NUTRIENTS AND ANTI-NUTRIENTS LEVELS, AND NUTRIENTS BIOACCESSIBILITY IN COOKED *Manihot esculenta* CRANTZ VARIETIES GROWN IN KILIFI AND BUSIA COUNTIES, KENYA

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DECLARATION

I hereby declare that this Thesis is my original work and has not been presented for award of a degree or any other award in any other university.

Signed……………………………………Date…………………………………………………

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We confirm that the work reported in this thesis was carried out by the candidate under our supervision as university supervisors.

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DEDICATION

I dedicate this thesis to the Almighty God, my wife, Selestiner Nabwire, my sons, Rian, Davian and Adriel Ogombe, my parents, Ogombe Semeo and Juma Consolata.

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Abbreviations and Acronyms

AAS	Atomic Absorption Spectroscopy
AES	Atomic Emission Spectroscopy
ANOVA	Analysis of Variance
AOAC	Association of Official Analytical Chemists
FAD	Flavin Mononucleotide
FAD	Flavin Adenine dinucleotide
FAO	Food Agriculture Organization
HCN	Hydrogen Cyanide
HPLC	High Performance Liquid Chromatography
KALRO	Kenya Agricultural and Livestock Research Organization
KARI	Kenya Agricultural Research Institute
KNBS	Kenya National Bureau of Statistics
NAD	Nicotinamide Adenine Dinucleotide
RDA	Recommended Dietary Allowance
RSD	Relative Standard Deviation
SGF	Simulated Gastric Fluid
SIF	Simulated Intestinal Fluid
SPSS	Statistical Package for Social Sciences
SSF	Simulated Salivary Fluid
USDA	United State Department of Agriculture
UV–Vis	Ultraviolet–visible spectroscopy
WHO	World Health Organization

Abstract

Manihot esculenta Crantz (cassava) is a staple crop, with different sweet and bitter varieties grown in developing nations. Bitter cassava varieties are primarily used for industrial purposes like making flour, starch, bioethanol, and adhesives after being processed to remove toxic cyanide. Sweet cassava is used for direct food consumption, where it is boiled, fried, or baked, and it can also be processed into various food products like flour and snacks. The study areas in Kenya, Kilifi County, grow *Kibandameno* and *Tajirika* varieties while *MM96/2480* and *Migyera* are cultivated in Busia County. The crop's roots and leaves are a source of protein, thiamine, riboflavin, ascorbic acid, niacin, potassium, calcium, zinc, iron, oxalate and phytate. Nutritional data has shown that Kilifi and Busia Counties have high prevalence of under nutrition, amidst relying on otherwise nutritious cassava-based diets. For consumption, the general cooking methods involves roots being boiled or deep-fried, while leaves are pounded then boiled. In view of malnutrition statistics in communities that otherwise consume cassava, it is unclear whether the underlying factor would be attributed to impact that cooking has on nutritional levels of both leaves and roots of cassava, or nutrient bioaccessibility. This was envisaged as a study gap. The concentrations of specific anti-nutrient and nutrients in cassava leaves and roots with age of the plant, impact that boiling and deep-frying tubers and boiling leaves of cassava has on nutritional and anti-nutrient levels, and finally the bioaccessibility of nutrients were determined. Static gastrointestinal digestion was performed after and prior to determination of protein (by kjeldahl), vitamins and anti-nutrients (by HPLC) and minerals (by AAS and AES). Nutrient levels (mg/100 g) in raw roots ranged from 17.00 - 114.00 (protein), 1.93 - 12.74 (vitamin C), and from 32.08 - 162.98 (Ca). Levels of anti-nutrients (mg/100 g) ranged from 390.37 - 561.28 (phytate), and from 4.72 - 613.46 (oxalate). Cooking resulted in a reduction of the levels ranging from 12% (K) to 98% (vitamin B1) in all nutrients and anti-nutrients studied, with boiling roots recording higher losses than deep-frying ($P<0.001$). The bioaccessibility of vitamins and minerals was lower in raw roots (15 - 72%), but significantly higher ($P<0.001$) in cooked roots, with deep-fried roots showing bioaccessibility between (20 – 79%) and boiled roots between (27 – 84%). A trend similar to the one observed in roots was observed in cooked cassava leaves with bioaccessibility ranges of vitamins in raw leaves (8 – 69%) being significantly lower ($P<0.001$) than in boiled leaves (11 – 81%). The study concludes that levels of ascorbic acid, thiamin, riboflavin, niacin, protein, calcium, zinc, iron and potassium, phytate and oxalate in *Manihot esculenta* Crantz varieties depend on the variety, part of the plant (roots or leaves), and harvesting age. Cooking significantly reduces levels of these nutrients and anti-nutrients with a higher reduction on boiling than deep-frying. The bioaccessibility of nutrients however significantly increased with cooking but the levels were lower than the recommended dietary allowance (RDA) for all the nutrients determined. The study recommends that cooking lowers anti-nutrients and increases nutrient bioaccessibility. Cassava roots should not be left in the farm for a longer period after maturity when nutrient levels are high and cooking by deep frying to retain high levels of the water-soluble nutrients.

CHAPTER ONE

INTRODUCTION

1.1 Background information

In countries which lie south of Sahara, after rice and maize, *Manihot esculenta* Crantz (cassava) ranks as 3rd most significant source of carbohydrates (Food Safety Network, 2014). Cassava is cultivated across approximately 29.95 million hectares, predominantly in the African continent (60.9%), followed by Asia (29.4%) and America (9.6%), with a total production exceeding 319 million tons of fresh roots. In 2021, a production of 319 million tons of fresh roots was reported, mostly concentrated in Nigeria, the Democratic Republic of Congo, Thailand, Ghana, Brazil, and Indonesia (Otekunrin, 2024). In Kenya cassava production is centered in the Western, Coastal, and Eastern regions (Githunguri *et al.*, 2017).

It is a famine reserve crop, and hence can survive in areas of low rainfall as those in parts of Kilifi and Busia Counties, Kenya. A number of varieties of cassava (sweet and bitter) including those improved by (KALRO) are grown. Other cassava cultivars could be developed using the great range and diversity shown by the different varieties (Berhanu *et al.*, 2023). The varieties in Kenya include; *Kibandameno*, *Tajirika, Shibe, Kaleso* grown in Kilifi County, and *Migyera*, *MM96/2480*, *Nasa 13, TME 14, Nasa 14, TME 2004, and* grown in Busia County (Kenya News Agency, 2024; Kidasi *et al.*, 2021). The *Tajirika* cultivar is favored because of its great yielding qualities and resistance to pests and diseases. On the other hand, *Kibandameno* is favored due to its sweet flavor, drought tolerance, and high cooking quality (Kidasi *et al.*, 2021).

Studies on nutritional composition of leaves and roots of cassava shows that they are an important source of vitamin C, B series and protein, minerals like Ca, Zn, Fe and K. Other studies have reported elevated amounts of phytate, oxalate, tannin (anti-nutrients) and poisonous cyanogenic glycosides in them (Akinpelu *et al.,* 2011). The nutritional composition would however depend on part of the plant (leaves or roots), plant age, variety, climatic conditions, soil fertility and methods of processing among other factors (Tagesse *et al.,* 2022; Oresegun *et al.*, 2016; Waluchio, 2016; USDA, 2008). In industries, cassava starch is a natural adhesive used for creating corrugated cardboard, paper bags, and remoistening gums for postage stamps and envelops. In pharmaceutical industry, cassava starch is used as a binder and filler in tablets. In cosmetics, modified starches can be used as a thicker in shampoo and as an emulsifier (Abotbina, 2022). The body needs nutrients; however anti-nutrients have an impact on the nutrients' bioaccessibility and consequently bioavailability.

Proteins are indispensable for numerous bodily functions including catalyzing metabolic reactions, transmitting signals and providing structural support. Vitamin C is essential to growth and maintenance of most tissues in the body including collagen. Collagen is needed for healing connective tissues and wounds, as well as helping bones and teeth stay strong and improve absorption of non-heme iron (Chambial *et al.*, 2013). An important micronutrient for the metabolism of glucose is vitamin B1. It helps in the functioning of the nerves, muscles and the heart. Vitamin B2 is necessary for FMN and FAD coenzyme which are essential for energy synthesis, cellular activity, growth and development, metabolism of lipids, medications, and steroids (Northrop-Clewes *et al.,* 2012). Every tissue in the body produces the coenzyme nicotinamide adenine dinucleotide (NAD), the main form of ingested vitamin B3 that is biologically active. More than 400 enzymes need this to catalyze bodily functions.

Potassium serves as an electrolyte, maintains the health of the heart, brain, kidney, muscle tissue, other vital organ systems and is a necessary mineral for the proper operation of all cells. Further, it stimulates neural activity, stabilizes blood sugar and blood pressure, strengthens muscles, controls the neurological system and controls fluid levels (Turck *et al.*, 2016). Calcium plays various roles, including; bone health, muscle contraction and cardiovascular systems where it is essential in blood clotting and also a co-factor for many enzymes. Numerous activities, such as immunological function, enzymatic reactions, wound healing, protein and DNA synthesis, growth and development and gene expression depend on zinc.

A key component for the production of blood is iron. Hemoglobin and myoglobin contain roughly 70% of the body's iron. While myoglobin absorbs, stores, transports and releases oxygen, hemoglobin is essential for delivering oxygen to the blood's tissues from the lungs. Phytate affects bioaccessibility and bioavailability of nutrients in human and mono-gastric animals. It accomplishes this by strongly chelating iron, zinc and calcium forming complexes that are not absorbed, hence exacerbating the minerals' deficiencies (Gupta *et al.*, 2015). Many mineral ions form insoluble precipitates with oxalate hence reducing their bioavailability, calcium oxalate being an example (Massey *et al.*, 2007). Cyanogenic glycosides are a class of secondary plant chemicals that contain nitrile and when broken down by enzymes, produce cyanide (cyanogenesis) (Ndubuisi *et al.*, 2018).

As already stated, processing would change the cassava leaves' and roots' nutritional makeup (Ujong *et al.,* 2020; Achidi, 2008). Processing methods for cassava roots include boiling, deep-frying, roasting and fermenting while leaves are pounded then boiled. In Nigeria cassava roots can be processed into products such as *gari, lafun, eba* and *fufu* (Adepoju, 2010). Even though when they are processed nutrient levels are affected, nutrients bioaccessibility is essential in addressing malnutrition. Bioaccessibility refers to the portion of a nutrient which is released from the matrix of food, and can be absorbed by the body. Bioaccessibility can therefore be used to predict nutrients bioavailability. Bioaccessibility depends on both nature of the matrix of food and digestion (Jakobsen, 2019). For vitamins and minerals, this has been attributed to inhibitors, like phytate, oxalate, polyphenols, fiber content and chemical forms of food's nutrients (Bertinato *et al.*, 2020; Platel *et al.*, 2016). Bioaccessibility is normally assessed either by *in vitro* or *in vivo* techniques. *In vitro* static simulated gastrointestinal digestion was employed (Etcheverry *et al.,* 2012). The method analyses the end points which entails nutrients quantity liberated from matrix of food when it is broken down by the digestive enzymes (Brodkorb *et al.*, 2019).

1.2 Statement of the problem and justification of the study

Cassava in Kenya, is mainly grown in coast where Kilifi County is located and western regions mainly Busia County. The sweet cassava varieties such as *Kibandameno, Tajirika, Migyera* and *MM96/2480* are predominant hence utilized in this study. Their roots are consumed as a meal or processed into flour while the leaves are served as vegetables. Nutritional composition of leaves and roots of cassava show them as nutritious. This would although, depend on plant age, variety, plant part, climatic conditions, soil fertility and method of processing among other factors (Tagesse *et al.,* 2022; Oresegun *et al.*, 2016; Waluchio, 2016; Latif & Muller, 2015; USDA, 2008). Boiling and deep-frying are common methods employed for cooking roots while leaves are boiled after pounding. These cooking methods are envisaged to affect nutrient and anti-nutrient content (Ujong *et al.*, 2020; Achidi, 2008).

In some studies, malnutrition has been associated with reliance on cassava-based diets (Nginya, 2015; Azikoyo *et al.*, 2012). Azikoyo *et al.*, (2012) reported malnutrition (26% stunted, 13% underweight and 10.1% wasting) among children less than five years in communities consuming cassava from Nambale Busia County, Kenya. The nutritional survey of children aged 2–5 years at the coastal part of Kenya showed that 22% were moderately stunted and 7% severely stunted (Nginya, 2015). The fact that literatures shows that cassava is nutritious on one hand and on the other hand over relying on it as the main source of nutrients leading to malnutrition creates a study gap. In view of malnutrition statistics in communities that otherwise consume cassava, it is unclear whether the underlying factor would be attributed to amount of anti-nutrients, harvesting age, impact of cooking on the amount of nutrients or bioaccessibility of nutrients from roots and leaves of cassava. While literature generally affirms that cooking affects nutrient content in foods, there lacks information on bioaccessibility of nutrients from cassava roots and leaves.

Bioaccessibility of nutrients has been reported in several food-stuffs including but not limited to processed cereals and pulses, spinach, kales and bananas (Akca, *et al.*, 2019; MacDonald *et al.*, 2016; Hemalatha *et al.*, 2007). This information cannot be applied to cassava since bioaccessibility depend on among other factors the levels of anti-nutrients which is more specific to given crops and even variety. There was therefore need to carry out this study which contributes to the data on bioacccessibility of nutrients in cooked cassava products. The study considered the various classes of nutrient that is macronutrients (protein), macro-elements (K and Ca), micro-elements (Fe and Zn) and water soluble vitamins because majority of the population which over relies on cassava may not afford cooking oil for frying. The four varieties were sampled based on their popularity in the study areas. The study areas were picked based on the importance of cassava in these areas (Kidasi *et al.,* 2021). Information on bioaccessibility of nutrients from cassava is very important in optimizing health and nutrition benefits of cassava, guiding food and supplement development and for predicting bioavailability.

1.3 Hypotheses

I. There is no significant difference in the levels of nutrients and anti-nutrients in raw roots and leaves of different varieties of *Manihot esculenta* Crantz harvested after 8 and 12 months in Kilifi and Busia Counties

II. There is no significant difference in levels of nutrients and anti-nutrients in cooked roots and leaves of the different varieties of *Manihot esculenta* Crantz harvested after 8 months in Kilifi and Busia Counties.

III. There is no significant difference in bioaccessibility of nutrients in cooked roots and leaves of different varieties of *Manihot esculenta* Crantz harvested after 8 months in Kilifi and Busia Counties.

1.4 General objective

To investigate nutrient and anti-nutrients levels, and nutrient bioaccessibility in cooked roots and leaves of *Manihot esculenta* Crantz varieties grown in Kilifi and Busia Counties, Kenya.

1.4.1 Specific objectives

i. To determine levels of protein, vitamins (C, B1, B2 and B3, minerals (Ca, Zn, Fe, K) and anti-nutrients (phytate and oxalate) in raw roots and leaves of 8- and

12-month plant of *Kibandameno, Tajirika, Migyera* and *MM96/2480 Manihot esculenta* Crantz varieties grown in Kilifi and Busia Counties.

ii. To determine the levels of protein, vitamins (C, B1, B2, and B3), minerals (Ca, Zn, Fe, K) and anti-nutrients (phytate and oxalate) in boiled roots and leaves, and deep-fried roots of 8- month plant of *Kibandameno, Tajirika*, *Migyera* and *MM96/2480 Manihot esculenta* Crantz varieties grown in Kilifi and Busia Counties.

iii. To determine the bioaccessibility of vitamins (C, B1, B2 and B3) and minerals (Ca, Zn, Fe and K) from deep-fried and boiled roots, and boiled leaves of *Kibandameno, Tajirika*, *MM96/2480* and *Migyera* varieties grown in Kilifi and Busia Counties.

1.5 Significance of the study

The findings of this study demonstrate variation of nutrients and anti-nutrients with age of the plant. This information helps the consumers to maximize the nutritional potential of cassava by getting the appropriate harvesting age. It indicates the extent to which nutrients and anti-nutrients nutrients are lost following cooking of leaves and roots of *Kibandameno, Tajirika, MM96/2480* and *Migyera* cultivars of cassava. Consequently, bioaccessible vitamins; C, B1, B2 and B3 and minerals Ca, Zn, Fe and K were studied. This not only contributes to knowledge on the nutritional efficacy of these cassava varieties in terms of being good sources of the studied nutrients but also provides information on the importance of their consumption to the consumers.

It is worth noting that the findings on nutrient bioaccessibility are important within the scope of nutrient availability, health, nutrient processing and risk assessment. In this study, understanding the bioaccessibility of the nutrients in cassava helps assess their availability for absorption by the human body. The information is also crucial in enabling assessment of the overall nutritional impact of cassava consumption in line with human health. The results show that the cooking methods can be optimized to maximize nutrient retention. In the academic field, the study helps bridge the knowledge gap by providing crucial data on bioaccessibility of nutrients from cassava roots and leaves which was missing.

1.6 Scope and limitations

Three factors that would affect quantity of protein, vitamins (C, B1, B2 and B3) and minerals (Ca, Zn, Fe and K) and anti-nutrients (phytate and oxalate) in cassava were considered namely the plant part, age and methods of cooking. The roots and leaves were harvested at 8 and 12 months and cooked by boiling and deep-frying which are the common cooking methods used in the study areas. The two cassava varieties cultivated in Kilifi County (*Tajirika, Kibandameno)* and another two varieties cultivated in Busia County (*Migyera, MM96/2480)*, were studied with the climatic conditions, soil fertility among other factors that affect the nutritional composition notwithstanding.

Although there are other anti-nutrients such as tannins and lectins, the study only considered phytate and oxalate. It is only bioaccessible vitamins; C, B1, B2 and B3 and minerals Ca, Zn, Fe and K that were determined. Although digestion can be achieved through *in vivo* and *in vitro* techniques, static gastrointestinal technique was adopted. This approach has drawbacks since it cannot assess quantities being absorbed, identify competition between nutrients and other dietary components at uptake site, or assess kinetics of transport as they occur *in vivo.* Furthermore, static *in vitro* assays cannot assess variables including nutritional status, age, physiological state (like obesity, pregnancy, and lactation), or chronic and acute infections that affect nutrient's or bioactive compound's bioavailability (Alegría *et al.*, 2015).

CHAPTER TWO

LITERATURE REVIEW

2.1 Food insecurity and malnutrition in Kenya

A situation where people have physical, financial and social access to adequate, nutritious free from contamination food is called food security. Lack of availability to enough food, or food of appropriate quality, to meet one's nutritional needs is known as food insecurity (WHO, 2022). Kenya continues to have significant levels of food insecurity, ranking 77th out of 119 countries with a score of 23.3 on the Global Hunger Index (2018). According to the survey, almost 20% of the population does not consume enough food to sustain a healthy life. Approximately 3.4 million Kenyans experienced acute food insecurity in 2017 (USAID, 2017), demonstrating the country's ongoing chronic food insecurity. Numerous factors contribute to the current food insecurity issues, such as the country's widespread droughts, the high cost of inputs, particularly fertilizers, the high cost of food globally, and the low purchasing power of a large portion of the population due to high poverty levels (USAID, 2017).

According to Hegazi *et al.*, (2024), malnutrition is defined as insufficiencies, more than enough, or lack of balances in an individual's nutrient and/ or energy consumption. There are two primary categories of malnutrition. Stunting (low height to age), underweight (low weight for age), wasting (low weight for height), and deficiencies of micronutrient are examples of "under nutrition". Non-communicable diseases related to the diet and obesity is the other (Hegazi *et al.*, 2024). Kenya is among the countries in the world where all forms of malnutrition are present. The triple burden of malnutrition in Kenya is characterized by the coexistence of under-nutrition as manifested by stunting, wasting and underweight, micronutrient deficiencies, and overweight and obesity. While more remains to be done, Kenya has made substantive progress in reducing the prevalence of stunting nationally, dropping from 26% in 2014 to 18% in 2022. Of the country's 6.3 million children under five, 1.13 million are stunted (18%), 631,196 (10%) are underweight and 189,359 (3%) are overweight (Nyakundi *et al.,* 2024). Stunting is highest among children in rural areas (20%) compared to those in urban areas (12%). Out of 47 counties, three have very high levels of stunting, 10 have high levels and 31 have medium levels, according to the World Health Organization's classification. While acute malnutrition (wasting) among children under five is relatively low nationally (5%), 15 counties have wasting rates above the national average (KDHS, 2022).

Stunting is the most common kind of malnutrition in Busia County, accounting for 22% of cases, while underweight and wasting affect 9.5% and 2.2% of children below five years, respectively (KDHS, 2014). Maternal and child survival are impacted by micronutrient status, which is extremely important. The frequency of micronutrient deficiencies has significantly decreased in Busia (Harika *et al.,* 2017), with the exception of zinc insufficiency, which remains a problem. In Kilifi County stunting levels stands at 32.2% with severe stunting at 8.7%. Underweight among children 6 – 59 months stand at 19.8% while severe underweight is at 3.4% (Kilifi County integrated smart survey report, 2023). These deficiencies are linked to a poor and limited dietary diversity, with a reliance on staple crops like cassava, and a shift away from traditional foods like African leafy vegetables. The problem is further compounded by factors like poverty and food insecurity. This study therefore intends to bridge the gap between nutritional levels of cassava shown in section 2.2 and its association to malnutrition when over relied on as a staple food.

2.2 Nutritional value of cassava

For at least 4,000 years, the indigenous Indian population in Latin America has been growing cassava (*Manihot esculenta* Crantz). After being found in the America, European traders brought the crop to Africa as a useful subsistence crop. Thereafter, Asians started growing it for starch extraction and as a crop for food security (Akinpelu *et al.*, 2011). With over 45 million metric tonnes produced annually, more cassava is produced in Nigeria than in any other nation worldwide (Otekunrin, 2024).

