±1.50^{abc}	651.00±24.25^{c}	74.25±6.99^{a}
		Top	15.66±9.65^{a}	22.07±8.79^{a}	.15±0.03^{bc}	2.48±0.16^{ab}	68.84±4.10^{abcd}	19.50±5.20^{a}	16.00±0.82^{abc}	704.50±55.62^{bc}	78.75±7.27^{a}
Tajirika	Raw	Bottom	8.06±0.01^{a}	17.99±0.32^{a}	.78±0.01^{a}	2.86±0.01^{a}	82.37±3.54^{ab}	21.50±2.12^{a}	19.50±0.71^{ab}	849.50±2.12^{ab}	71.00±2.83^{a}
		Top	4.41±0.59^{a}	14.05±5.22^{a}	.60±0.03^{ab}	2.74±0.13^{ab}	79.55±12.56^{abc}	21.50±2.12^{a}	20.50±0.71^{a}	750.00±2.83^{bc}	71.50±9.19^{a}
	Spontaneous	Bottom	10.97±3.04^{a}	26.44±10.^{37a}	.47±0.20^{abc}	2.72±0.10^{ab}	64.83±6.18^{abcd}	19.33±1.21^{a}	16.33±1.86^{abc}	711.17±26.01^{bc}	79.00±4.29^{a}
		Top	9.93±1.72^{a}	22.11±10.86^{a}	.45±0.19^{abc}	2.70±0.07^{ab}	61.21±10.54^{bcde}	20.50±4.23^{a}	17.00±1.79^{abc}	717.83±34.80^{bc}	76.17±3.66^{a}
	Salt- Sugar	Bottom	10.97±3.19^{a}	20.92±8.88^{a}	.44±0.20^{abc}	2.30±0.65^{abc}	64.81±9.73^{abcd}	19.67±3.27^{a}	15.83±1.47^{bc}	736.67±64.94^{bc}	74.67±4.23^{a}

	Top	9.96±3.02[a]	20.81±10.77[a]	.43±0.18[abc]	2.19±0.55[abcd]	64.31±11.86[abcd]	20.33±3.01[a]	15.50±1.38[bc]	689.83±63.51[c]	78.67±4.46[a]

Means± standard deviations with the same superscript letters along the column for respective chemical changes are not significantly different at the P≤0.05 level (Tukey's HSD test).

Top and bottom means top leaves and bottom leaves on the cassava plant

4.3.2 Chemical changes in cassava leaves under different treatments

The low consumption of cassava leaves in Kenya is attributed to the low production and the high anti-nutrient content of the leaves. Cassava leaves contain tannins, oxalates and cyanogenic glycosides which cause poisoning. These anti-nutrients are reduced by several treatments including fermentation, pounding and drying.

4.3.2.1 Nutritional changes

Beta carotene content of cassava leaves was highest in fermented-sundried and fermented oven-dried leaves (Table 4. 3). Fermented sundried leaves had the highest vitamin C (30.8 mg/100g). Additionally, leaves that were fermented only also had a higher content of vitamin C (20.57 mg/100g) compared to raw leaves (Table 4.3). Fermented oven-dried leaves had the lowest vitamin C content of 14.77mg/100g. There were no significant ($p>0.001$) differences of zinc content of cassava leaves in the different treatments. Raw cassava leaves had the highest calcium (830.17 mg/100g) while the lowest was in fermented sundried leaves (710.63 mg/100g). The iron content of the leaves was highest in raw cassava leaves (21.33mg/100g) and fermented only leaves (19.92 mg/100g) (Table 4.3). Iron levels were highest in fermented sundried and fermented oven-dried leaves. Treatment of the leaves had a significant effect on beta carotene, vitamin C, tannins, oxalates, cyanide and iron levels. Interactions between samples and treatments significantly influenced beta carotene, tannins, oxalates, iron and calcium. Sample and treatment interactions did not have a significant effect on cyanide, zinc and vitamin C levels of the leaves.

Table 4.1: Chemical changes in cassava leaves under different treatments

TREATMENT	Beta Carotene(mg/100g) DM	Vitamin C mg/100g DM	Tannins(g/100g)	Oxalates (mg/100g) DM	Cyanide (mg/kg) DM	Iron mg/100g DM	Zinc mg/100g DM	Calcium mg/100g DM	Moisture %
Fermented	8.93±1.61^{c}	20.57±5.86^{b}	0.34±0.22^{b}	2.58±0.22^{ab}	72.23±3.3.40^{b}	19.92±3.37^{ab}	16.33±1.55^{a}	718.75±76.74^{ab}	77.13±6.17^{a}
Fermented-Oven-dried	12.56±4.18^{b}	14.77±2.41^{c}	0.20±0.01^{d}	2.07±0.65^{c}	49.62±4.81^{d}	18.29±2.33^{b}	16.29±1.60^{a}	751.71±56.89^{c}	10.58±5.93^{d}
Raw	7.36±1.79^{d}	19.54±8.09^{bc}	0.48±0.18^{a}	2.71±0.23^{a}	80.17±6.89^{a}	21.33±2.50^{a}	16.67±2.96^{a}	830.17±69.40^{a}	78.50±6.59^{a}
Fermented-Sundried	16.32±5.42^{a}	30.82±8.38^{a}	0.28±0.20^{c}	2.55±0.20^{b}	65.16±3.64^{c}	18.92±3.13^{b}	16.21±1.61^{a}	710.63±36.41^{b}	12.92±7.13^{c}

Means± standard deviations with the same superscript letters along the column for respective chemical changes are not significantly different at the P≤0.05 level (Tukey's HSD test.

4.3.2.2 Anti-nutrient levels of cassava leaves

The tannins content were high in the raw leaves (0.48 mg/100g) and fermented only leaves (0.34 mg/100g). Drying reduced the tannins content of the cassava leaves with the fermented oven-dried and fermented sun-dried leaves recording the lowest amounts, 0.20 mg/100g and 0.28 mg/100g respectively. A combination of fermentation and drying of cassava leaves significantly ($p<0.001$) decreased the cyanide content of cassava leaves. Fermented cassava products have lower cyanide compared to unfermented, this is why the raw cassava leaves had higher cyanide compared to fermented ones. Oxalates were low in fermented oven-dried (2.07 mg/100g) and fermented sundried leaves (2.55 mg/100g).

4.4 Sensory characteristics of fermented cassava leaves

Fermented cassava leaves were assessed for appearance, color, flavor, texture and overall appearance. Most panelists liked appearance and color of the leaves. Additionally, the overall acceptance was rated high. However, panelists generally disliked the flavor and texture of fermented cassava leaves (Table 4.4). Most consumers do not like mushy texture. Color, texture and appearance of the leaves was not significantly ($p<0.05$) different among the samples. Color of cassava leaves usually darkens during fermentation compared to the color of unfermented

leaves (Barati et al., 2019a). The flavor of fermented leaves was significantly ($p<0.001$) different for all samples. Most of the panelists rated highly the overall acceptance

