SIMULATED BIOACCESSIBILITY STUDIES OF BETA-CAROTENE AND VITAMIN B SERIES IN SELECTED LEAFY AFRICAN INDIGENOUS VEGETABLES IN KISII COUNTY, KENYA

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DECLARATION

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

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This work is dedicated to my loving husband David Kengere and our children Donlin Mogoi Kengere, Adah Nyanchama Kengere, Olga Nyamoita Kengere and Amber Nyang'arisa Kengere.

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ABBREVIATIONS AND ACRONYMS

AIDS	Artificial Immune Deficiency Syndrome
LAIVs	Leafy African Indigenous Vegetables
ANOVA	Analysis of Variance
FAO	Food and Agriculture Organization
GDP	Gross Domestic Product
HPLC	High Performance Liquid Chromatography
pH	Potential of Hydrogen
UV	Ultra Violet
WHO	World Health Organisation
SNK	Student Neuman Keul's
PLP	Pyridoxal 5' – phosphate
PMP	Pyridoxamine 5' – phosphate
PNG	Pyridoxine 5' - glucoside

ABSTRACT

Over two billion people worldwide are affected by various vitamin deficiencies resulting in poor health, low gross domestic product and high mortality rates. Deficiency of vitamins remain a public health problem in the developing countries affecting mostly expectant and lactating mothers as well as children under five years of age. Despite a number of approaches taken in managing these deficiencies, the nutritional approach is still most preferred and especially employing leafy African indigenous vegetables (LAIVs), which can be consumed either fresh or processed. In Kenya, LAIVs are locally available and inexpensive. Although they are good sources of vitamins and other nutrients, the LAIVs are underutilized and face postharvest losses in seasons of high availability. Sun drying and oven drying are among the methods employed to address postharvest losses. The research gap presented is on the *in vitro* bioaccessibility of beta-carotene (BC) and vitamin B series to support promotion of LAIVs when consumed as individual fresh, processed or as recipes of mixtures and formulations, among the forms in which they can be consumed. This would inform on the amount of the same nutrients that are potentially available for absorption in the gastrointestinal tract once ingested. *In vitro* methods are preferred for being accurate, reproducible and have no ethical constrains among others. The general objective was to investigate the bioaccessibility of beta-carotene (BC) and vitamin B series in LAIVs, recipes and formulations using an *in vitro* method. Levels of vitamins were determined in the fresh and processed LAIVs; *Cleome gynandra*, *Vigna unguiculata*, *Amaranthus viridis*, *Basella alba* and *Cucurbita maxima* by UV-Vis spectrophotometry for BC and high performance liquid chromatography (HPLC) for vitamin B series. Gastrointestinal digestion was performed for the gastric and intestinal phases. The data obtained was analyzed by SPSS employing ANOVA to compare the mean levels and percentage bioaccessibilities of the vitamins. The mean (n=3) levels (mg/100g DW) of BC and the vitamin B series were sufficient to meet the recommended dietary allowances (RDAs) and provided the mean range of % bioaccessibility of BC of 61.36±1.87 to 97.23±0.06 (fresh), 65.67±5.53 to 84.31±0.27 (boiled) and 16.97±0.02 to 48.19±0.82 (boiled-fried) and for vitamin B ranged from 5.17±0.93 to 100.00±0.01 (fresh), 5.18±0.87 to 100.00±0.01 (boiled) and 14.23±3.69 to 100.00±0.01 (boiled-fried). In recipes the range of the mean % bioaccessibility of BC was 69.09±1.29 to 88.97±0.22 (RCP1) and 17.45±6.47 to 25.30±1.05 (RCP2) while that of vitamin B was 19.97±3.57 to 100.00±0.00 (RCP1) and 5.57±1.18 to 92.57±0.62 (RCP2). In formulations the range of the mean % bioaccessibility of BC ranged from 48.28±3.66 to 70.45±0.78 while that of vitamin B was 6.28±0.21 to 97.20±2.01. Statistical differences ($p<0.05$) were observed between processes, recipes and formulations. Both the fresh and processed vegetables were found to contain levels of BC and vitamin B series that meet the RDAs as set by the World Health Organization (WHO). The findings show there is sufficient *in vitro* bioaccessible levels of BC and vitamin B series with processing of LAIVs therefore promoting their consumption would play a significant role in addressing malnutrition and food security.