Cassava thrives in warm, tropical climates. Temperatures should ideally stay between 20^{o}C and 32^{o}C. Frost temperatures below 10^{o}C can stress the plant. It requires full sun for the majority of the day to produce the best yield. The best soil is deep, fertile, and loamy, as it holds water well and is easy to till. Cassava is very tolerant of poor soils and can grow in sandy or low-fertility conditions. Good drainage is crucial because cassava does not tolerate flooding or standing water. Waterlogged soil can cause root rot. While the crop is drought-tolerant, it should be watered until established and during dry spells. A minimum of 1000 mm of annual rainfall is ideal, with at least 50 mm per month for a minimum of six months. Cassava is tolerant of a wide range of soil pH levels from 4 to 8 but is most productive in the ranges of 5.5 to 6.5. Cassava should be planted at the beginning of the rainy season to take advantage of moisture for root development. It is highly susceptible to weed competition in the first 3-4 months. The first weeding should be done within the first month. Repeat weeding at 8 and 12 weeks, depending on weed pressure. Final weeding should be done between 20 and 24 weeks after planting. If soil fertility is high, fertilizer may not be needed. For extra yield, a high-potassium fertilizer can be used, applied when the soil is not waterlogged.

It is vitally regarded for its role in reducing poverty, ensuring food security, and providing feedstock for industries connected to agriculture. Approximately 30% of the Kenyan population regularly consumes cassava, particularly in regions such as Western, Nyanza, and Coastal areas (Khasoa, 2024). Cassava leaves and roots are a nutrient-dense food because they are high in protein, vitamins, and several essential minerals like iron and zinc (Table 2.1) (USDA, 2008).

Table 2.1**:** Composition of nutrients in cassava leaves and roots

	Cassava roots (100 g)	**Cassava leaves (100 g)**
Thiamine (mg)	0.03 – 0.28	0.06 - 0.31
Riboflavin(mg)	0.03 – 0.06	0.21 - 0.74
Niacin (mg)	0.6 – 1.09	1.3 - 2.8
Ascorbic acid (mg)	14.9 – 50	60 -370
Calcium (mg)	19 – 176	34 – 708
Iron (mg)	0.3 – 14	0.4 – 8.3
Potassium (%)	0.25 – 0.72	0.35 – 1.23
Zinc(ppm)	14 – 41	71 – 249

Source: National Nutrient Data base (USDA, 2008)

Researchers further agreed to the invaluable nutritional composition of cassava (Tagesse *et al.*, 2022; Waluchio, 2016; John *et al.*, 2012). Tagesse and co-workers (2022), reported a range (mg/100 g) of 52.49 – 54.90 (Ca), 15.33 – 18.61 (Zn), 14.28 – 16.65 (Fe), 292.38 – 296.77 (K) being levels in raw and cooked cassava roots. The authors also reported a range (mg/100 g) of 499.85 – 545.36 (Ca), 119.46 – 132.11 (Zn), 89.36 – 121.96 (Fe), and 340.53 – 1193.37 (K) in raw and cooked cassava leaves. The difference in soil fertility was thought to be the cause of the variations in mineral levels. Waluchio, (2016) reported crude protein levels of between 20% and 31.6% in cassava leaves. These were described as being high, and that could mitigate protein needs in the predominantly cassava roots diet.

According to John *et al.* (2012), variety *KME 61* had the greatest iron concentration (59.69 ppm), whereas variety *SS4* had the lowest (29.89 ppm). *Migyera* had the lowest zinc levels (64.56 ppm) and *KME 1* had the highest (93.07 ppm), but all varieties at this location fared very well because their zinc concentration was higher than 64 ppm at the Kericho site. Zinc concentrations in roots varied greatly, with *KME 61* possessing the greatest concentration (118.10 ppm) and *MM96/7151* possessing the lowest (46.78 ppm) at the Nakuru location. The oxalate levels in cassava leaves and roots varied from 30.59 to 154.7 and 1.35 to 2.88 g/100 g, respectively (Waluchio, 2016). According to the same report, the amount of phytic acid in roots ranged from 661.33 to 3040 mg/kg. The various cultivars and growth conditions were attributed for the variation in the cassava quality indicators examined.

Age of the plant at harvesting is another factor that has been shown to affect nutrients and anti-nutrients levels in cassava. The Technical Bulletin No 21, cassava post-harvest care and market preparation (2004), observed that as the cassava roots age beyond a year, they become woody and fibrous hence less nutritious. The decline was ascribed to uptake of nutrients by the plants more quickly when they are young, as they age, the rate of absorption reduces. Table 2.2 displays the nutrient content of different foods in comparison to that in leaves of cassava. According to the table, leaves of cassava have nutrient levels that are comparable to those of common vegetables such as carrots and spinach (USDA, 2008).

Table 2.2**:** Comparison between nutrient levels in leaves of cassava and various foods (per 100 g)

Food	Water (g)	Energy (Kcal)	Protein (g)	Total lipid (g)	Ash (g)	Dietary fiber (g)
Cassava leaves	64.8 – 88.6	91	1.0 - 10	0.2 – 2.9	0.7 – 4.5	0.5 - 10
Green beans	90.27	31	1.82	0.12	0.66	3.4
Carrots	88.29	41	0.93	0.24	0.97	2.8
Lettuce green leaf	95.07	15	1.36	0.15	0.62	1.3
Soya beans green	67.5	147	12.95	6.8	1.7	4.2
Spinach	94	14	1.5	0.2	1.8	-

Source: National Nutrient Data base (**USDA, 2008**)

According to a study by Satheesh *et al.,* (2020), kale is considered to be an excellent supply of vitamin C, which varied from 62.27 to 969 mg/100 g. The same study reported amounts of thiamine, riboflavin, and niacin to be 0.110 – 0.9, 0.13 – 0.9, and 1.00 mg/100 g, in that order. Iron levels varied from 1.1 to 12.19 mg/100 g, and those of zinc from 0.045 to 394 mg/100 g. Due to the presence of anti-nutrient and the poisonous cyanogenic glycosides in both cassava roots and leaves, various cooking methods as described in section 2.4 are used to prepare them before consumption.

2.3 Anti-nutrient factors in cassava

The presence of toxic and anti-nutritional substances in cassava has limited its consumption even though it is an important food source (Bayata, 2019). Anti-nutritional factors may either be in form of synthetic or natural compounds that interfere with absorption of nutrient and hence affect their bioavailability (Vikram *et al.,* 2020). The magnitude of anti-nutrients in raw roots of some cassava varieties render them unsafe for consumption until prepared (Ndie *et al.,* 2018). Some of the anti-nutrients in cassava include; phytates, oxalates, tannins and cyanogenic glycosides. Waluchio, (2016) reported oxalate levels of between 30.59 and 154.7 g/100 g in cassava leaves. A study done by Sarkiyayi *et al.,* (2010) reported phytate values of 2160 mg/kg and 3040 mg/kg for sweet and bitter cassava varieties respectively. Manano *et al.,* (2018) on the study chemical composition of major cassava varieties in Uganda, targeted for industrialization reported phytate levels ranging between 661.33 mg/kg and 984.64 mg/kg. Studies have indicated that these levels depend on among other factors: varieties, climatic conditions and soil fertility (Ndubuisi & Chidiebere, 2018).

Phytates: Phytate (Inosital hexakisphosphate) controls the intracellular signaling and forms the phosphate storage part in most cereal grains, legumes, nuts, oilseeds, and tubers (Pandey *et al.,* 2021). It is a saturated cyclic acid.

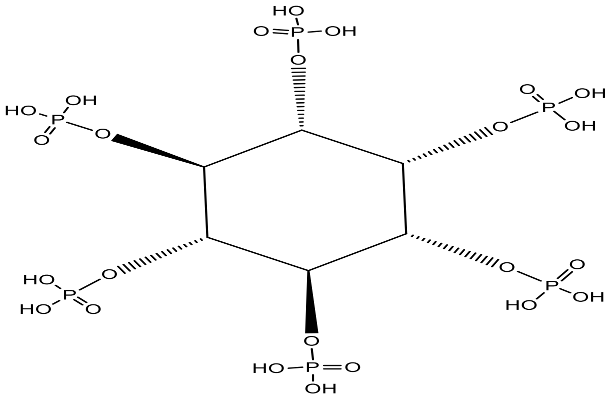

Figure 2.1: Phytate

Phytate has anti-nutritional activities in human and mono-gastric animal diets through its strong chelation of calcium, iron and zinc to form insoluble complexes that are not absorbed hence contributing to the deficiency of these minerals. The anti-nutritional effect of phytate in the human diet is caused by the inability of the human digestion system to degrade it because of the absence of the intestinal phytase enzyme in humans. The phosphate groups in phytate are double charged hence strongly bind cations and impede their absorption (Gupta *et al.,* 2015). The inhibitory effect of phytate on zinc and iron absorption is dose dependent. It is assumed that for foods the bioavailability of iron is affected by a molar ratio for phytate: iron above 1. The phytate: zinc molar ratio greater than 15 is likely to compromise zinc bioavailability (Rahman & Shaheen, 2022).

Oxalates: Oxalates are dicarboxylic acid $[(COO)_2^{2-}]$ that are present in plant-based foods normally synthesized by the incomplete oxidation of carboxylates (Salgado *et al.*, 2023).

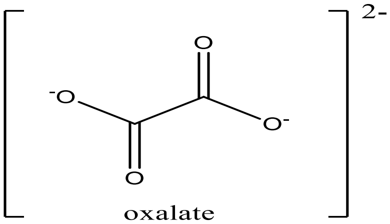

Figure 2.2: Oxalate

Many mineral ions form insoluble precipitates with oxalate hence reducing their bioavailability, a prominent example being calcium oxalate, the primary constituent of most common kidney stones (Khan *et al.,* 2016). Impact that oxalates have on health of human is highly dependent on the calcium available and the oxalate levels consumed.

Cyanogenic glycosides: The cyanogenic glycosides are a group of nitrile-containing plant secondary compounds that yield cyanide (cyanogenesis) following their enzymatic breakdown (Ndubuisi & Chidiebere, 2018). The mechanism is as shown in Figure 2.3. Two kids passed on in Makueni in the wake of eating inappropriately prepared cassava in 2011 (Njue *et al.,* 2011). Cyanide intake is known to exerberate goiter which is associated with iodine deficiency (Pearce & Braverman, 2017).

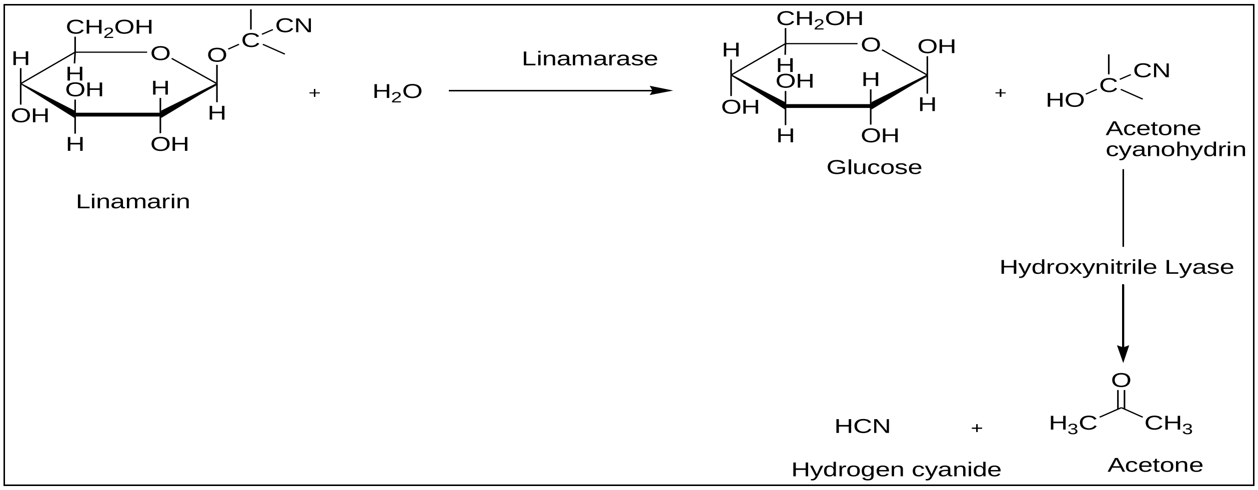

Figure 2.3: Hydrolysis of cynogenic glycoside.

2.4 Effects of cooking and other processing methods on nutrients and ant-nutrient levels

Boiling and deep-frying are common methods employed for processing cassava roots, while leaves are pounded then boiled. Anti-nutrients and nutrients levels are affected by both approaches (Ujong *et al.,* 2020; Achidi, 2008). Ujong and co-workers (2020) reported that while the minerals were significantly kept after microwaving cassava leaves (sweet and bitter types); steaming and boiling significantly reduced amounts of zinc, iron, potassium, magnesium and calcium. The considerable mineral losses observed with the boiling procedure were linked to the enormous amounts of boiling water and steaming water vapor, which may have caused these minerals to leach. Achidi (2008) reported that pounding cassava leaves before cooking them, led to higher loss of vitamins. When the leaves were pounded, the reduction was ascorbic acid (78.61%) and thiamine (38.95%) than when they were not pounded [vitamin C (76.87%) and vitamin B1 (35.14%)]. The reduction was because pounding exposes nutrients leading to increased leaching of these nutrients in boiling water.

Thermal processing significantly altered the vitamin content in vegetables ($P< 0.001$). According to Onyambu *et al.,* (2021), the levels both rose (+11.14 to +425.96) and fell (−6.02 to −100.00).

Adepoju (2010) examined how processing techniques affected the retention of nutrients and how cassava contributed to Nigerian consumers' dietary intake. The findings showed that processing cassava roots into various products improved the nutrients' bioavailability. For example, potassium improved from (234 mg in fermented *gari* - 473.2 mg in *Lafun* fermented), iron (1.0 - 4.3 mg), calcium (22.7 mg *eba* - 67.3 mg in *Lafun* fermented), zinc (2.5 - 6.7 mg), and protein increased from (1.3 g in *gari* - 2.6 g in *fufu* and *amala*). According to the same study, leaching significantly reduced the mineral content of fresh cassava tubers after they were soaked for longer than two days. The study came to the conclusion that nutrients, particularly minerals, were less retained the longer the soaking period. The minerals were more likely to leach easily through draining water due to processing techniques that required grating.

Kasaye *et al.,* (2018) reported a reduction of 37.4%, 0.05%, and 4.31%, in amount of phytic acid, protein and oxalate respectively on boiling cassava flour. The same study reported a reduction of 34.62%, 26.46% and 27.02% of calcium, iron and zinc respectively on fermentation of cassava flour table 2.3. This can be attributed to the fact that processing cassava into flour involves a combination of techniques like peeling, crushing, fermenting, and drying to reduce anti-nutrients. These techniques lead to the loss of nutrients through leaching of water-soluble nutrients, degradation of vitamins and microbial action of fermentation.

Table 2.3**:** Effect of fermentation and boiling on phytate, oxalate, protein, calcium, iron, and zinc levels in cassava flour (mg/kg)

Flour	**Phytate**	**Oxalate**	**%protein**	**Ca**	**Fe**	**Zn**
Raw cassava flour	809.48	2.32	1.02	734.88	30.38	4.7
Boiled cassava flour	506.34	2.22	0.97	554.42	30.59	4.53
Fermented cassava flour	416.86	1.58	0.71	480.44	22.34	3.43

*Source: (*Kasaye *et al.,* 2018*)*

Table 2.4 displays the findings of a study conducted by Waluchio (2016) on the impacts of pounding, fermentation, and solar drying on anti-nutrients and nutrients levels in leaves of *Tajirik*a and *Kibandameno* cassava cultivars that are cultivated along Kenya's coast. According to the findings, the *Tajirika* variety had lower quantities of crude protein than the *Kibandameno* variety. The genetic variations among the various cassava cultivars have been attributed for this. Protein level decreased in all the preparation methods which was attributed to leaching effect during boiling stage. *Kibandameno* had higher levels of vitamin C, iron and zinc than *Tajirika* and that most of the nutrients are reduced by the processing methods used. Due to its high sensitivity to drying and cooking, as well as its thermolability at mild heating, vitamin C was reduced during the solar drying process (Faber & Van Jaarsveld, 2007). Due to leaching during the pounding and boiling process, the iron, zinc, and calcium levels in pounded-boiled cassava leaves were the lowest (Ujong *et al.,* 2020; Achidi, 2008).

Table 2.4: Mineral content in prepared cassava leaves from *Kibandameno* and *Tajirika* varieties grown in Kenya (mg/100 g)

Varieties	**Cassava leaves preparation**	**% Protein**	**Vitamin C**	**Iron**	**Zinc**	**Calcium**	**Oxalate**	**Phytate**
Kibandameno	uncooked	38.40	96.28	58.78	31.58	1031	78.61	509.6
	Solar dried	32.14	26.64	96.47	24.05	1157.5	33.56	144.5
	Fermented	29.73	14.51	60.85	17.02	1073.1	29.65	351.8
	Pounded	32.65	53.47	12.45	27.44	214.4	67.97	47.8
Tajirika	Raw	27.40	30.33	45.62	15.61	1590	37.27	319.2
	Solar dried	27.29	16.65	81.15	13.79	1686	23.56	220.6
	Fermented	23.49	11.62	48.62	6.17	1294	21.17	264.0
	Pounded	22.74	22.65	23.71	11.51	412	34.76	45.0

Source: Waluchio (2016)

A variety of techniques such as boiling, baking, steaming, deep frying and sun-drying both blanched and raw chips are adopted for processing roots for consumption (Bolarinwa *et al.*, 2016). Residual levels of cyanogenic glucosides and their toxic degradation products, cyanohydrins and free cyanide, in processed cassava depend upon the original levels of cyanogenic glucosides, and on the nature and duration of the method used for processing. Up to 80% of the glucosides are removed by boiling and sun-drying, while only about 20% is eliminated by frying, baking and steaming. Reduction in total cyanogens is effected by enzymatic decomposition of cyanogenic glucosides and/or leaching of cyanogens in the water, in which the roots are boiled, with liberation of volatile HCN. The process of crushing and pounding fresh roots followed by sun-drying eliminates as much as 95% of the cyanogens. This is the most effective of the methods used in India for reducing the cyanogen content of cassava roots. Boiling of leaves removes about 85% of the cyanogenic glucosides (Bolarinwa *et al.*, 2016). Ojiambo *et al.*, (2017) observed that cassava leaves from Busia County contained cyanide levels that were higher than the recommended WHO levels when raw and these were significantly reduced by boiling more so by the duration of boiling. That notwithstanding longer duration of boiling the leaves with prior processing's of pounding and that of pounding and soaking is promoted to minimize the risks associated with cyanide poisoning.

Based on the literature reviewed in this section, it comes out clearly that all the processing methods used significantly affect levels of nutrients and anti-nutrients in both cassava tubers and leaves. It's worth noting that despite the reductions, not all the nutrients that remain after processing are bioaccessible hence the need to look at bioaccessibility section 2.5.

2.5 Nutrient Bioaccessibility

Bioaccessibility in this context can be defined as fraction of the consumed nutrient which is available for absorption after cassava leaves and roots have been digested through gastrointestinal digestion (Galanakis, 2017). In order to determine whether dietary intake of nutrients is enough, one has to know how much of each nutrient is available for absorption against the consumed amount. Bioaccessibility differ from bioavailability which refers to the proportion of the ingested nutrient that is absorbed from the diet and utilized for normal body functions (Lynch *et al.*, 2018).

Bioaccessibility can however be used to predict bioavailability through various methods, most commonly by using *in vitro* assays that simulate digestion and absorption. These assays use models like the Caco-2 human intestinal cell line to measure the fraction of a compound that can pass through an intestinal barrier. By establishing a strong correlation between bioaccessibility measurements and actual bioavailability from animal studies (or human data), simpler and more cost-effective lab-based tests are used to predict bioavailability without the need for expensive or ethically complex *in vivo* (animal or human) (Santos *et al.*, 2019).

Information on bioaccessibility is very important in optimizing health and nutrition: Understanding bioaccessibility helps individuals and health professionals make informed choices about diet and supplementation to achieve better health outcomes. Guiding food and supplement development: Food scientists and technologists use bioaccessibility information to develop strategies for improving the health benefits of foods and supplements, ensuring the body can actually absorb the intended nutrients. Predicting bioavailability: Bioaccessibility assays, which simulate digestion, are essential tools for predicting how a nutrient will be absorbed in the body, especially for complex compounds like phytonutrients in supplements and functional foods. Evaluating effectiveness: This information is vital for determining if a therapeutic treatment or a dietary change is effective, as it links the amount of nutrient ingested to the amount that is actually absorbed and available for use by the body.

Information on bioaccessibility is essential for evaluating health-promoting properties of bioactive compounds from plants and other sources. Without proper bioaccessibility, these compounds may not reach their target organs to exert their beneficial effects. Overcoming limitations of *in vivo* studies: *In vitro* bioaccessibility methods provide a cost-effective and ethical alternative to *in vivo* studies for assessing factors that influence nutrient absorption, such as food matrix effects of different processing methods. Factors including the dietary matrix, chemical type of nutrients and inhibitors typically have an impact on bioaccessibility as discussed in section 2.5.1 and 2.5.2.

