EFFECT OF CASSAVA LEAVES PREPARATION METHODS ON SENSORY QUALITY, RETENTION OF PROXIMATE COMPONENTS, SELECTED VITAMINS AND CYANOGENIC GLYCOSIDES IN COASTAL REGION CASSAVA VARIETIES.

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ABSTRACT

A survey was conducted at Mkuranga and Rufiji districts in the Coastal Region of Tanzania. A total of 10 samples comprised of two from each of 5 varieties were subjected to three preparation methods (pounded cassava leaves as preparation method 1, boiled pounded leaves for 30 minutes as preparation method 2, and pounded leaves in hot water then boiled for 30 minutes as preparation method 3). These samples were analyzed for proximate components, β-carotene, vitamin C and cyanide content. The data was subjected to analysis of variance (ANOVA) to compare the effect of preparation methods, location and variety on the above mentioned components. Means were separated by DMRT and tested for significant by differences attributed to preparation methods and variety. The findings indicate that cassava leaves from Kiroba variety is mostly cultivated by farmers in the study area which was significantly attributed to its being tasty and high yield, following significance test by DMRT at $p<0.05$. Locational mean difference were subjected to t test ($p< 0.05$). Mpira variety was found to contain higher moisture content (82.40%) than other varieties ($p<0.05$). Kizimbani variety was found to contain higher protein (31.78% DM) than other varieties. Nyakamgile contained significantly higher ash content (10.48% DM) compared to other varieties. Crude fiber was significantly higher in Cheupe (25.13% DM) than other varieties all mean differences were significant at $p<0.05$ level. Crude fat was higher in Kizimbani variety (3.91% DM) while Mpira variety contained significantly higher carbohydrate (36.10% DM) content ($p<0.05$). The cyanide content on cassava leaves was found to be higher in Kiroba (185.51mgHCN/kg) DM and lower in Nyamkagile variety (54.17mgHCN/kg) DM, cyanide content for the preparation method 1 was 376.31mgHCN/kg DM while

that of preparation method 2 was 12.37mgHCN/kg DM and preparation method 3 was 9.25mgHCN/kg DM and for which the difference was significant at $p<0.05$.Cheupe variety had significantly higher β carotene content while vitamin C content was significantly higher in the Kizimbani variety. The preparation methods had no effect on fat and protein content, but changes were observed by increased in moisture and ash content of cassava leaves prepared with method 2 and 3, while there was reduction in crude fiber, carbohydrate, β- carotene, vitamin C and cyanogenic potential levels during preparation method 2 and 3. The observed variability in cassava leaves composition was significantly attributed to preparation methods, varieties and location ($p<0.05$). In this study preparation methods and varieties had no effect on colour, taste, aroma and general acceptability. Preparation method 2 had more effect on aroma compared to cassava leaves prepared with method 3.The texture of cassava leaves was affected by the variety used while it will remain the same irrespectively of any preparation method used. Based on these finding, African preparation methods 2 and 3 reduce the cyanide content with considerable nutrient retention. There should therefore be no fear in utilization of cassava leaves for direct consumption.

DECLARATION

I, Mwanahamisi Fadhili Msangi do hereby declare to the Senate of the Sokoine University of Agriculture that this dissertation is my own original work done within the period of registration and that it has not been submitted in any other Institution.

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DEDICATION

This work is dedicated to a broad spectrum of personalities including my beloved husband Mr. Baraka Mshindo Muyagga who has always been my source of inspiration, my parents Mr & Mrs Fadhili Msangi; daughter Malaika B. Muyagga and son Daudi B. Muyagga; young brother Said Msangi and his wife Zaina, young brother Yusuph Msangi and young sister Neema Msangi; father and mother inlaw Mr. Mshindo Muyagga and Ms. Mary Gaspar. It is through their combined patience and support that constructed direct and indirect motivation that culminated to successful completion of my study at the Sokoine University of Agriculture.

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LIST OF ABBREVIATIONS

AOAC	Association of Official Analytical Chemists
CPB	Cereals and Other Produce Board Of Tanzania
DMRT	Duncan's Multiple Range Test
DW	Dry Weight
DM	Dry Matter
FAO	Food and Agriculture Organization
FW	Fresh Weight
g	Gram
HCN	Hydrogen Cyanide
IFAD	International Fund for Agriculture Development
IITA	International Institute of Tropical Agriculture
Kg	Kilogram
LSD	Least Significant Difference
m	Million
mg/100g	Milligram per hundred grams
SUA	Sokoine University of Agriculture
TFDA	Tanzania Food and Drugs Authority
WFP	World Food Programme
WHO	World Health Organization

CHAPTER ONE

1.0 INTRODUCTION

Cassava is a perennial woody shrub with an edible root, which grows in tropical and subtropical areas of the world. Cassava originated from tropical America and was first introduced into Africa in the Congo basin by the Portuguese around 1558 (IITA, 2009).

Cassava is grown predominantly by small-scale farmers with limited resources in marginally fertile soils. The world top five cassava producers are Nigeria, Brazil, Indonesia, Thailand and the Democratic Republic of Congo, which together produce up to 53% of world production (FAOSTAT, 2012).

This crop is drought resistant and is also important for both rural and urban food supplies. Today, cassava is a staple food and animal feed in tropical and subtropical Africa, Asia, and Latin America, with an estimated total cultivated area greater than 13 million hectares, of which more than 70% is in Africa and Asia (EL-Sharkawy, 2003). Hence, globally approximately 500 million people depend on it as a major source for carbohydrate (Montagnac *et al.,* 2009).

In Tanzania, cassava is an important crop grown for food and income generation. The crop is especially important in the Lake, Western, Southern and Eastern Zones (Msabaha *et al.,* 1986; Kapinga *et al.,* 1996). Cassava is largely cultivated for its root

and the leaves are consumed as vegetable. Cassava tubers are poor in protein and other nutrients while leaves are good source of protein that is rich in lysine notwithstanding its deficiency in sulphur containing amino acids notably methionine and possibly cystine (Julie *et al.*, 2009).

1.2 Problem Statement and Justification

In Tanzania cassava leaves from different varieties are traditionally prepared into a wide range of products including "kisamvu". In various parts of the country, cassava leaves are prepared for both domestic consumption and marketing.

Preparation effectiveness is constrained by lack of technical support or advice, and consequently its nutritional quality enhancement is not realized. It is also unfortunate that there are no reported data on proximate components and quality of prepared cassava leaves in Tanzania but Umuhozariho *et al.*, 2011 did utilization of Cassava Leaves as a Vegetable in Rwanda in which cassava species from which leaves are harvested as vegetable and identify leaf preparation methods, consumption rate, price variation, storability and perception of post-harvest losses. Lack of information to consumers on quality of prepared leaves is a factor that derails its market value compared to other vegetables such as cowpea, sweet potato and other traditional leafy vegetables. Quality, nutritional and toxicity aspects of "kisamvu" have not received due attention with a view of promoting consumption based on its endowed importance in human nutrition.

This study aims at comparing cassava leaves prepared by three methods commonly practiced by Tanzania's farmers to produce "kisamvu" and analyze its proximate components and quality attributes. Alongside the proximate components and selected vitamin following preparation, this study will also assess toxicity levels attributed to retained HCN in prepared cassava leaves.

Assessment of proximate components, sensory quality and toxicity reduction in traditionally prepared cassava leaves will provide valuable information to consumers who indeed will shed fears on cassava leaves consumption on grounds of toxicity. Boosting cassava leaves utilization will in turn contribute to improve household food security and livelihood.

1.3 Research Objectives

1.3.1 Overall objective

The overall objective of this study was to analyse the effect of cassava leaves traditional preparation methods on sensory quality, retention of proximate components, selected vitamins and cyanogenic glycosides in coastal region cassava varieties.

1.3.2 Specific objectives

a) Identify traditional cassava varieties that are locally grown in Mkuranga and Rufiji districts.
b) To determine proximate components, vitamin C and β carotene of prepared cassava leaves from different varieties.
c) To determine the relative reduction of cyanogenic glycosides in cassava leaves prepared from different varieties.

d) To assess effect of preparation methods on proximate components, β carotene and vitamin C retention

e) To determine the effect of preparation methods on sensory evaluation of cassava leaves.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Cassava Leaves

Cassava (*Manihot esculenta*, Crantz) is a seasonal crop used as food in several parts of Africa, Asia and Latin America (Longe, 1980; Rosling, 1987; Bradbury *et al.*, 1991). Nigeria is currently producing over 14 metric tonnes annually, representing about 25% of sub-saharan Africa's output (FAO, IFAD and WFP, 2015). It is the third most important food source in the tropical world after rice and maize, it provides calories for over 160 million people in Africa (Polsen and Spencer, 1991).

Cassava (*Manihot esculenta*, *Crantz*), forms a significant component of staple food supply in the tropics (Montagnac *et al.,* 2009). In addition to having non–food uses, cassava also has an important economic role in African countries, for it significantly contributes to basic food requirements in urban and rural areas. Cassava food value depends on the specific tissue (root or leaf) and on several other factors including; variety, plant age, growth location and prevailing weather conditions. The roots and leaves, which respectively constitute 50% and 6% of the mature cassava plant, are the nutritionally valuable parts of cassava (Tewe and Lutaladio, 2004).

2.2 Cassava Leaves Productivity

The potential yield of cassava leaves varies considerably, depending on climate, soil fertility, cultivar, age of the plant, plant density and harvesting frequency (Ravindran, 1992). Leaves harvesting, however has been reported to lower root crop yields by almost one half of the normal (Ravindran, 1993).

Cassava leaves yields are usually relatively high on harvesting during the growing season (Ravindran and Rajaguru, 1988). Never the less there are claims that root yields can be adversely affected by its leaf harvesting regiment. There are several studies (Ravindran and Rajaguru, 1988) that support the ideal of harvesting cassava leaves during the growth stage and yet maintaining acceptable root yields. When cassava cultivation is exclusively aimed at leaf production, the plant density could be increased and the harvesting frequency can be shortened. Cassava plant can withstand defoliation for several years, should the foliage be harvested after four mouths growth period with this regiment being repeated within cycles of 60 – 75 days in as long irrigation and fertilizer management are adhered to (Hahn *et al.,* 1992).

2.2.1 Cassava Leaves Harvesting Method

The appropriate harvesting method under the current practice of small scale cassava production is to manually harvest the foliage, including tender stems. However, mechanical harvesting equipment would be used if large scale defoliation is envisaged. According to the development stages of the cassava plant presented by (Alves, 2002) emergence of sprouting normally takes place from 5 to 15 days after planting, leaf development begins at 15 to 90 days after planting along with the formation of root system and the establishment of canopy happens through well-developed stems and leaves at approximately 90 to 180 days after planting and carbohydrate translocation to roots occurs mainly during the period 180 to 300 d after planting followed by dormancy at 300/360 days (Alves, 2002).

Under tropical conditions, the maximum growth rate is attained at 3 to 5 months while, under less favorable conditions this occurs later (Hue *et al*., 2012) The harvests at 3 and 6 months after planting take place at the beginning and end of the period of

canopy establishment, respectively. This assumption is confirmed by the fact that the yield at the 6 month harvest has been reported to be relatively higher during the 3 harvesting regiment normally associated with the relatively high leaf and stem growth rate. The harvest at 9 months would be during the phase when a large amount of carbohydrates are deposited in the roots. It is also the stage during which the leaf senescence increases. The lower DM yield at 9 months in the 3-harvest systems is therefore not surprising (Hue *et al.*, 2012).

2.3 Cassava Leaves as an indigenous Vegetable

Cassava leaves are widely consumed in most African countries as a vegetable, which is among the top three African indigenous vegetables rich in nutrients. They are the second in β-carotene after *Moringa oleifera*, the second in vitamin C after *Moringa stenopetala*, the third in vitamin E after *M. stenopetala* and *M. oleifera*, the third in zinc after *Pterocarpus mildbraedii* and *M. oleifera*, the third in antioxidant activity after *Adansonia digitata* and *Rorippa madagascariensis* and the third in total phenolic after *R. madagascariensis* and *A. digitata* (Shackleton *et al.*, 2009).

For healthy and nutrition importance, cassava leaves consumption may be improved by methods of preparation that can increase its availability and quality. Cassava leaves, like many other leafy vegetables, are generally seasonal with surpluses in the rainy season and scarcity during dry season, and therefore causing price hikes (Umuhozariho *et al.*, 2013).

2.4 Cassava Leaves Preparation Methods

There are three famous techniques for preparing cassava leaves, in the first approach, cassava leaves with 30 cm lengths (measured from the apex) are harvested from the

plants (Numfor and Ay, 1987). The hard petioles are removed and the blades and young petioles are pounded using a pestle in a mortar. Alternatively, the leaves are blanched before pounding. The resulting pulp is then boiled for about 30-60 minutes. In some countries, the water is discarded after decanting and the leaves are re boiled for 10 minutes prior to adding pepper, palm-oil and other spicing ingredients (coconut milk, tomatoes). The mixture is then boiled for 30 minutes.

2.5 Uses of Cassava Leaves

Cassava leaves are used as one of the preferred vegetables in most cassava growing countries, particularly Zaire, Congo, Gabon, Central African Republic, Angola, Sierra Leone, and Liberia (Hahn and Keyser, 1985). In most African countries cassava leaves are prepared and known by variety of traditional names with “kisamvu” being the most popular in Tanzania. They are mostly served as a sauce which is eaten with chickwangue, ugali, fufu, and boiled cassava (Hahn and Keyser, 1985).

2.6 Cassava Leaves Nutritional Value

Cassava leaves as a vegetable constitute a very significant source of dietary protein, minerals and vitamins (Gil and Buitrago, 2002). The nutritive value of cassava leaves has been found to be comparable with that from soybean, maize and amaranth but with higher vitamins and mineral levels (Lancaster and Brooks, 1993).

2.6.1 Protein Content

The level of these nutrients compare favorably with that of other green vegetables rich in protein. With the exception of methionine its, amino acid composition and values exceed those for the FAO reference protein (Lancaster and Brooks, 1993).

Many researchers showed that cassava leaves have relatively high protein content (from 16.7 to 39.9%), (Ravindran, 1991).The crude protein content is related to that of fresh egg 10.9% DM and the amino acid profile of cassava leaf protein is well balanced compared to that of the egg protein (Montagnac *et al.,* 2009). Indeed, furthermore, cassava leaves have an essential amino acid content higher than soybean protein and FAO's recommended reference protein intake (Okigbo 1980; West *et al.*,1988).

2.6.2 Carbohydrates Content

According to Gil and Buitrago (2002) the carbohydrate content in cassava leaves ranges from 7 to 18 g/100 g which is comparable to that of green-snap beans (7.1 g/100 g), carrots (9.6 g/100 g), or green soybeans (11.1 g/100 g), and it is higher than those of leafy vegetables such as green leaf lettuce (2.8 g/100 g) and New Zealand spinach (2.5 g/100 g).

2.6.3 Minerals Content

Cassava leaves are rich in iron, zinc, manganese, magnesium, and calcium (Wobeto *et al*., 2006). The variations in mineral content for cassava leaf meal (CLM) have been reported: from 61.5 to 270 mg iron/kg DM, 30 to 63.7 mg zinc/kg DM, 50.3 to 263 mg manganese/kg DM, 6.2 to 50 mg copper/kg DM, 2.3 to 3 g sulfur/kg DM, 2.6 to 9.7 g magnesium/kg DM, 0.4 to 16.3 g calcium/kg DM, and 8 to 16.9 g potassium/kg DM (Gomez and Valdivieso, 1985; Nwokolo, 1987; Ravindran *et al*.,1992; Aletor and Adeogun, 1995; Awoyinka *et al.,* 1995; Chavez *et al.,* 2000; Madruga and Câmara, 2000).

Cassava leaf meal is rich in iron which is equal to that of liver (121 mg/kg FW) and egg yolk (58.7 mg/kg FW) and iron from plant origin is generally less bioavailable than iron from foods of animal origin (Wobeto *et al.,* 2006). Iron and zinc content in CLM are higher than those reported for sweet potato leaves and peanut leaves (Lancaster and Brooks, 1993) Calcium content is equal to those of peanut and broccoli, and magnesium content surpasses that of broccoli but is below those of peanut and sweet potato. Thus, mineral content of CLM is equal to that of other leaves (Wobeto *et al.*, 2006).

2.6.4 Vitamins Content

In terms of vitamin content, cassava leaves is richer in thiamin (vitamin B_1, 0.25 mg/100 g) than legumes and leafy legumes, except for soybeans (0.435 mg/100 g) (Montagnac *et al.,* 2009). The leaves have more thiamin than several animal foods including fresh egg, cheese, and 0.325 mg/100g fat whole milk. The riboflavin (vitamin B_2) content of cassava leaves (0.60 mg/100 g) surpasses that of legumes, leafy legumes, soybean, cereal, egg, milk, and cheese (Wobeto *et al.,* 2006).

The niacin content (2.4 mg/100 g) is equal to that of maize (2 mg/100 g), and surpasses those reported for legumes and leafy legumes, milk, and egg. The vitamin A content of cassava leaves is equal to that of carrots and surpasses those reported for legumes and leafy legumes (Montagnac *et al.,* 2009). The cassava leaves Vitamin C content (60 to 370 mg/100 g) is higher than the values reported for other vegetables (Wobeto *et al.,* 2006). Thus, the overall vitamin content in the leaves is equal and in certain cases higher than those reported for most grains and their leaves legume, cereals and cheese (Watanabe *et al.,* 2014).

2.6.5 Fat Content

There are several factors that influence the fat content in cassava leaves including plant variety, age and stage of harvest as well as numerous environmental conditions. The lipid content is 10 times higher in leaves than in roots (Hue *et al.,* 2012). Although the lipids and lipid-soluble components such as chlorophyll, resin, and xanthophylls are much more concentrated in leaves, some of them, such as volatile fatty acids, chlorophyll, and resin, do not bring significant energy to the diet. Therefore, the energy density of the lipid is lower in leaves than in roots (Gil and Buitrago, 2002).

2.6.6 Fiber Content

The fiber content in cassava leaves is relatively higher than that in grain and leafy legumes, where it ranges between 1 and 10 g/100 g FW. Dietary fiber is considered part of a healthy diet and can reduce constipation problems (Montagnac *et al.,* 2009).