Table 4.4: Sensory scores for cassava leave samples

	Organoleptic characteristic				
Sample	**Appearance**	**Colour**	**Flavour**	**Texture**	**Overall acceptability**
Kaleso fresh	4.25±1.66^{a}	4.50±1.62^{a}	3.00±1.54^{ab}	3.25±1.91^{a}	3.75±1.71^{ab}
KMP fresh	3.83±1.47^{a}	3.83±1.47^{a}	3.17±1.64^{ab}	3.08±1.98^{a}	3.33±1.44^{ab}
Tajirika fresh	4.08±1.51^{a}	4.00±1.65^{a}	3.25±1.71^{ab}	3.17±1.70^{a}	3.50±1.83^{ab}
Kaleso solar dried	4.25±1.36^{a}	4.42±1.51^{a}	4.08±1.56^{ab}	3.00±1.54^{a}	3.67±1.44^{ab}
Kaleso sun dried	4.42±1.56^{a}	4.50±1.17^{a}	4.42±1.73^{ab}	3.58±1.68^{a}	4.33±1.56^{ab}
Kaleso oven- dried	4.33±1.56^{a}	4.33±1.61^{a}	4.00±1.54^{ab}	3.58±1.38^{a}	4.00±1.21^{ab}
KMP solar dried	4.50±1.45^{a}	4.17±1.53^{a}	3.50±1.62^{ab}	3.33±1.50^{a}	3.33±1.61^{ab}
KMP sun dried	4.08±1.38^{a}	4.00±1.48^{a}	3.92±1.31^{ab}	3.08±1.31^{a}	3.58±1.24^{ab}
KMP oven-dried	4.67±1.72^{a}	4.75±1.48^{a}	4.92±1.16^{a}	4.00±1.48^{a}	4.83±1.34^{a}
Tajirika solar dried	3.58±1.51^{a}	4.08±1.24^{a}	3.75±1.22^{ab}	2.92±1.68^{a}	3.33±1.37^{ab}
Tajirika sun dried	3.50±1.24^{a}	3.50±1.38^{a}	4.17±1.75^{ab}	3.50±1.68^{a}	3.50±1.62^{ab}
Tajirika oven dried	4.08±1.38^{a}	3.92±1.31^{a}	3.92±1.44^{ab}	3.75±1.66^{a}	3.50±1.31^{ab}
Kaleso fermented	3.58±2.07^{a}	3.67±1.83^{a}	3.50±1.98^{ab}	3.75±1.91^{a}	3.50±1.88^{ab}
KMP fermented	3.08±1.31^{a}	3.17±1.19^{a}	2.17±1.19^{b}	2.25±1.60^{a}	2.42±1.16^{b}
Tajirika fermented	3.50±1.24^{a}	3.58±1.08^{a}	2.75±1.29^{ab}	2.75±1.42^{a}	2.67±1.23^{ab}
P value	**0.503**	**0.450**	**0.0301***	**0.21**	**0.0042****
CV(%)	**38.43%**	**36.81%**	**46.27%**	**50.35%**	**43.49%**

Values (means± standard deviation) with different superscripts along a column are statistically different (Tukey's test)

4.4 Discussion

4.4.1 Chemical profile of cassava leaves

The cassava leaves were rich in vitamin A and C which boost the immune system and high beta carotene content which is a recognized source of vitamin A (Siqueira et al., 2007). Cassava leaves are rich in iron and highly recommended in pregnant women diet as it is associated with

increased milk production. The leaves provide minerals, proteins and vitamins (Hawashi et al., 2019). It also contains high contents of vitamins, B1, B2, C, carotenoids and minerals like phosphorous, magnesium, potassium and calcium but low contents of manganese, zinc, iron, copper and sodium (Latif and Müller, 2015). The level of potassium, magnesium, phosphorous, zinc and manganese decreases (Siqueira et al., 2007) while calcium, sodium and iron increase with the maturity of leaves (Latif and Müller, 2015). However, cassava leaves are known to contain anti-nutrients which are associated with cassava leaves poisoning. Freshly harvested cassava leaves contain cyanide levels between 137 and 1515 ppm depending on the variety (Kobawila et al., 2005). Fermented cassava leaves are found to have lower levels of cyanide of 16-23ppm (Estiasih *et al.*, 2018).

4.4.2 Chemical changes in cassava leaves under different treatments

The increase in beta carotene content in fermented sundried and oven-dried attributed to the concentration of constituents of the leaves due to water removal. Beta carotene and vitamin C content of fermented, sundried and oven-dried were significantly ($p<0.001$) from those of the raw cassava leaves. This is due to vitamin C degradation at high temperatures. A study conducted by Natukunda, Muyonga and Kaaya, (2004) indicated that oven drying had a significant effect on beta carotene content of the leaves. Vitamin C is known to be highly volatile and is lost during heating of foods (Igwemmar et al., 2013).

4.4.3 Changes in the anti-nutrient levels of cassava leaves

Fermentation of cassava leaves is a method used to lower the tannins and phytates to acceptable levels (Hawashi et al., 2019). Sun-drying is the most common method used to detoxify cassava leaves and other cassava products by reducing tannins, cyanide and phyatate content (Adamafio et al., 2010). Fermentation is known to reduce cyanide content in cassava through microbial degradation (Estiasih *et al.*, 2018). Cyanide is broken down by linamarase enzyme which is produced by the cassava lactic acid bacteria (Kobawila et al., 2005). Cyanide is removed

through thermal processes (cooking) or fermentation. Hydrocyanic acid is easily eliminated when the leaves are exposed to the sun or in endothermic processes involving the absorption of heat, due to fact that it is a highly volatile compound (Barati et al., 2019a; Imungi and Lamuka, 2007). Lactic acid fermentation can be used to detoxify cassava leaves making it safe for consumption (Barati et al., 2019b). During fermentation, the cyanide levels significantly decrease (Kobawila et al., 2005). The cyanide level was lower than 10ppm hence regarded as safe for consumption (Estiasih *et al.*, 2018). Sun- drying and oven drying reduces the cyanide content to lower levels compared to undried leaves (Udensi et al., 2005). Cassava leaves oxalates and other anti-nutrients are reduced during fermentation and drying processes (Adamafio et al., 2010). This is because during fermentation microorganisms breakdown anti-nutrients reduces their quantities and making the fermented leaves safer compared to unfermented leaves (Barati et al., 2019b; Kobawila et al., 2005).

4.4.4 Sensory characteristics of fermented cassava leaves

Cassava fermentation aids in elimination of cassava anti-nutrients which usually are the main causes of cassava bitterness. This leads to changes in the flavor of the fermented leaves (Itoua et al., 2018). Additionally, cassava fermentation is a lactic acid fermentation which involves lactic acid bacteria that produce lactic acid leading to increased acidity of the final product (Barati et al., 2019b). Increase in acidity contributes to changes in taste and flavor of the cassava leaves. Most people do not like the tartness that comes with increased acidity hence most panelists disliked the flavor (Alphonce and Kaale, 2020). Fermentation of cassava leaves alters the texture causing the leaves to soften and appear mushy due to breakdown of pectin on the cell wall (Staack et al., 2019).

4.5 Conclusion

Raw cassava leaves are highly nutritious with high content of iron, vitamin C, beta carotene and protein. Fermentation followed by drying was the most effective method in reducing toxic

cyanide and antinutrient compounds in cassava leaves. Fermentation greatly influences the sensory attributes of cassava leaves. The likes or dislikes of consumers to fermented cassava are based on the changes on sensory attributes of the leaves. The color and appearance of leaves was preferred by the panelists however, the panelists disliked the texture and flavor of the leaves. Consumer preferences affect their choices based on likes and dislikes. The sensory scores of the fermented leaves averaged at 5 points on a seven-point hedonic scale (Sanni and JaJi, 2003) stating, that they were likeable in comparison to the non-fermented samples.

CHAPTER FIVE

CHANGES IN MICROBIAL POPULATION DURING FERMENTATION OF CASSAVA LEAVES

5.0 Abstract

Consumption of fermented cassava products has a history in Africa especially in West Africa. Fermented cassava roots and flour is the most common. In Kenya, cassava root is fermented in most households; however, fermentation of cassava leaves has not largely been adopted. The objective of this study was to investigate the effect of microorganisms involved in fermentation of cassava leaves. Four top most cassava leaves at 6 months maturity were harvested and fermented for 16 days. Isolated lactic acid bacteria (LAB) starter culture was used for optimal fermentation. Changes in microbial population were assessed during fermentation. The results of this study indicate that LAB was the predominant microorganisms in cassava leaves fermentation. The LAB increased significantly ($p<0.001$) with increase in days of fermentation. Additionally, fungi and coliforms counts were significantly high ($p<0.001$) in the fermented cassava leaves. The mean logCFU/ml of yeasts and molds, LAB and coliforms were 6.96, 7.99 and 8.70 respectively. Leaf position and cassava leaves variety significantly ($p<0.001$) influenced microbial load during fermentation. Since LAB is the predominant microorganism in cassava leaves fermentation there is need for isolation of its pure cultures.

5.1 Introduction

Cassava (*Manihot escutenta, Crantz*), has been one of the crops promoted by Ministry of Agriculture, livestock, Fisheries and Irrigation through pillar two of The Big Four agenda on Food Security because it's high yielding, drought and disease resistant. Additionally, it performs better than cereals under environmental stresses of poor soil conditions and unreliable

rainfall. Cassava leaves are high in crude protein content and a well-balanced amino acid profile comparable to fresh egg although methionine, lysine and isoleucine are absent. They are also high in vitamins, minerals and fibre (Iglesias et al., 2015). Cassava roots and leaves also have anti-nutrients that bind minerals and other nutrients making them indigestible and unavailable for absorption in humans (Wobeto et al., 2006). They include cyanogenic glucosides, phytate, nitrate, polyphenols, oxalate and saponins (Siritunga and Sayre, 2003). Cyanogens occur in three forms: cyanogenic glucosides (95% linamarin and 5% lotaustratin), cyanohydrins and free cyanide. Cassava leaves have 10 times cyanide content of roots (Bradbury and Denton, 2014). Acute poisoning associated with consumption of 50 to 100 mg of cyanide are rare but long term consumption of small amounts of cyanide can cause severe health problems which include konzo (spastic paraparesis) , glucose intolerance, tropical neuropathy and goitre (Ernesto et al., 2002).