2.5.1 Food matrix and chemical form of nutrients

For nutrients to be bioavailable, they first have to be released from the matrix of food and be transformed such that it can hold onto and enter the gut cells or move between them. Nutrients become bioaccessible by chewing and the enzymatic breakdown of food in the mouth. After swallowing, food is mixed with the acid in gastric juice and other enzymes before being released into primary absorption area (small intestine). Food matrices' digestibility is facilitated by preparation techniques in addition to the body's natural masticatory and enzyme processes. Cooking, for example, may make the proteins binding calcium more digestible, which would boost the mineral's release from any protein complexes (Repo-Carrasco *et al.,* 2010).

The different chemical forms nutrients and minerals found in food have an impact on their bioavailability. For instance iron; heme iron is mostly derived from the molecules of myoglobin and hemoglobin, which are in charge of storing and transporting oxygen in the muscles and blood, respectively. The heme molecule surrounds the center iron atom like a protective ring when it is freed from the food matrix. As a result, it prevents the iron from interacting with other food ingredients, maintains its solubility in the intestinal fluid, and ensures that its absorption if wholly via a specific route on the gut cell's surface (Hu *et al.,* 2023).

2.5.2 Impact of inhibitors on nutrient bioaccessibility

According to Repo-Carrasco *et al., (*2010), by binding nutrients in a way that the intestinal cells' absorption mechanisms do not recognize, inhibitors can reduce their bioavailability. As a result, the nutrients become insoluble and cannot be absorbed or compete for the same absorption pathway. Phytic acid, which is abundant in certain plant-based dishes including whole grain cereals, nuts and seeds, form complexes which are insoluble or soluble by binding minerals such as zinc, iron and calcium, which hinder their absorptivity (Repo-Carrasco *et al.,* 2010). One example of competition for the same absorption mechanism is the relationship between calcium and non-heme iron. A transporter on the surface of intestine absorptive cells is where both minerals bind but calcium essentially remains in the doorways and prevents additional iron access, while non-heme iron enters the cells in this manner (Abebe *et al.*, 2007).

Bioavailability can be predicted using the molar ratio of minerals to anti-nutrients. There is proof that iron absorption is negatively impacted by phytate to iron molar ratios greater than 1; 0.4 is the recommended molar ratio (Magallanes-Lopez *et al.,* 2017; Hurrell and Egli, 2010). According to Magallanes-Lopez *et al.,* (2017), a molar ratio of phytate : zinc >15 is linked to below average bioavailability, while Phytate: zinc which ranges from 5 to 15 and below 5 is linked to average and above average bioavailability, with actual absorption of zinc equivalent to respectively 15, 30, and 50%. Foods high in calcium may make phytate's detrimental effects on zinc absorption worse. Zinc benefits from a 100:1 molar ratio of Ca: Zn, where at neutral pH, stability of Ca-Zn-phytate complexes increases. Consequently, reports show that Phytate.Ca: Zn molar ratio is more exact pointer of absorption of zinc (Magallanes-Lopez *et al.,* 2017).

Hemalatha *et al.,* (2007) examined zinc bioaccessibility from a range of grains and pulses and observed that in pulses whole green grams had the lowest 27% and decorticated chickpeas had the highest 56.5%. In grains sorghum had the lowest 5.5% while rice had the highest 21.4%. Generally, bioaccessibility of zinc was highest in pulses compared to cereals. This was explained by the fact that zinc bioaccessibility in pulses was less negatively impacted by a number of inherent variables, including dietary fiber, calcium, and phytate (Hemalatha *et al.,* 2007). Zinc from grains had a higher bioaccessibility than iron; this difference is especially noticeable when it comes to pulses. According to Bhavyashree *et al.*, (2009), bioaccessibility of iron was 1.5% for finger millet-based meal, 2.5% for rice-based meal, 3.5% for sorghum, and 4.7% for wheat-based meal. Conversely, meals based on rice had a higher (8.5%) zinc bioaccessibility, 5.6% in meals based on finger millet (0.31%) in meals based on sorghum.

By making the matrix of food loose and soft, heat processing is known to increase the accessibility of macronutrients like proteins and carbohydrates to the digestive enzymes, by improving their digestibility. Iron and other protein-bound minerals are thought to be released when the food matrix softens, making it easier for the body to absorb them. Additionally, heat processing may change natural components like phytate and soluble dietary fiber that prevent minerals from being absorbed, increasing the minerals' bioavailability. Iron and zinc bioaccessibility have been found to be affected differently by heat processing of cereal grains (Hamalatha *et al.,* 2007).

Iron from cereals and pulses were more bioavailable after cooking by pressure and heating using microwave, whereas these cooking methods decreased bioaccessibility of zinc to a higher extent. In comparison to the same raw cereals, iron bioaccessibility from ceraels cooked using pressure was substantially higher, ranging from 7% for wheat to 12% for rice. Pulses cooked using pressure and those heated using microwaves showed comparable patterns (Hemalatha *et al.*, 2007). For pulses however, zinc bioaccessibility from dietary grains reduced on cooking using pressure. Cooking using Pressure decreased the bioaccessibility of zinc in rice and finger millet by 57 and 63%, respectively. Although practically all of the pulses under investigation showed this decline in zinc bioaccessibility, the percentage loss varied from 11.4% in whole chick pea to 63% in cow pea (Hamalatha *et al.,* 2007).

Phytic acid and other intrinsic factors which reduce bioavailability of minerals can be reduced by fermentation and germination (Nkhata *et al.*, 2018). Bioavailability of calcium from different foods sources are as follows respectively; cow's milk, almolnds dry roasted, cabbage green, chinese spinach, kale, orange juice, spinach, sweet potatoes and yoghurt ranged between 8.36% to 64.9% (Weaver, 2009). Low amounts of oxalate dietary fiber, and phytate were present in these vegetables. Leaf samples from Indian mulberry and Sesbania showed calcium bioavailability range from 11 to 18% and oxalate levels from 290 to 580 mg/100 g. Both young and mature cooked soybean seeds had average phytate levels (290 – 400 mg/100 g) and low oxalate levels, but they had medium levels of dialyzable calcium. White and black sesame seeds, amaranth, and pak have high oxalate content (680 – 2620 mg/100 g) and poor dialyzable calcium content (2 – 7%). Phytate and dietary fiber were also abundant in sesame seeds. The calcium bioavailability of plant meals may be restricted by the high or medium quantities of inhibitory substances particularly oxalate.

While the bioavailability of vitamin C from spinach, kale, bananas, and kiwis ranges from 80 to 90% (Carr and Vissers, 2013), the bioavailability of potassium from same foods ranges from 60 to 85% (MacDonald-Clarke *et al.,* 2016). In baby diets based on cereal, bioaccessible (percentage) of ascorbic acid, thiamine, riboflavin, and niacin when the pH in the gastric was 1.5

was 81, 79, 39, and 51%, respectively and all reduced when the pH was raised (Akca *et al.*, 2019). Temperature, levels of fiber in the diet, stability, gastrointestinal tract pH, and bindings with polysaccharides and polypeptides all have a major impact on these vitamins' bioaccessibility (Akca, *et al.*, 2019). As can be seen from literature, bioaccessibility varies across the different plants and even varieties. Given the fact that both cassava roots and leaves are a common meal in the areas of study, and malnutrition in these areas have been associated with over reliance on cassava best meals, there was need to look into whether the nutrients from these cassava (both raw and processed) are bioaccessible. Bioaccessibility can be determined using different methods discussed in section 2.6.

2.6 Determining bioaccessibility

Bioaccessibility refers to the fraction of the ingested nutrient that is released from the food matrix and become available for absorption. For bioaccessibility of nutrients to be determined by *in vitro* method, the food substance is first exposed to simulated gastrointestinal digestion (Etcheverry *et al.*, 2012**)**. There are two types of simulated gastrointestinal digestion i.e. a static digestion method and dynamic digestion method. For every stage of digestion, the static digestion approach maintains a steady pH and constant meal-to-digestive fluid ratios. Because of this, the method is easy to apply, but it is not appropriate for simulating the kinetics of digestion (Xavier *et al.*, 2021).

Using this method, food samples are sequentially digested in the mouth, stomach, and intestines, and available physiological data is used to determine parameters including enzymes, bile dilution, pH digestion time and electrolytes. By measuring digestive products (such as simple sugar, fatty acids, and amino acids/peptides) and assessing how micronutrients are released from the matrix of food, this method can be utilized to evaluate the end points arising from food digestion (Brodkorb *et al.*, 2019). It has been demonstrated that the majority of dynamic models are appropriate for modeling how food and medications are absorbed by various demographic groups and for various applications (Dupont *et al.*, 2018). However, the majority of food researchers might not have access to these models due to their relative complexity, high setup and maintenance costs, and other limitations (Dupont *et al.*, 2018).

Static *in vitro* digestion models have been shown to be highly successful in prediction of the outcomes of *in vivo* digestion (Bohn *et al.*, 2017). The digestion procedure involves three steps; the sample is prepared, digested, treated followed by subsequent analysis. The quantities of bile salts and the activity of all digestive enzymes are empirically determined during the preparation phase using the suggested standard tests for lipase, pepsin, amylase (pancreatic and gastric), chymotrypsin and trypsin. Preparation step is crucial, and if enzyme activity is not accurately assayed, component digestion rates may be off, which could alter the food's total digestive process (Egger *et al.*, 2016).

The process of digestion entails exposing the food to the oral, stomach, and intestinal phases in succession. Simulated salivary fluid (SSF) is combined with food in the oral phase (1:1, w/w).

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The SSF contains 1.5 mM calcium chloride. Salivary amylase is then added if necessary (75 U/ml). Incubation of the mixture is done at 37^{o}C and pH 7 for 2 minutes while mixing. Oral boluses are combined with simulated gastric fluid (SGF) in the gastric stage (1:1, v/v). The SGF contains 0.15 mM calcium chloride. Gastric lipase and pepsin are added (2000, 60 U/ml). Following mixing, incubation was done for 2 hours at 37°C and pH 3.0. The gastric chime is combined with 10 mM bile salts and simulated intestinal fluid (SIF) (1:1, v/v) during the intestinal stage. SIF includes 0.6 mM calcium chloride. Pancreatin (trypsin activity 100U/ml) is added and incubation done while shaking for 2 hours at 37ºC, pH 7.0. To stop intestinal digestion a dialysis membrane or centrifugation tubes having a cut off of 3-10 KDa are used (Egger *et al.*, 2016).

Once the food has been digested by *in vitro* digestion bioaccessibility and/ or bioavailability can then be determined by solubility, dialyzability or using Caco-2-cell model. The intestinal digest is centrifuged in the solubility model used in this investigation to produce a precipitate and supernatant. The nutrients or compounds present in the supernatant are measured using (ICP-AES), (HPLC) or (AAS) which represent the soluble components. The ratio of soluble chemicals to the total number of compounds in the test sample is known as percentage solubility, according to Etcheverry *et al.,* (2012).

Dialyzability assay was introduced by Miller *et al.*, (1981) as a mean to estimate iron bioaccessibility from food. The model which measures soluble minerals of low molecular weight is based on an equilibrium dialysis. It involves the addition of a dialysis tubing of a certain molecular weight cut off (MWCO), following gastric digestion. The dialysis tubing or bag contains a buffer such as sodium bicarbonate that slowly diffuses out of the bag and neutralizes the peptic digest. After incubation, pancreatin/ bile is added and following another incubation total dialyzable iron can thus be determined by measuring the amount of mineral present in the dialysate. The whole premise of dialyzability methods is that dialyzable compounds will be available for absorption in the small intestine. This method has been applied and slightly modified to study the bioaccessibility of a number of micronutrients including Ca, Zn and Mg among others (Etcheverry *et al.*, 2012).

A number of institutions and commercial groups have developed sophisticated gut models to simulate the human digestive system (Vardokou *et al.*, 2011). One commercial gastrointestinal model (TIM), which has been developed by The Netherlands Organization (TNO) for applied science research. TNO's intestinal model (TIM) is a very sophisticated model since many parameters of the human digestive system are simulated e.g. body temperature, flow of saliva, gastric and pancreatic juice including digestive enzymes and bile, peristalsis and churning, gastrointestinal transit times, regulation of gastric and intestinal pH etc.

The model consists of two computer-controlled chambers named TIM1 and TIM2. TIM1 comprises four compartments that represent the stomach, duodenum, jejunum and ileum. Secretion of digestive juices and pH adjustment in each section are simulated according to physiological data. A dialysate component collects compounds and they represent the bioaccessible fraction. The material that exits the model represents on the other hand the non-bioaccessible fraction and is used to study colonic fermentation products in the TIM2 (Jagadeesan, 2019). One of the main advantages of the TIM system is the possibility of collecting samples at any level of the gastrointestinal tract and at any time during digestion (Etienne, *et al.,* 2011). Although this model measures bioaccessibility, bioavailability can also be measured if the food digest at the end of the TIM1 digestion is added to human intestinal cells and nutrient uptake is assessed.

Bioavailability (or more correctly components of bioavailability) can be assessed through the determination of nutrient uptake, transport or both by Caco-2 cell. Caco-2 cells belong to a human epithelial cell line derived from a human colonic adenocarcinoma. Even though they have a colonic origin, the cells behave very much like intestinal cells upon culture (Perfecto *et al.*, 2017). Uptake studies are performed with cells grown on the surface of the plastic dishes or wells, or alternatively, if transport will also be measured, on transwell inserts. Transwell inserts all the collection and measurement of nutrients that have been absorbed through the apical membrane and then released through the basolateral membrane. Following the gastric digestion of the food, pancreatin/ bile is added and the digest is added to the cells. *In vivo*, cellular integrity is maintained through the presence of an intestinal mucus layer. However *in vitro*, one of the several methods must be used to prevent the enzymatic degradation of the cells. One method is the introduction of a dialysis membrane secured with a silicon O-ring to a plastic insert, which is placed on top of the dialysis membrane thus preventing the enzymes from reaching the cells (Glahn, 2009).

Another method involves heat treating the intestinal digest for 4 minutes at 100^{o}C in order to inhibit the enzymes added during experiment (Frontela *et al.*, 2009). This step however imposes a short coming in the methodology because heating the sample at 100^{o}C will also likely denature food proteins thus impacting (either positively or negatively) bioavailability. Other methods involve the inactivation of the enzymes by acidifying the intestinal digest to pH 2 (Frontela-

Saseta *et al.,* 2011) or by lowering the temperature of the digests and subsequently filtering the samples. However, these steps are not physiologically representative of *in vivo* conditions. The *in vitro* co-culture of Caco-2 cell and HT29MTX, a human mucus producing cell line, might represent a more physiological and realistic approach to in vivo conditions (Mahler *et al.,* 2009), as the generated mucus layer would protect the Caco-2 cells from digestive enzymes. Cellular calcium and zinc uptakes have been determined by measuring cell uptakes via AAS. However, in this method one cannot differentiate the Ca and Zn originally present in cells from the minerals that have been absorbed from the digested food, since one is measuring total mineral content.

The *in vitro* method that has been used the most for determination of zinc bioaccessibility is the dialyzability method. Both solubility and dialyzability method aims to estimate bioaccessibility or the fraction of the mineral available for absorption. Chiplonkar *et al.,* (1999) found that *in vitro* zinc dialyzability strongly correlate with *in vivo* human data. In his study Chiplonkar *et al.,* (1999) used meals (n = 23) from different published human studies and compared the human absorption data to their own zinc dialyzability results. The different meals contained rice, fruit, milk, legumes, cheese, peanut oil, sugar etc. The result showed that the *in vitro* dialyzability method matched the human absorption data with a correlation coefficience of 0.925 ($p < 0.001$) (Fairweather-Tait *et al.,* 2005).

In this study, the solubility model was used in which the intestinal digest is centrifuged to separate it into a precipitate and a supernatant. The nutrients or compounds present in the supernatant are then quantified using analytical techniques such as Inductively Coupled Plasma–Atomic Emission Spectroscopy (ICP-AES), High-Performance Liquid Chromatography (HPLC), or Atomic Absorption Spectroscopy (AAS) as described in section 3.5.12. These measured values represent bioaccessible fraction (Etcheverry *et al.*, 2012).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Research design

The study involved setting up 2 cassava plots, one in Kilifi County and another in Busia County and planting two commonly consumed cassava varieties in each of the two Counties. According to Kidasi *et al.*, (2021), *Kibandameno* is the preferred variety followed by *Tajirika* as the second preferred variety. *Kibandameno* was preferred for its sweet taste by 75.6% farmers while *Tajirika* was preferred by 52.4% farmers because of the high yielding capacity in Kilifi County. *Migyera* and *MM96/2480* are preferred in Western region where Busia County is found (Githunguri & Rangwe, 2017). Harvesting both roots and leaves was done at 8 and 12 months and then laboratory work done to establish quantities of anti-nutrients, nutrients, and nutrients bioaccessibility. According to a study by Waluchio (2016), many improved varieties are considered early maturing and the harvesting age for most of them ranges between 8 and 12 months hence the choice of age.

3.2 Study area

Plots established in Kenya's Kilifi and Busia Counties provided samples of cassava used for analysis. 0˚ 28' 0"N/ 34˚ 6' 0"E is the coordinate of Busia County. There are 893,681 people living there, and it occupies 1695 km² (KNBS, 2019). With a poverty index of 67.6%, the County's economy is mostly dependent on agriculture and fishing, with the main subsistence crops being cassava, sweet potatoes, millet, maize and bean. Although the northern and center regions of the county are covered in dark clay soils, the majority of the county is covered with sandy loam soils; however, there are clays and sandy clays in other locations. The County receives between 750 and 2000 mm of rain annually. According to Jeatzold *et al.*, (2010), the county's average temperature is between 21 and 27ºC. Kenya's biggest cassava-producing county is Busia County. 3,250,859 (90-kg) bags of KES 6.5 billion were produced in 2014 across 19,580 hectares (GoK, 2015).

Within the coordinates 3˚ 38' 0"S/ 39˚ 51' 0"E is Kilifi County. With a population of 1,453,787, it occupies 12,245.9 km² (KNBS, 2019). Its main sources of income are fishing and tourism. However, roughly 68.5% of the households work in agriculture, with cowpeas, maize, cassava, and green grams being the primary crops farmed for subsistence. In Kilifi County, The hinterland receives 300 mm of rainfall on average each year, whereas the shore receives 1100 mm. The hinterland gets temperatures between 30°C - 34°C, while at the coastal belt it ranges 30°C - 21°C. This county's soil types include loamy soils, which make up 39.1%, clay soil, which makes up 32.7%, sandy soil, which makes up 21.3% of the total area, and extremely clay soil, which make up 6.9%.

3.3 Land preparation and planting

Two plots of 19 m by 19 m were established one at KALRO Mtwapa and another in Busia County. Ploughing was done using a tractor in June 2020 a period when the two Counties were receiving rainfall. Cuttings of *MM96/2480* and *Migyera* varieties obtained from KALRO Busia were planted in the plot established in Busia while cutting of *Kibandameno* and *Tajirika* obtained from KALRO Mtwapa were planted in Kilifi County. The varieties planted were selected based on people’s preference in the study areas. Standard procedures for planting that is; 1 m by 1 m were observed. No fertilizers were utilized during the whole growing period. Farm management which mainly entailed weeding was done after every four weeks as per Hauser *et al.*, (2014).

3.4 Sampling and sample preparations

Sample size was calculated using Nassiuma (2000) formula, equation (3.1).

$$n = \frac{Nc2}{C2+(N-1)e2} \qquad \textbf{(3.1)}$$

Where: n = sample size

N = accessible population

c = coefficient of variance (30%)

e = standard error (2%)

After eight and twelve months, first 3 leaves from the tips and roots of the 144 randomly selected plants were collected (72 from each of the two varieties grown in the same plot) and two composite samples made. Both cassava roots and leaves were prepared and cooked using home-based procedures that entailed; washing roots to do away with particles of soil, peeling, longitudinally splitting them into halves and cutting them into 4 cm long pieces. After washing the piece with tap water, they were partitioned in to three.

The first portion was packed raw in plastic containers, the second portion weighing (0.5 kg) was boiled in water (0.5L) in a cooking pan at 98°C for about 20 minutes to make them tender allowed to cool, then packed in plastic containers. The third portion (0.5 kg) was deep-fried in vegetable cooking oil (1L) until they became brown cooled then packed in plastic containers. Each variety's leaves were separated into two portions after being macerated by pounding in a mortar and pestle. While 2nd portion (0.5kg) was packed in plastic containers after being cooked for 45 minutes at 98°C in 1 L of water, the 1st portion was packed raw. All the samples were stored in a cooler box at about $0^{o}C$. They were then taken to Jomo Kenyatta University of Agriculture and Technology (JKUAT) Department of Food Science and Technology labs for laboratory analysis.

3.5 Laboratory procedures

3.5.1 Equipment and chemicals

High Performance Liquid Chromatography (Shimadzu 20A series), with a column Hypsil C18 measured; ascorbic acid, thiamin, riboflavin, niacic acid, oxalate and phytate. Protein levels were determined using Kjeldahl apparatus; Ca, Fe, K and Zn were determined using Atomic Absorption Spectrophotometer (Shimadzu AA-7000) Ca, Zn, and Fe. K was determined by use of AES. H_2SO_4, $FeCl_3$, NaOH, CH_3COONa, HCl, HNO_3, K_2SO_4, $CuSO_4$, CH_3OH, KH_2PO_4, alpha amylase, NaCl, KCl, KH_2PO_4, pepsin, $NaHCO_3$, pancreatin, bile salt, lipase. Sigma-Aldrich in Germany was the supplier of all chemicals and standards.

3.5.2 Cleaning of glassware and sample containers

To get rid of any stains, all glassware was soaked in a solution made up of equal parts 3% hydrogen peroxide and 3% sulfuric acid. Using warm water mixed with detergent and soap, all the glassware, sample containers made of plastic together with their lids were washed. Following a rinse with distilled and deionized water, they were allowed to air dry. Upon drying, they were kept in lockers as they awaited usage.