Although recent evidence is mixed, fiber may help prevent colon cancer. The high fiber in cassava leaves may minimize intestinal peristalsis and bolus progression (Rock, 2007). Nevertheless excess dietary fiber has negative effects in humans and especially reduction of trace elements bioavailability. Fiber can be a nutritional concern because it can decrease nutrient absorption in the body (Baer *et al.,* 1996). Excess fiber has also been reported to increase fecal nitrogen, intestinal irritation, and decrease macronutrient digestibility, especially protein (Baer *et al.,* 1996).

2.7 Cassava leaves Toxicity

Cyanide is the most toxic factor restricting consumption of cassava leaves. Cassava, particularly the bitter varieties, have cyanide levels higher than the FAO/WHO (1991) threshold recommendations, which is up to 10 mg cyanide equivalents/kg DM. Cassava leaves have indeed been reported to contain cyanide level ranging from 53 to 1,300 mg cyanide equivalents/kg of DM (Siritunga and Sayre, 2003; Wobeto *et al.*, 2007).

2.7.1 Cyanogenic Glucosides

Cassava root and cassava leaves contain cyanide, in the form of cyanogenic glucosides, primarily linamarin and small lotaustralin (Uyoh *et al*., 2007). The cyanogenic glucosides are distributed throughout the cassava plant, with highest levels in leaves (Etonihu *et al*., 2011). Under high temperature, pressure, and use of enzyme (linamarase), or mineral acids, cyanogenic glucosides are decomposed into acetone cyanohydrins which, at pH above 5 or temperatures above 35°C, is spontaneously broken down, into hydrogen cyanide (HCN) which is extremely toxic (Siritunga and Sayre, 2004). Cyanohydrins are the most dangerous form of the cyanide because at the elevated human body pH and temperatures, it rapidly decomposes to release the poisonous hydrogen cyanide (ACGIH, 1996).

The cassava leaves cyanide content has been studied extensively. The normal range of cyanide content is from 20 to 80 mg HCN per 100g fresh leaf weight, but samples containing as low as 8 mg/100g or over 400 mg/100g have also been reported (Ravindran, 1992). On a dry basis (assuming 25% DM in fresh leaves), the normal

range of HCN content would correspond to 800 to 3200 mg/kg. These levels are substantially higher than the normal range of HCN reported for fresh cassava roots (Ravindran, 1992).

The extensive variability observed in leaf cyanide levels may be attributed to genetic, physiological, edaphic and climatic differences and to some degree inherent problems arising from its assay methods (Bokanga, 1994). According to leaf maturity state is perhaps a major factor causing variation in the cyanide content (Nhassico *et al*., 2008). As in other cyanogenic plants, the glucosides concentration in cassava leaves decrease with age. Cyanide level in the leaves are also influenced by the plants nutritional status. According to Bokanga (1994) leaf cyanide levels were increased by nitrogen fertilizers and yet potassium fertilizer and farmyard manure had the opposite effect.

Continued consumption of high dietary cyanogens has been linked with a number of chronic health disorders, and occasionally death, depending on the level of cyanogens, frequency of cyanogens exposure and, quality and quantity of protein intake (Cliff *et al*., 2011; Nhassico *et al*., 2008; CCDN, 2007). For the human body detoxification, unbound cyanide is converted to less toxic thiocyanate (SCN) and is excreted in the urine. The synthesis of thiocyanate requires sulphur containing amino acids, as a consequence of protein intake (CCDN, 2007).

2.7.2 The Symptoms of Cyanide Poisoning

In humans, the clinical signs of acute cyanide intoxication include rapid respiration, drop in blood pressure, high pulse rates, dizziness, headache, stomach pain, vomiting,

diarrhoea, mental confusion, twitching and convulsions (Mlingi, 1995). Death due to cyanide poisoning can occur when the cyanide level exceeds the limit an individual is able to detoxify which ranges from 0.5 to 3.5 mg per kilogram of body weight. Children are particularly at risk because of their smaller body size (Rosling, 1987).

Chronic cyanide intoxication may lead to development of certain conditions including disturbance of thyroid function and neurological disorders (Mlingi *et al.,* 1993). Continued consumption of high dietary cyanogens has been linked with a number of chronic health disorders, and occasionally death, depending on the level of cyanogens, frequency of cyanogens exposure and, quantity and quality of protein intake (Cliff *et al*., 2011; Nhassico *et al*., 2008; CCDN, 2007). For the human body detoxification, unbound cyanide is converted to less toxic thiocyanate (SCN) and is excreted in the urine (CCDN, 2007).The synthesis of thiocyanate requires Sulphur containing amino acids, as a consequence of protein intake (Nhassico *et al.,* 2008) .

Several health disorders and diseases have been reported in cassava consuming communities. Consumption of 50 to 100 mg of cyanide has been associated with acute poisoning and has been reported to be lethal to adults (Lee *et al.*, 2012). Intake of lower cyanide level are not lethal but long-term intake could cause severe health problems such as tropical neuropathy (Osuntokun, 1994), glucose intolerance and konzo (spastic paraparesis) (Ernesto *et al*., 2002), and when combined with low iodine intake there culminate to goiter and cretinism (Delange *et al*., 1994).

2.8 Effect of Preparation methods on Nutrient Content of Cassava Leaves

The nutritional composition in cassava leaves products will differ depending on the nature and duration of the methods of preparation. In the case of many small scale

farmers in developing countries the preparation duration is limited. Even though researchers have successfully fabricated equipment for preparation of cassava leaves that accelerates the preparation of cassava leaves (Hahn *et al.*, 1987). The newly fabricated equipment for preparation of cassava leaves have been reported to tremendously reduce the antinutrients with minimal nutrient loss (Montagnac *et al.*, 2009). Some scholars have pointed out that, there should be no fear in the utilization of cassava leaves in man's own direct consumption as a good source of leafy vegetables (Achidi *et al.*, 2008). Despite the presence of the poisonous component, numerous reports have provided evidence on potential contribution of cassava leaves to human nutrition through protein, minerals and vitamins, provision and that depending on preparation techniques (Ayodeji, 2005 ; Mulokozi *et al.*, 200; Akinwale *et al*., 2010). Faber and Van Jaarsveld, (2007) revealed that improved handling, such as optimizing thermal treatment and drying process duration, so long as preliminary proper preparations approaches are adhered with a view of food quality retention.

2.8.1 Main Cassava Leaves Preparation Methods

2.8.1.1 Boiling

According to Nambisan (1994) at 100 ℃, linamarase, a heat-labile β-glucosidase, is denatured and linamarin cannot then be hydrolyzed into cyanohydrin. The inefficiency of this preparation method is due to the low temperatures. Bokanga (1994) reported that bound glucosides were reduced between 45% and 50% after 25 min of boiling at 100 ℃. They concluded that, boiling is not an effective method for cyanide removal (50%) in cassava leaves. Boiling further reduces cyanogenic glucosides to 1% (Nambisan, 1994).

2.8.1.2 Pounding/Crushing

Pounding or crushing cassava leaves and then boiling in water is an efficient preparation methods for removal of cyanogens while other nutrients are retained at considerable level. About 97% of cyanogenic glucosides are removed and cyanohydrin and free cyanide are completely removed (Nambisan, 1994). Pounding leaves reduces cyanogen content by 63% to 73% and promotes a rapid break down of cyanogenic glucosides because linamarase activity is much higher than in roots (Montagnac *et al.,* 2009).

2.8.1.3 Drying methods

There are two drying methods for cassava leaves namely oven and sun. Sun drying is used to extend the shelf life of the final product (Umuhozariho *et al.,* 2011). The direct exposure to sunlight is known to cause leaf discolouration and reduction of natural vitamins notably pro Vitamin A (β carotene) and Vitamin C (MMA, 2008).

2.8.2 Effect of Preparation Methods on Cassava Leaves

2.8.2.1 Proximate Components

African preparation methods tremendously reduce the proximate components with minimal loss in the nutrients. Different preparation methods exist to reduce or retain proximate components and their effectiveness depends on the preparation steps and the sequence utilized, and it often is time dependent. (Achidi *et al.*, 2008). Emmanuel *et al.* (2012) analyzed chemical composition of cassava varieties. The proximate components were determined using standard methods. The different cultivars had moisture content (33.14-45.86%), protein (1.17–3.48%), ash (1.71–2.34%), crude fibre (1.38-3.20%), fat (0.74-1.49%) and carbohydrate (83.42-87.35%).

A study by Achidi *et al.* (2008), found no significant changes in ash, lipid, protein, starch, fiber and carbohydrate contents for non-processed, pounded and cooked, ground and cooked cassava leaves. Nevertheless there was significant decrease in the free sugar content. Achidi *et al* (2008) reported a reduction in the free sugar content which was between 9.93 and 21.73% with a mean of 15.83% for pounded leaves, while the decrease was from 26.30 to 34.83% with a mean of 30.56% for the ground leaves. Average total reduction for both pounded and ground leaves was 23.20%.

2.8.2.2 Minerals

Studies have found significant effect of preparation methods on minerals in cassava leaves, Preparation methods had no effect on calcium, magnesium, potassium, sodium, phosphorus, copper, zinc and manganese (Achidi *et al.*, 2008). Mineral contents were 0.60-1.60, 1.35-1.58 and 1.06-2.13 mg/100g for Ca, Mg and P respectively, and 0.16-0.24, 0.021-0.030, 0.04-0.13, 0.25-0.36 and 0.25-0.37 mg/100g for Fe, Mn, Zn, K and Na respectively (Emmanuel *et al.*, 2012).

A study by Achidi *et al.* (2008) on the effect of preparation methods on nutrient content of cassava showed that Magnesium and sodium values for pounded and cooked leaves were higher than the corresponding values for the non-processed and the ground and cooked leaves, even though, in the these differences were not significant ($p<0.05$). However, no consistent pattern was observed for calcium, potassium, copper and manganese during processing. There were small reductions in phosphorus and zinc but the reductions were not significant. Iron content increased in the ground and cooked leaves compared to the non-processed or pound and cooked leaves (Achidi *et al.*, 2008).

2.8.2.3 Vitamins

Studies has shown that pounding or grinding cassava leaves before cooking brought about a reduction in the ascorbic acid and thiamine content of the cooked leaves; however, the loss in the ground leaves was slightly higher than in the pounded and cooked leaves but the difference was not significant (Achidi *et al.*, 2008). According to Umuhozariho *et al.,* (2011), vitamin C was eliminated to almost zero after boiling for 15 minutes and 30 minutes.

2.8.2.4 Cyanide Potential

A study on the utilization of cassava leaves as a vegetable in Rwanda by Umuhozariho *et al.* (2011) analyzed the effect of preparation procedures before and after boiling. It was noticeable that for undried and dried samples, before boiling, wild species had the highest concentrations in cyanide, after boiling for 30 minutes, the results showed a reduction in cyanide content. Cyanogenic potential ranged from 0.08-0.12 mgHCN/kg. Wide variations existed in chemical composition of the improved and traditional cassava cultivars but all possesed safe levels of cyanogenic potential and were safe for human consumption.

A study on nutrient composition and effects of preparation methods on cassava leaf antinutrients, leaves in 3 genetically improved varieties of cassava plants were harvested and subjected to different preparation methods including sun-drying (SND), oven-drying (OVD), steaming (STM), shredding (SHD) and steeping (STP) and a combination of these methods to deliberately reduce the high level of cyanogenic glucosides present in the leaves (Fasuyi, 2005). A combination of SHD and SND

(SHD+SND) seemed to be the most effective technique of reducing the cyanide content. Sun-drying cassava leaves was quite efficient with 6.8% of total cyanogens retained, shredding cassava leaves and then sun-drying removed 95.2% of cyanogens (Nambisan, 1994). However, oven-drying cassava leaves seemed to be relatively inefficient because 60% to 75% of cyanogens were retained. On an evaluation of the effect of various processing techniques on cyanogen content reduction in cassava, Nambisan (1994) found out that sun drying resulted in a greater loss of cyanogens.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Area

The study was conducted in December 2015 to January 2016 at Mkuranga and Rufiji districts in the Coastal Region. Preparation of cassava leaves, sensory evaluation and laboratory analysis were done from February 2016 to June 2016 at the International Institute of Tropical Agriculture (IITA)-Tanzania.

3.2 Controlled Study Design

3.2.1 Research design

Three out of the most outstanding traditional preparation methods 1, 2 and 3 identified using the survey method were subjected to a simulated trial in a Completely Randomized Design (CRD) while incorporating three principal factors visualized from the survey data.

The effect of these factors on proximate, vitamins (β carotene and vitamin C), sensory properties and cyanogenic glycosides were determined.
Mathematical expression is shown in equation in here under.

$Yijkt = \mu + tijk + eijkt$ …………………………………………………..……………. (1)

Where Yijkt is observation for ith treatment appearing in jth row, kth column and tth row μ is a general mean effect, tijk is the effect of ith treatment appearing in jth row and kth column and eijkt is error term. The effect of these three factors (Variety,

Preparation methods and Location) on proximate, cyanide level, β carotene, vitamin c and sensory properties were determined.

3.3 Sample Collection

A cross sectional survey was conducted at Mkuranga and Rufiji districts in the Coastal region, with a view of identifying dominant cassava varieties and traditional preparation methods. Exactly 50 farmers were interviewed using a structured questionnaire (25 farmers from each district).

Based on preliminary data analysis three of the most outstanding methods, a total of 10 cassava leaves samples were randomly collected (5 from each district) for proximate, carotene, vitamin C, cyanide and sensory assessment. Focused on three identified prominent traditional preparation methods; three of these methods were subjected to stimulated controlled trials so as to compare their relative effectiveness on nutrient retention, sensory and toxicity reduction.

3.3.1 Fresh Cassava Leaves Sample Collection

Fresh cassava leaves samples were randomly collected from selected respondents, sealed in closed polyethylene bags and taken to the IITA - Food Quality Laboratory, and stored in the ice cold box containers until required for Product Development, proximate components, β carotene, vitamins C, cyanide and sensory evaluation based on the three identified traditional preparation methods. A total of 30 samples were subjected to proximate analysis, relative retention of vitamin A precursor (β carotene and vitamins C), reduction of cynogenic glycocides and sensory evaluation.

3.4 Preparation Methods Used to Prepare Cassava leaves

The methods used to prepare leaves from each cassava varieties were based on responses from the farmers. The study revealed three dominant preparation methods for cassava leaves to get "Kisamvu".The three methods of preparation code named raw (preparation method 1, preparation methods 2 and 3) in this study are schematically summarized/illustrated in figure 1.

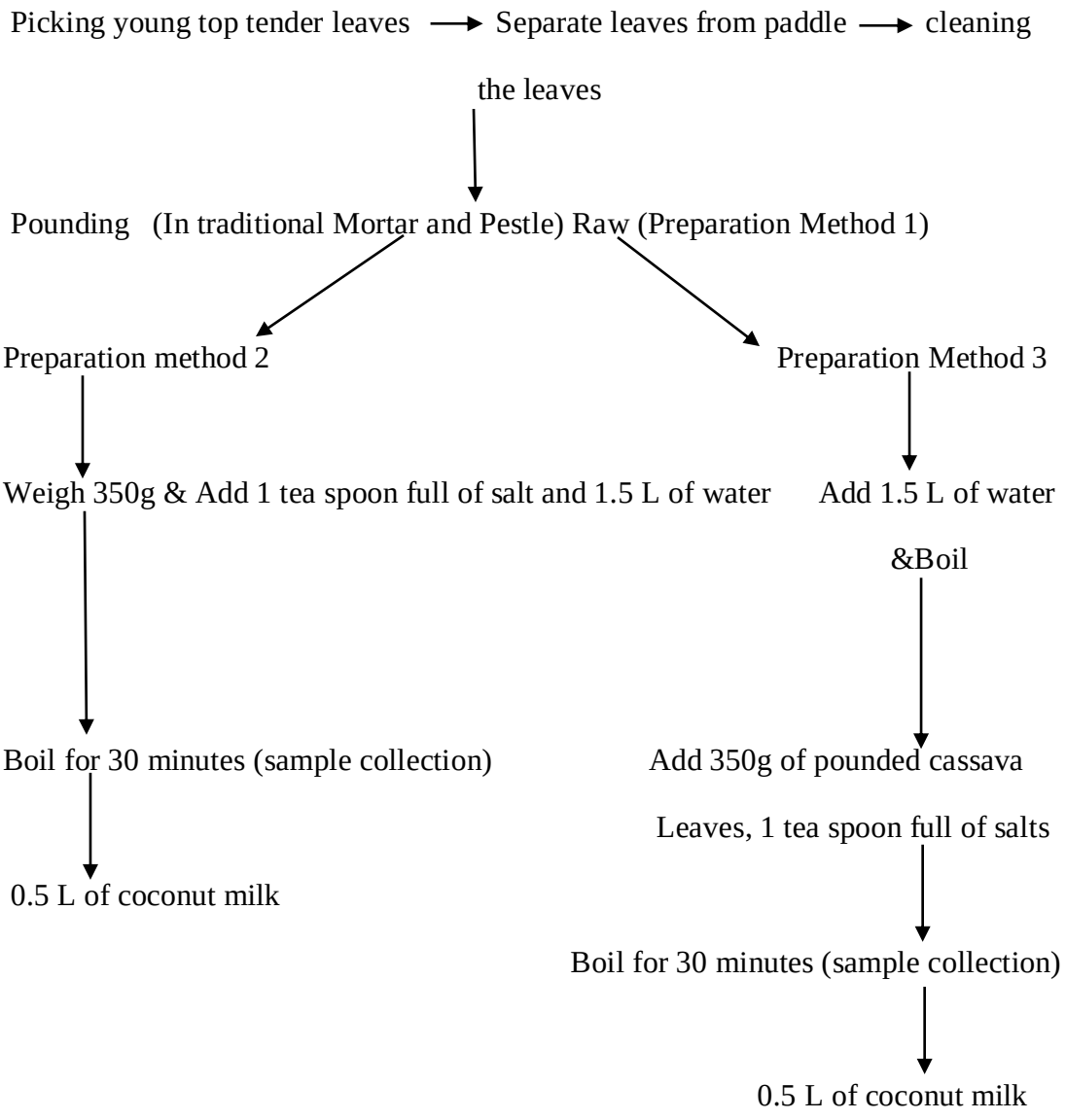

Figure 1: Flow diagram illustrating preparation methods of cassava leaves.

3.5 Chemical Analysis

3.5.1 Proximate analysis

The proximate analysis (moisture, ash, fat, crude fiber, crude protein) of the prepared cassava leaves were analyzed according to standard AOAC (1995).