Different processing methods are used to reduce cyanogenic compound and other anti-nutrients to allowable consumption levels. They include fermentation, boiling, drying, sun drying, and oven drying, shredding and soaking. Combination of two or more of these processes improves effectiveness and also improves nutrients retention. Fermentation, for instance increases the shelf-life, safety, palatability and sensory quality of the raw product, reduces undesired and toxic compounds, and may increase the availability of proteins and vitamins. Furthermore, some Lactic Acid Bacteria strains are well-known probiotics and it has been postulated that lactic fermented foods may also have positive effects on human gastrointestinal health (Mathara and Trierweiler, 2016).

Fermentation can be spontaneous or controlled fermentation. Natural fermentation focuses on creating conditions which are most favourable for the growth of the more suitable microorganism responsible for fermentation, while at the same time, killing of all other microorganisms. On the other hand, controlled fermentation is used in cases where natural

fermentation is not viable (Heuberger, 2005). Hence, it is necessary to isolate, characterize, and preserve these specific microbial strains which can be used as starter cultures. In the optimum growth conditions, these cultures can be used either, singularly or in combination with other cultures, allowing predictability in the end product in terms of organoleptic and nutritional characteristics (Heuberger, 2005).

The major micro-organisms that have been isolated in the fermenting pulp for Gari are mainly Leuconostoc and yeasts. In fermentation of fufu, *Candida utilis* strain and *Saccaromyces Cerevisiae,* which had been isolated from a wine brewed from sorghum were used for this experiment (Sobowale and Oyewole, 2008). The total viable count increased in this case, with counts of lactic acid bacteria and fungi increasing in the last fermentation stages. Other bacteria that were associated with the fermentation process include; (*Bacillus, Staphylococcus, Klebsiella, Escherichia, Streptococcus, Lactobacillus, Leuconostoc, Corynebacterium) while fungi strains of Penicillium, Aspergillus, Fusarium, Mucor*, *Rhizopus, candida, Saccharomyces, Hansenula, Rhodotorula*) (Sobowale and Oyewole, 2008).

Fermentation is usually in the first process in the development of flavor in both cassava leaves and roots. Cassava leaves and roots, especially the traditional and indigenous varieties usually have a bitter taste to it which sometimes lead to undesirable quality in the end product (Kehinde, 2013). In a study to determine the effect of fermentation on cassava leaves, lactic fermentation was carried out. During the process, there is an increase in progressive acidification of the leaves, and a reduction in total reducing sugars in both varieties. Also, there are significant changes in the microbial population during cassava leaves fermentation. This study aimed at assessing changes in microbial population during the fermentation process.

5.2 Materials and Methods

5.2.1 Procurement of cassava leaves

Samples of fresh cassava leaves were purchased from Kilifi and Taita Taveta Counties and transported by road to the Department of Food Science Nutrition and Technology of the University of Nairobi laboratories for microbial profile analysis. Cassava leaves at 6 months of maturity were preferred due to their optimal content of fermentable sugars (Nekesa, 2016). 1 kilogram of 4 top most leaves and 1 kilogram of top 5-8 leaves from each variety were harvested separately. The leaves were transported to DFSNT laboratory in cooler boxes.

5.2.2 Experimental design

The experiment was arranged in a completely randomized design with **4 main treatments**. For each treatment **2 replications** were done. A preliminary experiment to determine the optimal percentage concentration of salt and sugar by weight and isolation of starter culture was performed. The optimal percentage concentration of salt, sugar and isolated starter culture was used as part of the treatment in the experiment. First treatment optimal percentage concentration of salt and sugar percentage by weight was added and the cassava leaves fermented; second treatment isolated starter culture was added and the cassava leaves fermented ; third treatment both optimal salt and sugar percentage concentration by weight and starter culture were added and the cassava leaves fermented and fourth treatment was the control. Spontaneous fermentation of cassava leaves was done for all batches.

The leaves were divided into 4 batches per treatment. Each batch contained 6 kilograms in total; 1 kilogram of top four leaves for each variety and 1 kilogram of top five to eight leaves for each variety. The experiment was repeated twice. Analytical evaluation was done to determine the microbial content (lactic acid bacteria, coliforms, mould and yeast) population during fermentation of cassava leaves. Cassava leaves were harvested from three most popular

varieties in Kilifi and Taita Taveta counties. The sample size with 4 main treatments (spontaneous fermentation, fermentation with optimal salt percentage concentration and optimal sugar percentage concentration , fermentation with starter culture, fermentation with sugar and starter culture) x 3 varieties each x 2 top leaves groups x 2 replicates which totaled to 48 samples. The experiment was conducted as indicated in Figure 5.1.

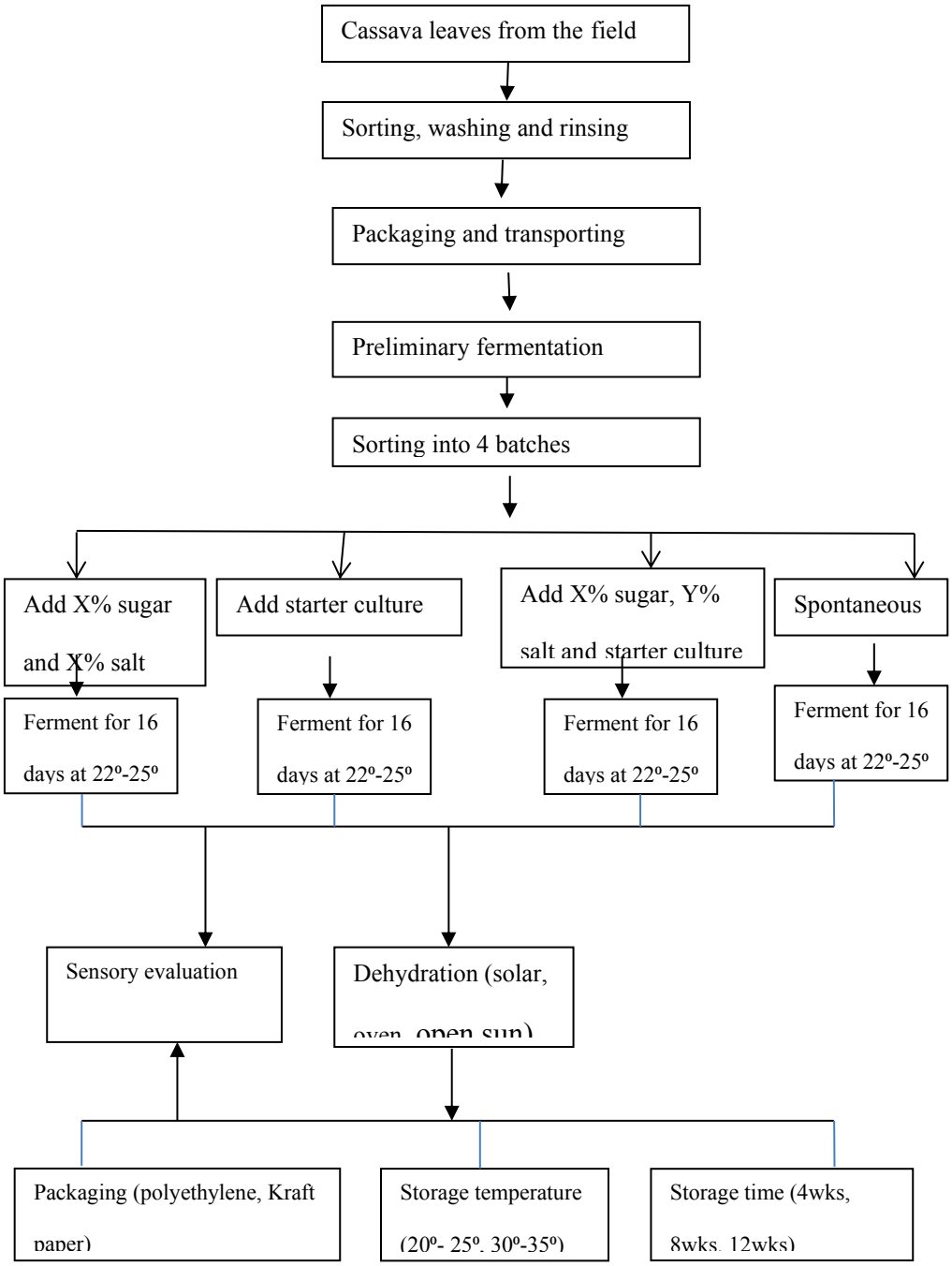

Figure 5.1: Fermented cassava leaves production diagram

5.2.3 Preparation of cassava leaves for fermentation

The fresh leaves were sorted, de-stemmed, washed in clean water and divided in 4 batches