3.5.3 Methods validation

The accuracy of the methods was determined by applying equation (3.2) to determine the analyte percentage recovery from the samples to which the analytes were added (Gonzalez & Herrador, 2007).

$$\% \text{ Recovery} = \frac{Cs - Cx}{Cadd} \times 100 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \mathbf{(3.2)}$$

Where: C_{add} = extra concentration as a result of added analyte to the samples

C_s = level found in the samples to which same analyte were added

C_x = level found in the sample to which no analyte were added

The methods' precision was determined in accordance with Lu Ning-wei *et al.*, (2016) relative standard deviation (RSD) and the mean of the triplicate measurements were computed using equation (3.3).

$$\text{RSD} = \frac{S}{X} \times 100 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (\mathbf{3.3})$$

Where: x = average levels of triplicate analysis of the samples

s = standard deviation of the triplicate analysis of the test sample.

The standard calibration curves were used to verify the linearity (Meghanathan, 2016).

3.5.4 HPLC calibration of vitamins

The stock solutions for vitamins C, B1, B2, and B3 were made in a 100 ml volumetric flask by dissolving 0.1 g of each standard in 50 mL of 0.1M HCl. Serial standards were made in the following µg/mL ranges after proper dilutions with 0.1M HCl: vitamin C (0 – 100), B1 (0 – 100), B2 (0 – 100), and B3 (0 – 100). The standards were run simultaneously for vitamins C, B1, B2 and B3.The mobile phase consisted of 0.1 mol/L KH_2PO_4 (pH 7) and methanol (9:1) (Ekinci and Kadakal, 2005). Prior to use, the mobile phase was degassed by sonication and filtered through a 0.45 µm membrane. The column elute was monitored using a photodiode array (PDA) detector for ascorbic acid (265 nm), thiamin (234 nm), riboflavin (266 nm), and niacin (261 nm). The column was run at a flow rate of 0.7 mL/min at room temperature (25°C). Plotting the peak area values of each vitamin as a function of concentration produced calibration curves.

Identification of compounds was achieved through comparison between retention periods and UV spectra's to those of standards.

3.5.5 Calibration of Ca, Zn, Fe and K

Calibration curves were made based on AOAC (2011.14) standard method (Poitevin, 2016). Standard stock solutions (1000 µg/mL) were made by weighing calcium chloride (0.692 g), ferric nitrate (1.083 g), zinc nitrate (0.724 g) and potassium chloride (0.477 g), in different beakers and separately dissolved them in distilled water (50 mL). After quantitatively transferring the resultant solution into a volumetric flask (250 mL), it was topped up using distilled water. Working standards were made by diluting the stock solutions appropriately. The absorbance of the standards was then measured at 213.9 nm Zn, 248.3 nm Fe, 422.7 nm Ca and 766 nm K by aspirating them into the AAS device. Plotting absorbance against the standards' corresponding concentrations produced standard calibration curves.

3.5.6 HPLC calibration for oxalate

Using a beaker, 0.381 g of sodium oxalate was dissolved in 100 mL of distilled water to create an oxalate stock solution. It was transferred into a volumetric flask (250 mL) the topped up using distilled water. Using suitable stock solution dilutions, working standards ranging from 0 - 200 µg/mL were created. The standards were run on a Hypsil C18 column (5 µm, 4.6 mm * 250 mm), 0.0025 M tetrabutyl ammonium hydrogen sulphate buffered at pH 2.0 with ortho-phosphoric acid and 0.25% dehydrogenate phosphate formed the mobile phase. The detector, a photo diode array detector, at 314 nm, rate of flow a flow 0.6 mL min^{-1}, and a pressure of 62 kgf was used. The peak area calibration curve was plotted versus the oxalate concentration.

3.5.7 Determination of protein

The levels of protein were determined using the kjeldahl technique (Maehre *et al.,* 2018). Triplicate samples of each treatment were made. 0.5 g of the prepared sample was transferred on a filter paper to the kjeldahl digestion flask. Copper (II) sulphate was used as a catalyst (0.5 g) and 2 mL of concentrated sulphuric (VI) acid were added to the flask. The Flask was then connected to the fume trap and attached to the pump. Sample was allowed to digest for four hours until a clear solution without black particles was obtained. Then the sample was cooled for one an hour. Blank digest was also carried out. Sample was dissolved in a minimum amount of ammonia free distilled water and transferred to a semi micro kjeldahl distillation apparatus which had previously been conditioned by passing steam for several minutes. Sodium hydroxide (8 mL) solution was then added to the kjeldahl apparatus. 5 mL of 4% boric acid solution and 3 drops of mixed indicator were added into a titration flask kept at the end of the apparatus to trap the ammonia liberated. Steam was passed through the flask until about 15 mL of distillate was received. The solution collected at the titration flask was titrated with standard hydrochloric acid solution. The end point was a pink color. Same procedure was applied to the blank sample and was done in triplicate. Equation 3.4 was used to obtain total nitrogen.

$$\text{N (\%)} = \frac{(mL\ 0.02\ M\ sample - mL\ 0.02\ M\ blank)\times 0.0014\ \times N\ HCl\ \times 100}{Weight\ of\ sample} \qquad \text{Equation } \mathbf{3.4.}$$

Protein content was determined from total nitrogen through multiplication of the concentration of nitrogen in food by 6.25, conversion factor.

3.5.8 Determination of vitamins

HPLC procedure described by Martin *et al.*, (2016) was used to determine levels of vitamins prior to and after *in vitro* digestion. All the steps were carried out three times. Deionized water (20 cm^3) was mixed with food sample (5 g) and homogenization of the mixture done followed by centrifugation at 14000 g for 1 minute. Ten milliliters of the centrifuged and homogenized food sample were loaded onto an activated stationary phase and elution done at a flow rate of 1 cm^3 min^{-1} using 10 cm^3 of water at pH 4.2, and 10 cm^3 of methanol. After being collected in a bottle, the eluent was dried by evaporation. A 0.45 µm millipore filter was used to filter the residue after it had been dissolved using the mobile phase. The RP-HPLC system was filled with 20 µL of the filtrate. At a flow-rate of 0.7 mL min^{-1} and 25^oC, the separation was accomplished using a C18 column (internal diameter 4.6 mm; pore size 3.5 µm) and a mobile phase of 0.1 M KH_2PO_4 (pH 7): methanol (9:1). Elutes for thiamin, ascorbic acid, niacin, and riboflavin were monitored at 234nm, 265 nm, 261 nm, and 266 nm respectively, using a photodiode array detector. By using the calibration curves to compare the peak regions of the compounds with those of standards with known concentrations, the compounds were identified and quantified.

3.5.9 Determination of minerals

Standards of potassium, iron, calcium, and zinc in the range of 0 – 2 µg/mL were used to create calibration curves. Absorbance of blank solutions made by following all the protocols for test portions, without the test sample was determined before standards were run. Every protocol was carried out three times. Dry, clean crucibles were laden with five grams of the sample. The crucibles were placed on a hot plate inside a fume hood, and the temperature was progressively increased until the smoke stopped and the samples were fully burned. After that, they spent an hour in a muffle furnace with the temperature gradually raised to 250^oC. After raising the temperature to 600°C it was burned for almost five hours then temperature lowered to 300°C. The crucibles were then taken out and allowed to cool to 25°C. All of the ash was moved to a 100 mL beaker, shaken, and allowed to settle using 0.5 M HNO_3 (20 mL). After that, 0.5 M HNO_3 was added to a 100 mL volumetric flask until the mark. After the insoluble particles were filtered out, the filtrate was labeled and stored in a bottle made of plastic bottle. The absorbance of the solutions at 248.3 nm, 422.7 nm, and 213.9 nm, for iron, calcium, and zinc, respectively, was then measured using an Atomic Absorption Spectrophotometer (AAS). AES measured potassium's emission at 766 nm, and each mineral's concentration was derived from its own calibration curve, which was created using its standard (AOAC (2011.14).

3.5.10 Determination of Oxalate

The sample method used to determine oxalate was based on Ruan *et al.*, (2013). According to the standard series for oxalate (0-200 µg/mL), standard calibration curves were developed. Every procedure was carried out three times. 4 mL of 0.5 M HCl was used to homogenize precisely 0.5 g of the sample's fresh weight from the freezer. It was then heated for 10 minutes at 80°C with periodic shaking, and 25 mL of distilled water was added. Before being injected onto the HPLC column, a 0.45 µm micro filter was used to filter the 1 mL of supernatant that was obtained from centrifuging a 3 mL aliquot of the solution for 10 minutes at 12,000 rpm. The photo diode array detector was utilized for HPLC detection, and the stationary phase was a Hypsil C18 column (5µ M, 4.6 mm * 250 mm) equipped with water 550. 0.0025 M tetrabutyl ammonium hydrogen sulphate and 0.25 percent dehydrogenate phosphate, buffered at pH 2.0 with ortho-phosphoric acid, made up the mobile phase.

3.5.11 Determination of phytate

The HPLC method was used to analyze the phytate in accordance with Camire and Clydesdale (2006). Phytate standard series (0-200 µg/mL) were used to generate standard calibration curves. Every technique was carried out three times. Ten milliliters of 3% H_2SO_4 were added to 0.5 grams of the material. After the contents were filtered, 3 mL of iron (III) chloride was added, and the filtrate was then left in a boiling water bath for five minutes (6 mg ferric iron per mL in 3% H_2SO_4). Precipitation of the complex of ferric phytate was completed by heating the content for 45 minutes. After centrifuging them for ten minutes at 2500 rpm, the supernatant was disposed of. Centrifuging was carried out and the solution disposed of after the precipitate had been cleaned with 30 milliliters of distilled water. The was mixed with 3 mL of 1.5 M NaOH, and the volume increased to 30 mL using distilled water. After 30 minutes of heating the contents in a boiling water bath, ferric hydroxide precipitated out. The samples were centrifuged once they had cooled, and the supernatant was then poured into a 50 mL volumetric flask. After centrifuging the precipitate and rinsing it with ten milliliters of distilled water, the supernatant was added to the volumetric flask's contents. HPLC analysis was performed once this was micro-filtered. Using distilled water and 0.005 M sodium acetate as the mobile phase, the detection was carried out at 500 nm.

3.5.12 Determining bioaccessibility of vitamins and minerals

Simulated static gastrointestinal digestion as described by Brodkorb *et al.,* (2019) was used. There were three steps to this technique: the intestinal, gastric, and oral phases. A solution of 137 mM NaCl, 2.7 mM KCl, and 1.8 mM KH_2PO_4 was used to dissolve 1.055 g of alpha amylase, and 1.5 mL of newly produced saliva was added to 1 g of the sample. For ten minutes, the mixture was incubated at 37^oC and pH 7 in a shaking water bath. After using HCl to get the pH down to 2.0, 2.5 mL of pepsin solution was added, and the mixture was incubated for two hours at 37^oC in a shaking water bath. The mixture was removed from the water bath, placed on ice for 10 minutes then its pH raised to 7.0 by adding 1 M $NaHCO_3$. 12.5 mL mixture of pancreatin, bile extract and lipase solution was added to the mixture, incubated in the shaking water bath at 37^oC for 2 hours, placed on ice for 10 minutes then the pH adjusted to 7.2 by addition of 0.5 M NaOH. Bioaccessible minerals were obtained from the supernatant by ultra-centrifugation using a Beckman L7-65 ultracentrifuge set to 70000×g for 120 minutes. Vitamins; B_1, B_2, B_3 and C in the supernatant were determined using HPLC as described in section 3.4.4. The minerals were determined in the solid that remained as described in section 3.4.5. Percentage bioaccessibility was calculated by dividing bioaccessible levels by the levels before digestion.

3.5.13 Data analysis

A statistical program called SPSS (version 29) was used for data analysis. The one-way ANOVA Student-Newman-Keuls (SNK) method was used to compare the mean anti-nutrient and nutrient levels in raw and cooked cassava leaves and roots, as well as the bioaccessible levels of ascorbic acid, thiamin, riboflavin, niacin, calcium, zinc, potassium and iron in leaves and roots of cassava under various cooking methods and in different varieties (Sawyer and Beebe, 2007).

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Methods Validation

Table 4.1 presents the outcomes of the method validation. The percentage recoveries, which varied from 98.000% to 100.002%, attest to the accuracy and suitability of the analysis techniques employed for each parameter compared to the recommended range of between 80% and 120% (Gonzalez & Herrador, 2007). The coefficient of variations (CV) fell within the adequately exact range of 0.6897 to 2.9851% against the recommended range of less than 5% (Zady, 2018).

Table 4.1: Methods validation parameters

Analyte	**Equation**	**R^2**	**% Recovery**	**%RSD**
Vitamin C	y= 42299x	0.9999	99.996	2.6862
Vitamin B_1	y= 33361x	0.9936	99.940	2.8375
Vitamin B_2	y= 128202x	0.9999	100.002	0.6897
Vitamin B_3	y= 211142x	1.0000	99.940	1.8585
Calcium	y= 0.06x+0.0104	0.9921	98.000	2.7280
Zinc	y= 1.065x-0.0109	0.9966	99.800	2.9851
Iron	y=0.1136x+0.0024	0.9975	99.200	2.6874
Potassium	y= 8572x+0.0399	0.9939	99.400	1.3605
Phytate	y= 12745x	0.9991	99.998	1.0191
Oxalate	y= 43493x	0.9992	99.994	2.8708

The correlation coefficient R^2 which ranged from 0.9921 to 1.0000 was used to show linearity of the relationship between the signal and concentration. This indicates a 99.21-100 % instrumental response to the concentrations and that established that the calibration curves used were linear over the respective range of the concentration of the standards. This is based on Meghanathan, (2016), interpretations of correlation coefficient which are: weak (0.20 – 0.39), moderate (0.40 – 0.59), strong (0.60 – 0.79), very strong (0.80 – 1.00). Figures 4.1 and 4.2, shows the calibration curves for calcium and vitamin C respectively, while the rest of the curves are given in Appendix I.

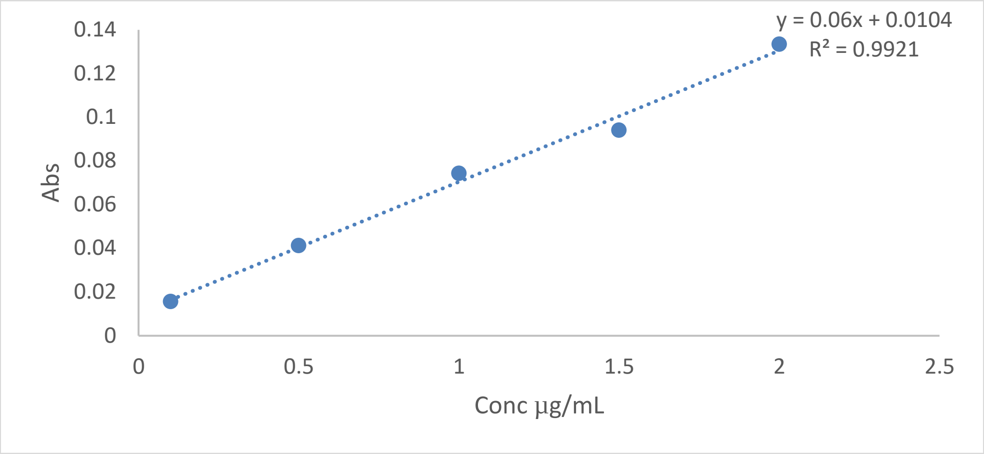

Figure 4.1: Calibration curve of calcium

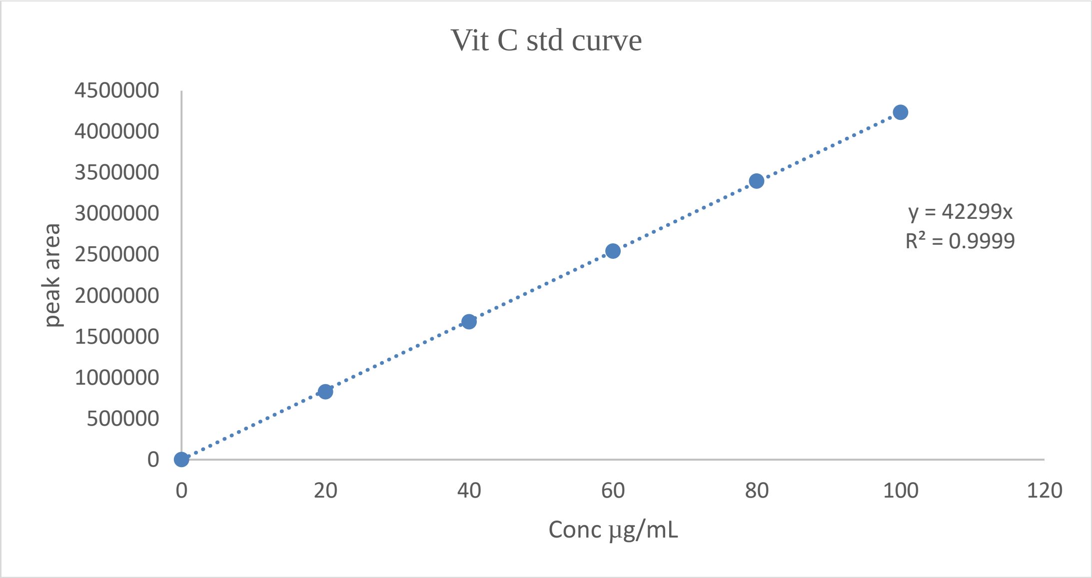

Figure 4.2: Calibration curve for vitamin C

4.2 Levels of nutrients and anti-nutrients in raw cassava roots and leaves

Mean levels of protein, vitamins; C, B1, B2 and B3 in raw cassava roots are shown in table 4.2. The levels which were within the ranges 17.00±1.00 - 114.00±3.00 mg/100 g (protein), 1.93±0.04 - 12.74±0.77 mg/100 g (vitamin C), 0.64±0.08 - 3.21±0.18 mg/100 g (vitamin B1), 0.12±0.01 - 0.57±0.01 mg/100 g (vitamin B2), 0.77±0.01 - 5.10±0.37 mg/100 g (vitamin B3) agree with those reported by Waluchio, (2016) and USDA (2008) in cassava roots in section 2.2 table 2.1.

A comparison of nutrient levels between varieties grown in Kilifi County and those grown in Busia County shows a significant difference (P<0.001) in levels of all the nutrients. *Tajirika* variety recorded significantly higher levels (P<0.001) of vitamins B1 3.21±0.18 mg/100 g compared to 1.19±0.02 mg/100 g recorded in *MM96/2480*. *Migyera* variety recorded significantly higher levels (P<0.001) of crude protein 114.00±3.00 mg/100 g than 27.00±0.01 mg/100 g recorded in *Kibandameno* variety. The variations in climatic conditions that impact nutrient levels can be the reason for the disparity in nutrient levels between the cassava varieties cultivated in Kilifi and Busia Counties (John *et al.*, 2012). *Kibandameno* and *Tajirika* varieties are grown at the coast while *Migyera* and *MM96/2480* are grown in western Kenya; with the two regions having different climatic conditions. Soil fertility is another factor that impacts on the nutrient levels though it was not considered in this study (John *et al.*, 2012).

A comparison between levels of nutrients in varieties grown in the same plot hence same climatic conditions indicates a significant difference (P<0.001) in the levels of all nutrients analyzed. For instance, levels of vitamin B3 (mg 100 g) ranged between 1.77±0.02 (*Kibandameno*) - 5.10±0.37 (*Tajirika*) for the varieties grown in Kilifi County and between

2.60±0.18 (*Migyera*) - 4.44±0.08 (*MM96/2480*) varieties grown in Busia County. The genetic difference between the many cassava varieties is the cause of this (Oresegun *et al.,* 2016; John *et al.,* 2012). Each cassava variety has a unique genetic makeup that dictates its potential to absorb nutrients and produce certain compounds. The other factor can be micro-environmental variations (Mustafa *et al.,* 2021). Even within a single plot, conditions are not perfectly uniform. Slight variations can lead to different nutrient uptake by plants. Patches of soil within a plot can differ in pH, texture, moisture, and organic matter content, which all affect nutrient availability. Variations in soil microbial populations can influence nutrient cycling and uptake. Mycorrhizal fungi, for example, are known to enhance phosphorus uptake in cassava. Different varieties planted in the same area may compete differently for resources like water and nutrients, which can affect their nutrient concentrations. *Kibandameno* and *Migyera* are local varieties while *Tajirika* and *MM95/2480* are improved varieties developed by KALRO. Given the difference in nutrient levels, different cassava varieties can be grown together and mixed during harvesting and consumption to help compensate for the low nutrients in some varieties.

In comparison to 8 months, the levels of all the minerals examined significantly decreased (P<0.001) when the cassava roots were harvested after 12 months. Levels of protein in *Migyera* decreased from 114.00±3.00 mg/ 100 g to 19.00±1.00 mg/100 g which translates to 83.7% decrease. Vitamin C in *Tajirika* variety decreased from 10.64±0.38 mg/100 g to 2.44±0.06 mg/100 g which is 77.1% decrease. The decrease of nutrients with age of cassava tubers agrees with the observation in the Technical Bulletin No 21, cassava post-harvest care and market preparation (2004), which observed that as the cassava tubers age beyond a year, they become woody and fibrous hence less nutritious. The decline is explained by the quick uptake of minerals by plants in their early stages of growth and the slow dilution that takes place as the plant ages. In essence, the plant's resources are largely dedicated to producing and storing carbohydrates for energy reserves as it matures, which inherently dilutes the concentration of other components like protein, vitamins, and certain minerals in the edible parts.