3.5.1.1 Determination of Crude Protein

Crude protein content of cassava leaves prepared from the three methods were determined by macro Kjeldahl's method number 920.87. Dry samples were ground using pestle and mortar. One gram portions of the dried sample (W) were weighed into digestion tubes followed by addition of 15ml of concentrated sulphuric acid and 5g Kjeltic tablets. The tubes was gently shaken to wet the samples. Samples were digested in a tecator block digester in the fume hood. Two blanks contained Sulphuric acid and Kjeltic tablets were prepared in the digested tubes separate without sample. The samples, standards and the blanks were digested for 2 hours at 400^{o}C.Samples were digested to a clear, blue solution.

The digest was cooled and then 20 ml of distilled water was added to dissolve the content. To the dilute digest 50 ml of 40% sodium hydroxide was added to the digest to facilitate the release of ammonia and distilled using macro-distillation apparatus (Kjeltec^{TM}8200 Auto Distillation Unit, 2012). Ammonia was extracted by steam distillation and collected in a 250 ml flask containing 30 ml of 4% boric acid. The distillate was titrated with 0.1M HCl standard solution using bromocresol green methyl red mixture as indicator to a faint purple colour as end point.

The amount of protein in the sample (dry matter basis) was calculated as follows

$$\% \text{ Protein} = \frac{(T-B)*M*14.007*100*6.25*MCF}{W}$$

T = Volume of the standard hydrochloric acid used in the sample titration (ml).

B = Volume of the standard hydrochloric acid used in the blank titration (ml).

M = Molarity of the acid in four digits.

W = mass of the sample in milligram used in the determination.

6.25 = factor for converting nitrogen to protein for plant material.

MCF = Moisture Correction Factor = 100/ (100-% Moisture)

3.5.1.2 Determination of Moisture Content

Moisture content in prepared cassava leaves was determined by method number 925.09 as described by AOAC (1995). Crucibles were washed and dried in an oven at 105^0C for three hours, cooled in desiccators and weighed. Two gram of the sample was weighed in the crucible. The sample was spread evenly in the crucible and dried in an oven at 105^0C for 24 hrs. After that period, the crucibles were transferred to the desiccators for cooling. The crucibles were reweighed after cooling. The percentage moisture content was then calculated using the following formula:-

$$\% \text{ Moisture content} = \frac{(W_1 - W_2)}{W_1} \times 100\ \%$$

Whereby;

W_1 is weight of sample (gm) before drying and W_2 is weight of sample (gm) after drying

3.5.1.3 Determination of Crude Fiber

Crude fiber in cassava leaves was determined by AOAC (1995), official method 920.86. One gram portion of each samples were weighed for crude fiber determination using Fibertec^{TM}1020 FOSS model 2012. The samples were first digested in dilute 0.125M sulphuric acid for 30 minutes and washed three times using hot water. The residue was then digested in 0.125M KOH alkali for another 30 minutes and then washed using hot water three times. The digested residue was dried in an oven for 5 hours then cooled and weighed. The residue was then placed in muffle furnace and incinerated for 2 hours at 550^0C, then cooled and reweighed. The percentage crude fiber content was calculated from the following relationship:

$$\% \text{ crude fiber} = \frac{W_1\,(g) - W_2\,(g) \times 100}{W\,(g)}$$

Whereby:

W_1 is weight of sample residue before incineration (g), W_2 is weight of sample residue after incineration (g) and W is weight of dry sample taken for determination (g)

3.5.1.4 Determination of Crude Fat

Crude fat of leaves prepared from three methods were determined by ether extraction using the Soxtec System (Soxhtec^{TM} 2055 FOSS model 2012) in accordance with the AOAC (1995) method number 920.65. The method involved extracting crude fat from the samples into petroleum spirit (40-60^{o}C), which was then evaporated, and the weight of the crude fat was determined. Two grams of pre-dried samples were weighed and placed into extraction thimble. The thimbles were covered with fat free cotton and placed in the central part of the Soxhlet apparatus. Thirty ml of petroleum ether were poured into the pre-dried and pre-weighed cups and adjusted to the

Soxhlet extractor where extraction process took place for approximately one hour. After extraction, the cups with fat extract were further dried in the oven at 115^{o}C for 30minutes, and then cooled in desiccators for 30 minutes and then weighed. Percentage crude fat content was calculated as follows:

$$\% \text{ Crude fat} = \frac{\text{Weight of crude fat (g)} \times 100}{\text{Weight of dry samples (g)}}$$

3.5.1.5 Determination of Ash Content

The ash content in cassava leaves samples was determined by using a muffle furnace as described in standard method (AOAC, 1995), official method 923.03.

The crucibles were dried in an oven at 110 ^{o}C for 1 hour and cooled in a desiccator. Triplicate samples of 2 grams were placed in a pre-weighed crucible. The samples were transferred to a muffle furnace and ashed at 550℃ for 6 hours. The crucibles were allowed to cool in desiccators and then weighed. Percentage ash content was calculated using the following relationship

$$\% \text{ Ash} = \frac{\text{Weight of ash (g)} \times 100}{\text{Weight of sample (g)}}$$

3.5.1.6 Determination of Carbohydrate

Carbohydrate content in prepared leaves from the three methods was calculated as percentage by difference (AOAC, 1995). The following formula was used.

% Carbohydrate = 100 – (% Protein + % Crude fibre +% Crude fat +% Ash content)

3.5.2 Determination of Cyanide Content

Cyanogenic glycosides level of cassava leaves prepared from the three methods was determined by the method developed by Cooke (1987) and improved by O'Brien *et al* (1991). A 50-70 g homogenized sample was taken and swirled gently in 250 ml of extracting medium (0.1M H3PO4, 25% V/V ethanol is optional),the mixture was mixed for 15 sec at low speed, followed by 2 X 1 min at high speed with a 1 min rest period in between. The mixture was centrifuged for 10 minute at 4000 g. The extracts were stored cool for 2 weeks.

Exactly 0.1 ml of Chloramine T was added to the 4 ml of buffered extracted in the test tubes, the mixture was mixed and left for 5min. The volume (0.6 ml) of the colour reagent was added. The mixture was left for 10 min (between 8 and 30 min was reasonable), the absorbance was determined at 605 nm. Cyanide was oxidized to a cyanogen halide by chloramine T, is the most important and most accurate colorimetric method. The cyanogen halide reacts with pyridine or a related compound to produce a dialdehyde, which is then coupled with primary amines or compounds with active methylene groups such as pyrazolone or barbituric acid to yield a coloured complex.

The cyanogen level was calculated in mg CN equivalent per kg sample on a dry weight as follows:

$$[CN] = \frac{x\,(v + s\,*(m/100))}{s\,(1 - (m/100))*\,d} * 0.026$$

Where: s = sample weight (g);

v = volume of extraction medium (ml);

d = volume of extracted assayed (ml);

m = moisture content (%)

x = quantity of cyanogen (nmol) in the tube

0.026 is the conversion factor to express results in mg CN equivalent, to express as mg HCN equivalents use 0.027.

3.5.3 Determination of Vitamin C

The vitamin C determination was done as described in AOAC (1990). Two point five gram of homogenized sample was weighed into polytron bottle, 25ml of 10% metaphosphoric acid was added. The polytron was used to extract the vitamin C content of the sample at 1000rpm for 1 minute. Using 5ml portions of 10% metaphosphoric acid, the blades of polytron rinsed na finelly the volume of extract made to 50ml with Metaphosphoric acid. The extract is then filtered using No. 1 whatman filter paper to obtain clear sample extract. Ten ml of clear sample extract was poured into 150ml erlenmeyer flask and titrated against the standardized 2,6-dichlorophenol indophenol, sodium salt (DCIP) until rose-pink color was obtained. The ascorbic acid content was calculated as follows:

$$\text{Mg Ascorbic acid per 100g} = \frac{V_1\,(\text{ml}) \times C\,(\text{mg/ml}) \times V_2\,(\text{ml}) \times 100}{\text{Sample wt (g)} \times V_3\,(\text{ml})}$$

Where;

V1 =Volume of DCIP used for sample, ml

V2 =Total extraction volume made, 50ml

V3 =Volume of clear sample extract taken for analysis, 10ml

C =mg equivalent of ascorbic acid per 1ml of DCIP

3.5.4 Determination of β Carotene

Determination of β-carotene content was done by a method described by Rodriguez-Amaya, (2001) with slight modifications. A 0.5 - 2gm of pounded sample were accurately weighed in duplicate, transferred to a 50ml capacity containing a small amount of hyflosupercel and homogenized with 30mL of cold acetone. Homogenized sample was filtered with suction through a sintered glass funnel. The extraction procedure was repeated three times until the residue was devoid of any colour and washings were colourless.

In the partition step, the acetone extract was poured into a separatory funnel containing 30mL of petroleum ether (b.p. 40 - 60^0C). Distilled water was added slowly along the walls of the funnel and two phases were allowed to separate. The lower aqueous acetone phase was discarded whereas the upper phase (petroleum ether) was washed four times with distilled water to remove acetone completely. The petroleum ether phase was collected in a volumetric flask of either 25mL, 50mL or 100mL depending on the carotenoid content of the sample (corresponding to the intensity of the colour), after passing through a glass funnel containing anhydrous sodium sulphate. Ten mL of the extract was put in a test tube and evaporated to

dryness under nitrogen. The alternative way of evaporating excess petroleum ether is to use a rotary vacuum evaporator to reduce the volume to about 2 mL and then transfer to a test tube for complete dryness under nitrogen. Just before injection, the dry extract was redissolved in 1mL HPLC grade acetone, filtered through a 0.22 µm syringe filter for High Performance Liquid Chromatography (HPLC) analysis and quantification of beta-carotene.Due to the risk of oxidation and isomerization of carotenoids the analysis was performed at relatively dark corner in a fume hood and by covering all glassware with an aluminium foil.

3.5.5 Analysis of β-Carotene by HPLC

β-carotene was determined by isocratic reverse phase HPLC using methanol: methyl-t-butyl ether: water (56:40:4) as the mobile phase. The HPLC system comprised a Waters 600 dual piston solvent delivery pump connected to a Waters 996 UV-Visible photodiode array detector and equipped with a C_{30} polymeric column (YMC, Inc., Wilmington, NC,USA). The polarity of the ligands in C_{30} column is optimized for separation of both polar and non-polar carotenoids and their isomers. Empower 3 software (Waters) was used to acquire, store and process spectra and chromatographic data. Absorption spectra of the carotenoids were recorded between 400 and 500 nm at the rate of 0.5 spectra /second and the resolution of 1.2 nm. Flow rate was 0.8mL/minute and injections were made with a 10µL loop.

3.5.5.1 Identification and quantification of β-carotene

Retention times and spectral comparison to β-carotene standard were used for identification of β-carotene pigment and peak resolution was confirmed by peak

purity feature of the diode array detection. β-carotene was quantified from the calibration curve according to the following formula:

β-carotene concentration (µg/g) = A x volume (mL)/∝/ sample weight (g)

A = Peak area, volume (volume of petroleum ether extract), ∝ = regression coefficient from standard (calibration) curve.

3.6 Sensory Evaluation

Developed samples of preparation method 2 and 3 were subjected to sensory evaluation using a 5 point hedonic scale ranging from dislike very much to like very much (Appendix 24). Thirty panelist members were selected randomly within International Institute of Tropical Agriculture (IITA) compound to perform the test. All evaluation sessions were held in the canteen of International Institute of Tropical Agriculture. All samples were presented before the panelists in the canteen under normal lighting conditions in white disposable plastic plates and coded with three-digit numbers. Spoons were provided to the panelists and drinking water was provided for oral rinsing. The samples attributes assed were color, texture, taste, aroma and overall general acceptability.

3.7 Statistical Data Analysis

Data obtained from the results of proximate analyses, cyanogenic level, vitamin C, β carotene and sensory were subjected to statistical analysis, using STATA 13 (Data Analysis and Statistical Software). Data were analyzed by descriptive analysis and Multiple ways (5 cassava varieties, preparation methods with three levels namely preparation methods 1, 2 and 3) analysis of variance (ANOVA) was applied in

proximate, cyanogenic, vitamin C and β carotene after assuming the normal distribution of the data (Appendix 1-17). For sensory evaluation 5 cassava varieties, preparation methods with two levels namely preparation methods 2 and 3) analysis of variance (ANOVA) was applied. Means and standard deviation were determined, two and three way ANOVA was used to determine the significant difference. The means were separated by LSD (Least significant difference) and t test where appropriate. The treatments were judged statistically significantly different at $p< 0.05$.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 Cassava varieties and preparation methods

From the cross sectional survey conducted at Mkuranga and Rufiji Districts, 74% of all interviewed respondents were female (Table 1). This suggests that female constitute the majority of farmers engaged in cassava production in the study area, this may be due to the fact that female headed households allocates a larger proportion of their food budget to cassava leaves than male headed households.

Table 1: Distribution of Respondents by gender and variety grown

Respondents characteristics	Frequency	Percent
Female	37	74
Male	13	26
Total	**50**	**100**
Variety grown		
Mpira	**15**	**30**
Kizimbani	**1**	**2**
Cheupe	**2**	**4**
Nyamkagile	**4**	**8**
Kiroba	**28**	**56**
Total	**50**	**100**

Cassava leaves from Kiroba variety is mostly grown by farmers (56%) than the other varieties (Mpira 30%, Nyamkagile 8%, Cheupe 4% and Kizimbani 2%) as shown in Table 1. Farmers were using three traditional preparation methods (identified as preparation method 1, 2 and 3) as discussed under section 4.3.

4.2 Cassava varieties and Preparation Methods

Among respondents, all respondent were using preparation method 1 and while more than 52% preferred both method 1 and method 2 and likewise the rest (48%) preferred the cassava leaves prepared by method 1 as well as method 3 as indicated in Table 2 below.

Table 2: Respondents Distribution by their interest with Preparation Methods and Variety Preference

Preparation Method	Percent
Preparation Method 1	50
Preparation Method 2	52
Preparation Method 3	48
Variety	
Mpira	20
Kizimbani	20
Cheupe	20
Nyamkagile	18
Kiroba	22

Frequencies are based on multiple responses

On the other hand Kizimbani, Cheupe and Mpira varieties were equally prefered by respondents while 18% of respondents prefered cassava leaves from Nyamkagile and 22% preferred Kiroba varieties (Table 2).

4.3 Proximate Components of Prepared Cassava Leaves

The results of effect of preparation methods on the proximate components in cassava leaves samples are presented and discussed in Table 4.

4.3.1 Moisture Content

The moisture content in cassava leaves was between 79.75% in Nyamkagile variety and 82.40 % in Mpira variety (Table 3). This indicates that the moisture content did

differ significantly among varieties at $p<0.05$.The moisture content of cassava leaves prepared with method 2 was significantly higher (84.11%) followed by method 3 (83.54%) and method 1 (75.24%) as depicted in Table 4. Also the mean moisture content of cassava leaves between Mkuranga (81.29%) and Rufiji (80.64%) did not differ significantly (Appendix 2).

The food moisture content is used as indicator of its shelf life, where by the higher moisture content the shorter shelf life (Fellows, 2000). Higher moisture content on Preparation method 2 and method 3 suggests that the cassava leaves will have a short shelf life compared to cassava leaves prepared with method 1. Cassava leaves moisture content is affected by location, variety and preparation methods ($p<0.05$) as indicated in Appendix 1. Preparation methods had effect on moisture content, as there were changes observed (increase) in moisture content of cassava leaves prepared with method 2 and 3 compared to preparation method 1.

Table 3: Proximate components (%) of cassava leaves varieties

Variety	Proximate Components (%) DM					
	Moisture	**Protein**	**Ash**	**Crude fiber**	**Crude fat**	**Carbohydrate**
Mpira	82.40[a]	31.54[a]	8.57[a]	20.14[a]	3.65[a]	36.10[a]
Kizimbani	80.66[b]	31.78[b]	9.42[b]	18.85[a]	3.91[b]	36.04[a]
Nyamkagile	79.75[b]	30.84[c]	10.48[c]	19.49[a]	3.57[a]	35.61[a]
Kiroba	80.17[a]	31.43[a]	9.65[b]	21.16[b]	3.62[a]	34.14[b]
Cheupe	81.82[c]	31.24[a]	7.85[d]	25.13[c]	3.60[a]	32.17[c]

Means within the same column superscripted by the same letter are not significantly different following separation by DMRT at the 5% level

Table 4: Effect of cassava leaves preparation methods in proximate components (%)

Method	Proximate Component (%) DM					
	Moisture	Protein	Ash	Crude fiber	Crude fat	Carbohydrate
Preparation Method 1	75.24[a]	31.508[a]	5.77[a]	22.97[a]	3.57[a]	36.19[a]
Preparation Method 2	84.11[b]	30.967[b]	10.73[b]	20.24[b]	3.87[b]	34.18[b]
Preparation Method 3	83.54[c]	31.629[a]	11.08[c]	19.65[b]	3.57[a]	34.07[b]

Means within the same column superscripted by the same letter are not significantly different following separation by DMRT at the 5% level

The finding agrees with those of previous researchers (Julie *et al*., 2009; Wobeto *et al*., 2006) who reported that the average moisture content for different varieties of cassava leaves were 77.7%, 70.46% and 76.7%.

4.3.2 Protein Content

The protein content of prepared cassava leaves for Kizimbani variety was significantly higher (31.78%) DM as indicated in Table 3 compared to other varieties under the study. However, Mpira, Kiroba and Cheupe varieties did not differ significantly ($p<0.05$).

The results for protein content is closer to Awoyinka *et al*. (1995) who reported a range of crude protein between 29.3% and 32.4% compared to a conventional vegetable. In this study it was observed that, preparation methods had no effect on protein content as the protein content was the same irrespective of the preparation methods used (Table 4).

However, results further indicated that preparation method 3 had significant higher protein content compared to preparation method 1 at $p < 0.05$ level of significant. The protein content between preparation method 1 and preparation method 3 did not differ significantly ($p<0.05$). The results further indicates that, the protein content of cassava leaves in Mkuranga is not different from the protein content of leaves in Rufiji at $p<0.05$ level of significance (Appendix 4).

However, the observed variation of protein content in cassava leaves were consistent with other studies (Ravindran, 1992; Ayodeji, 2005; Nagib and Antonio, 2005). According to these researchers, this wide variability was attributed to differences in cultivars, stage of maturity, sampling procedure, soil fertility and climate. The findings are consistent with a report by Fasuyi (2005) who pointed out that the wide variability protein in leaves content is related to cultivars, preparation methods and climate differences.