5.2.4 Optimization of cassava leaves fermentation conditions

5.2.4.1 Determination of optimal concentration of added sugar and salt

The sorted cassava leaves were divided into equal 4 portions and fermented in lots of 250g, each lot was mixed thoroughly with 0,1, 2, 3 % concentration respectively of table salt (Kensalt, Kenya) followed by tight packing in 2-litre plastic beakers. They were allowed to stand for 20 minutes after which a polythene bag full of water was placed inside each container as a weight to press down the leaves with sugar and ensure that the experiment was air tight during fermentation. Fermentation was carried out at ambient temperatures (22º-26º C). During fermentation, samples of the fermenting liquor were withdrawn at regular intervals of 1, 4, 8 and 16 days for PH and total titratable acidity (TTA) determination. Fermentation lasted 16 days. The preliminary experiment was replicated twice. The sugar concentration that gave the highest total titratable acidity (TTA) and lowest PH was used for the other treatments in the experiment.

5.2.4.2 Determination of starter culture levels

The sorted cassava leaves were divided into 3 equal portions and fermented in lots of 250g as in the first preliminary above. Each portion contained the different cassava leaves varieties. Isolation and enumeration of most dominant Lactobacillus species (*Lactobacillus plantarum, Lactobacillus Fermentum or Lactobacillus brevis*) was done by plate count on Lactobacilli de Man, Rogosa, and Sharpe (MRS) agar. Colonies were counted as viable numbers of microorganisms (cfu/g) per gram. The dominant microorganisms were cultured and isolated and were used as starter culture for the other fermentation treatments. The amount of starter

culture determined was varied to determine the percentage of starter culture that gives highest total titratable acidity (TTA) and lowest PH. This level was used for the other treatments in the experiment. Different treatments as indicated in Table 5.1.

Table 5.1: Treatment of cassava leaves for fermentation

	Treatments	T1	T 2	T 3	T 4
1	Sugar (% concentration)	0	1	2	3
2	Salt (% concentration)	0	1	2	3
3	Sugar + salt (% concentration)	0 + 3	1+ 2	2 + 1	3+ 0
4	Starter culture (% concentration)g/l	0.05	0.1	0.15	0.2

5.2.6 Fermentation of cassava leaves

The cleaned leaves were cut into 5 millimetres thickness and weighed. 1 kilogram of each of the 3 popular varieties top 4 leaves and 1 kilogram of top 5-8 leaves was weighed. Batch 1 was emptied into an air tight bucket and the bucket and covered with a sheet of polyethylene paper (fermentation vessel). The bucket was kept at ambient temperatures of 22º- 25º for 16 days (spontaneous fermentation). For Batch 2, one kilogram of each of the 3 popular varieties top 4 leaves and 1 kilogram of top 5-8 leaves was weighed. The percentage concentration of sugar and the percentage concentration of salt by weight determined to give the lowest PH and highest total titratable acidity (TTA) in 3.2.2.2 was added to the mixture. The mixture was gently mixed and the bucket covered with a sheet of polyethylene paper. The bucket was kept at ambient temperatures of 22º- 25º for 16 days. Batch 3, one kilogram of each of the 3 popular varieties top 4 leaves and 1 kilogram of top 5-8 leaves was weighed. Selected starter culture from 3.2.2.4 above (*Lactobacillus plantarum, Lactobacillus Fermentum or Lactobacillus brevis*) at the rate of 2mg/Kg was added. The mixture was gently mixed and the bucket covered with a sheet of polyethylene paper. The bucket was kept at ambient temperatures of 22º- 25º for 16days. Batch 4, one kilogram of each of the 3 popular varieties top 4 leaves and 1 kilogram

of top 5-8 leaves was weighed. Selected starter culture from 3.2.2.4 above (*Lactobacillus plantarum, Lactobacillus Fermentum or Lactobacillus brevis*) at the rate of 2mg/Kg and optimal percentage concentration of sugar concentration and salt optimal percentage concentration by weight from 3.2.2.4 above were added. The mixture was gently mixed and the bucket covered with a sheet of polythene paper. The bucket was kept at ambient temperatures of 22º- 25º for 16 days.

5.2.7 Microbiological analysis

Samples of cassava leaves (10 g) were blended with 90 millimetres of sterilized water, and serially diluted in sterilized water.

5.2.7.1 Lactobacilli

The number of LAB enumerated by plate count on Lactobacilli de Man, Rogosa, and Sharpe (MRS) agar. Colonies were counted as viable numbers of microorganisms (cfu/g) per gram as per the enumeration of mesophilic lactic acid bacteria according to ISO 15214:1998.

5.2.7.2 Coliforms

Coliform bacteria were detected and enumerated according to ISO 4831: 2006 Horizontal methods for the detection and enumeration of coliforms –MPN technique. Colonies were counted as viable numbers of microorganisms (cfu/g) per gram.

5.2.7.3 Moulds and Yeast

Moulds and yeast were detected and enumerated viable numbers of microorganisms (cfu/g) per gram according to ISO 21527:2008 for enumeration of yeasts and moulds in products.

5.2.8 Data analysis

Descriptive statistics i.e. means, standard deviation was used to analyze the mean microbial counts of the lactobacilli, coliforms, moulds and yeasts. T-test and analysis of variance was

done using R package for statistical computing, Agricolae package (R Core Team, 2019) and SPSS version 25 to analyse for significant differences between the samples. The mean number of colonies was expressed as colony forming units (CFU/ml) and converted to log CFU for analysis.

5.3 Results

The microbial profile of fermented cassava leaves differed significantly ($P<0.05$) among the specific groups of targeted microorganisms (Table 5.2). Spontaneous fermentation recorded the lowest microbial counts as compared to leaves fermented with starter culture only, combined salt and sugar and starter culture (Table 5.3). Fermentation using starter cultures only and fermentation with a combination of salt, sugar and starter culture were the best because the fermentation optimized at day seven.

Table 5.2: Summary statistics of microbial profile of cassava leaves

	Log Coliforms	**Log Yeasts/ Molds**	**Log Lactic Acid Bacteria**
Minimum	6.431	2.322	2.724
Mean	8.695	6.956	7.999
Maximum	10.991	10.785	10.964