Table 4.2: Mean levels of protein and vitamins in raw roots of *Manihot esculenta* Crantz varieties grown in Kilifi and Busia Counties (mg/100 g)

Nutrients	**County**	**varieties**	**Mean ± SD (mg/100 g) n = 3**		**P value**
			8 months	**12 months**	
Protein	Kilifi	*Kibandameno*	27.00 ± 0.01^{bA}	21.00 ± 1.00^{aC}	
		Tajirika	35.00 ± 0.01^{bB}	23.00 ± 0.01^{aD}	
	Busia	*Migyera*	114.00 ± 3.00^{bD}	19.00 ± 1.00^{aB}	**<0.001**
		MM96/2480	56.00 ± 1.00^{bC}	17.00 ± 1.00^{aA}	
			P<0.001		
Vitamin C	Kilifi	*Kibandameno*	12.74 ± 0.77^{bD}	11.92 ± 0.64^{aD}	
		Tajirika	10.64 ± 0.38^{bC}	2.44 ± 0.06^{aB}	
	Busia	*Migyera*	4.95 ± 0.22^{bB}	2.79 ± 0.08^{aC}	**<0.001**
		MM96/2480	3.94 ± 0.14^{bA}	1.93 ± 0.04^{aA}	
			P<0.001		
Vitamin B1	Kilifi	*Kibandameno*	3.06 ± 0.26^{bC}	1.70 ± 0.01^{aD}	
		Tajirika	3.21 ± 0.18^{bB}	1.04 ± 0.02^{aC}	
	Busia	*Migyera*	1.16 ± 0.03^{bA}	0.64 ± 0.08^{aA}	**<0.001**
		MM96/2480	1.19 ± 0.02^{bA}	0.75 ± 0.01^{aB}	
			P<0.001		
Vitamin B2	Kilifi	*Kibandameno*	0.57 ± 0.01^{aD}	0.53 ± 0.02^{aD}	
		Tajirika	0.41 ± 0.03^{bC}	0.23 ± 0.01^{aB}	
	Busia	*Migyera*	0.190 ± 0.01^{bA}	0.12 ± 0.01^{aA}	**<0.001**
		MM96/2480	0.40 ± 0.04^{bB}	0.30 ± 0.01^{aC}	
			P<0.001		
Vitamin B3	Kilifi	*Kibandameno*	1.77 ± 0.02^{bA}	1.40 ± 0.07^{aB}	
		Tajirika	5.10 ± 0.37^{bD}	2.11 ± 0.03^{aC}	
	Busia	*Migyera*	2.60 ± 0.18^{bB}	1.40 ± 0.03^{aB}	**<0.001**
		MM96/2480	4.44 ± 0.08^{bC}	0.77 ± 0.01^{aA}	
			P<0.001		

There is a significant difference between the mean values of the same nutrient followed by different capital letters in the same column and different small letters in the same row (One-Way ANOVA, SNK test, α = 0.05).

Mean levels of protein, vitamins; C, B1, B2, B3 in raw cassava leaves are shown in table 4.3. The levels which ranged between 178.00±1.32 - 208.00±2.00 mg/100 g (protein), 11.66±0.30 - 76.37±0.48 mg/100 g (vitamin C), 3.31±0.07 - 13.35±0.14 mg/100 g (vitamin B1), 0.61±0.01 - 1.052±0.003 mg/100 g (vitamin B2), 1.92±0.13 - 11.49±0.11 mg/100 g (vitamin B3) are within the ranges reported in kale which is one of the most common green leafy vegetables according to Satheesh *et al.*, (2020) as seen in section 2.2.

A comparison between nutrient levels in varieties grown in Kilifi and Busia Counties shows a significant difference (P<0.001) in most of the nutrients analyzed. Vitamin C in varieties in Kilifi County ranged from 76.37±0.48 (*Kibandameno*) - 34.22±0.38 mg/100 g (*Tajirika*) while in varieties grown in Busia County it ranged from 23.86±0.49 (*Migyera*) - 14.95±0.61 mg/100 g (*MM96/2480*). The difference can be attributed to the different climatic conditions experienced along the Coastal region where Kilifi County is located and Western region where Busia County is located. There was a significant difference (P<0.001) in levels of nutrients in varieties grown in the same plots and hence same climatic conditions. For instance, vitamin B1 in varieties grown in Kilifi ranged from 6.71±0.11 (*Kibandameno*) - 4.26±0.07 mg/100 g (*Tajirika*). This can be attributed to the high genetic diversity of cassavas (Berhanu *et al.,* 2023). *Kibandameno* is a local variety while *Tajirika* is an improved variety developed by KALRO.

The levels of protein and vitamins; C, B1, B2 and B3 in leaves of cassava harvested after 8 months are significantly higher (P<0.001) than those harvested after 12 months. For instance, vitamin B_2 in *MM96/2480* decreased from 1.052±0.003 to 0.395±0.002 mg/ 100g. The decrease can be attributed to the fact that plants uptake minerals more rapidly during early growth and the gradual dilution that occurs as the plant matures.

Table 4.3: Mean levels of protein and vitamins in raw leaves of *Manihot esculenta* Crantz varieties grown in Kilifi and Busia Counties (mg/100 g)

Nutrients	**County**	**varieties**	**Mean ± SD (mg/100 g) n = 3**		**P value**
			8 months	**12 months**	
Protein	Kilifi	*Kibandameno*	192.00 ± 1.00^{bC}	181.00 ± 1.00^{aB}	
		Tajirika	188.00 ± 2.00^{bA}	178.00 ± 1.32^{aA}	
	Busia	*Migyera*	190.00 ± 1.24^{bB}	183.00 ± 1.15^{aC}	**<0.001**
		MM96/2480	208.00 ± 2.00^{bD}	204.00 ± 1.36^{bD}	
		P<0.001			
Vitamin C	Kilifi	*Kibandameno*	76.37 ± 0.48^{bD}	74.94 ± 2.47^{aD}	
		Tajirika	34.22 ± 0.38^{bC}	25.59 ± 0.57^{aC}	
	Busia	*Migyera*	23.86 ± 0.49^{bB}	19.20 ± 0.86^{aB}	**<0.001**
		MM96/2480	14.95 ± 0.61^{bA}	11.66 ± 0.30^{aA}	
		P<0.001			
Vitamin B1	Kilifi	*Kibandameno*	6.71 ± 0.11^{bC}	4.97 ± 0.02^{aC}	
		Tajirika	4.26 ± 0.07^{bB}	3.31 ± 0.07^{aB}	
	Busia	*Migyera*	13.35 ± 0.14^{bD}	8.15 ± 0.54^{aD}	**<0.001**
		MM96/2480	3.55 ± 0.66^{bA}	3.33 ± 0.01^{aA}	
		P<0.001			
Vitamin B2	Kilifi	*Kibandameno*	0.72 ± 0.01^{bB}	0.62 ± 0.01^{aC}	
		Tajirika	0.81 ± 0.01^{bC}	0.61 ± 0.01^{aC}	
	Busia	*Migyera*	0.49 ± 0.00^{bA}	0.12 ± 0.00^{aA}	**<0.001**
		MM96/2480	1.05 ± 0.00^{bD}	0.40 ± 0.00^{aB}	
		P<0.001			
Vitamin B3	Kilifi	*Kibandameno*	8.71 ± 0.05^{bB}	1.92 ± 0.13^{aA}	
		Tajirika	11.49 ± 0.11^{bC}	8.54 ± 0.54^{aD}	
	Busia	*Migyera*	8.88 ± 0.07^{bB}	6.55 ± 0.16^{aC}	**<0.001**
		MM96/2480	7.78 ± 0.16^{bA}	6.02 ± 0.11^{aB}	
		P<0.001			

There is a significant difference between the mean values of the same nutrient followed by different capital letters in the same column and different small letters in the same row (One-Way ANOVA, SNK test, α = 0.05).

Mean levels of calcium, zinc, iron, potassium, phytate and oxalate in raw cassava roots harvested after 8 and 12 months are shown in table 4.4. The levels which ranged; 32.08±0.88 - 162.98±3.76 mg/100 g (Ca), 0.32±0.01 - 1.91±0.26 mg/100 g (Zn), 0.31±0.01 - 2.24±0.08 mg/100 g (Fe), 152.07±2.74 - 485.78±6.61 mg/100 g (K), 390.37±2.40 - 561.28±5.72 mg/100 g (phytate), 4.72±0.04 - 613.46±17.61 mg/100 g (oxalate) are in the same range as those reported by Tagesse *et al.*, (2022); Waluchio, (2016); USDA (2008) in cassava roots.

The levels of mineral elements and anti-nutrients just like those of protein and vitamins differ significantly (P<0.001) with climatic conditions. In varieties grown in Kilifi County levels of Ca ranged between 162.98±3.76 - 96.22±0.28 mg/100 g while for those grown in Busia County it ranged between 68.73±2.06 - 56.79±5.09 mg/100 g. This can be attributed to the different soil fertility of the two plots though not within the scope of this study. The levels of anti-nutrients also varied with climatic conditions. The levels of oxalate in varieties grown in Kilifi ranged between 12.24±0.24 - 9.90±0.25 mg/100 g while varieties grown in Busia it ranged from 613.46±17.61 - 413.86±8.08 mg/100 g. A significant difference (P<0.001) in levels of nutrients and anti-nutrients in varieties grown within the same climatic conditions was noted. The levels of calcium ranged between 96.22±0.28 (*Kibandameno*) - 162.98±3.76 mg/100 g (*Tajirika*) harvested after 8 months in the same plot. The difference can be attributed to the varietal differences (Oresegun *et al.*, 2016).

The levels of both minerals and anti-nutrients in cassava roots harvested after 8 months and 12 months varied significantly (P<0.001). Generally, both minerals and anti-nutrients decreased with increase in harvesting age. The level of Zn in *Kibandameno* decreased from 1.59±0.03 -

0.40±0.01 (mg/100 g) while in *Tajirika* the level decreased from 1.41±0.08 - 0.32±0.01 (mg/100 g). The decline is explained by the quick uptake of minerals by plants in their early stages of growth and the slow dilution that takes place as the plant ages. The decrease in anti-nutrients between 8 and 12 months is advantageous as high levels of anti-nutrients affects bioavailability of nutrients.

Table 4.4: Mean levels of mineral elements and anti-nutrients in raw roots of *Manihot esculenta* Crantz varieties grown in Kilifi and Busia Counties (mg/100 g)

Nutrients	**County**	**varieties**	**Mean ± SD (mg/100 g) n = 3**		**P value**
			8 months	**12 months**	
Calcium	Kilifi	*Kibandameno*	96.22 ± 0.28^{bC}	58.19 ± 0.95^{aC}	**<0.001**
		Tajirika	162.98 ± 3.76^{bD}	110.26 ± 2.90^{aD}	
	Busia	*Migyera*	68.73 ± 2.06^{bB}	32.08 ± 0.88^{aA}	
		MM96/2480	56.79 ± 5.09^{bA}	47.46 ± 0.634^{aB}	
			P<0.001		
zinc	Kilifi	*Kibandameno*	1.59 ± 0.03^{bC}	0.40 ± 0.01^{aB}	**<0.001**
		Tajirika	1.41 ± 0.08^{bB}	0.32 ± 0.01^{aA}	
	Busia	*Migyera*	1.91 ± 0.26^{bD}	0.74 ± 0.02^{aD}	
		MM96/2480	0.59 ± 0.05^{bA}	0.54 ± 0.00^{aC}	
			P<0.001		
Iron	Kilifi	*Kibandameno*	2.06 ± 0.10^{bC}	0.31 ± 0.01^{aA}	**<0.001**
		Tajirika	2.24 ± 0.08^{bD}	0.37 ± 0.01^{aB}	
	Busia	*Migyera*	1.65 ± 0.21^{bB}	0.71 ± 0.02^{aC}	
		MM96/2480	0.96 ± 0.09^{bA}	0.72 ± 0.02^{aC}	
			P<0.001		
Potassium	Kilifi	*Kibandameno*	304.19 ± 22.07^{bB}	169.96 ± 2.70^{aB}	**<0.001**
		Tajirika	229.89 ± 19.70^{bA}	152.07 ± 2.74^{aA}	
	Busia	*Migyera*	485.78 ± 6.61^{bD}	382.47 ± 12.86^{aD}	
		MM96/2480	355.19 ± 27.29^{bC}	326.41 ± 5.04^{aC}	
			P<0.001		
Phytate	Kilifi	*Kibandameno*	458.70 ± 4.64^{bB}	412.99 ± 35.19^{aB}	**<0.001**
		Tajirika	394.18 ± 6.82^{bA}	390.37 ± 2.40^{aA}	
	Busia	*Migyera*	561.28 ± 5.72^{bD}	425.91 ± 3.82^{aC}	
		MM96/2480	472.10 ± 7.10^{bC}	463.77 ± 5.75^{aD}	
			P<0.001		
Oxalate	Kilifi	*Kibandameno*	9.90 ± 0.25^{bA}	7.36 ± 0.07^{aB}	**<0.001**
		Tajirika	12.24 ± 0.24^{bB}	4.72 ± 0.04^{aA}	
	Busia	*Migyera*	613.46 ± 17.61^{bD}	229.41 ± 0.56^{aD}	
		MM96/2480	413.86 ± 8.08^{bC}	110.91 ± 0.19^{aC}	
			P<0.001		

There is a significant difference between the mean values of the same nutrient followed by different capital letters in the same column and different small letters in the same row (One-Way ANOVA, SNK test, α = 0.05).

The mean levels of mineral elements and anti-nutrients in leaves of cassava are shown in table 4.5. The levels ranged from 39.15±0.38 - 287.99±5.68 mg/100 g (Ca), 0.79±0.03 - 2.10±0.12

mg/100 g (Zn), 0.92±0.01 - 4.79±0.43 mg/100 g (Fe), 192.95±5.01 - 435.07±12.57 mg/100 g (K), 433.55±32.31 - 861.32±22.77 mg/100 g (phytate), 38.73±0.03 - 526.98±0.17 mg/100 g (oxalate). The levels of nutrients compare well to those reported in kales and spinach (Satheesh *et al.*, 2020). This means cassava leaves could be used as a substitute for kales and spinach if it were not for the high levels of anti-nutrients and the poisonous cyanogenic glycosides.

The levels of minerals and anti-nutrients significantly varied (P<0.001) with the climatic conditions. The levels of Ca in varieties grown in Kilifi ranged between 287.99±5.68 - 130.55±3.51 mg/100 g while in those grown in Busia it ranged from 125.46±8.83 - 94.99±8.73 mg/100 g. The levels of oxalate ranged from 49.01±0.69 - 39.70±0.19 mg/100 g for the varieties grown in Kilifi and between 526.98±0.17 - 284.27±6.29 mg/100 g for varieties grown in Busia. Minerals and anti-nutrients levels varied significantly with variety for those grown within the same environmental conditions (Oresegun *et al.*, 2016). Fe ranged from 3.35±0.09 (*Tajirika*) - 1.69±0.01 mg/100 g (*Kibandameno*). In varieties grown in Busia oxalate ranged between 526.98±0.17 (*MM96/2480*) - 284.27±6.29 mg/100 g (*Migyera*). Leaves of *Tajirika* variety had higher levels of Ca, those of *Kibandameno* variety had higher levels of K and Phytate while those of *Migyera* had higher levels of Zn and Fe. This means that mixing cassava leaves from different varieties is better than consuming leaves from one variety.

There was a significant decrease in levels of minerals and anti-nutrients as the plant age increased from 8 to 12 months. Levels of Ca in *Kibandameno* decreased from 130.55±3.51- 46.67±3.73 (mg/100 g), which is 64.3% decrease. Fe in *Tajirika* decreased from 3.35±0.09 - 1.27±0.41 (mg/100 g), which translates to 62.1% decrease. This decrease can be attributed to the rapid uptake of minerals by plants during early growth and the gradual dilution that occurs as the plant matures. This means that leaves of a younger cassava plant are more nutritious than those of an ageing one. The levels of anti-nutrients are however higher in young cassava plant which contributes to low bioaccessibility and bioavailability of these nutrients. For instance, levels of oxalate in *MM96/2480* variety decreased from 526.98±0.17 - 321.64±1.30 (mg/100 g), which is 39 % decrease between 8 and 12 months.

Table 4.5: Mean levels of mineral elements and anti-nutrients in raw leaves of *Manihot esculenta* Crantz varieties grown in Kilifi and Busia Counties (mg/100 g)

Nutrients		**varieties**	**Mean ± SD (mg/100 g) n = 3**		**P value**
			8 months	**12 months**	
Calcium	Kilifi	*Kibandameno*	130.55±3.51^{bC}	46.67±3.73^{aB}	
		Tajirika	287.99±5.68^{bD}	143.03±12.20^{aD}	
	Busia	*Migyera*	125.46±8.83^{bB}	50.13±0.20^{aC}	**<0.001**
		MM96/2480	94.99±8.73^{bA}	39.15±0.38^{aA}	
			P<0.001		
Zinc	Kilifi	*Kibandameno*	1.21±0.02^{bA}	0.91±0.06^{aB}	
		Tajirika	1.23±0.02^{bA}	0.79±0.03^{aA}	
	Busia	*Migyera*	2.10±0.12^{bC}	1.16±0.01^{aD}	**<0.001**
		MM96/2480	1.36±0.34^{bB}	1.01±0.02^{aC}	
			P<0.001		
Iron	Kilifi	*Kibandameno*	1.69±0.01^{bA}	0.92±0.01^{aA}	
		Tajirika	3.35±0.09^{bC}	1.27±0.41^{aB}	
	Busia	*Migyera*	4.79±0.43^{bD}	2.38±0.03^{aD}	**<0.001**
		MM96/2480	3.14±0.02^{bB}	1.83±0.19^{aC}	
			P<0.001		
Potassium	Kilifi	*Kibandameno*	435.07±12.57^{bD}	192.95±5.01^{aA}	
		Tajirika	350.62±18.35^{bB}	240.43±4.19^{aC}	
	Busia	*Migyera*	375.80±3.77^{bC}	245.81±9.92^{aD}	**<0.001**
		MM96/2480	298.40±3.10^{bA}	213.25±3.21^{aB}	
			P<0.001		
phytate	Kilifi	*Kibandameno*	861.32±22.77^{bD}	763.77±45.47^{aD}	
		Tajirika	783.50±6.91^{bC}	761.39±10.10^{aC}	
	Busia	*Migyera*	555.99±5.72^{bB}	433.55±32.31^{aA}	**<0.001**
		MM96/2480	542.77±13.92^{bA}	490.62±47.19^{aB}	
			P<0.001		
oxalate	Kilifi	*Kibandameno*	39.70±0.19^{bA}	38.73±0.03^{aA}	
		Tajirika	49.01±0.69bB	41.27±0.78aB	
	Busia	*Migyera*	284.27±6.29^{bC}	193.93±0.10^{aC}	**<0.001**
		MM96/2480	526.98±0.17^{bD}	321.64±1.30^{aD}	
			P<0.001		

There is a significant difference between the mean values of the same nutrient followed by different capital letters in the same column and different small letters in the same row (One-Way ANOVA, SNK test, α = 0.05).

4.3 Levels of nutrients and anti-nutrients in cooked roots and leaves.

Table 4.6 displays the average amounts of protein and vitamins C, B1, B2, and B3 in both cooked and raw cassava roots. Boiling and deep-frying caused a significant ($P<0.001$) decrease in vitamins and protein levels. These nutrients on boiling reduced in the ranges of 40.0% - 92.0% (protein), 50.0% - 92.0% (vitamin C), 72.0% - 98.0% (vitamin B1), 68.0% - 88.0% (vitamin B2), and 21.0% - 74.0% (vitamin B3). Frying reduced nutrient levels in roots by 26.0% - 89.0% (protein), 40.0% - 82.0% (vitamin C), 17.0% - 92.0% (vitamin B1), 43.0% - 78.9% (vitamin B2), 3.0% - 58.9% (vitamin B3). A comparison between deep-frying and boiling on levels of protein and vitamins; C, B1, B2 and B3 shows a significant reduction during boiling than deep-frying. Protein and all the analyzed vitamins are water soluble therefore leaches in water during boiling (Ujong *et al.,* 2020; Achidi, 2008). The reduction of vitamin C during boiling can be attributed to the fact that it is a water-soluble and temperature-sensitive vitamin, so is easily degraded during cooking, and elevated temperatures and long cooking times have been found to cause particularly severe losses of vitamin C (Tian *et al.,* 2016). Because deep-frying preserves a comparatively higher amount of water-soluble nutrients than boiling, it is therefore a better technique of cooking tubers. Malnutrition cases in populations that depend on a diet based on cassava may be exacerbated by the significant nutritional losses that occur during cooking therefore there is need to optimize these cooking methods to maximize on the nutritional potential of cassava.

The loss of protein, and vitamins analyzed was dependent on the climatic conditions under which the cassava was grown. The reduction of protein from varieties grown in Kilifi County ranged between 44% - 40% while for those grown in Busia County it ranged between 92% - 84%.

Vitamin B_3 reduced in the range 33% - 21% in the varieties grown in Kilifi and between 74% - 24% in varieties grown in Busia. Varietal difference significantly affected the reduction of nutrients in both boiling and deep-frying processing methods. The loss in protein for instance, during boiling *Migyera* and *MM96/2480*, both grown in Busia ranged between 92% (*Migyera*) – 84% (*MM96/2480*). The reduction in vitamin B3 ranged between 74% - 24% in the same varieties grown in Busia. This can be attributed to the genetic difference of the different cassava varieties (Oresegun *et al.*, 2016), since these two cassava varieties were grown in the same plot hence same climatic conditions.