4.3.3 Ash Content

The ash content of processed cassava leaves for the five varieties in the two locations with the three preparation methods was found to be (10.48%) DM for Nyamkagile variety which was significantly higher at $p <0.05$ level compared to other varieties under the study, while the ash content for Kiroba (9.65%) DM and Kizimbani (9.42%) DM did not differ statistically at $p <0.05$ level of significance (Table 3).

As indicated in Table 4, preparation method 3 had significant higher ash content (11.08%) DM compared to preparation method 2, (10.73%) DM and preparation method 1 (5.77%) DM at $p<0.05$ level of significance. From the results obtained,

preparation methods had effect on ash content of cassava leaves, the ash content of the cassava varieties used were also significantly ($p<0.05$) by the 3 preparation methods used. Once again the preparation method 2 and 3 significantly increase the ash content of the samples.

The findings agrees with Awoyinka *et al*., (1995) who reported that ash content was 4.6 %–6.4% in cassava leaf samples and Ravindran, (1992) who reported that ash content of processed cassava leaves was 5.7 % - 12.5%. However, the observed variation of ash content in cassava leaves is consistent with other studies (Ayodeji, 2005; Bui *et al*., 1996).

4.3.4 Crude Fiber

Crude fiber content for the five varieties of cassava leaves in the two locations with the three preparation methods was found to be (25.13%) DM for Cheupe variety was significantly higher than Kiroba variety (21.16%) DM, while the mean crude fiber content for Mpira was (20.14%) DM, Nyamkagile (19.49%) DM and Kizimbani (18.85%) DM varieties did not differ significantly ($p<0.05$) as indicated in Table 3. The crude fiber comparison by preparation methods indicated that, preparation method 1 gave significant higher crude fiber content (22.97%) DM at $p <0.05$ level compared to other methods. However, the crude fiber content between preparation method 2 and preparation method 3 did not differ significantly ($p<0.05$) as depicted in Table 4. Thus, the data provide evidence to conclude that preparation method had effect on crude fiber content, as preparation method 2 and 3 slightly decrease the fiber content of cassava leaves.

Following the result obtained, the crude fiber content of cassava leaves in Mkuranga (19.47%) DM and Rufiji (22.44%) DM were different. Cassava leaves in Rufiji had higher mean crude fiber content than Mkuranga at $p < 0.05$ level of significance. Other researchers (Awoyinka *et al*., 1995; Ravindran, 1992; Ayodeji, 2005) have documented mean crude fiber content between 26.9% and 39%.

4.3.5 Crude Fat

Crude fat content of cassava leaves for Kizimbani variety (3.91%) DM was significantly different and higher than the crude fat content for other varieties ($p<0.05$). The test further indicates that the crude fat content did not differ significantly among the four varieties of Mpira, Kiroba, Cheupe and Nyamkagile at $p<0.05$ level as indicated in Table 3.

For preparation methods indicated that the crude fat content for preparation method 2 was significantly higher (3.87%) DM compared to other methods. However, the crude fat content for the two preparation methods; preparation method 3 and preparation method 1 did not show significant difference at $p < 0.05$ level (Table 4). Thus, the data do provide evidence to conclude that preparation methods had no effect on crude fat content, crude fiber content will remain the same irrespective of the methods used. The crude fat content of cassava leaves in the two study was somehow similar between Rufiji (3.56%) DM and Mkuranga (3.78%) DM as indicated in Appendix 9. The crude fat content of cassava leaves revealed in this study is closer to Ravindran (1992) who reported an average of 5.5% crude fat and Ayodeji (2005) who reported an average of 70g/kg DM crude fat.

4.3.6 Carbohydrate

The carbohydrate content for the five varieties in the two locations with the three preparation methods was found to be (36.1%) DM for Mpira variety which was significantly higher as indicated in Table 3 at $p<0.05$ compared to other varieties under investigation. Despite the higher carbohydrates content for Mpira variety, the finding indicated that carbohydrates content did not differ significantly between Cheupe, Kizimbani and Nyamkagile varieties at $p<0.05$ (Table 3). The results indicates that the carbohydrate content on cassava leaves is influenced by variety ($p<0.05$), preparation methods ($p<0.05$) and location ($p<0.05$) (Appendix 10).

As indicated in Table 4, preparation method 1 of cassava leaves had significant higher carbohydrates content (36.19%) DM compared to other methods ($p<0.05$). The test further indicated that the carbohydrate content did not differ significantly between preparation method 2 and preparation method 3 at $p<0.05$ level. Preparation method had effect on carbohydrates content of cassava leaves, as preparation method 2 and 3 reduce the carbohydrates content.

The carbohydrates content in cassava leaves for the two locations under the study were different. The cassava leaves in Mkuranga contained higher carbohydrates in comparison to Rufiji. The finding has revealed higher value of carbohydrates content in cassava leaves compared to the findings by Oresegun *et al*. (2016) who reported that cassava leaves from six varieties had values ranging from 27.31 % to 38.71%.

4.4 Cyanide Content

Cyanide content for kiroba variety was (185.51mgHCN/kg) DM, it has the highest cyanide content compared to other varieties (Table 5). All variety had significantly greater cyanide content for preparation method 1. The mean comparison by cassava leaves preparation methods indicated that at the $p < 0.05$ level, preparation method 1 (376.31mgHCN/kg) DM has significantly higher cyanide content than other methods (Table 6). However, preparation method had effect on cyanide content, as preparation method 2 and preparation method 3 were significantly different in reducing the cyanide content to relatively low level (Table 6). These level are below and above the recommended level (10mgHCN/kg) for foods, in which were safe with regard to cyanide toxicity based on the fact that the vegetable was served in small quantities as side food. The mean cyanide content in cassava leaves for the two locations under the study did not differ (Appendix 13).

Studies by Umuhozariho *et al.* (2011) have reported the cyanide content in cassava leaves from three species that ranged from 32 - 50 mg HCN/kg (dry matter basis) after boiling for 30 min. Whereby the levels of cyanide in fresh leaves in the studied species were 1905, 1480 and 2179 mg HCN/kg respectively, for bitter, sweet and wild. Other studies (Fukuba *et al*., (1982) have reported the values ranging from 189 to 2466 mg HCN/kg fresh weight. These finding are related with those reported by Ayodeji (2005) who reported 52.9 mgHCN/100g average cyanide in the leaves and ranging from 40.2 mg HCN/100g to 60.6 mg HCN/100g.

Table 5: Cassava leaves varietal differences in Cyanide, β carotene and Vitamin C on Dry Matter Basis

Variety	Analyte beyond proximate components		
	Cyanide(mg/HCN kg),	β carotene(µg/100g)	Vitamin C(mg/100g)
Mpira	145.46[a]	516.68a	0.83[a]
Kizimbani	178.62[b]	842.64[b]	1.01[b]
Nyamkagile	54.17[c]	1128.61[c]	0.77[c]
Kiroba	185.51[d]	898.04[d]	0.90[a]
Cheupe	100.16[e]	1333.15[e]	0.78[d]

Means within the same column superscripted by the same letter are not significantly different following separation by DMRT at the 5% level

Table 6: Cassava leaves Cyanide content (mg/HCNkg), β carotene (µg/100g) and Vitamin C (mg/100g), mean separation test by preparation methods

Method	Cyanide	β carotene	Vitamin C
Preparation Method 1	376.31[a]	1359.24[a]	1.10[a]
Preparation Method 2	12.37[b]	582.14[b]	0.74[a]
Preparation Method 3	9.68[c]	890.09[c]	0.73[b]

Means within the same column superscripted by the same letter are not significantly different following separation by DMRT at the 5% level

4.5 β Carotene Content

The β carotene content among the five varieties at the $p < 0.05$ level, Cheupe variety had significantly greater β carotene content compared to other varieties ($p<0.05$) as depicted in Table 5. The mean comparison by preparation methods of cassava leaves indicated that at the $p<0.05$ level, preparation method had effect on β carotene content, as preparation method 1 (1359.24 µg/100g) DM had significantly higher β carotene content than other methods (Table 6).

However, processing method 2 and processing method 3 were significantly different in reducing the β carotene content to relatively moderate level (Table 6). Variation in β carotene content may be due to the difference in the enzymatic oxidation during preparation, occurrence of less values could be due to leaching of vitamin A precursor as in thermo chemical reaction occurring during boiling times (Vimala *et al.,* 2011).

Moreover, β carotene content was found to be higher in Rufiji (1129.4 µg/100g) DM than in Mkuranga (758.2 µg/100g) DM at p (0.05) level of significance (Appendix 15). These results were higher compared to the findings of by Umuhozariho *et al.* (2011), who reported the values of β-carotene ranging from 406 to 804 mg/kg and that of Oresegun *et al. (*2016), who reported a range of 298 to 816.82 µg/100g. However, Priadi *et al.,* (2009), who reported the lowest content of β-carotene of 298.95 ppm and the highest was 517.72 ppm.

4.6 Vitamin C Content

Vitamin C content was significantly higher in Kizimbani (1.0 mg/100g) DM than other varieties. However, Vitamin C content in Kiroba and Mpira varieties did not differ significantly at $p < 0.05$ level (Table 5).

On the contrary vitamin C was reduced to extremely low values by all preparation methods, changes was observed during preparation method 2 and 3.The concentration of ascorbic acid (the predominant form of vitamin C) in green vegetable generally was decreased after boiling. Water and heat are known destructive agents for vitamin

C in vegetables, this means that boiling represents the biggest threat of vitamin loss, because it uses both water and heat.

Lower vitamin C levels were found in this study, when compared to Wobeto *et al.* (2006) who, analyzing cassava leaves at three plant ages and found levels of 55.72 and 64.12 mg/100 g of dry matter (DM) respectively.

The mean comparison by methods of cassava leaves preparation indicated at the $p < 0.05$ level, Preparation method 1 (1.10 mg/100g) DM has significantly higher vitamin C content than other methods (Table 6) although, vitamin C content did not differ significantly between Preparation method 1 and Preparation method 3 at $p< 0.05$ level. Moreover, vitamin C content was found to be higher in Mkuranga (0.94mg/100g) DM than in Rufiji (0.78mg/100g) DM at 5% level of significance (Appendix17).

4.7 Sensory Evaluation

4.7.1 Effect of varieties and preparation methods on sensory attributes

The effect of varieties and two preparation methods on sensory attributes (colour, taste, aroma, texture and general acceptability) are presented and discussed.

4.7.1.1 Colour

Variety and preparation methods had no significantly effect on colour (Table 7), Even though the colour of Nyamkagile variety was mostly liked by the panelist while kiroba was least likely by the panelist (Table 7). The colour of cassava leaves from

preparation method 2 was most preferred by the panelist compared to the preparation method 3 (Table 8). The colour of the cassava leaves will remain the same irrespective of any variety or preparation methods were used.

4.7.1.2 Taste

The implication of the findings is that, the taste of cassava leaves is not affected by varieties and preparation methods, it have no significantly at $p< 0.05$ (Table 7). Thus, taste will remained the same irrespective of the cassava variety and preparation method used.

The taste of nyamkagile variety was mostly liked by the panelist compared to other variety under the study (Table 7). The interaction between variety and preparation method was significantly at $p<0.05$ (Table 9), nyamkagile and preparation method 2 had more significantly effect on taste while kiroba and preparation method 3 had least significant on taste.

4.7.1.3 Aroma

Variety had no effect on aroma (Table 7), preparation methods had significantly different at $p<0.05$, cassava leaves prepared by method 2 had more effect on aroma than cassava leaves prepared by method 3 (Table 8). The interaction of variety and preparation method had significantly different at $p<0.05$, nyamkagile variety and method 2 had more effect on aroma compared to other interactions between variety and preparation method (Table 10).

4.7.1.4 Texture

Cassava variety had significantly different on texture at $p<0.05$, the texture of cassava leaves will be changed depends on variety used, nyamkagile variety was mostly liked

by the panelist while mpira and kiroba was the least liked by the panelist (Table 7). Preparation methods had no effect on texture, the texture of the cassava leaves will remain the same irrespective of the preparation methods used (Table 8). The texture of cassava leaves from preparation method 2 was most preferred by the panelist compared to the preparation method 3.

4.7.1.5 General Acceptability

Variety and preparation methods have no significantly effect on general acceptability, the general acceptability will remain the same irrespective of any variety or preparation methods were used. Kizimbani variety was mostly acceptable by the panelist while kiroba was least acceptable by the panelist (Table 7). Cassava leaves from preparation method 2 was most acceptable by the panelist compared to the preparation method 3 (Table 8).

The finding of this study relates to results obtained by (Umuhozariho *et al.,* 2013), who did sensory evaluation of different preparations of cassava leaves from three species as a leafy vegetable.

Table 7: Means for sensory characteristics for leaves from different cassava varieties

Parameter		Sensory attributes and their scores				
		Colour	**Taste**	**Aroma**	**Texture**	**General Acceptability**
Variety	Kizimbani	4.03[a]	3.91[a]	3.78[a]	3.76[a]	3.93[a]
	Kiroba	3.80[a]	3.61[a]	3.60[a]	3.36[b]	3.60[a]
	Mpira	3.85[a]	3.68[a]	3.55[a]	3.36[b]	3.70[a]
	Cheupe	3.91[a]	3.91[a]	3.81[a]	3.55[ab]	3.81[a]
	Nyamkagile	4.21[a]	4.01[a]	3.71[a]	3.85[a]	3.91[a]

For each sensory characteristics, within column, values with the same letter were not statistically significant ($p<0.05$)

Table 8: Means for sensory characteristics for leaves from different preparation methods.

Parameter		Sensory attributes and their scores				
		Colour	**Taste**	**Aroma**	**Texture**	**General Acceptability**
Preparation Methods	Preparation Method 2	3.98[a]	3.92[a]	3.82[a]	3.61[b]	3.87[a]
	Preparation Method 3	3.94[a]	3.74[a]	3.56[b]	3.54[b]	3.71[a]

For each sensory characteristics, within column, values with the same letter were not statistically significant following and unpaired t test ($p<0.05$)

Table 9: Methods and variety interaction on Taste

Variety	**Method**	
	2	3
Kizimbani	4.06[ab]	3.76[bc]
Kiroba	3.76[bc]	3.46[c]
Mpira	3.46[c]	3.90[bc]
Cheupe	3.86[bc]	3.96[abc]
Nyamkagile	4.43[a]	3.60[bc]

Means within the table superscripted with the same lower case letter where not statistically significant ($p<0.05$)

Table 10: Methods and variety interaction on Aroma

Variety	**Method**	
	2	3
Kizimbani	3.96[a]	3.60[abc]
Kiroba	3.80[abc]	3.40[bc]
Mpira	3.33[c]	3.76[abc]
Cheupe	3.90[ab]	3.73[abc]
Nyamkagile	4.13[a]	3.30[c]

Means within the table superscripted with the same lower case letter where not statistically significant ($p<0.05$)

CHAPTER FIVE

5.0 CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

From the cross sectional survey conducted at Mkuranga and Rufiji Districts, it is possible to conclude that; Kiroba variety is mostly grown by farmers than other varieties. Its high preference is probably attributed to its being tasty and culminates to high yields.

In comparison of proximate composition based on variety showed that, Mpira variety was found to contain higher moisture content than other varieties. While Kizimbani variety was found to contain higher protein than other varieties. Nyakamgile contained significantly higher ash content compared to other varieties. Crude fiber was significantly higher in Cheupe than other varieties. On the other hand, Crude fat was higher in Kizimbani variety than other varieties. While Mpira variety contained significantly higher carbohydrate compared to other varieties under the study. The cyanide content on cassava leaves was found to be higher in Kiroba and lower in Nyamkagile variety. Cheupe variety has significantly greater β carotene content compared to other varieties while vitamin C content was significantly higher in Kizimbani in comparison to other varieties.

Also this review is intended to reduce the uncertainly about how the three preparation methods affect the proximate composition, β carotene, vitamin C and cyanogenic glycosides of prepared cassava leaves. According to this review, it is possible to conclude that, the increased in moisture content and ash content of prepared cassava

leaves was observed in preparation method 2 and 3.No changes was observed in protein content and fat content, The cyanide content was reduced in preparation method 2 and 3,hence this methods of preparation was advisable. Slightly reduction of crude fiber, carbohydrates content, β carotene content and vitamin C was observed during preparation method 2 and 3.

Based on the sensory evaluation, variety and preparation methods had no effect on colour, taste, aroma and general acceptability. The taste and colour of Nyamkagile variety was mostly preferred by the panelists while Kizimbani variety was mostly acceptable by the panelist. Preparation method 2 had more effect on aroma compared to other cassava leaves prepared with method 3.The texture of cassava leaves was affected by the variety used while it will remain the same irrespectively of any preparation method used. However, the interaction effect among cassava leaves varieties and preparation methods, was found to have a significant effect on the cassava leaves aroma and taste.

As preparation methods of cassava leaves eliminate numerous anti-nutrients while either maintaining or improving kisamvu, its food value, there is therefore a need to ensure that boiling practices maximize nutrient retention in cassava leaves. It is obvious from this study that cassava leaves are endowed with enormous contribution to the world food supplies and promote food security, especially in developing countries. Hence the use of cassava leaves as a readily available food source.

5.2 Recommendations

Pounding cassava leaves followed by preparation described under method 2 and 3 is a more effective method for cyanide reduction. However, considering the small quantities by serving of green vegetables as side food.

Cassava varieties with low levels of cyanide in leaves should be released for use as a vegetable for human consumption with a view of alleviating micronutrient deficiencies, notably β-carotene and iron.

Farmers needs to be sensitized on consumption of cassava leaves from Kizimbani variety due to its higher cassava leaves protein contents. From nutrition point of view, protein content is of interest and cassava leaves are affordable by poor people whose access to protein rich foods such as milk, meat and fish is difficult, the leaves can be helpful. However, time of boiling (methods of preparation) should be extended to improve reduction of cyanide level in cassava leaves relishes.

Nevertheless vitamin C is very low in cooked cassava leaves subjected to the described preparation methods, therefore a complement vitamin C rich food is necessary to accompany cassava leaves meal.

REFERENCES

ACGIH (American Conference of Governmental Industrial Hygienists). 1996. Acetone Cyanohydrin. Supplements to the Sixth Edition Documentation of the Threshold Limit Values and Biological Exposure Indices. American Conference of Governmental Industrial Hygienists, Cincinnati, OH.pp.1-6

Achidi, A. U., Olufunmike, A. A, Dixon, B. M. and Bokonga. M. (2008). The effect of processing on the nutrient content of cassava (*Manihotesculenta*crantz). *Journal of Food Processing and Preservation* 32: 486 – 502.