Table 5.3: Microbial profile of spontaneously fermented cassava leaves

Fermentation treatment	Variety	Day	Leaf Position	logCFU/ml COLIFORMS	logCFU/ml LAB	LogCFU/ml Yeasts/moulds
Spontaneous	Kaleso	0	Bottom	6.77±0.01^{tuv}	3.52±0.02^{z}	3.79±0.01^{wx}
			Top	7.24±0.02^{rs}	4.46±0.02^{u}	3.50±0.02^{z}
		4	Bottom	6.82±0.01^{tuv}	3.81±0.00^{x}	4.35±0.01^{rs}
			Top	7.54±0.00^{pq}	4.46±0.02^{u}	4.67±0.01^{op}
		8	Bottom	9.16±0.02^{rstu}	8.26±0.00^{f}	5.20±0.00^{lm}
			Top	8.19±0.02^{jk}	8.24±0.02^{f}	5.34±0.00^{kl}
		12	Bottom	9.41±0.02^{opq}	9.93±0.04^{ghi}	6.32±0.00^{c}
			Top	9.93±0.01^{hijk}	10.29±0.02^{c}	5.74±0.01^{gh}
		16	Bottom	8.47±0.01^{hi}	10.02±0.03^{fg}	6.74±0.06^{z}
			Top	9.41±0.02^{opq}	10.87±0.04^{a}	6.76±0.01^{z}
	KMP	0	Bottom	6.79±0.00^{tuv}	2.73±0.01^{z}	4.54±0.01^{pq}
			Top	7.40±0.02^{qr}	3.51±0.01^{z}	3.94±0.01^{uv}
		4	Bottom	8.56±0.01^{fghi}	2.98±0.00^{z}	5.93±0.01^{ef}
			Top	8.71±0.01^{cdef}	4.70±0.01^{s}	5.75±0.01^{gh}
		8	Bottom	9.65±0.01^{lmn}	8.60±0.01^{cd}	6.04±0.00^{de}
			Top	10.06±0.03^{ghi}	9.39±0.12^{qr}	4.19±0.02^{t}
		12	Bottom	9.78±0.01^{jkl}	10.95±0.00^{gh}	7.65±0.07^{v}
			Top	10.66±0.01^{cd}	9.55±0.01^{mno}	7.39±0.12^{w}
		16	Bottom	9.82±0.00^{hijk}	10.35±0.01^{c}	6.97±0.00^{xy}
			Top	9.91±0.00^{hijk}	9.60±0.00^{lmn}	6.92±0.00^{y}
	Tajirika	0	Bottom	6.67±0.01^{uvw}	3.89±0.00^{x}	2.34±0.03^{z}
			Top	7.18±0.04^{s}	3.67±0.01^{y}	3.88±0.01^{vw}
		4	Bottom	6.74±0.01^{tuv}	4.31±0.01^{v}	3.99±0.00^{uv}
			Top	7.86±0.01^{lm}	4.57±0.02^{t}	3.98±0.00^{uv}
		8	Bottom	8.83±0.01^{zabc}	6.04±0.00^{o}	4.52±0.01^{q}
			Top	8.41±0.04^{hi}	6.38±0.00^{n}	5.77±0.01^{gh}
		12	Bottom	10.59±0.01^{cde}	9.47±0.01^{opq}	5.57±0.02^{ij}
			Top	10.78±0.01^{bc}	9.25±0.03st	6.64±0.01^{za}
		16	Bottom	8.48±0.00ghi	10.50±0.01^{b}	5.29±0.02^{lm}
			Top	9.50±0.01^{nop}	10.48±0.00^{b}	4.49±0.02^{qr}

5.3.1 Coliforms

The highest coliform load was in samples that had a combination of salt and sugar and salt, sugar and starter culture (Table 5.4, 5.5). Variety had a significant ($p<0.001$) effect on coliforms. KMP variety of cassava leaves had more coliforms compared to Kaleso and Tajirika varieties. Leaf position significantly ($p<0.001$) affected the number of coliforms loads during fermentation of cassava leaves. Fermented top leaves recorded higher numbers of coliforms compared to bottom leaves. Additionally, the day of fermentation had a significant($p<0.05$) effect on the CFU with an increase in number of fermentation days leading to a substantial increase in number of coliforms with the highest numbers recorded on the 12^{th} and 15^{th} day of fermentation (Table 5.2, 5.3, 5.4, 5.5).

Table 5.4: Microbial profile of cassava leaves fermented using starter culture

Fermentation treatment	Variety	Day	Leaf Position	logCFU/ml coliforms	logCFU/ml LAB	LogCFU/ml Yeasts/moulds
Starter culture	**Kaleso**	0	Bottom	8.87±0.04^{wxyzabc}	4.37±0.01^{uv}	6.67±0.01^{za}
			Top	9.04±0.06^{stuvwxy}	4.45±0.03^{u}	6.06±0.03^{de}
		4	Bottom	9.23±0.04^{qrs}	9.60±0.01^{lmn}	9.85±0.00^{de}
			Top	9.83±0.01^{jkl}	9.93±0.00^{ghi}	10.62±0.01^{b}
		8	Bottom	9.06±0.03^{stuvwx}	9.13±0.02^{uv}	9.61±0.01^{ghi}
			Top	9.10±0.02^{stuv}	8.59±0.02^{d}	9.94±0.01^{d}
		12	Bottom	8.86±0.01^{xyzabc}	8.99±0.00^{wx}	9.56±0.01^{hij}
			Top	8.84±0.00^{yzabc}	9.50±0.02^{nop}	9.33±0.04^{kl}
		16	Bottom	8.45±0.02^{hi}	8.94±0.01^{wxyz}	8.97±0.01^{mn}
			Top	8.48±0.01^{ghi}	8.96±0.01^{wxy}	8.96±0.00^{mn}
	KMP	0	Bottom	7.78±0.01^{mno}	6.80±0.00^{l}	6.96±0.01^{xy}
			Top	8.00±0.00^{kl}	7.49±0.02^{i}	5.83±0.01^{fg}
		4	Bottom	10.08±0.05^{gh}	9.89±0.01^{hi}	10.78±0.01^{a}
			Top	10.76±0.01^{bc}	9.68±0.03^{kl}	10.35±0.01^{c}
		8	Bottom	8.15±0.21^{k}	8.60±0.01^{cd}	8.60±0.00^{qr}
			Top	7.67±0.01^{mnop}	9.47±0.01^{opq}	8.69±0.12^{pqr}
		12	Bottom	7.64±0.01^{nop}	8.40±0.11^{e}	8.58±0.02^{qr}
			Top	7.52±0.04^{pq}	8.96±0.01^{wxy}	8.72±0.03^{pq}
		16	Bottom	6.50±0.02^{wx}	7.98±0.00^{g}	7.96±0.00^{tu}
			Top	6.72±0.03^{tuv}	8.92±0.00^{xyz}	7.98±0.01^{tu}
	Tajirika	0	Bottom	8.74±0.06^{bcdef}	7.89±0.02^{g}	4.77±0.01^{o}
			Top	8.15±0.21^{k}	7.42±0.01^{i}	4.54±0.09^{pq}
		4	Bottom	9.73±0.01^{klm}	9.95±0.01^{gh}	9.08±0.05^{m}
			Top	9.77±0.01^{jkl}	9.59±0.02^{lmn}	10.60±0.01^{b}
		8	Bottom	9.33±0.01^{pqr}	9.47±0.01^{opq}	8.91±0.01^{no}
			Top	9.53±0.02^{mnop}	9.34±0.03^{rs}	9.52±0.01^{hij}
		12	Bottom	8.98±0.01^{uvwxyza}	8.93±0.00^{wxyz}	8.62±0.01^{qr}
			Top	9.06±0.03^{stuvwx}	8.89±0.00^{xyz}	8.35±0.07^{s}
		16	Bottom	8.61±0.01^{defgh}	8.91±0.01^{xyz}	7.98±0.00^{tu}
			Top	8.98±0.00^{uvwxyza}	8.87±0.01^{yz}	7.93±0.01^{u}

Table 5.5: Microbial profile of cassava leaves fermented with salt and sugar

Fermentation treatment	Variety	Day	Leaf Position	Log CFU/ml coliforms	Log CFU/ml LAB	Log CFU/ml Yeasts/moulds
Salt and sugar	Kaleso	0	Bottom	6.88±0.01^{tu}	3.69±0.01^{y}	3.67±0.01^{xy}
			Top	7.49±0.02^{pq}	4.08±0.05^{w}	2.91±0.00^{l}
		4	Bottom	6.74±0.01^{tuv}	3.89±0.01^{x}	4.34±0.00^{s}
			Top	7.18±0.04^{s}	4.45±0.03^{u}	3.96±0.01^{uv}
		8	Bottom	9.13±0.02^{rstuv}	7.78±0.01^{h}	4.93±0.04^{n}
			Top	9.41±0.02^{opq}	7.08±0.00^{k}	5.04±0.06^{n}
		12	Bottom	10.54±0.02^{de}	10.27±0.02^{cd}	5.43±0.02^{jk}
			Top	10.25±0.03^{fg}	9.46±0.02^{opq}	6.42±0.01^{bc}
		16	Bottom	8.39±0.12^{ij}	8.54±0.09^{d}	7.39±0.12^{w}
			Top	9.94±0.01^{hij}	9.04±0.06^{vw}	7.65±0.07^{v}
	KMP	0	Bottom	6.91±0.01^{t}	4.08±0.00^{w}	4.04±0.00^{u}
			Top	7.61±0.01^{nop}	4.36±0.00^{uv}	4.48±0.00^{qrs}
		4	Bottom	7.86±0.01^{lm}	4.89±0.01^{r}	6.15±0.00^{d}
			Top	7.69±0.01^{mnop}	5.62±0.01	4.34±0.03^{s}
		8	Bottom	9.89±0.00^{hijk}	6.58±0.02^{p}	5.66±0.01^{hi}
			Top	9.86±0.00^{ijk}	6.41±0.02^{n}	4.78±0.00^{o}
		12	Bottom	10.99±0.01^{a}	9.55±0.01^{mno}	8.10±0.02^{t}
			Top	10.93±0.01^{ab}	9.65±0.01^{lm}	7.87±0.04^{u}
		16	Bottom	8.59±0.01^{fghi}	10.48±0.01^{b}	7.96±0.01^{tu}
			Top	8.55±0.01	9.27±0.02st	7.86±0.02^{u}
	Tajirika	0	Bottom	6.85±0.01	5.33±0.01^{q}	2.82±0.01^{l}
			Top	7.58±0.02	4.83±0.01^{r}	3.57±0.02^{wx}
		4	Bottom	7.65±0.03	5.33±0.01^{q}	3.88±0.01^{vw}
			Top	8.68±0.01^{cdefg}	5.26±0.00^{q}	5.20±0.00^{lm}
		8	Bottom	8.74±0.01^{bcdef}	7.23±0.00^{j}	5.00±0.00^{n}
			Top	9.08±0.00^{stuvw}	7.06±0.03^{k}	5.56±0.02^{ij}
		12	Bottom	9.20±0.08^{rst}	9.32±0.03^{rs}	7.10±0.02^{x}
			Top	10.40±0.02^{ef}	9.42±0.01^{pqr}	7.99±0.01^{tu}
		16	Bottom	7.54±0.09^{pq}	10.96±0.00^{a}	7.37±0.01^{w}
			Top	8.54±0.09^{fghi}	9.46±0.02^{opq}	6.54±0.02^{ij}