Table 4.6: Mean levels of protein and vitamins in raw and cooked roots of *Manihot esculenta* Crantz varieties grown in Kilifi and Busia Counties (mg/100 g)

Nutrients	**Variety**	**Mean ± SD (mg/100 g) n =3**			**P value**
		Raw	**Boiled**	**Deep-fried**	
Protein	*Kibandameno*	27.00 ± 0.01^{cA}	15.01 ± 0.01^{aB} (44%)	18.01 ± 0.04^{bC} (33%)	
	Tajirika	35.02 ± 0.01^{cB}	21.01 ± 0.01^{aC} (40%)	26.03 ± 0.01^{bD} (26%)	
	Migyera	114.00 ± 0.01^{cD}	9.02 ± 0.01^{aA} (92%)	13.01 ± 0.01^{bB} (89%)	**<0.001**
	MM96/2480	56.02 ± 0.01^{cC}	9.01 ± 0.01^{aA} (84%)	11.02 ± 0.01^{bA} (80%)	
			P<0.001		
Vitamin C	*Kibandameno*	12.74 ± 0.64^{cD}	2.41 ± 0.05^{aD} (81%)	6.24 ± 0.06^{bD} (51%)	
	Tajirika	10.64 ± 0.06^{cC}	1.69 ± 0.02^{aC} (84%)	1.82 ± 0.06^{bC} (82%)	
	Migyera	2.79 ± 0.07^{cB}	1.50 ± 0.03^{aB} (50%)	1.69 ± 0.03^{bB} (40%)	**<0.001**
	MM96/2480	2.14 ± 0.03^{cA}	0.16 ± 0.01^{aA} (92%)	1.22 ± 0.01^{bA} (43%)	
			P<0.001		
Vitamin B1	*Kibandameno*	1.70 ± 0.06^{cD}	0.10 ± 0.00^{aB} (94%)	0.13 ± 0.00^{bA} (92%)	
	Tajirika	1.04 ± 0.02^{cA}	0.02 ± 0.00^{aA} (98%)	0.33 ± 0.00^{bB} (67%)	**<0.001**
	Migyera	1.16 ± 0.03^{cB}	0.31 ± 0.01^{aC} (73%)	0.69 ± 0.01^{bC} (41%)	
	MM96/2480	1.19 ± 0.02^{cC}	0.33 ± 0.01^{aC} (72%)	0.99 ± 0.02^{bD} (17%)	
			P<0.001		
Vitamin B2	*Kibandameno*	0.57 ± 0.06^{cD}	0.18 ± 0.00^{aD} (68%)	0.21 ± 0.03^{bD} (64%)	**<0.001**
	Tajirika	0.23 ± 0.06^{cB}	0.07 ± 0.00^{aC} (70%)	0.13 ± 0.02^{bC} (43%)	
	Migyera	0.15 ± 0.01^{cA}	0.03 ± 0.00^{aA} (79%)	0.07 ± 0.00^{bA} (54%)	
	MM96/2480	0.40 ± 0.01^{cC}	0.05 ± 0.00^{aB} (88%)	0.09 ± 0.00^{bB} (79%)	
			P<0.001		
Vitamin B3	*Kibandameno*	1.77 ± 0.01^{cC}	1.39 ± 0.02^{aC} (21%)	1.72 ± 0.01^{bD} (3%)	**<0.001**
	Tajirika	2.11 ± 0.03^{cD}	1.42 ± 0.02^{aD} (33%)	1.51 ± 0.01^{bC} (32%)	
	Migyera	1.39 ± 0.02^{cB}	0.36 ± 0.01^{aA} (74%)	0.56 ± 0.01^{bA} (59%)	
	MM96/2480	0.77 ± 0.01^{cA}	0.59 ± 0.01^{aB} (24%)	0.61 ± 0.01^{bB} (20%)	
			P<0.001		

There is a significant difference between the mean values of the same nutrient followed by different capital letters in the same column and different small letters in the same row (One-Way ANOVA, SNK test, α = 0.05).

Mean levels of protein, vitamins; C, B1, B2 and B3 in raw and cooked cassava leaves are shown in table 4.7. The levels of these nutrients significantly reduced (P<0.001) on boiling. Protein reduced in the range 6% (*Migyera*) – 16% (*Tajirika),* vitamin C 57% (*Kibandameno*) – 90% (*Migyera*), vitamin B1 83% (T*ajirika*) – 93% (*Migyera*), vitamin B2 19% (*Kibandameno*) – 98% (MM96/2480) and vitamin B3 47% (*Kibandameno*) – 77% (*MM96/2480*). This agrees with the ranges reported by Onyambu *et al.,* (2021) in thermally processed leafy African indigenous vegetables for vitamin B series. The reduction can be attributed to leaching of the vitamins in the boiling water. The reduction of protein and the analyzed vitamins significantly differed with the climatic condition in which the cassava was grown.

Varieties grown in Kilifi recorded a significantly lower percentage reduction in vitamins; C, B2 and B3 than those varieties grown in Busia. The reduction in vitamin C ranged between 77% - 57% in varieties grown in Kilifi compared to 90% - 82% in varieties grown in Busia. The reduction in vitamin B3 in varieties grown in Kilifi ranged from 58% - 47% compared to between 77% - 69% in varieties grown in Busia. The reduction in protein and analyzed vitamins significantly varied with the variety for those grown in the same climatic conditions. For instance, vitamin C in leaves of *Kibandameno* variety reduced by 57% on boiling while that of *Tajirika* reduced by 77%. Vitamin B2 in *Kibandameno* reduced by 19% while that *Tajirika* reduced by 78%. According to Oresegun *et al.*, (2016), this is explained by the genetic differences across the various varieties cassava.

Table 4.7: Mean levels of protein and vitamins in raw and cooked leaves of *Manihot esculenta* Crantz varieties grown in Kilifi and Busia Counties (mg/100 g)

Nutrient	**Variety**	**Mean ± SD (mg/100 g) n = 3**		**P value**
		Raw	**Boiled**	
Protein	*Kibandameno*	192.10 ± 0.03^{bC}	181.12 ± 0.01^{aC} (6%)	
	Tajirika	188.06 ± 0.01^{bB}	158.21 ± 0.02^{aA} (16%)	**$P<0.001$**
	Migyera	183.01 ± 0.02^{bA}	172.03 ± 0.01^{aB} (6%)	
	MM96/2480	279.46 ± 0.01^{bD}	204.01 ± 0.02^{aD} (7%)	
		$P<0.001$		
Vitamin C	*Kibandameno*	76.37 ± 0.48^{bD}	32.81 ± 0.65^{aD} (57%)	
	Tajirika	34.22 ± 0.38^{bC}	7.88 ± 0.06^{aC} (77%)	**$P<0.001$**
	Migyera	23.86 ± 0.49^{bB}	2.42 ± 0.05^{aB} (90%)	
	MM96/2480	11.66 ± 0.30^{bA}	2.08 ± 0.05^{aA} (82%)	
		$P<0.001$		
Vitamin B1	*Kibandameno*	6.70 ± 0.1^{bC}	0.65 ± 0.01^{aC} (90%)	
	Tajirika	3.31 ± 0.07^{bA}	0.57 ± 0.01^{aB} (83%)	**$P<0.001$**
	Migyera	13.35 ± 0.14^{bD}	0.92 ± 0.01^{aD} (93%)	
	MM96/2480	4.21 ± 0.03^{bB}	0.39 ± 0.01^{aA} (91%)	
		$P<0.001$		
Vitamin B2	*Kibandameno*	0.72 ± 0.01^{bC}	0.59 ± 0.01^{aC} (19%)	
	Tajirika	0.40 ± 0.07^{bA}	0.09 ± 0.00^{aB} (78%)	**$P<0.001$**
	Migyera	0.49 ± 0.01^{bB}	0.02 ± 0.00^{aA} (96%)	
	MM96/2480	0.39 ± 0.01^{bA}	0.01 ± 0.00^{aA} (98%)	
		$P<0.001$		
Vitamin B3	*Kibandameno*	8.71 ± 0.05^{bB}	4.59 ± 0.02^{aC} (47%)	
	Tajirika	11.49 ± 0.11^{bD}	4.88 ± 0.03^{aD} (58 %)	**$P<0.001$**
	Migyera	8.87 ± 0.07^{bC}	2.77 ± 0.03^{aB} (69%)	
	MM96/2480	6.02 ± 0.11^{bA}	1.37 ± 0.01^{aA} (77%)	
		$P<0.001$		

The mean values of the same nutrient followed by different capital letters in the same column and different tiny letters in the same row differ significantly. (One-Way ANOVA, SNK test, $\alpha = 0.05$).

Table 4.8 displays the average mineral and anti-nutrient values in raw and cooked roots. Boiling and deep-frying considerably ($P<0.001$) reduced the elements and anti-nutrients. Boiling reduced calcium by 50% - 79%, zinc by 17% - 23%, iron by 14% - 44%, potassium by 12% - 32%, oxalate by 17% - 74%, and phytate by 27% - 74%. However, deep-frying decreased calcium by 30% - 44%, zinc by 3% - 16%, iron by 7% - 18%, potassium by 0.02% - 9%, oxalate by 0.3% - 65% and phytate by 1% - 52%. The percentage reductions were higher on boiling than deep-frying. The high reduction on boiling can be attributed to leaching in the boiling water (Ujong *et al.,* 2020; Kasaye *et al.,* 2018; Adepoju, 2010). The reduction of minerals and anti-nutrients was dependent on climatic conditions under which the cassava was grown. For instance, the reduction of Ca in varieties grown in Kilifi ranged from 57% - 50% while for those grown in Busia ranged from 79% - 78%. K reduced from 18% - 12% for the varieties grown in Kilifi and between 32% - 22% for the varieties grown in Busia. Phytate reduced in the range 39% - 27% in varieties grown in Kilifi and between 74% and 59% for those grown in Busia. The reduction of minerals and anti-nutrients on cooking was affected by variety for those grown in the same plot hence same climatic conditions. For instance, on boiling roots the reduction ranged between 17% (*Kibandameno*) – 23% (*Tajirika*) Zn both of which were grown in Kilifi and between 14 % (*Migyera*) – 44 % (*MM96/2480*) Zn both grown in Busia. The genetic distinctions between the various cultivars can be the cause of the variations (Oresegun *et al.,* 2016).

Table 4.8 indicates that oxalate and phytate in cassava roots are significantly reduced by deep-frying and boiling. On boiling, the reduction of both oxalate and phytate was highest in *Migyera* at 74% and lowest in *Kibandameno* variety at 17% and 27% oxalate and phytate respectively. The reduction in anti-nutrients though high, the levels that remained still gave higher than the recommended molar ratios of phytate: zinc < 15, phytate: iron < 1 and phytate: Calcium < 0.24 (Magallanes-Lopez *et al.,* 2017; Hurrell and Egli, 2010) which therefore affects the bioaccessibility and bioavailability of these minerals. Boiling may cause phytate to dissolve in water, which could result in losses. Phytate is comparatively heat stable at the typical home boiling temperature of 100^{o}C, but it's lost more when high temperatures are utilized in commercial processes like canning or extrusion cooking.

Table 4.8: Mean levels of mineral elements and anti-nutrients in raw and cooked roots of *Manihot esculenta* Crantz varieties grown in Kilifi and Busia Counties (mg/100 g)

Nutrient	**Variety**	**Mean ± SD (mg/100) n = 3**			**P value**
		Raw	**Boiled**	**Deep-fried**	
Calcium	*Kibandameno*	58.19±0.95^{cC}	29.34±0.88^{aC} (50%)	40.63±0.04^{bC} (30%)	
	Tajirika	110.26±2.89^{cD}	47.50±0.99^{aD} (57%)	61.91±1.36^{bD} (44%)	**<0.001**
	Migyera	32.08±0.87^{cA}	6.64±0.14^{aA} (79%)	21.65±0.53^{bA} (33%)	
	MM96/2480	47.46±0.63^{cB}	10.57±0.12^{aB} (78%)	29.99±0.21^{bB} (37%)	
			P<0.001		
Zinc	*Kibandameno*	0.39±0.01^{cB}	0.32±0.01^{aB} (17%)	0.38±0.04^{bB} (5%)	
	Tajirika	0.32±0.01^{cA}	0.24±0.01^{aA} (23%)	0.27±0.03^{bA} (15%)	**<0.001**
	Migyera	0.74±0.02^{cD}	0.61±0.01^{aD} (18%)	0.62±0.01^{bD} (16%)	
	MM96/2480	0.54±0.01^{cC}	0.42±0.01^{aC} (23%)	0.53±0.01^{bC} (3%)	
			P<0.001		
Iron	*Kibandameno*	0.32±0.01^{c}	0.24±0.01^{aA} (22%)	0.26±0.04^{bA} (18%)	
	Tajirika	0.37±0.01^{c}	0.31±0.01^{aB} (15%)	0.33±0.06^{bB} (13%)	**<0.001**
	Migyera	0.71±0.01^{c}	0.61±0.01^{aD} (14%)	0.61±0.01^{bC} (13%)	
	MM96/2480	0.72±0.02^{c}	0.40±0.01^{aC} (44%)	0.67±0.01^{bD} (7%)	
			P<0.001		
Potassium	*Kibandameno*	169.96±2.69^{c}	149.46±1.18^{aB} (12%)	169.86±1.53^{bB} (0.05%)	
	Tajirika	152.07±2.73^{c}	125.25±1.98^{aA} (18%)	145.24±1.65^{bA} (4%)	**<0.001**
	Migyera	485.78±6.61^{c}	328.57±3.75^{aD} (32%)	485.69±3.77^{bD} (0.02%)	
	MM96/2480	326.43±5.03^{c}	254.47±3.28^{aC} (22%)	298.21±1.28^{bC} (9%)	
			P<0.001		
Oxalate	*Kibandameno*	7.36±0.07^{c}	6.09±0.06^{aB} (17%)	7.33±0.14^{bB} (0.3%)	
	Tajirika	12.24±0.23^{c}	4.10±0.08^{aA} (66%)	4.19±0.11^{bA} (65%)	**<0.001**
	Migyera	613.46±17.61^{c}	157.41±2.30^{aC} (74%)	252.27±5.49^{bC} (59%)	
	MM96/2480	413.86±8.08^{c}	282.25±2.85^{aD} (32%)	361.53±7.18^{bD} (13%)	
			P<0.001		
Phytate	*Kibandameno*	458.69±4.63^{c}	334.04±4.05^{aD} (27%)	453.01±3.62^{bD} (1%)	
	Tajirika	390.36±2.39^{c}	236.77±2.72^{aC} (39%)	257.13±1.61^{bB} (34%)	**<0.001**
	Migyera	561.28±5.72^{c}	146.34±0.94^{aA} (74%)	271.16±2.48^{bC} (52%)	
	MM96/2480	463.77±5.75^{c}	192.23±3.64^{bB} (59%)	244.51±2.78^{bA} (47%)	
			P<0.001		

The mean values of the same nutrient followed by different capital letters in the same column and different tiny letters in the same row differ significantly. (One-Way ANOVA, SNK test, α = 0.05).

Table 4.9 displays the average concentrations of minerals and anti-nutrients in both cooked and raw cassava leaves. Boiling was the cooking method that considerably reduced the elements and anti-nutrients (P<0.001). The reduction ranged between 16% - 60% Ca, 53% - 70% Zn, 38% - 55% Fe, 39% - 58% K, 44% - 59% oxalate, and 52% - 93% phytate. Because of the leaching caused by crushing before boiling, the leaves saw a noticeably greater reduction in the elements and anti-nutrients. Cassava varieties grown in different climatic conditions experienced varied reduction of minerals and anti-nutrients on boiling. For instance, the reduction in Fe ranged from 55% - 53% for varieties grown in Kilifi and between 43% - 38% for the varieties grown in Busia. Phytate reduced in the range 53% - 52% for the varieties grown in Kilifi and between 93% - 89% for the varieties grown in Busia. Variety is another factor that was seen to affect reduction in minerals and anti-nutrients. For instance, boiling leaves of *MM96/2480* variety reduced levels of Ca 16%. The same procedure reduced Ca levels in *Migyera* by 60%. Since these two types were cultivated on the same plot, the same environmental factors applied.

The difference can be attributed to the genetic difference between varieties (Oresegun *et al.*, 2016). Overall, the findings indicate that while raw cassava leaves and tubers are rich in protein, vitamins, and minerals, a greater proportion of nutrients are lost during the two primary cooking methods of preparing cassava for human consumption, boiling and deep-frying. Therefore, it is not possible to rely wholly on either, boiled or deep-fried cassava tubers and leaves to reduce malnutrition. There is therefore need for fortification or consuming them with other food products like vegetables.

Table 4.9: Mean levels of mineral elements and anti-nutrients in cooked and raw leaves of *Manihot esculenta* Crantz varieties grown in Kilifi and Busia Counties (mg/100 g)

Nutrient	**Variety**	**Mean ± SD (mg/100 g) n = 3**		**P value**
		Raw	**Boiled**	
Calcium	*Kibandameno*	130.54±3.51^{bC}	80.99±1.50^{aC} (38%)	
	Tajirika	287.99±5.68^{bD}	128.12±2.50^{aD} (56%)	**P<0.001**
	Migyera	50.13±0.20^{bB}	20.29±0.22^{aA} (60%)	
	MM96/2480	39.15±0.38^{bA}	33.02±0.24^{aB} (16%)	
		P<0.001		
Zinc	*Kibandameno*	1.21±0.02^{bC}	0.52±0.01^{aC} (56%)	
	Tajirika	1.22±0.02^{bC}	0.56±0.01^{aD} (53%)	**P<0.001**
	Migyera	1.16±0.01^{bB}	0.35±0.01^{aA} (70%)	
	MM96/2480	1.01±0.02^{bA}	0.47±0.01^{aB} (54%)	
		P<0.001		
Iron	*Kibandameno*	1.69±0.01^{bA}	0.76±0.01^{aA} (55%)	
	Tajirika	3.35±0.09^{bD}	1.58±0.01^{aC} (53%)	**P<0.001**
	Migyera	2.38±0.03^{bB}	1.48±0.01^{aB} (38%)	
	MM96/2480	3.14±0.03^{bC}	1.78±0.01^{aD} (43%)	
		P<0.001		
Potassium	*Kibandameno*	192.94±5.01^{bA}	117.98±2.27^{aB} (39%)	
	Tajirika	240.43±4.19^{bB}	99.83±1.62^{aA} (58%)	P<0.001
	Migyera	375.79±3.77^{bD}	185.45±2.09^{aD} (51%)	
	MM96/2480	298.39±3.10^{bC}	163.30±2.54^{aC} (45%)	
		P<0.001		
Oxalate	*Kibandameno*	38.74±0.01^{bA}	17.30±0.29^{aA} (55%)	
	Tajirika	41.27±0.78^{bB}	17.84±0.26^{aB} (57%)	P<0.001
	Migyera	284.27±6.29^{bC}	115.56±3.47^{aC} (59%)	
	MM96/2480	526.98±0.17^{bD}	294.28±4.12^{aD} (44%)	
		P<0.001		
Phytate	*Kibandameno*	861.32±22.77^{bD}	417.60±0.49^{aD} (52%)	
	Tajirika	783.50±6.91^{bC}	369.33±3.65^{bC} (53%)	P<0.001
	Migyera	555.99±5.71^{bB}	61.52±0.78^{aB} (89%)	
	MM96/2480	452.77±13.92^{bA}	32.09±0.33^{aA} (93%)	
		P<0.001		

The mean values of the same nutrient followed by different capital letters in the same column and different tiny letters in the same row differ significantly. (One-Way ANOVA, SNK test, α = 0.05).

4.4 Bioaccessibility of nutrients in cooked cassava roots and leaves.

The mean bioaccessible levels and percentage bioaccessibility of vitamins C, B1, B2 and B3 in roots and leaves are shown in Table 4.10. Percentage bioaccessibility was based on the amounts of nutrients ingested and that which is bioaccessible. The percentage bioaccessibility of vitamin

C ranged between 43% (raw *Kibandameno* leaves) - 90% (boiled *Migyera* roots). These falls below the percentage bioaccessibility ranges of between 80 – 90% from spinach, kale, banana and kiwi reported by MacDonald *et al.,* 2016; Carr and Vissers, 2013). For the analyzed vitamin B series, bioaccessibility of vitamin B1 which ranged between 39% (raw *Kibandameno* leaves) - 85% (boiled *Migyera* roots) was higher than that of B2 and B3. These B1 values fall within the low range of 39–81% reported by Akca *et al.,* (2019) in baby diets based on cereal. Since cassava is a plant-based diet, its high fiber content and poor protein digestibility would predict that its bioaccessibility of vitamins B1, B2, and B3 would be low (Akca *et al.,* 2019).

Cooking significantly (P<0.001) increased percentage bioaccessibility of vitamins in both roots and leaves being highest in boiled, followed by deep-fried and lowest in raw. For instance, bioaccessibility of vitamin C in boiled tubers of *Kibandameno* variety was 83%, 60% in deep-fried and 45% in raw. In leaves, percentage bioaccessibility of vitamin C in *Tajirika* variety was 80% in boiled and 57% in raw. The reason for this is that foods processed by heating have their macronutrients such as proteins and carbohydrates more digestible when the matrix of food are loosened and softened. The contact between digestive enzymes and the nutrients is therefore increased (Hamalatha *et al.,* 2007).