Adewusi, S. R. A. and Bradbury, J. H. (1993).Carotenoid in cassava: comparison of open column and HPLC methods of analysis. *Journal of the Science of Food and Agriculture* 62: 375 – *3*83.

Akinwale, M. G., Aladesanwa, R. D., Akinyele, B. O., Dixon, A. G. O. and Odeyi, A. C. (2010). Inheritance of β-carotene in cassava (*Manihotesculenta*crantz). *International Journal of Genetic Molecular Biology* 2(10): 198 – 201.

Alves, A. A. C. (2002). Botany and physiology. In: Cassava: biology, production and utilization. (Edited by Hillocks, R. J., Thresh, J. M. and Belotti, A. C.), Commonwealth for Agriculture Bureau International, Wallingford, London. pp. 67 – 89.

Amey, M. A. (1987). Some traditional methods of Cassava conservation and processing in Uganda. *Paper Presented at the Third East and Southern Africa Crops Workshop,* 7 - 11 December 1987, Mzuzu, Malawi. 7pp.

AOAC (1990). *Official Methods of Analysis of the Association of Official Analytical Chemists.* (15th Ed.), Association of Official Analytical Chemists, Arlington, pp. 1058 – 1059.

AOAC (1995). *Official Methods of Analysis.* (16th Edition), Association of Official Analytical Chemists, Washington DC.

AOAC (2000). *Official Methods of Analysis.* (17th Edition), Association of Official Analytical Chemists, Gaithersburg.

Awoyinka, A. F. Abegunde, V. O. and Adewusi, S. R. A. (1995). Nutrient content of young cassava leavesand assessment of their acceptance as a green vegetable in Nigeria. *Plant Foods Hum NutrDordrecht* 47: 21 – 28.

Ayodeji, O. F. (2005). Nutrient composition and processing effects on cassava leaf (*Manihotesculenta*, Crantz). *Pakistan Journal of Nutrition* 4(1): 37 – 42.

Bokanga, M. (1994). Processing of cassava leaves for human consumption. Acta Horticulturae 375: 203–207.

Bradbury, J. H., Egan, S. M. and Lynch, M. J. (1991). Analysis of cyanide in cassava using acid hydrolysis of cyanogenic glucosides. *Journal of Science Food Agriculture* 55: 277 – 290.

Bui HuyNhuPhuc, P. T. R., Ogle, B. and Lindberg, J. E. (1996). The nutritive value of sun-dried and ensiled cassava leaves for growing pigs. *Livestock Research for Rural Development* 8(3): 26- 33

Buitrago, J., Ospina, B., Gil, J. L. and Aparicio, H. (2002). Cassava root and leaf meal as the main ingredient in poultry feeding: Some experiences in Colombia. In: (Edited by Howeler, R. H.). *Cassava Research and Development in Asia: Exploring New Opportunities for and Ancient Crop 7th Regional Cassava Workshop,* October 28 – November 1, 2002 Bangkok, Thailand. 523-541.

Cassava Cyanide Diseases and Neurolathyrism Network CCDN (2007). Working together to eliminate cyanide poisoning, konzo and Tropical Ataxic Neuropathy (TAN) Issue No. 9. 4 pp.

Cliff, J., Muquingue, H., Nhassico, D., Nzwalo, H. and Bradbury, J. H. (2011). Konzo and neurolathyrism: similarities and dissimilarities between these crippling neurodegenerative diseases. *Toxic* 49(3): 631 – 635.

Cooke, R. D. (1978). An enzymatic assay for the total cyanide content of cassava. *Journal of the Science of Food and Agriculture* 29: 345 – 352.

Delange, F., Ekpechi, L. O. and Rosling, H. (1994).Cassava cyanogenesis and iodine deficiency disorders. *ActaHorticulturae* 375: 289 – 293.

El-Sharkawy, M. A. *(*2003). Cassava biology and physiology. *Plant Molecular Biology* 53*:* 621 – *6*41.

Emmanuel, O. A., Clement, A., Agnes, S. B., Chiwona-Karltun, L. and Drinah, B. N. (2012). Chemical composition and cyanogenic potential of traditional and high yielding CMD resistant cassava (Manihot esculenta Crantz) varieties, International Food Research Journal 19(1): 175-181

Ernesto, M., Cardoso, A. P., Nicala, D., Mirione, E., Massaza, F. and Cliff, J. (2002). Persistent konzo and cyanogen toxicity from cassava in Northern Mozambique. *Acta Tropical* 82: 357 – 362.

Essers, A. A., Bosvel, M., Van der Grift, R. and Voragen, A. (1993). Studies on the quantification of specific cyanogens in cassava products and introduction of a new chromogen.*Journal of the Science of Food and Agriculture* 63: 287 – 296.

Etonihu, A. C., Olajubu, O., Ekanem, E. O. and Bako, S. S. (2011). Titrimetric evaluation of cyanogens in parts of some Nigerian cassava species. *Pakistan Journal of Nutrition* 10(3): 260 – 263.

Faber, M. and Van Jaarsveld, P. J. (2007). The production of pro vitamin A-rich vegetables in home gardens as a means of addressing vitamin A deficiency in rural African communities. *Journal of Science Food Agriculture* 87: 366 – 377.

FAO (2012). FAOSTAT agricultural data database. Rome, Italy. [http://faostat.fao.org/ site/609/default.aspx#ancor] site visited on 9/6/2015.

FAO, IFAD and WFP (2015), the State of Food Insecurity in the World 2015. Meeting the 2015 international hunger targets: taking stock of uneven progress, Food and Agriculture Organization. Publications, Rome.

FAO/WHO (1973). Energy and protein requirements: Report of a joint FAO/WHO ad hoc expert committee. *Teacher Rep Series* 522: 1–118.

FAO/WHO *(*1991). *Food Standards Programme*. Food and Agriculture Organization, Rome, Italy. 292 pp.

Fasuyi, A. O. (2005). Nutrient composition and processing effects on cassava leaf (*Manihotesculenta*, Crantz) antinutrients. *Pakistan Journal of Nutrition* 4*:* 37 – 42.

Fellows, P. J. (2000). *Food Processing Technology*, Principles and Practice. Wood Head Publishing, Cambridge. 591pp.

Fukuba, H., Igarashi, O., Briones, C. M. and Mendoza, E. M. T. (1982). Determination and detoxification of cyanide in cassava and cassava products. *Journal of Crop Science* 7(3): 170 – 175.

Hahn, S. K. and Keyser, J. (1985). Cassava: A basic food of Africa. *Outlook on Agriculture* 4: 95 – 100.

Hahn, S. K. Reynolds, L and Egbunike, G. N. (1992). Cassava as livestock feed in Africa. Proc. IITA/ILCA/Univ. of Ibadan Workshop on the Potential Utilization of Cassava as Livestock Feed in Africa, 14-18 November 1988, Ibadan, Nigeria.

Hahn,S.K. Mahungu, N.M., Yamaguchi Y, and Almazan,A.M. (1987). Reduction of cyanide during processing of cassava to some traditional African foods. Journal of Food and Agriculture 1: 11 - 15.

Hue, K. T., Van, D. T. T., Ledin, I., Spörndly, E. and Wredle, E. (2012). Effect of harvestingfrequency,variety and leaf maturity on nutrient composition,hydrogen cyanide content and cassava foliage yield. *Livestock Science* 2012: 1691–1700.

International Institute of Tropical Agriculture (2009). -Cassava. [http://www .iita.org/cassava] site visited on 20/6/2015.

Jacquot R. *(*1957). *The Food efficicacy factors. In:* Tropical Food and Nutrition. *Vol 1, AO editions. Rome , Italy : FAO.*

Julie, A. M., Christopher, R. D. and Sherry, A. T. (2009).Nutritional Value of Cassava for Use as a Staple Food and Recent Advances for Improvement.

Kapinga, R. (1996). *Multiplication and Distribution of Clean Healthy Planting Materials of Cassava: Experiences from Tanzania.* Ukiriguru Research and Training Institute Lake Zone. Mwanza, Tanzania. 15pp.

Kutner, M. H., Nachtsheim, C. N., Neter, J. and Li, W. (2005). Applied linear statistical models. (4^{th} Edition), Mc Graw-Hill.56pp.

Lambert, J. L., Ramasamy, J., and Paukstelis, J. V. (1975). Stable reagents for the colorimetric determination of cyanide by modified Konig reactions. *Analytical Chemistry* 47: 916 – 918.

Lancaster, P. A. and Brooks, J. E. (1983). Cassava leaves as human food. *Economic Botany* 37(3): 331 – 348.

Laucess, H. T. and Heyman, H. (2010).*Sensory Evaluation of Food.Principal and Practices.* Second Edition17pp.

Lee, S. K., Rhee, J. S. and Yum, H. S. (2012). Cyanide poisoning deaths detected at the national forensic service headquarters in seoul of Korea: a six year survey (2005~2010). *Toxicological Research*. 28(3):195-9.

Longe, O. G. (1980). Effect of processing on the chemical composition and energy value of cassava. 21: 819 – 829.

Mlingi, N. V., Assey, V. D., Swai, A. B. M., Mclarty, D. G., Karlen, H. and Rosling, H., (1993): Determinants of cyanide exposure from cassava in a konzo-affected population in northern Tanzania. International Journal of Food Sciences & Nutrition 44(2): 137-144.

Mlingi, N.L.V. (1995). Cassava Processing and Dietary Cyanide Exposure in Tanzania,vol. 571. Acta Universitatis Uppsaliensis, Uppsala, p. 69.

Montagnac, J. A., Davis, C.R., and Tanumihardjo, S.A. (2009). Nutritional value of cassava for use as a staple food and recent advances for improvement Comprehensive Reviews in Food Science and Food Safety, 8 (3), pp. 181-194.

Montaldo, A. (1977).Whole plant utilization of cassava for animal feed. In: *Cassava as Animal Feed*. (Edited by Nestel, B. and Graham, M.). International Research Development Centre, Ottawa. pp. 95 – 106.

Msabaha, M. A. M., Kepakepa, V. M. and Laswai, H. S. M. (1986). Cassava production and consumption in the United Republic of Tanzania. *Paper Presented at the International Institute of tropical agriculture Meeting,* Morogoro, Tanzania. 65pp.

Mulokozi, G., Mugyabuso, J. and Modaha, F. (2007). Potential of cassava and sweet potato leaves to contribute to the vitamin A requirements. In: *Proceedings of the 13th* International Society for Tropical Root Crops *(ISTRC) symposium*, Arusha, Tanzania. pp. 755 – 762.

Nagib, M. A. N. and Antonio, O. M. (2005). Cassava Leaves as a Source of protein. *Depatamento de Geneica e Morfologia and Departamento de Nutricao respectivelyUniversidade de Brasília*, Brasília, Brazil. *Journal of Food, Agriculture and Environment Vol.4 (1):187-1888.*

Nambisan B. (1994). Evaluation of the effect of various processing techniques on cyanogen content reduction in cassava. *ActaHortic* 375*:* 193–201.

Nhassico, D., Muquingue, H., Cliff, J., Cumbana, A. and Bradbury, J. H. (2008). Rising African cassava cyanide initake and controle measures. *Journal Science Food Agriculture* 88: 2043 – 2049.

Normanha, E. S. (1962). Meals of stalks and leaves of cassava. *Agro-nomica Venezuela* 14: 16 – 19.

Numfor, F. A. and P. Ay (1987). *Postharvest Technologies of Root and Tuber Crops in Cameroon: A Survey. Memoires Ettravaux*. Cameroon. 54pp.

O'Brien, G. M. O., Taylor, A. J. and Poulter, N. H. (1991). Improved enzymic assay for cyanogens in fresh and processed cassava. *Journal of the Science of Food and Agriculture* 56: 277 – 289.

Oguche, Gladys, H. E. (2011). Effect of drying methods on chemical composition of Spinach "Aieifo" (Amaranthus aquatic), and Pumpkin leaf (Telfairiaoccidentalis) and their soup meals. *Pakistan Journal Nutrition* 10 (11): 1061 – 1065.

Okigbo, B. N. (1980). Nutritional implications of projects giving high priority to the production of staples of low nutritive quality. In the case for cassava (*Manihotesculenta*, Crantz) in the humid tropics of West Africa. *Food Nutrition Bulletin* 2:1–10.

Oresegun, A., Fagbenro, A., Ilona, P. and Bernard,E (2016). Nutritional anti nutritional composition of cassava leaf protein concentrate from six cassava varieties for use in aqua feed. *Cogen Food and Agriculture* 2: 147 – 323.

Polsen, R. A. and Spencer, D. S. C. (1991). The technology adoption process in subsistence agriculture: The case for cassava in Southwestern. *Nigeria Agriculture System* 36: 65 – 78.

Priadi, D., Permana, D. R., Dona. S. E., Hartati. S. and Sudarmonowati, E. (2009). Selection of Indonesia cassava (*Manihotesculenta* Crantz) genotype as source of β-carotene. *Biodiversitas* 10(1): 6 – 11.

Ravindran, V. (1992). Preparation of cassava leaf products and their use as animal feed. Roots, tubers, plantains and bananas in animal feeding. *Animal Production and Health* 95: 111 – 122.

Ravindran, V. (1993).Cassava leaves as animal feed: Potential and limitations. *Journal of the Science of Food Agriculture* 61(2):141–150.Web. http://dx.doi.org/10.1002/jsfa.

Ravindran, V. and Rajaguru, A. S. B. (1988). Effect of stem pruning on cassava root yield and leaf growth Sri Lankan. *Journal of Agricultural Science* 25(2): 32 – 37.

Rock, C. L. (2007). Primary dietary prevention is the fiber story over? *Recent Results Cancer Research* 174*:* 171 – 177.

Rodriguez-Amaya, D. B. (2001). A *Guide to Carotenoid Analysis*. Human Nutrition Institute, Washington DC, USA. 20005-5802: 64.

Rosling, H. (1987). *Cassava Toxicity and Food Security*. TryckKontakt, Uppsala. 40pp.

Shackleton, C. M., Pasquini, M. W. and Drescher, A. W. (2009). *African Indigenous Vegetables in Urban Agriculture*. Earthscan. London, UK. 298 pp.

Siritunga, D. and Sayre, R. T. (2003). Generation of cyanogen free transgenic cassava. *Planta* 217: 367 – 373.

Siritunga, D. and Sayre, R. T. (2004). Engineering cyanogen synthesis and turnover in cassava (*Manihotesculenta*). *Plant Molecular Biology* 56: 661 – 669.

Tewe, O. O. and Lutaladio, N. *(*2004). *Cassava for Livestock Feed in Sub-Saharan Africa*. Food and Agriculture Organization, Rome, Italy. 21pp.

Thompson, B. and Amoroso, L. (Eds) (2011). *Combating Micronutrient Deficiencies: Food-Based Approaches.* Commonwealth for Agriculture Bureau International, Wallingford, UK. 432pp.

Umuhozariho, M. G., Shayo, N. B., Msuya, J. M. and Sallah, P.Y. K. (2011). Utilization of cassava leaves as a vegetable in Rwanda. *Journal of Agriculture Science* 24: 15 – 27.

Umuhozariho, M. G., Shayo, N. B., Msuya, J. M. and Sallah, P.Y. K. (2014). Cyanide and selected nutrients content of different preparations of leaves from three cassava species. African Journal of Food Science 8(3): 122-129.

Umuhozariho, M. G., Shayo, N. B., Msuya, J. M. and Sallah, P.Y. K. (2013). Sensory evaluation of different preparations of cassava leaves from three species as a leafy vegetable. African Journal of Food Science 12(46): 6452-129.

Uyoh, E. A., Udensio, Natwi, V. and Urea, I. (2007). Effect of different processing methods on cyanide content of garri from cultivars of cassava. *Journal of Food Agriculture Environment* 3(4): 105 – 107.

Vimala,B., Nambisan B and Hariprakash B. (2011). Retention of carotenoids in orange-fleshed sweet potato during processing. *Journal of Food Science Technology* 48(4):520-4.

Watanabe, F., Yabuta, Y., Bito, T and Teng, F. (2014).Vitamin B12 containing plant food source for vegetarians. Nutrients 6(5): 1861-1873.

West, C. E., Pepping, F. and Temalilwa, C. R. (1988). *The Composition of Foods Commonly Eaten in East Africa*. Wageningen Agricultural University, The Netherlands.

Wobeto, C., Corrêa, A. D., De Abreu, C. M. P., Dos Santos, C. D. and De Abreu, J. R. (2006). Nutrients in the cassava (*Manihotesculenta, Crantz)* leaf meal at three ages of the plant. *Science Technology Aliment* 26*:* 865 – 869.

Yeoh, H. H. and Chew, M.Y. (1976). Protein content and amino acid composition of cassava. Leaf Photochemistry 15: 1597 – 1599.