Table 2.6: Microbial profile of cassava leaves fermented with addition of salt, sugar and starter culture

Fermentation treatment	Variety	Day	Leaf Position	log CFU/ml coliforms	log CFU/ml LAB	Log CFU/ml Yeasts/moulds
Salt, sugar and starter culture	Kaleso	0	Bottom	8.72±0.01^{bcdef}	4.79±0.00^{rs}	5.19±0.02^{m}
			Top	8.81±0.05^{zabcd}	4.34±0.03^{v}	5.54±0.09^{ij}
		4	Bottom	9.93±0.04^{hijk}	9.84±0.01^{ij}	8.82±0.00^{op}
			Top	10.54±0.02^{de}	10.87±0.00^{a}	9.90±0.00^{d}
		8	Bottom	8.54±0.09^{fghi}	10.18±0.04^{de}	9.31±0.01^{i}
			Top	8.57±0.02^{fghi}	10.08±0.00^{ef}	9.89±0.00^{d}
		12	Bottom	8.56±0.01^{fghi}	10.02±0.03^{fg}	8.99±0.00^{mn}
			Top	8.49±0.02^{ghi}	9.95±0.00^{gh}	8.97±0.01^{mn}
		16	Bottom	8.15±0.04^{k}	9.98±0.00^{fgh}	8.91±0.01^{no}
			Top	8.06±0.03^{kl}	9.92±0.01^{ghi}	8.91±0.01^{no}
	KMP	0	Bottom	8.15±0.21^{k}	7.89±0.00^{g}	5.81±0.01^{fg}
			Top	8.93±0.04^{vwxyzab}	6.62±0.01^{m}	5.72±0.01^{gh}
		4	Bottom	10.77±0.01^{bc}	9.68±0.03^{kl}	10.24±0.02^{c}
			Top	9.20±0.04^{rst}	9.90±0.00^{hi}	10.37±0.01^{c}
		8	Bottom	9.19±0.02^{rst}	10.95±0.01^{a}	9.75±0.01^{efg}
			Top	8.39±0.12^{ij}	8.84±0.00^{za}	9.47±0.01^{ijk}
		12	Bottom	8.73±0.01^{bcdef}	10.94±0.01^{a}	9.65±0.01^{fgh}
			Top	7.79±0.00^{mn}	8.84±0.00^{za}	9.45±0.02^{jkl}
		16	Bottom	6.45±0.02^{x}	10.91±0.00^{a}	8.98±0.00^{mn}
			Top	6.62±0.01^{vwx}	8.73±0.01^{AB}	8.93±0.01^{no}
	Tajirika	0	Bottom	9.00±0.00^{tuvwxyz}	8.70±0.01^{BC}	3.77±0.01^{wx}
			Top	8.87±0.04^{wxyzabc}	7.02±0.03^{K}	4.54±0.09^{pq}
		4	Bottom	9.20±0.04^{rst}	9.77±0.02^{jk}	9.57±0.01^{hij}
			Top	9.82±0.01^{jkl}	9.95±0.01^{gh}	10.25±0.03^{c}
		8	Bottom	9.08±0.05^{stuvw}	9.69±0.01^{kl}	9.59±0.01^{hij}
			Top	9.55±0.06^{mno}	9.18±0.04^{tu}	9.74±0.01^{ef}
		12	Bottom	8.95±0.00^{vwxyza}	9.33±0.04^{rs}	8.62±0.01^{qr}
			Top	8.97±0.01^{uvwxyza}	8.96±0.01^{wxy}	9.50±0.03^{ij}
		16	Bottom	8.70±0.01^{cdef}	8.98±0.00^{wx}	7.91±0.01^{u}
			Top	8.79±0.00^{abcde}	8.92±0.01^{xyz}	7.94±0.01^{u}

5.3.2 Yeast and Moulds

Yeasts and moulds were the least in numbers compared to coliforms and lactic acid bacteria. However, yeasts and moulds were highest in cassava leave samples that were fermented using starter cultures only, salt and sugar only and salt, sugar and starter culture. Cassava variety significantly ($p<0.001$) affected the yeasts and moulds loads during fermentation. KMP and Kaleso cassava varieties had more yeast and moulds loads compared to Tajirika variety (Table 5.4, 5.5, 5.6). Leaf position significantly ($p<0.001$) caused changes in the number of yeasts and moulds during fermentation. Most of the yeasts and moulds were found among the top leaves as compared to the bottom leaves because most microorganisms are found in soil and the top most leaves are the most exposed to dust and water splashes. Additionally, where irrigation is done using contaminated water, the top leaves will always have the highest microbial load. Additionally, the day of fermentation significantly ($p<0.001$) affected yeasts and moulds whereby the number of yeasts and moulds decreased with increase in fermentation days with the initial fermentation days recording more CFUs compared to day 12 and 15 (Table 5.4, 5.5, 5.6). Yeasts and moulds decreased due to the increase in lactic acid during the fermentation which inhibited their multiplication.

5.3.4 Lactic Acid Bacteria (LAB)

Lactic acid bacteria were the highest in all samples. Cassava variety had a significant ($p<0.001$) effect on LAB with KMP recording the highest CFUs compared to Kaleso and Tajirika varieties. Leaf position also significantly ($p<0.001$) caused changes in the number of LAB with bottom leaves having the highest number of CFUs compared to top leaves. Moreover, the day of fermentation significantly ($p<0.001$) influenced changes in LAB loads during fermentation with the last days (12 and 15) recording more LAB compared to the initial days of fermentation (Table 5.3, 5.4, 5.5, 5.6). This is because microbial multiplication increases with increases with increase in days of fermentation (Tefera *et al.*, 2014). Fermentation extends the shelf life of

cassava leaves by producing lactic acid that preserves by inhibiting the growth of microorganisms.

5.4 Discussion

Addition of starter culture fastened the fermentation by increasing the rate of sugar conversion to lactic acid thus shortening the fermentation period (Tefera, Ameha and Biruhtesfa, 2014). This culture can used in industrial fermentation of cassava leaves to reduce the time required to ferment the leaves. Cassava fermentation has been reported to be dominated by lactic acid bacteria especially the Bacillus spp and some fungi species (Perera et al., 2018).

5.4.1 Changes in microbial profile of fermented cassava leaves

Presence of coliforms in fermented cassava leaves is an indicator of contamination of the leaves with feacal matter (Olopade et al., 2014). The load increased with increase in days of fermentation due to multiplication of the cells of the lactic acid bacteria as the dominant microorganisms in lactic acid fermentations (Tefera, Ameha and Biruhtesfa, 2014). Lactic acid bacteria can tolerate acidic environments and multiplies during fermentation. Increased growth of coliforms in the fermented cassava leaves is undesirable and could be attributed to water contamination or poor hygiene (Inyang and Akpapunam, 2009).