The percentage bioaccessibility of vitamins from both raw and boiled tubers was observed to be significantly higher (P<0.001) than that of leaves (table 4.10**)**. Roots of both *Kibandameno* and *Tajirika* varieties had between 2 - 6% higher percentage bioaccessibility of the nutrients than their leaves. According to USDA (2008), the amount of fiber in leaves of cassava is higher than that in roots which explains the lower bioaccessibility levels observed. Boiled roots of *Migyera* variety had higher percentage bioaccessibility of vitamins; C and B1, those of *Kibandameno* variety had higher percentage bioaccessibility of vitamin B2 while those of *Tajirika* variety had higher percentage bioaccessibility of vitamin B3. Findings support the fact that bioaccessibility would be attributed to genetic differences between cassava varieties (Oresegun *et al.*, 2016).

Table 4.10: Mean bioaccessible levels of vitamins in raw and cooked roots and leaves of *Manihot esculenta* Crantz varieties grown in Kilifi and Busia Counties (mg/100 g)

Nutrient	**Variety**	**Mean ± SD (mg/100 g) n = 3**		
		Raw	**Boiling**	**Deep-frying**
Vitamin C	*Kibandameno* roots	4.04±0.04^{cD} (45%)	2.01±0.01^{aE} (83%)	3.84±0.07^{bC} (60%)
	Kibandameno leaves	33.16±0.86^{bH} (43%)	25.34±0.71^{aH} (77%)	
	Tajirika roots	1.46±0.02^{cB} (60%)	1.44±0.03^{bD} (85%)	1.38±0.02^{aB} (76%)
	Tajirika leaves	19.57±0.48^{bG} (57%)	6.30±0.054^{aG} (80%)	
	Migyera roots	1.76±0.05^{bC} (63%)	1.36±0.12^{aB} (90%)	1.38±0.21^{aB} (82%)
	Migyera leaves	14.56±1.95^{bF} (61%)	2.65±0.53^{aF} (69%)	
	MM96/2480 roots	1.30±0.11^{cA} (61%)	0.14±0.02^{aA} (87%)	0.87±0.02^{bA} (71%)
	MM96/2480 leaves	7.59±0.38^{bE} (65%)	1.40±0.34^{aC} (67%)	
		P<0.001		
Vitamin B1	*Kibandameno* roots	0.70±0.01^{bB} (41%)	0.08±0.00^{aA} (80%)	0.08±0.00^{aB} (58%)
	Kibandameno leaves	2.64±0.02^{bE} (39%)	0.51±0.01^{aD} (78%)	
	Tajirika roots	0.58±0.01^{cA} (56%)	0.26±0.00^{bB} (81%)	0.02±0.00^{aA} (79%)
	Tajirika leaves	1.68±0.032^{bC} (50%)	0.46±0.01^{aC} (81%)	
	Migyera roots	0.58±0.02^{cA} (50%)	0.27±0.04^{aB} (85%)	0.39±0.01^{bC} (55%)
	Migyera leaves	5.95±0.09^{bF} (44%)	0.67±0.01^{aE} (72%)	
	MM96/2480 roots	0.58±0.07^{cA} (48%)	0.28±0.01^{aB} (83%)	0.53±0.01^{bD} (53%)
	MM96/2480 leaves	1.74±0.14^{bD} (41%)	0.27±0.01^{aB} (70%)	
		P<0.001		
Vitamin B2	*Kibandameno* roots	0.22±0.01^{cE} (39%)	0.14±0.00^{bC} (79%)	0.11±0.00^{aD} (52%)
	Kibandameno leaves	0.25±0.01^{aF} (34%)	0.44±0.00^{bD} (75%)	
	Tajirika roots	0.09±0.00^{bB} (37%)	0.05±0.00^{aB} (75%)	0.09±0.00^{cC} (70%)
	Tajirika leaves	0.14±0.00^{bC} (35%)	0.05±0.00^{aB} (73%)	
	Migyera roots	0.06±0.01^{cA} (43%)	0.03±0.01^{bA} (50%)	0.02±0.01^{aA} (38%)
	Migyera leaves	0.20±0.01^{bD} (40%)	0.02±0.01^{aA} (70%)	
	MM96/2480 roots	0.09±0.01^{cB} (21%)	0.03±0.01^{aA} (69%)	0.04±0.01^{bB} (50%)
	MM96/2480 leaves	0.07±0.02^{bA} (18%)	0.01±0.01^{aA} (63%)	
		P<0.001		
Vitamin B3	*Kibandameno* roots	0.62±0.01^{aC} (35%)	0.77±0.01^{bC} (55%)	0.84±0.02^{cD} (49%)
	Kibandameno leaves	2.68±0.02^{bG} (30%)	2.31±0.04^{aG} (50%)	
	Tajirika roots	0.64±0.02^{aD} (30%)	0.98±0.02^{bE} (69%)	0.70±0.01^{aC} (51%)
	Tajirika leaves	3.10±0.06^{aH} (27%)	3.28±0.07^{bH} (67%)	
	Migyera roots	0.24±0.01^{aB} (17%)	0.24±0.01^{aA} (67%)	0.26±0.07^{bA} (45%)
	Migyera leaves	1.39±0.02^{aF} (15%)	1.67±0.07^{bF} (60%)	
	MM96/2480 roots	0.12±0.01^{aA} (15%)	0.37±0.03^{bB} (62%)	0.29±0.06^{cB} (48%)
	MM96/2480 leaves	0.74±0.02^{aE} (12%)	0.82±0.06^{bD} (59%)	
		P<0.001		

The mean values of the same nutrient followed by different capital letters in the same column and different tiny letters in the same row differ significantly. (One-Way ANOVA, SNK test, α = 0.05).

Mean bioaccessible levels and percentage bioaccessibility of Ca, Zn, Fe and K in roots and leaves are shown in Table 4.11. Bioaccessibility of calcium ranged between 12% (raw *Migyera* leaves) and 55% (boiled *Tajirika* tubers) similar to that found in kales, Chinese cabbage and soybean sprouts (Weaver, 2009; Kamchan *et al.*, 2004). These levels described as medium (Kamchan *et al.,* 2004), are caused by anti-nutrients like oxalate which forms calcium oxalate complex that is insoluble hence hindering its bioaccessibility (Gupta *et al.,* 2015). The bioaccessibility of zinc ranged between 10% (raw *Kibandameno* leaves) and 31% (boiled *Tajirika* roots). These levels are similar to those found in cereals and pulses (Hemalatha *et al.*, 2007) where, bioaccessibility was 8.5% in rice, 0.31% in sorghum, 5.8% in finger millet and 1.6% in wheat-based meals.

This range falls below 16 – 50% range which is the expected absorption of dietary zinc in human, which may be attributed to presence of phytate and high amount of calcium (Bertinato *et al.,* 2020). The bioaccessibility of iron ranged between 8% (raw *Kibandameno* leaves) and 30% (boiled *Tajirika* roots*)*. This is greater than the ranges found in pulses and cereals by Hemalatha *et al.,* (2007), iron (1.5%) finger millet, rice (2.5%), sorghum (3.5%), and wheat-based meal (4.7%). The low bioaccessibility of iron may be due to the high concentrations of anti-nutrients and the non-digestible iron-storing cellular components like mitochondria and chloroplasts. Bioaccessibility of potassium ranged between 50 % (raw *Tajirika* leaves) and 84% (boiled *Tajirika* tubers) falling within the range found in spinach, kale, banana and kiwi (MacDonald and co-workers (2016). The authors explain that to some extent, matrix effects reduce potassium absorption from unprocessed vegetables (Macdonald *et al.,* 2016).

Bioaccessibility (percentage) of minerals was significantly higher (P<0.001) in cooked roots than in cooked leaves (Table 4.11) whilst noting cassava leaves have higher levels of anti-nutrients than cassava roots (Latif & Muller, 2015)**.** For example, boiled roots of *Kibandameno* variety, percentage bioaccessibility of minerals in roots and leaves was; 47% tubers, 25% leaves (Ca), 29% roots, 12% leaves (Zn), 27 % roots, 11 % leaves (Fe) and 82 % roots, 70 % leaves (K). Bioaccessibility was seen to be dependent on the variety. For instance, percentage bioaccessibility of most minerals in roots was significantly higher (P<0.001) in *Kibandameno* variety than in *Tajirika* variety. The percentage bioaccessibility of minerals in raw tubers was 27% *Kibandameno*, 22% *Tajirika* (Ca), 21% *Kibandameno*, 19% *Tajirika* (Zn), 20% *Kibandameno*, 15% *Tajirika* (Fe) and 70% *Kibandameno*, 72% *Tajirika* (K). This can be explained by the genetic differences in cassava varieties (Oresegun *et al.,* 2016), which affects among other things their anti-nutrient contents. From Table 4.4, roots of *Kibandameno* variety have the lowest levels of oxalate and second lowest level of phytate after *Tajirika* variety. Findings imply that *Kibandameno* variety is a better source of minerals than *Tajirika* variety for the varieties grown in Kilifi. The bioaccessibility of most minerals was higher in *MM96/2480* variety than *Migyera* for the varieties grown in Busia County. This can be a result of lower phytate and oxalate levels in *MM96/2480* variety compared to those in *Migyera*.

Percentage bioaccessibility of minerals in cooked roots and leaves was significantly higher (P<0.001) than in raw. Compared to raw food, cooking allows the body to absorb a far higher percentage of the nutrients (Platel *et al.,* 2016). Inhibitors are reduced during cooking, which may lower nutrient bioaccessibility by competing for the same uptake system, making the nutrient insoluble and therefore not absorbable, or attaching it to the intestinal cell surface in a way that the uptake systems do not recognize. Cooking may make the proteins that contain calcium more digestible, which would boost the mineral's release from any protein complexes (Repo-Carrasco *et al.*, 2010).

Table 4.11: Mean bioaccessible levels of mineral elements in raw and cooked roots and leaves of *Manihot esculenta* Crantz varieties grown in Kilifi and Busia Counties (mg/100 g)

Nutrient	**Variety**	**Mean ± SD (mg/100 g) n = 3**		
		Raw	**Boiled**	**Deep-fried**
Calcium	*Kibandameno* roots	15.75±0.33^{cE} (27%)	13.81±0.09^{bE} (47%)	11.39±0.15^{aC} (28%)
	Kibandameno leaves	19.59±0.37^{aF} (15%)	20.32±0.23^{bF} (25%)	
	Tajirika roots	24.38±0.17^{bG} (22%)	32.80±0.14^{cH} (55%)	18.09±0.20^{aD} (29%)
	Tajirika leaves	37.57±0.46^{bH} (13%)	30.94±0.22^{aG} (24%)	
	Migyera roots	4.86±0.21^{bA} (15%)	2.19±0.48^{aA} (33%)	5.21±0.36^{cA} (24%)
	Migyera leaves	6.04±0.15^{bC} (12%)	3.06±0.09^{aB} (15%)	
	MM96/2480 roots	8.12±0.31^{cD} (17%)	3.17±0.12^{aC} (30%)	6.94±0.84^{bB} (23%)
	MM96/2480 leaves	5.10±0.23^{aB} (13 %)	5.63±0.15^{bD} (17 %)	
		P<0.001		
Zinc	*Kibandameno* roots	0.08±0.00^{aB} (21%)	0.10±0.00^{bC} (29%)	0.10±0.00^{bB} (25%)
	Kibandameno leaves	0.12±0.00^{bC} (10%)	0.06±0.00^{aA} (12%)	
	Tajirika roots	0.06±0.00^{aA} (19%)	0.08±0.00^{bB} (31%)	0.06±0.00^{aA} (23%)
	Tajirika leaves	0.12±0.00^{bC} (10%)	0.09±0.00^{aC} (16%)	
	Migyera roots	0.09±0.00^{aB} (12%)	0.18±0.00^{cE} (30%)	0.13±0.00^{bD} (21%)
	Migyera leaves	0.13±0.05^{bC} (11%)	0.05±0.00^{aA} (13%)	
	MM96/2480 roots	0.08±0.00^{aB} (15%)	0.12±0.00^{bD} (28%)	0.11±0.00^{bC} (20%)
	MM96/2480 leaves	0.12±0.00^{aC} (12%)	0.07±0.00^{aB} (15%)	
		P<0.001		
Iron	*Kibandameno* roots	0.06±0.00^{aA} (20%)	0.07±0.00^{aA} (27%)	0.06±0.00^{aA} (24%)
	Kibandameno leaves	0.14±0.00^{bC} (8%)	0.08±0.00^{aB} (11%)	
	Tajirika roots	0.06±0.00^{aA} (15%)	0.10±0.00^{bC} (30%)	0.07±0.00^{aA} (20%)
	Tajirika leaves	0.30±0.01^{bE} (9%)	0.24±0.01^{aE} (15%)	
	Migyera roots	0.08±0.00^{aB} (11%)	0.17±0.00^{cD} (27%)	0.11±0.00^{bB} (17%)
	Migyera leaves	0.24±0.00^{bD} (10%)	0.18±0.00^{aD} (12%)	
	MM96/2480 roots	0.09±0.00^{aB} (13%)	0.10±0.00^{aC} (25%)	0.11±0.00^{aB} (16%)
	MM96/2480 leaves	0.35±0.008^{bF} (11%)	0.25±0.00^{aE} (14%)	
		P<0.001		
Potassium	*Kibandameno* roots	119.08±1.23^{aC} (70%)	122.62±1.67^{bD} (82%)	128.09±1.44^{cB} (75%)
	Kibandameno leaves	133.59±2.34^{bD} (69%)	82.688±1.14^{aA} (70%)	
	Tajirika roots	110.12±2.02^{bB} (72%)	105.495±2.16^{aC} (84%)	115.77±1.63^{cA} (79%)
	Tajirika leaves	72.74±1.13^{aA} (50%)	98.30±0.99^{bB} (75%)	
	Migyera roots	323.39±0.80^{cH} (66%)	274.34±0.60^{bH} (83%)	263.13±1.62^{aD} (70%)
	Migyera leaves	209.94±1.55^{bF} (62%)	136.04±0.57^{aF} (73%)	
	MM96/2480 roots	214.08±1.85^{bG} (65%)	215.77±1.03^{cG} (85%)	204.21±0.71^{aC} (68%)
	MM96/2480 leaves	189.58±0.41^{bE} (63%)	118.37±0.33^{aE} (72%)	
		P<0.001		

The mean values of the same nutrient followed by different capital letters in the same column and different tiny letters in the same row differ significantly. (One-Way ANOVA, SNK test, α = 0.05).

CHAPTER FIVE

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

The study determined protein, thiamine, riboflavin, niacin, calcium, zinc, iron, potassium, oxalate and phytate levels in raw and processed leaves and roots of *Kibandameno*, *Tajirika*, *MM96/2480* and *Migyera* cassava varieties. The bioaccessibility of the vitamins and minerals in processed and raw leaves and roots of these cassava varieties was also determined. The findings draw the following conclusions;

i) Raw roots and leaves (8 and 12 months) of *Kibandameno*, *Tajirika*, *Migyera* and *MM96/2480 Manihot esculenta* Crantz varieties grown in Kilifi and Busia Counties contained protein (208.00 – 17.00 mg/100 g), vitamins (C, B1, B2 and B3) (76.37 – 0.12 mg/100 g), minerals (Ca, Zn, Fe, K) (485.78 – 0.32 mg/100 g), and anti-nutrients (phytate and oxalate) (861.32 – 4.72 mg/100 g). These levels are dependent on; variety of the cassava; *Migyera* had higher levels of protein, Zn, K, phytate and oxalate. Vitamins; C and B2 were higher in *Kibandameno*, while vitamins; B1 and B3, Ca and Fe were higher in *Tajirika* variety. Climatic conditions; Compared to Busia, Kilifi County-grown cultivars showed greater concentrations of vitamins and minerals, including calcium and iron. Age at harvesting; there was a decrease in nutrients and anti-nutrients with age.

ii) Both roots and leaves have much lower quantities of nutrients and anti-nutrients after cooking. For roots, deep-frying is preferable to boiling because it preserves a comparatively larger concentration of water-soluble nutrients. The reduction of anti-nutrients though higher in boiling than in deep-frying was not sufficient to lower their levels to the recommended molar ratios for Zn and Fe. This means that the levels of anti-nutrients that remain after cooking are still able to affect the bioaccessibility of nutrients hence causing undernourishment in populations that depend on a diet high in cassava. The *Migyera* variety exhibited a larger reduction of most nutrients and anti-nutrients, showing varietal difference in how they respond to the various cooking methods. Climate; Compared to varieties grown in Kilifi County, those grown in Busia County showed a greater percentage loss of most nutrients.

iii) Bioaccessibility of nutrients significantly increased on cooking of cassava roots and leaves. Boiling significantly increased bioaccessibility than deep-frying. Bioaccessibility of nutrients was higher in roots than in leaves. Bioaccessibility was dependent on variety; bioaccessibility of most nutrients was higher in *Tajirika* variety.

5.2 Recommendations from this study

The study's conclusions lead to the following recommendations.

i. Cassava roots should not be left in the farm for a longer period after maturity as this lowers the nutrient levels.

ii. Cassava roots should be cooked by deep frying as it retained a bigger percentage of the water-soluble nutrients.

iii. To help lower anti-nutrients and increase nutrient bioaccessibility, cassava roots and leaves should be cooked before eating.

5.3 Recommendations for further studies

Given the study's limitations and conclusions, it is suggested that more research be done in the following areas:

i) Study on anti-nutrients and nutrients levels in *Kibandameno, Tajirika, Migyera and MM96/2480* cassava varieties with soil fertility and climatic conditions taken into consideration.

ii) Impact of methods of processing like roasting, fermenting, sun-drying on bioaccessibility of nutrients in *Kibandameno, Tajirika*, *Migyera* and *MM96/2480* cassava varieties.

iii) *In vivo* study of bioaccessibility of nutrients in *Kibandameno, Tajirika*, *Migyera* and *MM96/2480* cassava varieties.

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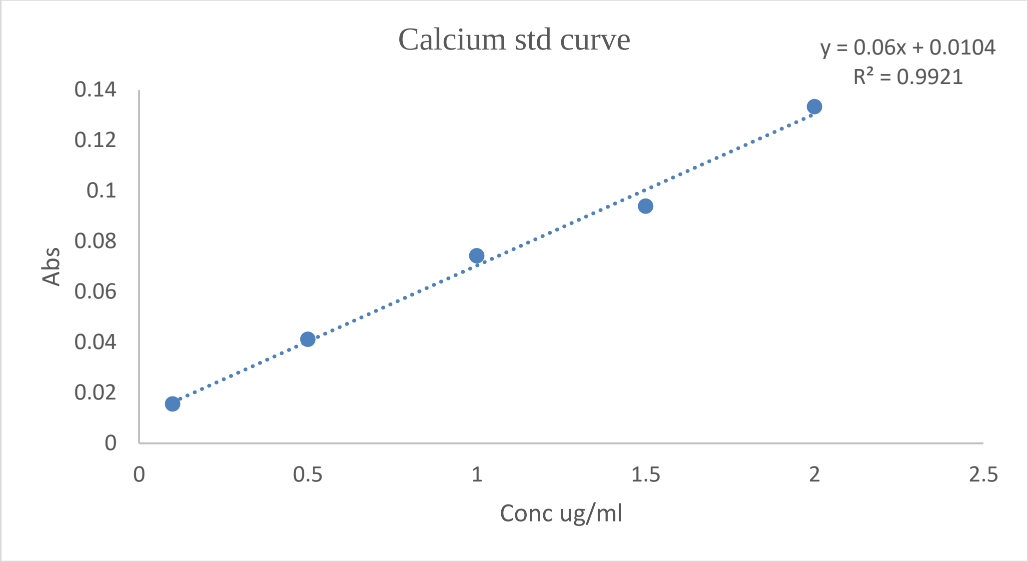
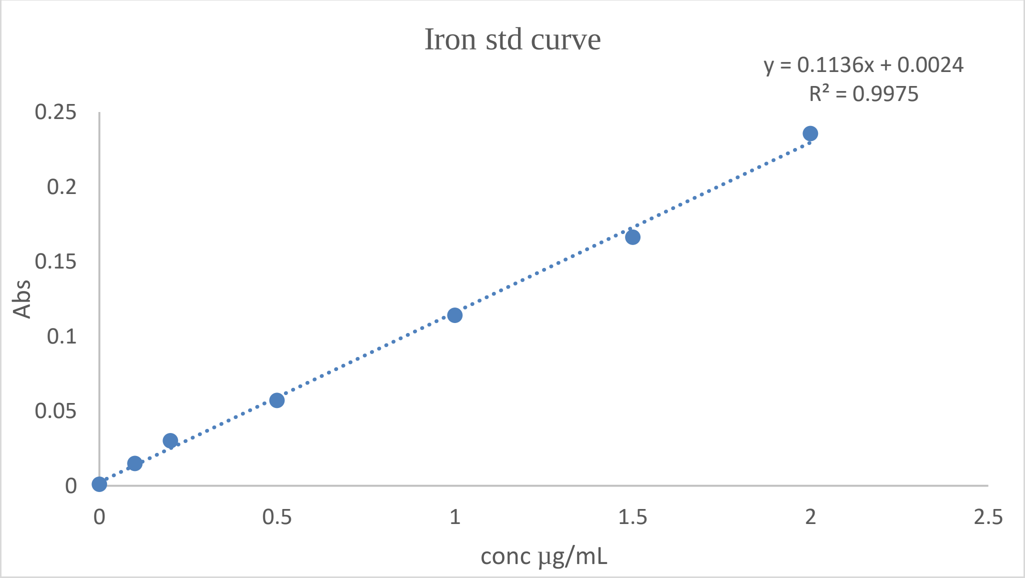
APPENDIX