APPENDICES

Appendix 1: Analysis of variance of mean moisture content of cassava variety by location and preparation methods

Source of Variation	df	Sum of Squares	Mean Square	F value	P value
Location	1	9.511	9.511	33.5844	0.0000
Variety	4	89.953	22.488	79.4103	0.0000
Location* Variety	4	19.744	4.936	17.4302	0.0000
Method	2	1478.218	739.109	2609.9284	0.0000
Location*Method	2	3.148	1.574	5.5578	0.0061
Variety*Method	8	87.795	10.974	38.7525	0.0000
Location*Variety*Method	8	84.707	10.588	37.3896	0.0000
Error	60	16.991	0.283		
Total	**89**	**1790.068**			

Appendix 2: T-Test for difference in mean moisture content between Mkuranga and Rufiji

```
Two-sample t test with equal variances
------------------------------------------------------------------------------
   Group |     Obs        Mean    Std. Err.   Std. Dev.   [95% Conf. Interval]
---------+--------------------------------------------------------------------
       1 |      45    81.28822    .6724673    4.511048    79.93295    82.64349
       2 |      45    80.63844    .6686407    4.485378    79.29089      81.986
---------+--------------------------------------------------------------------
combined |      90    80.96333    .4727399    4.484805    80.02401    81.90266
---------+--------------------------------------------------------------------
    diff |            .6497772    .9483104               -1.23479    2.534345
------------------------------------------------------------------------------
    diff = mean(1) - mean(2)                                      t =   0.6852
Ho: diff = 0                                     degrees of freedom =       88

    Ha: diff < 0                 Ha: diff != 0                 Ha: diff > 0
 Pr(T < t) = 0.7525         Pr(|T| > |t|) = 0.4950          Pr(T > t) = 0.2475
```

Appendix 3: Analysis of variance of mean protein content of cassava variety by location and preparation methods

Source of Variation	df	Sum of Squares	Mean Square	F value	P value
Location	1	3.784	3.784	8.8125	0.0043
Variety	4	8.983	2.246	5.2295	0.0011
Location* Variety	4	63.390	15.848	36.9039	0.0000
Method	2	7.447	3.724	8.6714	0.0005
Location*Method	2	69.542	34.771	80.9711	0.0000
Variety*Method	8	222.084	27.761	64.6456	0.0000
Location*Variety*Method	8	175.866	21.983	51.1920	0.0000
Error	60	25.766	0.429		
Total	**89**	**25.766**			

Appendix 4: T-Test for difference in mean protein content between Mkuranga and Rufiji

```
Two-sample t test with equal variances
------------------------------------------------------------------------------
   Group |     Obs        Mean    Std. Err.   Std. Dev.   [95% Conf. Interval]
---------+--------------------------------------------------------------------
Mkuranga |      45    31.57333    .4894265    3.283173    30.58696    32.55971
  Rufiji |      45    31.16289    .2234454    1.498918    30.71256    31.61321
---------+--------------------------------------------------------------------
combined |      90    31.36811    .2683778    2.546055    30.83485    31.90137
---------+--------------------------------------------------------------------
    diff |            .4104445    .5380206               -.6587584    1.479647
------------------------------------------------------------------------------
    diff = mean(Mkuranga) - mean(Rufiji)                          t =   0.7629
Ho: diff = 0                                     degrees of freedom =       88

    Ha: diff < 0                 Ha: diff != 0                 Ha: diff > 0
 Pr(T < t) = 0.7762         Pr(|T| > |t|) = 0.4476          Pr(T > t) = 0.2238
```

Appendix 5: Analysis of variance of mean ash content of cassava variety by location and preparation methods

Sourceof Variation	**Df**	**Sum of Squares**	**Mean Square**	**F Value**	**Prob**
Location	1	0.451	0.451	2.1332	0.1494
Variety	4	73.985	18.496	87.5157	0.0000
Location* Variety	4	116.551	29.138	137.8658	0.0000
Method	2	530.360	265.180	1254.7045	0.0000
Location*Method	2	3.236	1.618	7.6559	0.0000
Variety*Method	8	63.340	7.918	37.4619	0.0000
Location*Variety*Method	8	146.790	18.349	86.8177	0.0000
Error	60	12.681	0.211		
Total	**89**	**947.395**			

Appendix 6: Analysis of variance of mean crude fiber content of cassava variety by location and preparation methods

Sourceof Variation	**Df**	**Sum of Squares**	**Mean Square**	**F Value**	**Prob**
Location	1	198.414	198.414	42.8354	0.0000
Variety	4	444.905	111.226	24.0125	0.0000
Location* Variety	4	94.763	23.691	5.1146	0.0013
Method	2	188.421	94.210	20.3390	0.0000
Location*Method	2	4.252	2.126	0.4590	0.6337
Variety*Method	8	713.073	89.134	19.2431	0.0000
Location*Variety*Method	8	221.159	27.645	5.9682	0.0000
Error	60	277.921	4.632		
Total	89	2142.906			

Appendix 7: T-Test for differences in mean crude fiber content between Mkuranga and Rufiji

```
Two-sample t test with equal variances
------------------------------------------------------------------------------
   Group |     Obs        Mean    Std. Err.   Std. Dev.   [95% Conf. Interval]
---------+--------------------------------------------------------------------
       1 |      45    19.47111    .7367771    4.942451    17.98623    20.95599
       2 |      45    22.44089    .6627999    4.446197     21.1051    23.77667
---------+--------------------------------------------------------------------
combined |      90      20.956    .5172536    4.907099    19.92823    21.98377
---------+--------------------------------------------------------------------
    diff |           -2.969778    .9910318               -4.939246   -1.00031
------------------------------------------------------------------------------
    diff = mean(1) - mean(2)                                      t =  -2.9967
Ho: diff = 0                                     degrees of freedom =       88

    Ha: diff < 0                 Ha: diff != 0                 Ha: diff > 0
 Pr(T < t) = 0.0018         Pr(|T| > |t|) = 0.0035          Pr(T > t) = 0.9982
```

Appendix 8: Analysis of variance of crude fat content of cassava variety by location and preparation methods

Sourceof Variation	Df	Sum of Squares	Mean Square	F Value	Prob
Location	1	1.021	1.021	19.2539	0.0000
Variety	4	1.320	0.330	6.2265	0.0003
Location* Variety	4	19.747	4.937	93.1326	0.0000
Method	2	1.840	0.920	17.3601	0.0000
Location*Method	2	7.017	3.509	66.1914	0.0000
Variety*Method	8	12.599	1.575	29.7115	0.0000
Location*Variety*Method	8	20.702	2.588	48.8185	0.0000
Error	60	3.180	0.053		
Total	**89**	**67.427**			

Appendix 9: T-Test for differences in mean crude fat content between Mkuranga and Rufiji

```
Two-sample t test with equal variances
------------------------------------------------------------------------------
   Group |     Obs        Mean    Std. Err.   Std. Dev.   [95% Conf. Interval]
---------+--------------------------------------------------------------------
       1 |      45    3.561778    .1474912    .9894013    3.264529    3.859027
       2 |      45    3.775111    .1086778    .7290331    3.556085    3.994137
---------+--------------------------------------------------------------------
combined |      90    3.668444    .0917861    .8707596    3.486067    3.850822
---------+--------------------------------------------------------------------
    diff |           -.2133334    .1832063               -.5774173    .1507506
------------------------------------------------------------------------------
    diff = mean(1) - mean(2)                                      t =  -1.1644
Ho: diff = 0                                     degrees of freedom =       88

    Ha: diff < 0                 Ha: diff != 0                 Ha: diff > 0
 Pr(T < t) = 0.1237         Pr(|T| > |t|) = 0.2474          Pr(T > t) = 0.8763
```

Appendix 10: Analysis of variance of mean carbohydrate content of cassava variety by location and preparation methods

Sourceof Variation	Df	Sum of Squares	Mean Square	F Value	Prob
Location	1	191.068	191.068	38.6343	0.0000
Variety	4	202.171	50.543	10.2198	0.0000
Location* Variety	4	87.929	21.982	4.4448	0.0033
Method	2	85.169	42.585	8.6107	0.0005
Location*Method	2	69.334	34.667	7.0098	0.0018
Variety*Method	8	576.140	72.018	14.5621	0.0000
Location*Variety*Method	8	544.073	68.009	13.7516	0.0000
Error	60	296.733	4.946		
Total	**89**	**2052.618**			

Appendix 11: T-Test for differences in mean carbohydrate content between Mkuranga and Rufiji

```
Two-sample t test with equal variances
------------------------------------------------------------------------------
   Group |     Obs        Mean    Std. Err.   Std. Dev.   [95% Conf. Interval]
---------+--------------------------------------------------------------------
       1 |      45    36.27044    .7398407    4.963002    34.77939     37.7615
       2 |      45    33.35622    .6268904    4.205309    32.09281    34.61964
---------+--------------------------------------------------------------------
combined |      90    34.81333    .5062641    4.802843     33.8074    35.81927
---------+--------------------------------------------------------------------
    diff |            2.914222    .9697194                .9871087    4.841336
------------------------------------------------------------------------------
    diff = mean(1) - mean(2)                                      t =   3.0052
Ho: diff = 0                     degrees of freedom =       88

    Ha: diff < 0                 Ha: diff != 0                 Ha: diff > 0
 Pr(T < t) = 0.9983         Pr(|T| > |t|) = 0.0035          Pr(T > t) = 0.0017
```

Appendix 12: Analysis of variance of mean cyanide content in cassava variety by location and preparation methods

Sourceof Variation	df	Sum of Squares	Mean Square	F Value	Prob
Location	1	6188.46	79447.30	119.89	0.0000
Variety	4	147432.34	6188.46	714.07	0.0000
Location* Variety	4	27182.12	36858.09	131.65	0.0000
Method	2	1779210.06	6795.53	17234.79	0.0000
Location*Method	2	7846.13	889605.03	76.00	0.0000
Variety*Method	8	272279.72	3923.07	659.38	0.0000
Location*Variety*Method	8	63832.84	34034.97	154.58	0.0000
Error	30	1548.50	7979.11		
Total	**59**	**2052.618**			

Appendix 13: T-Test for differences in cyanide content between Mkuranga and Rufiji

```
Two-sample t test with equal variances
------------------------------------------------------------------------------
   Group |     Obs        Mean    Std. Err.   Std. Dev.   [95% Conf. Interval]
---------+--------------------------------------------------------------------
       1 |      30      142.94    38.81173    212.5806     63.5611    222.3189
       2 |      30    122.6283     33.7129    184.6532     53.6777     191.579
---------+--------------------------------------------------------------------
combined |      60    132.7842    25.52013    197.6781    81.71851    183.8498
---------+--------------------------------------------------------------------
    diff |            20.31166    51.40924               -82.59511    123.2184
------------------------------------------------------------------------------
    diff = mean(1) - mean(2)                                      t =   0.3951
Ho: diff = 0                                     degrees of freedom =       58

    Ha: diff < 0                 Ha: diff != 0                 Ha: diff > 0
 Pr(T < t) = 0.6529         Pr(|T| > |t|) = 0.6942          Pr(T > t) = 0.3471
```

Appendix 14: Analysis of variance of mean β carotene content of cassava variety by location and preparation methods

Sourceof Variation	df	Sum of Squares	Mean Square	F value	P value
Location	1	2067349.78	2067349.78	1243.96	0.0000
Variety	4	4566010.47	1141502.62	686.86	0.0000
Location* Variety	4	4332907.97	1083226.99	651.79	0.0000
Method	2	6125380.83	3062690.41	1842.87	0.0000
Location*Method	2	1918910.22	959455.11	577.32	0.0000
Variety*Method	8	3815974.82	476996.85	287.02	0.0000
Location*Variety*Method	8	3738798.69	467349.837	281.21	0.0000
Error	30	49857.46	1661.91536		
Total	**59**	**26615190.2**			

Appendix 15: T-Test for differences in β Carotene Content between Mkuranga and Rufiji

```
Two-sample t test with equal variances
------------------------------------------------------------------------------
   Group |     Obs        Mean    Std. Err.   Std. Dev.   [95% Conf. Interval]
---------+--------------------------------------------------------------------
       1 |      30    758.2006    51.43286    281.7094    653.0086    863.3926
       2 |      30    1129.446     159.908    875.8522    802.3976    1456.495
---------+--------------------------------------------------------------------
combined |      60    943.8234     86.7088    671.6434    770.3195    1117.327
---------+--------------------------------------------------------------------
    diff |           -371.2456    167.9759               -707.486    -35.0053
------------------------------------------------------------------------------
    diff = mean(1) - mean(2)                                      t =  -2.2101
Ho: diff = 0                                     degrees of freedom =       58

    Ha: diff < 0                 Ha: diff != 0                 Ha: diff > 0
 Pr(T < t) = 0.0155         Pr(|T| > |t|) = 0.0311          Pr(T > t) = 0.9845
```

Appendix 16: Analysis of variance for vitamin C content of cassava variety as influenced by location and preparation methods

Sourceof Variation	df	Sum of Squares	Mean Square	F value	P value
Location	1	0.3605	0.3605	63.54	0.0000
Variety	4	0.4825	0.1206	21.26	0.0000
Location* Variety	4	0.1473	0.0368	6.49	0.0007
Method	2	1.8263	0.9131	160.94	0.0000
Location*Method	2	0.3632	0.1816	32.01	0.0000
Variety*Method	8	1.1405	0.1426	25.13	0.0000
Location*Variety*Method	8	0.1954	0.0244	4.31	0.0015
Error	30	0.1702	0.0057		
Total	**59**	**4.6859**			

Appendix 17: T-test for differences in Vitamin C content between Mkuranga and Rufiji

```
Two-sample t test with equal variances
------------------------------------------------------------------------------
   Group |     Obs        Mean    Std. Err.   Std. Dev.   [95% Conf. Interval]
---------+--------------------------------------------------------------------
       1 |      30    .9356479    .0589129    .3226793    .8151575    1.056138
       2 |      30    .7806207    .0387428    .2122032    .7013827    .8598587
---------+--------------------------------------------------------------------
combined |      60    .8581343    .0363827    .2818195    .7853326     .930936
---------+--------------------------------------------------------------------
    diff |            .1550272    .0705105                .013885    .2961694
------------------------------------------------------------------------------
    diff = mean(1) - mean(2)                                      t =   2.1986
Ho: diff = 0                     degrees of freedom =       58

    Ha: diff < 0                 Ha: diff != 0                 Ha: diff > 0
 Pr(T < t) = 0.9840         Pr(|T| > |t|) = 0.0319          Pr(T > t) = 0.0160
```

Appendix 18: Analysis of variance for Colour

K Value	Source	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Prob
2	Factor A	4	6.647	1.662	1.6939	0.1514
4	Factor B	1	0.083	0.083	0.0850	
6	AB	4	4.367	1.092	1.1128	0.3506
-7	Error	289	283.500	0.981		
	Total	298	294.597			

Appendix 19: Analysis of variance for Taste

K Value	Source	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Prob
2	Factor A	4	7.013	1.753	1.9767	0.0981
4	Factor B	1	2.430	2.430	2.7395	0.0990
6	AB	4	13.653	3.413	3.8481	0.0046
-7	Error	290	257.233	0.887		
	Total	299	280.330			

Appendix 20: Analysis of variance for aroma

K Value	Source	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Prob
2	Factor A	4	3.187	0.797	0.8936	
4	Factor B	1	5.333	5.333	5.9825	0.0150
6	AB	4	12.733	3.183	3.5708	0.0073
-7	Error	290	258.533	0.891		
	Total	299	279.787			

Appendix 21: Analysis of variance for Texture

K Value	Source	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Prob
2	Factor A	4	11.980	2.995	3.3083	0.0114
4	Factor B	1	0.333	0.333	0.3682	
6	AB	4	8.233	2.058	2.2737	0.0615
-7	Error	290	262.533	0.905		
	Total	299	283.080			

Appendix 22: Analysis of variance for General Acceptability

K Value	Source	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Prob
2	Factor A	4	4.887	1.222	1.4869	0.2061
4	Factor B	1	1.920	1.920	2.3369	0.1274
6	AB	4	4.113	1.028	1.2516	0.2893
-7	Error	290	238.267	0.822		
	Total	299	249.187			

Appendix 23: Questionnaire

Household Survey Questionnaire

Name of interviewer ----------

Date of interview (dd-mm-yyy)---------- 1.3

Village----------

Name of the respondent ----------

Sex of the respondent 0= female---------- 1=male ----------

Cassava Processing

2.0 What are the cassava varieties do you grow?.....Local or improved?

2.1 Are the cassava variety sweet, bitter or wild?

2.2 Do you process cassava leaves (Kisamvu) in your household? Yes or No

2.3 What are the age of that cassava varieties which you are used for processing cassava leaves (Kisamvu)?……

2.4 What are the processing methods/procedures are you using for preparing processing cassava leaves.

2.5 Which Equipment are you using during processing of cassava leaves?

Traditional mortar and pestle, Yes or No

Appendix 24: Sensory Evaluation Form

Sex……..

Age…….

Time……..

Date……..

Please look and taste each of the (3) coded samples. Indicate how much you like or dislike each sample by checking the appropriate sample attribute and indicate your reference (1-5) in the column against each attribute. Put the appropriate number against each attribute.

5- Like very much

4- Like Moderately

3- Neither like nor dislike

2- Dislike moderately

1- Dislike very much

Attributes	Sample 1	Sample 2	Sample 3
Appearance/ colour			
Flavor/ taste			
Aroma			
Consistence/texture			
General acceptability			

Comments

……

……

……

Appendix 25: Proximate components, vitamin C,β carotene and cyanide (DM) of cassava leaves

Variety	Location	Method	Lab_Rep	%MC	% DM	% Ash	%Protein	% Fat	% Fiber	% Cabohydrates	Cyanide mg HCN/kg	B carotene	Vit C
Mpira	Mkuranga	Preparation method 1	1	77.36	22.64	5.97	33.96	5.86	22.65	31.56	510.40	960.5438	0.870171
Mpira	Mkuranga	Preparation method 1	2	77.35	22.65	6.05	34.64	5.67	23.01	30.64	519.36	972.3333	0.922521
Mpira	Mkuranga	Preparation method 1	3	77.45	22.55	5.80	33.75	5.40	22.13	32.92			
Kizimbani	Mkuranga	Preparation method 1	1	79.12	20.88	6.14	34.90	3.50	16.98	38.48	442.21	1218.071	1.159178
Kizimbani	Mkuranga	Preparation method 1	2	78.81	21.19	6.39	35.70	3.64	18.80	35.47	425.93	1221.787	1.220524
Kizimbani	Mkuranga	Preparation method 1	3	79.02	20.98	6.12	35.58	3.28	15.05	39.97			
Cheupe	Mkuranga	Preparation method 1	1	75.08	24.92	5.67	29.98	3.45	31.68	29.22	350.28	796.6625	1.372308
Cheupe	Mkuranga	Preparation method 1	2	76.14	23.86	5.04	28.70	3.44	26.21	36.61	347.94	813.0918	1.721532
Cheupe	Mkuranga	Preparation method 1	3	75.88	24.12	5.19	26.39	3.52	34.22	30.68			
Kiroba	Mkuranga	Preparation method 1	1	73.91	26.09	6.32	35.52	2.90	19.28	35.98	614.43	733.1184	1.602266
Kiroba	Mkuranga	Preparation method 1	2	73.88	26.12	6.27	35.40	3.09	23.00	32.24	607.09	914.557	1.757584
Kiroba	Mkuranga	Preparation method 1	3	74.00	26.00	6.39	34.15	2.79	24.15	32.53			
Nyamkagile	Mkuranga	Preparation method 1	1	72.99	27.01	5.57	30.62	3.36	13.90	46.55	103.32	1204.795	1.079325
Nyamkagile	Mkuranga	Preparation method 1	2	72.79	27.21	4.56	31.02	3.11	11.09	50.22	105.35	1198.876	1.149931
Nyamkagile	Mkuranga	Preparation method 1	3	73.56	26.44	5.18	30.42	3.15	15.87	45.38			
Mpira	Rufiji	Preparation method 1	1	75.92	24.08	5.39	29.66	2.91	25.25	36.79	302.79	428.9561	0.710506
Mpira	Rufiji	Preparation method 1	2	76.22	23.78	4.84	29.86	3.19	22.01	40.10	308.11	410.2808	0.735803
Mpira	Rufiji	Preparation method 1	3	75.85	24.15	4.06	29.38	3.14	24.13	39.29			
Cheupe	Rufiji	Preparation method 1	1	72.85	27.15	6.16	29.84	3.00	33.97	27.03	233.65	3381.989	1.161982
Cheupe	Rufiji	Preparation method 1	2	72.90	27.10	6.39	30.60	3.28	34.27	25.47	218.91	3459.844	1.245303
Cheupe	Rufiji	Preparation method 1	3	73.81	26.19	6.01	30.07	2.98	32.97	27.97			
Kizimbani	Rufiji	Preparation method 1	1	74.54	25.46	6.23	28.65	3.32	23.35	38.44	552.57	1872.497	0.676018
Kizimbani	Rufiji	Preparation method 1	2	74.78	25.22	5.86	27.90	2.65	26.69	36.90	584.02	1859.123	0.820982