Yeasts and moulds were the least in numbers compared to coliforms and lactic acid bacteria. This is due to the fact that most yeasts and moulds are aerobic in nature and the fermentation was anaerobic hence reducing their multiplication rate (Estiasih *et al.*, 2018). Yeasts and moulds have been found in fermented cassava products and are associated with flavor development (Sobowale and Oyewole, 2008). Yeasts have been found to reduce cyanide content in cassava roots and leaves hence beneficial (Tefera, Ameha and Biruhtesfa, 2014). Sugar is a suitable substrate for yeasts and moulds growth hence the high numbers when the substrate is adequate (Estiasih *et al.*, 2018). However, increased growth of fungi in fermented

cassava leaves is because fungi can survive in acidic conditions hence the increase in numbers. However, presence of fungi in fermented products can be due to poor hygiene and handling practices or contamination (Inyang and Akpapunam, 2009). Additionally, yeasts and moulds are considered contaminants in fermented cassava products and affect the safety and quality of the product (Olopade et al., 2014).

Lactic acid bacteria were the highest in all samples. This is because the cassava leaves fermentation is a lactic acid fermentation which involves LAB (Estiasih *et al.*, 2018). Addition of starter culture had a significant ($p<0.001$) effect on cassava leaves fermentation. These findings are similar to results of Tefera *et al.* (2014) which indicated that *L. plantarum* which belongs to the lactic acid bacteria is the main fermenter of cassava leaves and reduces the cyanide content in the leaves. The number of LAB increased with fermentation due to presence of fermentable sugars increasing acidity of the media which in turn inhibits the growth of other unwanted microorganisms. This affords the preservation of the leaves (Kobawila et al., 2005).

5.5 Conclusion

Fermentation of cassava leaves has a great impact on the shelf life of the leaves through increase in acidity which inhibits the growth of spoilage microorganisms. Fermentation can be used for cassava leaves value addition during times of glut to extend shelf life of the leaves for use during periods of scarcity.

CHAPTER SIX

GENERAL CONCLUSIONS AND RECOMMENDATIONS

6.1 General Conclusions

There was lack of proper harvesting and postharvest handling practices of cassava leaves in Kilifi and Taita Taveta counties and this is attributed to the low consumption levels due to presence of anti-nutrients in the leaves. Fermentation of cassava leaves contributed to significant changes on beta carotene, ascorbic acid, iron, zinc, calcium, cyanide, oxalates, tannins and microbial population. Fermentation influenced the sensory characteristics of fermented cassava leaves making them generally acceptable by sensory panellists.

6.2 Recommendations

Providing the farmers with effective storage facilities and training on cassava leaves value addition would provide an avenue for its improved utilization and appropriate postharvest handling. Improved value chain for cassava from leaves to the roots will increase production, consumption and marketability of cassava in the region. Optimization cassava leaves fermentation as an effective method for cassava leaves fermentation to extend its shelf life. Additionally, production of cassava leaves fermentation starter cultures to optimize the process and reduce the risk of growth of unwanted microbes during the fermentation process.

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APPENDICES

APPENDIX 1: QUESTIONNAIRE 1

Project Title: RU/2018/CARP+/04:

Capacity building for micro propagation and certification of cassava planting materials to enhance productivity, incomes and food nutrition security for small holder farmers in Coastal Kenya

Introduction:

The goal of the project is to increase cassava productivity and reduce the effect of major cassava diseases caused by viruses and bacteria. The current practice is that farmers acquire planting materials from each other or KALRO centres and in the process this has been a very effective method of distributing infected or diseased planting materials. In addition, many cassava producing countries in Africa including Kenya have no protocol to produce and certify healthy cassava planting materials. Thus, the integration of greenhouse technology as a protected environment will allow KEPHIS to certify cassava planting materials emanating from these greenhouses to ensure that the multiplication and distribution of these materials are disease free.

QUESTIONNAIRE 2: MODES OF UTILISATION AND METHODS OF PREPARATION OF CASSAVA LEAVES IN KILIFI COUNTY AND TAITA TAVETA COUNTY.

INTRODUCTION AND VERBAL CONSENT TAKING

My name is SAMUEL MWATHI undertaking this research on behalf of the University of Nairobi and RUFORUM on Capacity building for micro propagation and certification of cassava planting materials to enhance productivity, income and food and nutrition security for

small holder farmers in Coastal Kenya. I would like to invite you on behalf of the University of Nairobi to take part in the study that is aimed and increasing productivity of cassava in this region. I am requesting you to help us learn more about cassava leaves modes of utilisation and methods of preparation. All that you will say will be confidential for purposes of this study and participation is voluntary. If you agree, I will ask you some questions

Yes ( ) No ( )

QUESTIONNAIRE NO: **INTERVIEW DATE:**

ENUMERATOR'S NAME:

A.DEMOGRAPHIC INFORMATION

1	County	
	Sub-County	
	Ward	
	Location	
	Village	
	GPS coordinates	Longitude (E)……. Latitude (S)…… Altitude ……..
2	Name of farmer:	
	Sex:	Male=1 Female=2
	Age in Years:	
		1= Youth (< 35 years) 2= Middle aged (36-50 years)
		3= Upper middle aged (51-60 years)
		4= Retiree > 60
3	Head of household (sex)	1= Male 2= Female
	Household size (Number of members)	
	Own farm size in acres	
		1= Less than 2 acres

		2= 2-5 acres
		3= 6-15 acres
		4= >15 acres
	Rented farm size(if any) in acres	
4	Respondent main Occupation	1=Salaried employee 2=Farmer 3=Self-employment/business 4=Casual labourer 5=Student 6=Housewife 7=Unemployed 8=Others (specify)
	Do you participate in other off-farm activities	1= Yes 2= No If 1 specify ……..
	Estimated annual income in Ksh	
5	Academic qualification Years of schooling	
	Level of education attained	1= None 2= primary 3= secondary 4= Tertiary
6	Marital status	1=Married 2=Separated 3=Widowed 4=Single 5=Divorced
7	Religion	1=Christian 2=Muslim 3=Traditionist 4=Others(specify)

B. CASSAVA HARVESTING AND HANDLING

No.	Questions	Responses
1	Do you grow cassava on your farm?	1=Yes 2= No
2	Why do you grow cassava.(Select more than 1)	1= Food 2= Income 3= Soil conservation = Others(specify)
2	What varieties have you grown for the last 2 years? (Select more than 1)	1= Tajirika 2= shibe 3= Kibanda Meno

		4= Nzalauka 5= Karibuni 6= Karembo 7=Girikacha 8= others(specify)
3	Rank the best 3 varieties(select 1 to 3)	
4	Why do you prefer these variety lists in order of importance? (Select more than 1)	1= High yield 2=Drought resistant 3= Disease and pest resistant 4= Low cyanide level 5= Taste 6= Others (specify)
4	Do you consume cassava leaves	1= Yes 2= No
5	Why do you consume cassava leaves? (Select more than 1)	1= Availability 2= Affordability 3= Nutrition value 4=Others(specify)
6a	Method of harvesting cassava leaves. (Select more than 1)	1= Piecemeal 2 = few leaves 3= all leaves(whole plant)
6b	If 6a is few leaves, what leaves are picked	1= top 2 2= top 3-4 3=top 5 and above
6c	What is the reason for picking the preferred number of top leaves? (Select more than 1)	1= nutritional value 2= anti nutrient content 3= Others(specify)
6d	Tools used in harvesting. (Select more than 1)	1= uprooting 2= jembe 3= panga 4= Hands picking 5= others(specify)
6e	Time of harvesting	1= 5am to 9 am 2= 9-12 3= 12-4 4 = after 4
6f	Reason for harvesting at this time.	1= market requirement 2= labour availability 3= temperature 4= other (specify)
7a	Do you sometime harvest your cassava before it matures very well	1= Yes 2= No
B	If yes why? (Select more than 1)	1= Money 2= Food 3= others(specify)
8a	Do you sort and grade cassava leaves after harvesting?	1= Yes 2= No

	If yes, what are the criteria for sorting or grading?	1= size 2= colour 3=shape 4= Damage 5 = others (specify)
C	Reason for grading/sorting	1= specific market 2= price consideration 3= storage 4= others (specify)
9a	What are the types of damages to leaves during harvesting?	1= mechanical damage 2= pest damage 3= Rot 4= others (specify)
9b	What do you do with the damaged leaves? (Select more than 1)	1= immediate boiling 2=immediate processing 3= livestock feed 4= others (specify)
10	Do you preserve cassava leaves	1= Yes 2= No
	If yes how do you preserve fresh leaves after harvesting	1= Fermentation 2= solar drying 3= others (specify)
	Where did you learn about the preservation method	1= farmers 2= KALRO 3= media 4= extension officer 5= others (specify)
	What is the length of preservation time(days)	1= 1-2 days 2= 3-5 days 3= 5-7 days 4= others (specify)