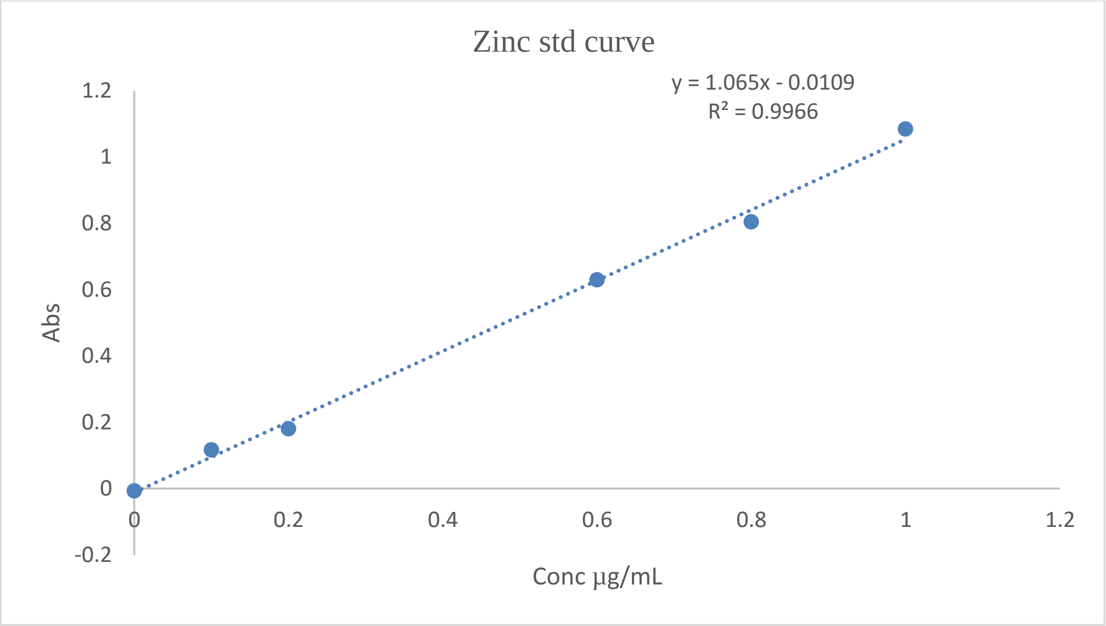
Appendix I: Calibration curves



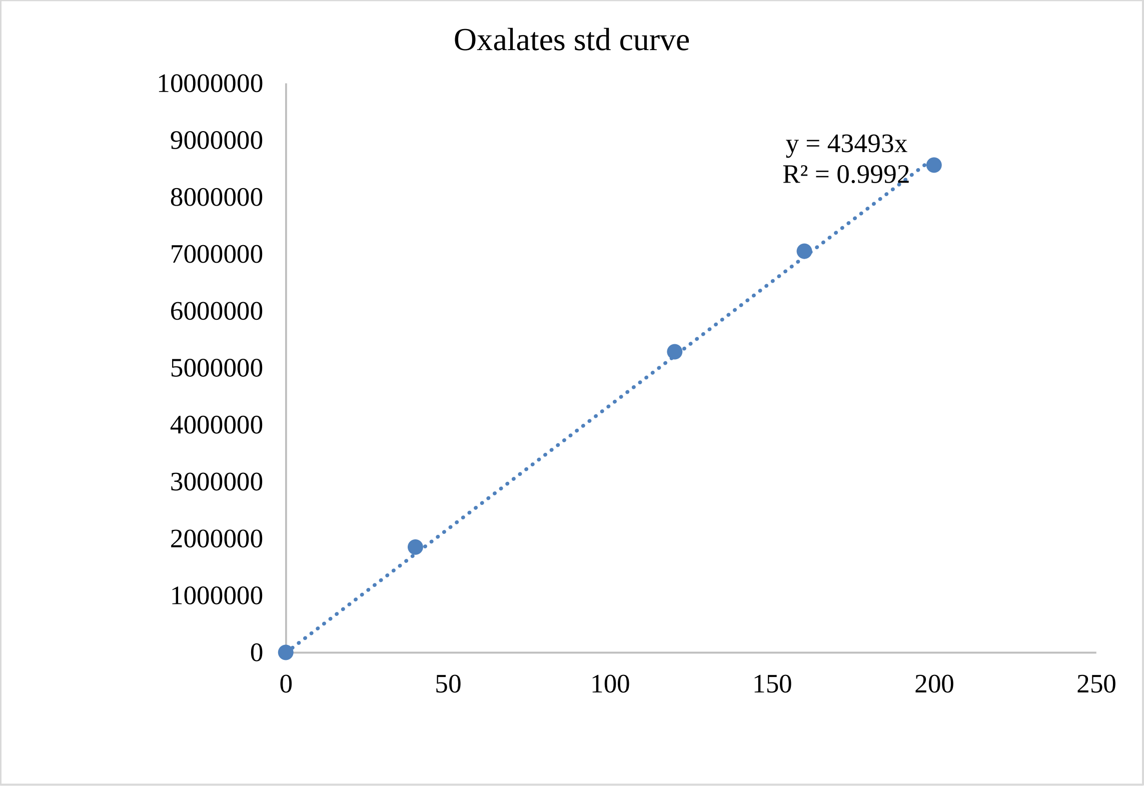
Appendix Figure 1: Calibration curve for calcium



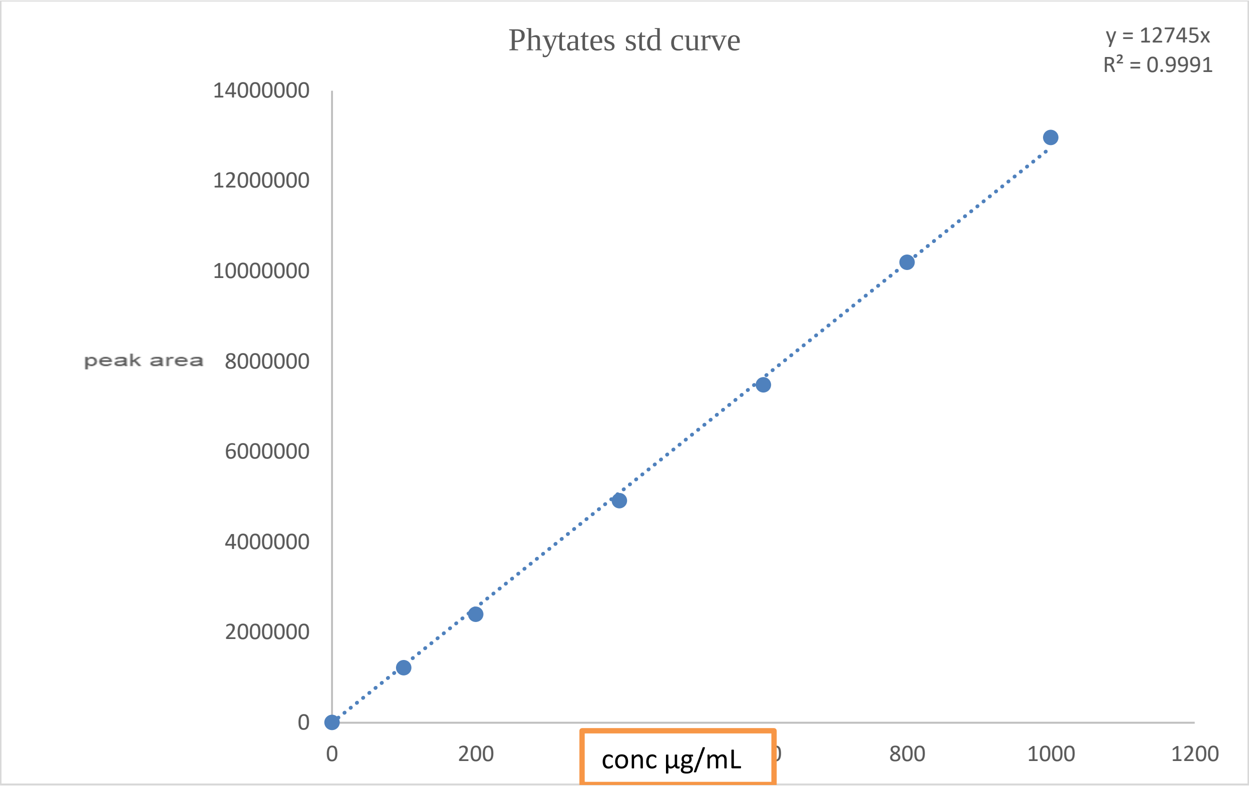
Appendix Figure 2: Calibration curve for iron



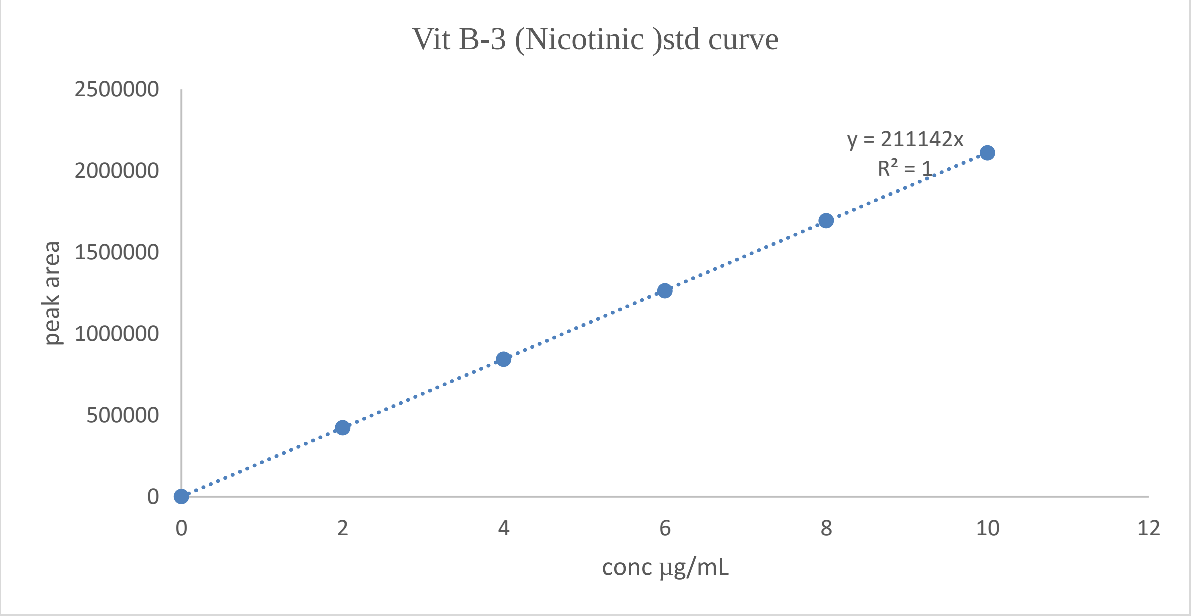
Appendix Figure 3: Calibration curve for zinc



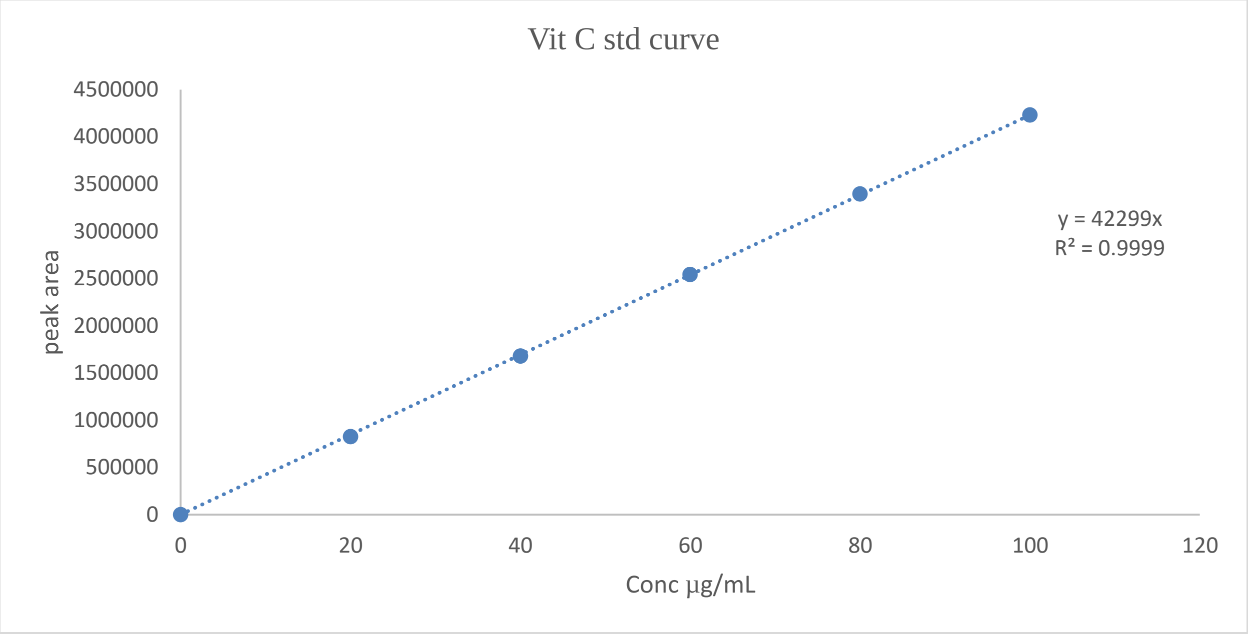
Appendix Figure 4: Calibration curve for oxalate



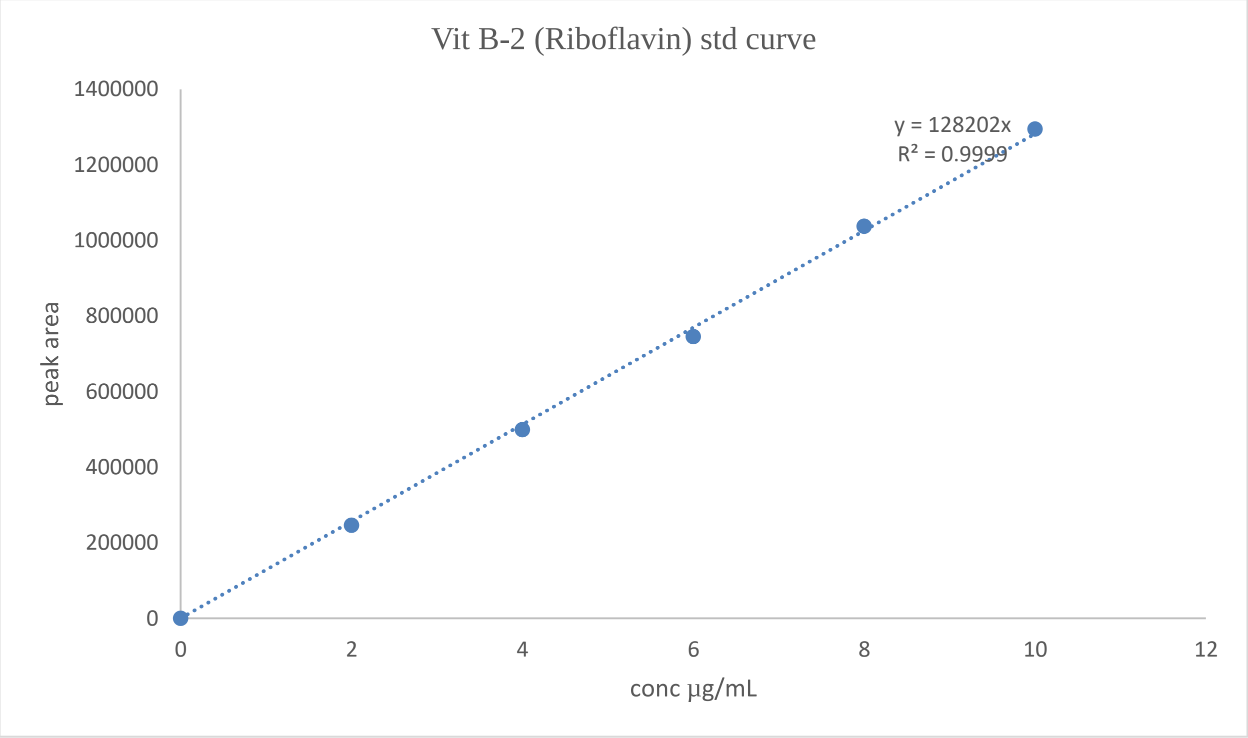
Appendix Figure 5: Calibration curve for phytate



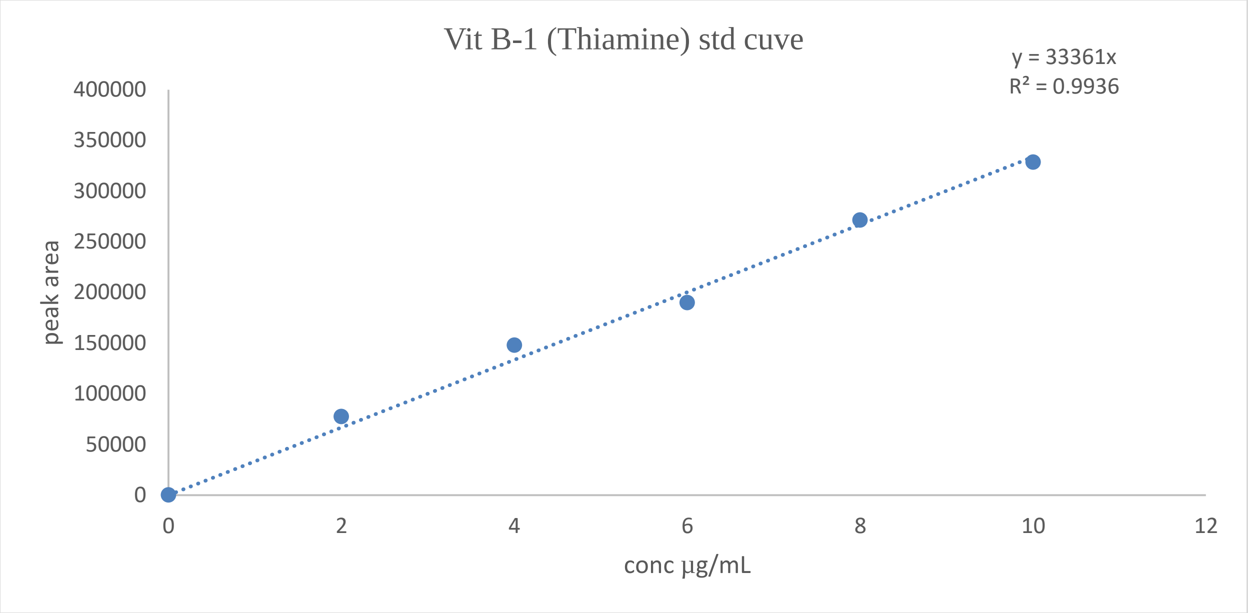
Appendix Figure 6: Calibration curve for vitamin B_3



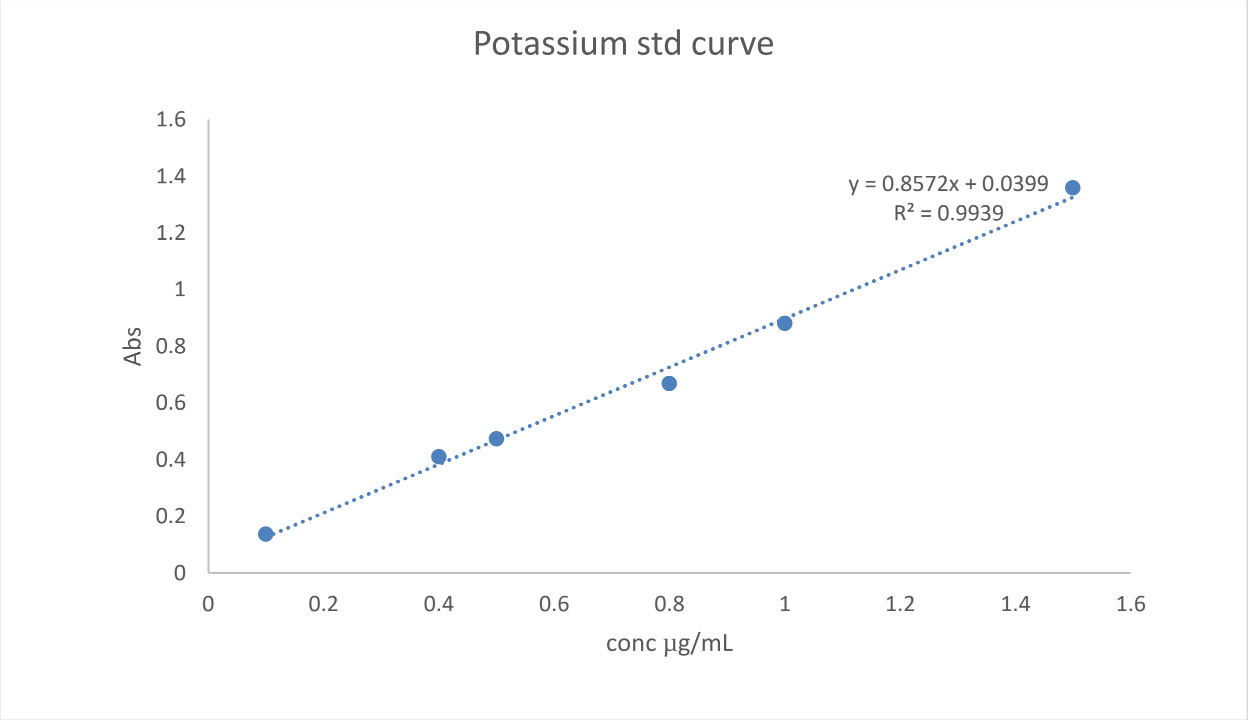
Appendix Figure 7: Calibration curve for vitamin C



Appendix Figure 8: Calibration curve for vitamin B_2



Appendix Figure 9: Calibration curve for vitamin B_1



Appendix Figure 10: Calibration curve for potassium