Kizimbani	Rufiji	Preparation method 1	3	74.96	25.04	6.10	27.57	2.95	22.00	41.38			
Nyamkagile	Rufiji	Preparation method 1	1	74.51	25.49	5.52	30.99	3.55	18.76	41.18	181.89	1572.463	0.692084
Nyamkagile	Rufiji	Preparation method 1	2	74.33	25.67	5.71	30.78	4.07	20.08	39.36	186.33	1513.522	0.750456
Nyamkagile	Rufiji	Preparation method 1	3	74.81	25.19	6.06	30.73	3.69	23.95	35.57			
Kiroba	Rufiji	Preparation method 1	1	74.83	25.17	6.00	32.59	3.90	20.84	36.67	483.72	1322.502	1.211802
Kiroba	Rufiji	Preparation method 1	2	75.16	24.84	5.96	33.08	4.09	20.88	35.99	447.89	1329.753	1.236755
Kiroba	Rufiji	Preparation method 1	3	74.42	25.58	6.05	32.81	4.07	22.03	35.04			
Kizimbani	Mkuranga	Preparation method 2	1	81.43	18.57	10.91	33.71	4.42	13.95	36.99	23.16	615.5784	0.674673
Kizimbani	Mkuranga	Preparation method 2	2	81.93	18.07	10.19	32.25	4.82	12.67	40.07	29.12	590.9712	0.699558
Kizimbani	Mkuranga	Preparation method 2	3	82.22	17.78	10.17	33.85	4.98	11.98	39.03			
Mpira	Mkuranga	Preparation method 2	1	86.01	13.99	5.73	32.91	4.03	17.55	39.78	14.36	566.9715	0.813223
Mpira	Mkuranga	Preparation method 2	2	86.20	13.80	6.44	31.11	4.35	17.53	40.57	15.08	512.3705	0.735803
Mpira	Mkuranga	Preparation method 2	3	86.66	13.34	5.84	32.67	4.06	18.26	39.17			
Cheupe	Mkuranga	Preparation method 2	1	86.63	13.37	12.07	33.24	2.39	18.78	33.53	8.58	693.451	0.710506
Cheupe	Mkuranga	Preparation method 2	2	87.09	12.91	11.43	33.59	2.39	17.75	34.84	3.43	659.012	0.735803
Cheupe	Mkuranga	Preparation method 2	3	87.20	12.80	11.16	33.17	2.55	15.54	37.58			
Kiroba	Mkuranga	Preparation method 2	1	81.12	18.88	11.57	24.60	5.14	22.73	35.97	2.15	328.095	0.620574
Kiroba	Mkuranga	Preparation method 2	2	81.01	18.99	11.67	24.00	3.98	20.56	39.79	2.93	327.7447	0.619374
Kiroba	Mkuranga	Preparation method 2	3	81.12	18.88	11.30	23.89	4.18	26.95	33.68			
Nyamkagile	Mkuranga	Preparation method 2	1	85.06	14.94	13.45	27.02	3.60	26.40	29.52	24.00	1059.287	0.885676
Nyamkagile	Mkuranga	Preparation method 2	2	84.83	15.17	11.35	27.23	3.57	24.02	33.82	25.55	1082.724	0.802806
Nyamkagile	Mkuranga	Preparation method 2	3	85.22	14.78	12.74	27.28	3.40	20.28	36.30			
Kizimbani	Mkuranga	Preparation method 3	1	81.52	18.48	10.72	30.41	3.52	19.98	35.36	17.77	478.2058	0.955544
Kizimbani	Mkuranga	Preparation method 3	2	82.00	18.00	10.70	30.23	3.77	19.46	35.84	14.60	488.8995	0.902874
Kizimbani	Mkuranga	Preparation method 3	3	82.09	17.91	10.76	32.50	3.45	15.58	37.71			
Mpira	Mkuranga	Preparation method 3	1	86.57	13.43	10.50	30.78	3.32	17.75	37.65	14.49	447.9424	0.845418

Mpira	Mkuranga	Preparation method 3	2	85.00	15.00	9.64	30.37	2.82	18.91	38.26	12.94	449.1685	0.71955
Mpira	Mkuranga	Preparation method 3	3	85.89	14.11	9.58	30.34	2.50	18.94	38.63			
Cheupe	Mkuranga	Preparation method 3	1	86.20	13.80	13.38	28.84	2.82	21.96	33.00	8.89	559.5463	0.823797
Cheupe	Mkuranga	Preparation method 3	2	85.41	14.59	12.69	29.11	3.01	19.82	35.37	11.63	694.0305	0.820982
Cheupe	Mkuranga	Preparation method 3	3	85.39	14.61	12.86	29.01	2.80	19.06	36.27			
Kiroba	Mkuranga	Preparation method 3	1	85.20	14.80	9.40	34.24	1.54	13.03	41.80	11.55	533.0671	0.507236
Kiroba	Mkuranga	Preparation method 3	2	85.19	14.81	9.48	35.63	1.70	13.16	40.04	15.40	547.5309	0.582753
Kiroba	Mkuranga	Preparation method 3	3	83.79	16.21	9.49	33.71	1.86	13.03	41.91			
Nyamkagile	Mkuranga	Preparation method 3	1	80.16	19.84	13.28	34.70	4.01	20.14	27.87	2.73	1020.598	0.835132
Nyamkagile	Mkuranga	Preparation method 3	2	81.39	18.61	13.45	34.39	4.56	19.50	28.11	3.53	1056.987	0.922816
Nyamkagile	Mkuranga	Preparation method 3	3	81.10	18.90	11.95	35.29	4.58	22.91	25.26			
Mpira	Rufiji	Preparation method 2	1	83.05	16.95	11.43	29.53	2.91	24.93	31.21	10.63	292.1565	0.763575
Mpira	Rufiji	Preparation method 2	2	84.01	15.99	12.94	31.74	2.95	19.86	32.51	9.51	308.5736	1.036261
Mpira	Rufiji	Preparation method 2	3	85.90	14.10	12.13	31.05	2.71	19.08	35.03			
Cheupe	Rufiji	Preparation method 2	1	83.98	16.02	7.08	34.22	5.10	20.06	33.54	4.93	483.118	0.946136
Cheupe	Rufiji	Preparation method 2	2	84.99	15.01	5.86	34.43	5.48	22.28	31.95	5.29	490.013	0.844675
Cheupe	Rufiji	Preparation method 2	3	84.79	15.21	5.93	34.04	5.01	21.83	33.19			
Kizimbani	Rufiji	Preparation method 2	1	84.99	15.01	11.17	31.95	4.34	17.65	34.89	19.37	218.8012	0.533718
Kizimbani	Rufiji	Preparation method 2	2	85.37	14.63	11.79	32.13	4.19	19.79	32.10	15.33	211.7552	0.649077
Kizimbani	Rufiji	Preparation method 2	3	86.59	13.41	11.44	30.84	4.07	21.78	31.87			
Nyamkagile	Rufiji	Preparation method 2	1	81.75	18.25	14.55	31.83	3.29	17.45	32.88	4.17	1209.01	0.707107
Nyamkagile	Rufiji	Preparation method 2	2	82.01	17.99	15.73	31.97	3.08	18.97	30.24	5.11	1100.01	0.72507
Nyamkagile	Rufiji	Preparation method 2	3	81.99	18.01	15.12	31.70	3.39	19.92	29.87			
Kiroba	Rufiji	Preparation method 2	1	83.06	16.94	10.18	31.43	3.85	24.66	29.87	11.73	453.4057	0.618577
Kiroba	Rufiji	Preparation method 2	2	83.20	16.80	10.30	30.76	3.99	25.32	29.63	12.91	439.8335	0.617583
Kiroba	Rufiji	Preparation method 2	3	83.81	16.19	10.37	30.88	3.89	28.74	26.12			

Mpira	Rufiji	Preparation method 3	1	84.27	15.73	13.17	32.34	3.07	15.98	35.44	13.94	425.3493	0.504971
Mpira	Rufiji	Preparation method 3	2	84.41	15.59	12.63	31.83	3.49	17.90	34.15	13.94	425.5507	0.53224
Mpira	Rufiji	Preparation method 3	3	85.15	14.85	12.05	31.85	3.32	16.67	36.11			
Cheupe	Rufiji	Preparation method 3	1	84.15	15.85	4.40	32.86	4.43	29.16	29.15	5.82	1980.001	0.831999
Cheupe	Rufiji	Preparation method 3	2	85.38	14.62	5.36	31.98	4.38	24.07	34.21	2.59	1987.01	0.895164
Cheupe	Rufiji	Preparation method 3	3	84.97	15.03	4.69	32.29	4.78	28.78	29.47			
Kizimbani	Rufiji	Preparation method 3	1	80.76	19.24	11.95	31.47	4.37	21.19	31.02	7.62	658.01	0.523398
Kizimbani	Rufiji	Preparation method 3	2	80.79	19.21	11.10	30.95	4.42	18.70	34.82	11.68	678.01	0.552434
Kizimbani	Rufiji	Preparation method 3	3	81.01	18.99	11.81	31.50	4.60	23.75	28.34			
Nyamkagile	Rufiji	Preparation method 3	1	83.80	16.20	11.42	29.33	3.02	15.82	40.41	4.60	765.1974	0.702199
Nyamkagile	Rufiji	Preparation method 3	2	82.19	17.81	11.52	30.64	3.52	23.42	30.90	3.45	759.8098	0.716599
Nyamkagile	Rufiji	Preparation method 3	3	83.06	16.94	11.53	29.21	3.30	18.35	37.61			
Kiroba	Rufiji	Preparation method 3	1	83.44	16.56	14.42	31.43	4.45	20.91	28.78	8.63	1990.975	0.740909
Kiroba	Rufiji	Preparation method 3	2	82.35	17.65	14.77	30.76	4.79	21.97	27.71	7.72	1855.867	0.73524
Kiroba	Rufiji	Preparation method 3	3	83.62	16.38	13.72	30.88	4.90	19.67	30.83			

Appendix 26: Sensory evaluation of cassava leaves

Sex	Age	Time	Variety	Method	Colour	Taste	Aroma	Texture	General Acceptability
FEMALE	23	1300	Kizimbani	Preparation Method 2	4	5	5	3	4
FEMALE	33	1300	Kizimbani	Preparation Method 2	4	5	5	4	4
FEMALE	30	1251	Kizimbani	Preparation Method 2	4	4	4	3	4
FEMALE	27	1230	Kizimbani	Preparation Method 2	2	4	3	2	2
FEMALE	26	1230	Kizimbani	Preparation Method 2	5	5	5	5	5
FEMALE	26	1230	Kizimbani	Preparation Method 2	4	4	3	3	4
MALE	28	1230	Kizimbani	Preparation Method 2	4	5	4	3	4
MALE	25	1230	Kizimbani	Preparation Method 2	3	2	4	4	4
MALE	45	1212	Kizimbani	Preparation Method 2	5	4	3	4	2
MALE	49	1150	Kizimbani	Preparation Method 2	5	4	5	5	5
MALE	28	1202	Kizimbani	Preparation Method 2	4	4	3	3	4
FEMALE	26	1209	Kizimbani	Preparation Method 2	5	4	4	5	5
MALE	20	1149	Kizimbani	Preparation Method 2	4	4	5	3	4
MALE	28	1149	Kizimbani	Preparation Method 2	4	5	4	5	4
FEMALE	25	1200	Kizimbani	Preparation Method 2	4	4	3	3	3
FEMALE	26	1203	Kizimbani	Preparation Method 2	5	5	5	5	5
FEMALE	23	1203	Kizimbani	Preparation Method 2	4	5	5	5	5
FEMALE	25	1200	Kizimbani	Preparation Method 2	5	4	4	5	5
MALE	28	1148	Kizimbani	Preparation Method 2	3	4	4	2	4
MALE	29	1150	Kizimbani	Preparation Method 2	4	3	3	4	4
FEMALE	25	1204	Kizimbani	Preparation Method 2	5	5	4	4	5
FEMALE	25	1148	Kizimbani	Preparation Method 2	4	5	3	4	4
MALE	29	1200	Kizimbani	Preparation Method 2	4	4	4	3	4

MALE	30	1230	Kizimbani	Preparation Method 2	4	3	5	3	3
MALE	25	1613	Kizimbani	Preparation Method 2	4	3	3	4	3
FEMALE	27	1611	Kizimbani	Preparation Method 2	5	2	3	3	3
FEMALE	26	1612	Kizimbani	Preparation Method 2	4	4	4	5	5
MALE	27	1542	Kizimbani	Preparation Method 2	4	4	4	4	4
FEMALE	25	1458	Kizimbani	Preparation Method 2	3	4	4	4	4
MALE	27	1453	Kizimbani	Preparation Method 2	5	4	4	3	4
MALE	40	1428	Kizimbani	Preparation Method 2	5	4	3	4	4
FEMALE	33	1300	Kiroba	Preparation Method 2	3	4	3	2	3
MALE	51	1520	Kiroba	Preparation Method 2	4	3	4	5	4
MALE	25	1518	Kiroba	Preparation Method 2	2	3	4	3	3
MALE	41	1515	Kiroba	Preparation Method 2	3	5	4	3	4
MALE	33	1514	Kiroba	Preparation Method 2	2	3	4	4	3
MALE	27	1349	Kiroba	Preparation Method 2	4	4	4	4	4
FEMALE	27	1430	Kiroba	Preparation Method 2	1	3	4	2	3
MALE	25	1430	Kiroba	Preparation Method 2	4	3	3	3	3
FEMALE	26	1427	Kiroba	Preparation Method 2	5	4	3	4	4
FEMALE	26	1429	Kiroba	Preparation Method 2	5	4	4	4	3
MALE	41	1418	Kiroba	Preparation Method 2	4	3	5	3	3
MALE	26	1340	Kiroba	Preparation Method 2	4	4	5	4	4
MALE	25	1348	Kiroba	Preparation Method 2	5	4	3	2	3
FEMALE	23	1230	Kiroba	Preparation Method 2	1	2	1	1	1
MALE	28	1238	Kiroba	Preparation Method 2	5	5	4	5	5
FEMALE	23	1236	Kiroba	Preparation Method 2	4	2	4	3	2
FEMALE	25	1330	Kiroba	Preparation Method 2	3	1	1	2	1
MALE	49	1220	Kiroba	Preparation Method 2	2	2	2	3	4

FEMALE	23	1225	Kiroba	Preparation Method 2	4	4	3	3	4
FEMALE	27	1221	Kiroba	Preparation Method 2	2	5	4	3	3
MALE	28	1259	Kiroba	Preparation Method 2	5	5	4	4	5
MALE	36	1232	Kiroba	Preparation Method 2	5	4	3	2	1
FEMALE	25	1210	Kiroba	Preparation Method 2	4	5	5	4	5
MALE	41	1205	Kiroba	Preparation Method 2	5	5	5	5	5
MALE	43	1200	Kiroba	Preparation Method 2	5	4	5	3	5
MALE	49	1200	Kiroba	Preparation Method 2	5	5	5	4	5
MALE	48	1210	Kiroba	Preparation Method 2	5	5	5	5	5
MALE	25	1400	Kiroba	Preparation Method 2	4	4	5	4	4
MALE	28	1489	Kiroba	Preparation Method 2	5	4	4	4	4
MALE	26	1320	Kiroba	Preparation Method 2	5	4	4	4	4
FEMALE	26	1356	Kiroba	Preparation Method 2	3	4	4	3	3
MALE	45	1434	Mpira	Preparation Method 2	3	3	3	3	2
MALE	49	1420	Mpira	Preparation Method 2	4	3	4	4	4
FEMALE	25	1420	Mpira	Preparation Method 2	1	3	3	3	3
MALE	38	1415	Mpira	Preparation Method 2	3	3	3	2	3
MALE	32	1413	Mpira	Preparation Method 2	5	3	3	3	4
MALE	27	1308	Mpira	Preparation Method 2	5	2	4	2	5
MALE	26	1308	Mpira	Preparation Method 2	3	4	3	3	4
MALE	25	1245	Mpira	Preparation Method 2	5	4	4	4	4
FEMALE	26	1232	Mpira	Preparation Method 2	4	4	3	3	3
MALE	33	1125	Mpira	Preparation Method 2	4	4	3	2	4
MALE	25	1156	Mpira	Preparation Method 2	4	3	4	4	4
MALE	25	1158	Mpira	Preparation Method 2	4	2	3	2	3
MALE	29	1156	Mpira	Preparation Method 2	2	3	2	3	3