C. CASSAVA LEAVES PREPARATION INFORMATION

1	Do you process cassava leaves on-farm.	1= Yes 2= No
1b	If yes , Reasons for processing	1= subsistence

		2= income
1c	What are the processed products in order of importance?	1= dried leaves 2= fermented leaves 3= boiled and pounded leaves 4= others (specify)
1d	What are the cassava varieties preferred (Select more than 1).	1= Tajirika 2= shibe 3= Kibanda Meno 4= Nzalauka 5= Karibuni 6= Karembo 7=Girikacha 8= others (specify)
1e	Reason for using variety to process the products above. (Select more than 1).	1= availability 2= less fibrous 3= better product quality 4=affordable 5= less toxic 6= others(specify)
2a	How do you process dried leaves? (Select more than 1).	1= Drying 2= fermenting 3= boiling and pounding 4= others (specify)
2b	Equipment/tools used during processing dried leaves. (Select more than 1).	1= chippers 2= graters 3= solar dryers

		4= pounding mortar 5= panga 5= knives 6=grinding mill 7= others (specify)
2c	Pre-treatment during drying leaves. (Select more than 1).	1= washing 2= fermenting 3= chipping 4= chopping 5= scraping 6= others (specify)
2d	Reason for drying leaves. (Select more than 1).	1= cleaning 2= detoxify 3=reduce bulkiness 4= ease of further processing 5= better taste 6= others (specify)
2e	Where the drying of leaves takes place. (Select more than 1).	1= designated place 2= open yard
3a	How do you process fermented leaves? (Select more than 1).	1= Drying 2= fermenting 3= boiling and pounding 4= others (specify)
3b	Equipment/tools used during fermenting leaves. (Select more than 1).	1= chippers 2= graters 3= solar dryers 4= pounding mortar

		5= panga 5= knives 6= grinding mill 7= others (specify)
3c	Pre-treatment during fermentation of leaves. (Select more than 1).	1= washing 2= chipping 3= chopping 4= scraping 5= others (specify)
3d	Reason for processing leaves. (Select more than 1).	1= cleaning 2= detoxify 3=reduce bulkiness 4= ease of further processing 5= better taste 6= others (specify)
3e	Where the drying of leaves takes place. (Select more than 1).	1= designated place 2= open yard
4a	How do you process boiled and pounded leaves? (Select more than 1).	1= Drying 2= fermenting 3= boiling and pounding 4= others (specify)
4b	Equipment/tools used during boiling and pounding leaves. (Select more than 1).	1= chippers 2= graters 3= solar dryers 4= pounding mortar 5= panga 5= knives

		6=grinding mill 7= others (specify)
4c	Pre-treatment during boiling and pounding of leaves. (Select more than 1).	1= washing 2= fermenting 3= chipping 4= chopping 5= scraping 6= others (specify)
4d	Reason for boiling and pounding leaves. (Select more than 1).	1= cleaning 2= detoxify 3=reduce bulkiness 4= ease of further processing 5= better taste 6= others (specify)
4e	Where the drying of leaves takes place	1= designated place 2= open yard
5a	Frequency of processing	1= daily 2= weekly 3= monthly 4= seasonal 5= on demand
5b	Factors for checking good quality. (Select more than 1).	1= colour 2= texture 3= taste 4= others (specify)

5c	Why do you prefer these products? (Select more than 1).	1= easy to process 2= high prices 3=consumption 4= others (specify

D. STORAGE

1a	Do you store cassava leaves products	1= Yes 2= No
1b	If yes, where is the product stored? (Select more than 1).	1= on the floor 2= on raised platform in house 3=in a granary 4= Others (specify)
1c	If yes, Storage container. (Select more than 1).	1= drums 2= baskets 3= canvas bags 4= jute bags 5=kraft paper 6= polyethylene paper 7= Others (specify)
1d	If yes, Length of storage	1= 1-7 days 2= between 8-14 days 3= Others (specify)
1e	If yes, Reason for storage	1= food security 2= income 3= Others (specify)
1f	If no, why don't you store cassava leaves products	1= lack of knowledge 2= available all year round 3= Others (specify)
2	Do you store cassava leaves in the same store with other products	1= Yes 2= No
2a	If yes, name the crops cassava is stored with. (Select more than 1).	1= beans 2= cowpeas 3=pigeon pea 4=green grams 5=maize 6= Others (specify)

3a	What are the causes of loss during storage? (Select more than 1).	1= pests 2= caked 3=thieves 4=broken 5= rots 6= Others (specify)
3b	What do you do with the damaged/spoilt cassava products? (Select more than 1).	1=cook 2=sell 3=throw 4= Others (specify)

E. UTILISATION AND LIFE STYLE PATTERNS

1a	What part of cassava do you consume and in what form	1= root 2= leaves
1b	Forms of consumption	1= raw 3=boiled 3=fermented 4= Others (specify)
1c	Frequency of consumption	1= daily 2= weekly 3= monthly 4= Others (specify)
1d	Do you incorporate any other type of food during preparation	1= Yes 2= No
1e	If yes, incorporated with other food. (Select more than 1).	1= cowpeas seeds 2= cowpeas leaves 3=pigeon peas 4= Others (specify)
1f	How do you incorporate	1= boiled 2= fried 3= both 4= Others (specify)
1g	Frequency of consumption	1= daily 2= weekly 3= monthly 4= Others (specify)
2a	Which variety do you consume? (Select more than 1).	1= Tajirika 2= shibe 3= Kibanda Meno 4= Nzalauka 5= Karibuni 6= Karembo 7=Girikacha

		8= others (specify)
2b	Is the variety involve above either bitter or sweet	1=Bitter 2= Sweet
3a	Which cassava leaves processed products do you frequently consume	1= dried leaves 2= fermented leaves 3= boiled and pounded leaves 4= flour (e.g. maize) 5= others (specify)
3b	How frequently do you consume each of the products	1= daily 2= weekly 3= monthly 4= others (specify)
3c	If flour is mentioned, what else do you incorporate with the flour?	1= cassava flour alone 2= cassava flour + maize flour 3= cassava flour + wheat flour 4= others (specify)
3d	Which are the common meals you prepare from flour or flour incorporate? (Select more than 1).	1= ugali 2= porridge 3= chapatti 4= bread 5= cake
4	Which month does your household consume cassava/cassava based products most, specify. (Select more than 1).	1= Jan 2= Feb 3= Mar 4= Apr 5=May 6= June 7= July 8= Aug 9= Sep 10= Oct 11= Nov 12= Dec
5a	In your household are there family members exempted from cassava – based meals?	1= Yes 2= No
5b	If yes, kindly list them. (Select more than 1).	1= elderly 2= expectant women 3= children 4= others (specify)
5c	Have you or anyone you know experienced negative reactions after consuming cassava roots/products?	1= Yes 2= No
5d	If yes, kindly list the reactions exhibited by these members. (Select more than 1).	1= constipation 2= skin rashes 3= diarrhoea

		4= vomiting 5= dizziness 6= faint 7= death 8= others (specify)
	How they were managed.	1= sought medical attention 2= the symptoms dissipated 3= Others (specify)
E	What is the probable cause of the negative reaction	
6.	How do you package the products you sell? (Select more than 1).	1= packaging Kraft paper 2= sacks/ Gunny bags 3= baskets 4= others (specify)
7.	How do you utilize cassava peels? (Select more than 1).	1= animal feed 2= drying 3=decompose 4= milling 5= others (specify)

APPENDIX 3: QUESTIONNAIRE 2

SENSORY EVALUATION QUESTIONNAIRE

HEDONIC SCALE SCORING

NAME _____

DATE_____

PRODUCT_____

INSTRUCTIONS

Please, observe and taste each sample in order from left to right. Use the scale provided below to indicate how much you like or dislike the sample you have tasted. Please, comment on your attitude. Remember you are the only one who can tell what you like. An honest expression of your personal feeling will help us.

DEGREE OF PREFERENCE	SCALE
Like very much	7
Like moderately	6
Like slightly	5
Neither like nor dislike	4
Dislike slightly	3
Dislike moderately	2
Dislike very much	1

ATTRIBUTES

Sample code	Appearance	Colour	Flavour	Texture	Overall acceptability

Comment_____
_____
_____