FEMALE	25	1200	Mpira	Preparation Method 2	4	4	5	2	4
FEMALE	26	1213	Mpira	Preparation Method 2	5	4	5	3	4
FEMALE	27	1214	Mpira	Preparation Method 2	3	3	4	3	3
FEMALE	26	1213	Mpira	Preparation Method 2	5	3	4	4	4
FEMALE	22	1158	Mpira	Preparation Method 2	4	3	3	2	4
FEMALE	37	1217	Mpira	Preparation Method 2	5	5	4	4	4
MALE	40	1400	Mpira	Preparation Method 2	2	4	2	5	4
MALE	25	1613	Mpira	Preparation Method 2	4	4	4	4	4
FEMALE	27	1611	Mpira	Preparation Method 2	4	3	4	4	4
FEMALE	26	1612	Mpira	Preparation Method 2	4	5	4	4	4
MALE	27	1542	Mpira	Preparation Method 2	4	4	4	4	4
FEMALE	25	1458	Mpira	Preparation Method 2	3	2	4	3	3
MALE	27	1453	Mpira	Preparation Method 2	5	4	3	3	4
MALE	40	1428	Mpira	Preparation Method 2	4	5	2	4	3
FEMALE	25	1408	Mpira	Preparation Method 2	2	3	1	2	4
FEMALE	29	1615	Mpira	Preparation Method 2	1	3	1	2	4
MALE	27	1559	Mpira	Preparation Method 2	5	4	5	4	4
MALE	34	1610	Mpira	Preparation Method 2	5	5	4	4	5
MALE	27	1628	Cheupe	Preparation Method 2	2	3	5	3	3
MALE	26	1625	Cheupe	Preparation Method 2	5	5	5	3	5
MALE	25	1603	Cheupe	Preparation Method 2	3	2	3	3	3
MALE	27	1633	Cheupe	Preparation Method 2	4	5	5	5	5
MALE	32	1521	Cheupe	Preparation Method 2	5	4	3	4	4
MALE	33	1517	Cheupe	Preparation Method 2	2	2	2	2	2
MALE	41	1516	Cheupe	Preparation Method 2	4	4	5	4	4
MALE	26	1516	Cheupe	Preparation Method 2	4	3	3	4	3

FEMALE	26	1558	Cheupe	Preparation Method 2	5	5	5	4	5
FEMALE	27	1623	Cheupe	Preparation Method 2	2	4	4	4	3
FEMALE	27	1543	Cheupe	Preparation Method 2	4	3	4	5	4
MALE	25	1550	Cheupe	Preparation Method 2	4	2	3	4	4
FEMALE	26	1600	Cheupe	Preparation Method 2	2	2	3	2	2
FEMALE	25	1435	Cheupe	Preparation Method 2	3	4	3	3	4
MALE	58	1228	Cheupe	Preparation Method 2	5	5	4	3	4
MALE	49	1228	Cheupe	Preparation Method 2	3	3	3	4	3
MALE	40	1247	Cheupe	Preparation Method 2	5	2	5	5	5
MALE	47	1247	Cheupe	Preparation Method 2	5	4	4	2	4
MALE	49	1245	Cheupe	Preparation Method 2	4	5	2	1	4
MALE	27	1431	Cheupe	Preparation Method 2	4	5	4	4	5
MALE	35	1630	Cheupe	Preparation Method 2	4	4	5	4	4
MALE	45	1434	Cheupe	Preparation Method 2	5	5	5	5	5
MALE	49	1420	Cheupe	Preparation Method 2	5	5	4	3	4
FEMALE	25	1420	Cheupe	Preparation Method 2	4	4	4	4	4
MALE	38	1415	Cheupe	Preparation Method 2	2	4	4	3	3
MALE	32	1413	Cheupe	Preparation Method 2	5	5	4	4	4
MALE	27	1308	Cheupe	Preparation Method 2	5	5	4	5	5
MALE	26	1308	Cheupe	Preparation Method 2	4	3	3	4	4
MALE	25	1245	Cheupe	Preparation Method 2	5	5	5	5	5
FEMALE	26	1232	Cheupe	Preparation Method 2	5	5	5	5	5
MALE	33	1125	Cheupe	Preparation Method 2	5	5	5	4	5
MALE	41	1205	Nyamkagile	Preparation Method 2	5	5	5	5	5
MALE	43	1200	Nyamkagile	Preparation Method 2	5	5	5	5	5
MALE	49	1200	Nyamkagile	Preparation Method 2	4	4	3	4	2

MALE	48	1210	Nyamkagile	Preparation Method 2	4	4	3	2	2
MALE	25	1400	Nyamkagile	Preparation Method 2	3	5	3	4	5
MALE	28	1489	Nyamkagile	Preparation Method 2	5	5	5	5	5
MALE	26	1320	Nyamkagile	Preparation Method 2	5	5	4	4	5
FEMALE	26	1356	Nyamkagile	Preparation Method 2	4	4	4	4	4
FEMALE	27	1400	Nyamkagile	Preparation Method 2	3	3	4	3	4
FEMALE	27	1358	Nyamkagile	Preparation Method 2	2	5	4	3	3
FEMALE	26	1359	Nyamkagile	Preparation Method 2	4	4	3	4	4
MALE	30	1535	Nyamkagile	Preparation Method 2	4	5	4	4	4
MALE	32	1537	Nyamkagile	Preparation Method 2	4	4	3	4	3
FEMALE	25	1532	Nyamkagile	Preparation Method 2	3	3	4	4	3
MALE	41	1524	Nyamkagile	Preparation Method 2	4	4	3	4	4
MALE	28	1533	Nyamkagile	Preparation Method 2	5	5	4	3	4
MALE	33	1515	Nyamkagile	Preparation Method 2	5	5	5	5	5
MALE	45	1434	Nyamkagile	Preparation Method 2	5	5	5	5	5
MALE	49	1420	Nyamkagile	Preparation Method 2	5	5	4	4	4
FEMALE	25	1420	Nyamkagile	Preparation Method 2	4	4	5	5	5
MALE	38	1415	Nyamkagile	Preparation Method 2	4	3	3	3	4
MALE	32	1413	Nyamkagile	Preparation Method 2	5	1	2	3	2
MALE	27	1308	Nyamkagile	Preparation Method 2	4	5	4	4	5
MALE	26	1308	Nyamkagile	Preparation Method 2	4	5	5	4	4
MALE	25	1245	Nyamkagile	Preparation Method 2	5	5	5	5	5
FEMALE	26	1232	Nyamkagile	Preparation Method 2	5	5	5	5	5
MALE	33	1125	Nyamkagile	Preparation Method 2	5	5	5	5	5
MALE	25	1156	Nyamkagile	Preparation Method 2	4	4	4	4	4
MALE	25	1158	Nyamkagile	Preparation Method 2	4	1	2	2	3

MALE	29	1156	Nyamkagile	Preparation Method 2	4	3	5	5	5
FEMALE	25	1200	Nyamkagile	Preparation Method 2	3	3	3	4	3
FEMALE	23	1300	Kizimbani	Preparation Method 3	4	2	3	4	3
FEMALE	33	1300	Kizimbani	Preparation Method 3	4	4	4	3	4
FEMALE	30	1251	Kizimbani	Preparation Method 3	4	3	4	3	4
FEMALE	27	1230	Kizimbani	Preparation Method 3	1	4	2	2	1
FEMALE	26	1230	Kizimbani	Preparation Method 3	5	4	4	4	4
FEMALE	26	1230	Kizimbani	Preparation Method 3	4	3	3	3	4
MALE	28	1230	Kizimbani	Preparation Method 3	4	4	4	3	5
MALE	25	1230	Kizimbani	Preparation Method 3	5	4	2	5	5
MALE	45	1212	Kizimbani	Preparation Method 3	4	4	3	4	2
MALE	49	1150	Kizimbani	Preparation Method 3	4	4	4	4	4
MALE	28	1202	Kizimbani	Preparation Method 3	3	3	4	4	4
FEMALE	26	1209	Kizimbani	Preparation Method 3	5	4	4	5	5
MALE	20	1149	Kizimbani	Preparation Method 3	4	3	3	2	3
MALE	28	1149	Kizimbani	Preparation Method 3	4	4	4	5	4
FEMALE	25	1200	Kizimbani	Preparation Method 3	4	5	2	2	5
FEMALE	26	1203	Kizimbani	Preparation Method 3	5	5	5	5	5
FEMALE	23	1203	Kizimbani	Preparation Method 3	5	5	5	5	5
FEMALE	25	1200	Kizimbani	Preparation Method 3	3	5	5	4	4
MALE	28	1148	Kizimbani	Preparation Method 3	4	5	4	4	5
MALE	29	1150	Kizimbani	Preparation Method 3	3	3	3	3	3
FEMALE	25	1204	Kizimbani	Preparation Method 3	5	5	5	5	5
FEMALE	25	1148	Kizimbani	Preparation Method 3	4	4	3	3	3
MALE	29	1200	Kizimbani	Preparation Method 3	4	3	4	4	4
MALE	30	1230	Kizimbani	Preparation Method 3	4	4	3	3	2

MALE	25	1613	Kizimbani	Preparation Method 3	4	5	4	5	5
FEMALE	27	1611	Kizimbani	Preparation Method 3	3	3	3	4	3
FEMALE	26	1612	Kizimbani	Preparation Method 3	4	4	5	4	4
MALE	27	1542	Kizimbani	Preparation Method 3	4	4	3	4	4
FEMALE	25	1458	Kizimbani	Preparation Method 3	3	3	4	4	4
MALE	27	1453	Kizimbani	Preparation Method 3	5	5	5	4	5
MALE	40	1428	Kizimbani	Preparation Method 3	5	2	3	4	4
FEMALE	33	1300	Kiroba	Preparation Method 3	4	5	4	3	4
MALE	51	1520	Kiroba	Preparation Method 3	4	2	2	4	4
MALE	25	1518	Kiroba	Preparation Method 3	4	4	3	4	4
MALE	41	1515	Kiroba	Preparation Method 3	3	4	3	5	5
MALE	33	1514	Kiroba	Preparation Method 3	2	4	4	4	3
MALE	27	1349	Kiroba	Preparation Method 3	4	4	4	4	4
FEMALE	27	1430	Kiroba	Preparation Method 3	4	5	4	4	4
MALE	25	1430	Kiroba	Preparation Method 3	4	4	4	4	4
FEMALE	26	1427	Kiroba	Preparation Method 3	3	3	4	2	3
FEMALE	26	1429	Kiroba	Preparation Method 3	5	3	4	3	4
MALE	41	1418	Kiroba	Preparation Method 3	4	3	5	3	4
MALE	26	1340	Kiroba	Preparation Method 3	5	4	4	4	4
MALE	25	1348	Kiroba	Preparation Method 3	4	3	3	3	3
FEMALE	23	1230	Kiroba	Preparation Method 3	1	3	2	2	2
MALE	28	1238	Kiroba	Preparation Method 3	4	2	3	1	4
FEMALE	23	1236	Kiroba	Preparation Method 3	4	4	5	4	4
FEMALE	25	1330	Kiroba	Preparation Method 3	3	2	1	2	3
MALE	49	1220	Kiroba	Preparation Method 3	2	3	1	2	3
FEMALE	23	1225	Kiroba	Preparation Method 3	3	3	3	3	3

FEMALE	27	1221	Kiroba	Preparation Method 3	2	4	4	2	3
MALE	28	1259	Kiroba	Preparation Method 3	4	4	2	4	4
MALE	36	1232	Kiroba	Preparation Method 3	5	4	3	2	1
FEMALE	25	1210	Kiroba	Preparation Method 3	4	3	2	4	4
MALE	41	1205	Kiroba	Preparation Method 3	5	5	5	5	5
MALE	43	1200	Kiroba	Preparation Method 3	5	1	3	2	2
MALE	49	1200	Kiroba	Preparation Method 3	4	4	4	4	4
MALE	48	1210	Kiroba	Preparation Method 3	4	4	2	4	4
MALE	25	1400	Kiroba	Preparation Method 3	4	4	4	4	4
MALE	28	1489	Kiroba	Preparation Method 3	4	5	5	5	5
MALE	26	1320	Kiroba	Preparation Method 3	4	3	3	4	3
FEMALE	26	1356	Kiroba	Preparation Method 3	3	4	4	4	4
MALE	45	1434	Mpira	Preparation Method 3	3	4	4	4	4
MALE	49	1420	Mpira	Preparation Method 3	5	4	2	3	3
FEMALE	25	1420	Mpira	Preparation Method 3	1	4	4	3	4
MALE	38	1415	Mpira	Preparation Method 3	3	4	4	2	3
MALE	32	1413	Mpira	Preparation Method 3	5	2	2	3	3
MALE	27	1308	Mpira	Preparation Method 3	4	4	4	4	4
MALE	26	1308	Mpira	Preparation Method 3	4	3	4	2	3
MALE	25	1245	Mpira	Preparation Method 3	5	4	4	4	4
FEMALE	26	1232	Mpira	Preparation Method 3	4	4	3	3	3
MALE	33	1125	Mpira	Preparation Method 3	4	3	3	2	3
MALE	25	1156	Mpira	Preparation Method 3	4	4	4	4	4
MALE	25	1158	Mpira	Preparation Method 3	4	3	4	3	3
MALE	29	1156	Mpira	Preparation Method 3	4	5	4	5	5
FEMALE	25	1200	Mpira	Preparation Method 3	5	4	5	4	4

FEMALE	26	1213	Mpira	Preparation Method 3	5	5	4	4	4
FEMALE	27	1214	Mpira	Preparation Method 3	3	3	4	3	3
FEMALE	26	1213	Mpira	Preparation Method 3	5	4	4	4	4
FEMALE	22	1158	Mpira	Preparation Method 3		4	3	3	4
FEMALE	37	1217	Mpira	Preparation Method 3	5	5	4	4	5
MALE	40	1400	Mpira	Preparation Method 3	4	3	2	2	2
FEMALE	33	1300	Mpira	Preparation Method 3	5	5	5	5	4
MALE	51	1520	Mpira	Preparation Method 3	4	4	5	4	4
MALE	25	1518	Mpira	Preparation Method 3	4	4	5	4	4
MALE	41	1515	Mpira	Preparation Method 3	3	4	4	4	4
MALE	33	1514	Mpira	Preparation Method 3	2	4	4	3	4
MALE	27	1349	Mpira	Preparation Method 3	4	4	4	4	4
FEMALE	27	1430	Mpira	Preparation Method 3	5	5	5	5	5
MALE	25	1430	Mpira	Preparation Method 3	4	4	3	3	4
FEMALE	26	1427	Mpira	Preparation Method 3	2	2	4	2	2
FEMALE	26	1429	Mpira	Preparation Method 3	5	5	5	5	5
MALE	41	1418	Mpira	Preparation Method 3	5	5	5	5	5
MALE	27	1628	Cheupe	Preparation Method 3	4	4	3	3	4
MALE	26	1625	Cheupe	Preparation Method 3	4	5	4	3	5
MALE	25	1603	Cheupe	Preparation Method 3	3	3	3	3	3
MALE	27	1633	Cheupe	Preparation Method 3	4	4	4	4	3
MALE	32	1521	Cheupe	Preparation Method 3	5	4	2	4	3
MALE	33	1517	Cheupe	Preparation Method 3	4	4	2	2	4
MALE	41	1516	Cheupe	Preparation Method 3	4	4	4	4	4
MALE	26	1516	Cheupe	Preparation Method 3	4	4	4	4	4
FEMALE	26	1558	Cheupe	Preparation Method 3	5	4	4	4	4

FEMALE	27	1623	Cheupe	Preparation Method 3	3	4	5	3	4
FEMALE	27	1543	Cheupe	Preparation Method 3	4	4	4	3	4
MALE	25	1550	Cheupe	Preparation Method 3	4	3	3	4	4
FEMALE	26	1600	Cheupe	Preparation Method 3	3	3	3	3	3
FEMALE	25	1435	Cheupe	Preparation Method 3	3	5	3	2	4
MALE	58	1228	Cheupe	Preparation Method 3	5	5	4	3	4
MALE	49	1228	Cheupe	Preparation Method 3	3	2	3	2	2
MALE	40	1247	Cheupe	Preparation Method 3	4	5	5	5	3
MALE	47	1247	Cheupe	Preparation Method 3	5	5	3	2	3
MALE	49	1245	Cheupe	Preparation Method 3	3	2	4	5	2
MALE	27	1431	Cheupe	Preparation Method 3	4	5	4	3	5
MALE	35	1630	Cheupe	Preparation Method 3	4	5	4	3	4
MALE	45	1434	Cheupe	Preparation Method 3	4	4	4	4	3
MALE	49	1420	Cheupe	Preparation Method 3	3	2	3	4	3
FEMALE	25	1420	Cheupe	Preparation Method 3	5	2	2	2	3
MALE	38	1415	Cheupe	Preparation Method 3	5	4	3	4	4
MALE	32	1413	Cheupe	Preparation Method 3	5	4	3	4	4
MALE	27	1308	Cheupe	Preparation Method 3	4	4	4	4	5
MALE	26	1308	Cheupe	Preparation Method 3	5	5	3	5	5
MALE	25	1245	Cheupe	Preparation Method 3	5	3	4	5	3
FEMALE	26	1232	Cheupe	Preparation Method 3	5	4	4	4	4
MALE	33	1125	Cheupe	Preparation Method 3	5	5	5	4	5
MALE	41	1205	Nyamkagile	Preparation Method 3	5	5	5	5	5
MALE	43	1200	Nyamkagile	Preparation Method 3	4	4	4	4	4
MALE	49	1200	Nyamkagile	Preparation Method 3	4	2	2	4	3
MALE	48	1210	Nyamkagile	Preparation Method 3	4	4	2	2	2

MALE	25	1400	Nyamkagile	Preparation Method 3	3	3	2	3	3
MALE	28	1489	Nyamkagile	Preparation Method 3	3	4	2	3	3
MALE	26	1320	Nyamkagile	Preparation Method 3	5	4	4	4	4
FEMALE	26	1356	Nyamkagile	Preparation Method 3	4	4	4	5	5
FEMALE	27	1400	Nyamkagile	Preparation Method 3	4	3	4	3	4
FEMALE	27	1358	Nyamkagile	Preparation Method 3	1	3	4	2	2
FEMALE	26	1359	Nyamkagile	Preparation Method 3	5	4	4	4	4
MALE	30	1535	Nyamkagile	Preparation Method 3	4	5	4	4	4
MALE	32	1537	Nyamkagile	Preparation Method 3	4	3	3	4	3
FEMALE	25	1532	Nyamkagile	Preparation Method 3	4	4	3	4	3
MALE	41	1524	Nyamkagile	Preparation Method 3	4	3	3	4	4
MALE	28	1533	Nyamkagile	Preparation Method 3	5	4	3	4	4
MALE	33	1515	Nyamkagile	Preparation Method 3	3	2	2	2	2
MALE	45	1434	Nyamkagile	Preparation Method 3	2	3	3	3	3
MALE	49	1420	Nyamkagile	Preparation Method 3	4	3	2	2	3
FEMALE	25	1420	Nyamkagile	Preparation Method 3	4	4	4	3	4
MALE	38	1415	Nyamkagile	Preparation Method 3	3	2	3	2	3
MALE	32	1413	Nyamkagile	Preparation Method 3	5	4	4	4	4