CHAPTER 1

1 INTRODUCTION

1.1Background

Malnutrition, defined as the deficit of nutrient, has been reported to affect more than two billion of the global population (Bwembya *et al*., 2014). Chronic nutrient deficiency affects about 215 million people worldwide, about 43 % of this population being in Sub-Saharan Africa (Kunyanga *et al.,* 2013). Chronic undernourishment has affected 161 million children aged five years and below while 51 million have been reported to be acutely undernourished globally (UNICEF/WHO, 2014). Notably, of all deaths in children aged five years and below in Kenya, 38 % are as a result of malnutrition, distributed between severe malnutrition (4 %) on one end and mild and moderate malnutrition (34 %) on the other (Kunyanga *et al*., 2013).

Deficiency of micronutrients in developing countries has mostly affected young women and children (Regan *et al.,* 2015), resulting in poor health, low worker productivity, high mortality rate and morbidity (Bwembya *et al.*, 2014). Deficiency of dietary proteins causes kwashiorkor while marasmus results when there is lack of a balanced diet (Younis *et al.,* 2015). Lack of minerals causes anemia (iron), goiter (iodine) and growth retardation (zinc) among others (Younis *et al.*, 2015). Deficiency of vitamin B series causes a number of deficiency diseases including but not limited to; vitamin B_1-beriberi, B_2-cardiac disorder, B_3-pellagra, B_5-impaired coordination, B_6-neurological disturbances and B_9-megaloblastic anemia (Schellack *et al.,* 2015). Most vitamin B series are not stored in the body necessitating their inclusion in dietary intake (Schellack *et al.*, 2015).

A number of approaches used to address malnutrition include fortification (addition of nutrients to processed foods), bio fortification (growing crops rich in nutrients) and supplementation (provision of nutrient supplements on top of the normal diet) (Nyambaka and Ryley, 2001; Nawiri *et al.,* 2013; Uraku *et al.,* 2015; Younis *et al.,* 2015). While fortification and supplementation are expensive, bio fortification is a sustainable solution to nutrient deficiencies in developing countries (FAO/WHO, 2003). Vitamins in food are a better alternative therapeutically, correcting deficiencies and treating diseases more safely than use of drugs which are toxic and have side effects (Nkafamiya *et al.,* 2010). Consistent consumption of diets rich in carotenoids and vitamins not only maintains good health but also addresses micronutrient deficiency status (WHO, 2002; WHO, 2003; Smith and Eyzaguirre, 2007; Hasan *et al.,* 2013). The World Health Organization recommends dietary allowances (RDAs) for vitamin A ranging from 0.3 to 1.3 mg/day and vitamin B series ranging from 0.1 – 20 mg/day (WHO/FAO, 2005).

Vitamins vary widely in their chemical and physiological functions (Ball, 2005), classified as either fat soluble (vitamin A, D, E and K) or water soluble (vitamin C and the B series). Apart from animal products, leafy African indigenous vegetables (LAIVs) are the main sources of vitamins and other nutrients (Nyambaka and Ryley, 2001; Nawiri *et al.,* 2013; Uraku *et al.,* 2015) such as minerals and proteins (Hotz and Gibson, 2007; Palafox-Carlos *et al*., 2011; Kamga *et al*., 2013). Vitamin A takes part in growth and repair of eyes for good vision among other functions while vitamin B series act as co-enzymes in metabolism of food to produce energy (Bwembya *et al.*, 2014).

Vitamin deficiencies are caused by poor eating habits, improper absorption of vitamins, alcoholism and emotional stress (Nkafamiya *et al.*, 2010). Lack of these vitamins cause deficiency diseases that affect children and lactating mothers. About 2.8 million pre-school children are at risk of blindness and acute deficiency of vitamins causes pneumonia and diarrhea (Nawiri *et al.,* 2013).

Vegetables are commonly consumed when cooked. In many households though, rather than considering nutrient retention, vegetables are prepared on the basis of convenience and taste (Yuan *et al.,* 2009). Steaming, microwaving, boiling, stir frying and stir frying followed by boiling are among the methods used for cooking vegetables (Yuan *et al.,* 2009). Vitamins in food undergo degradation (Ball, 2005). The degradation is mainly due to high temperature, exposure to air (oxygen), light, moisture content, water capacity, pH, degradative enzymes and metal trace elements mainly iron (Fe) and copper (Cu) (Ball, 2005; Yuan *et al.,* 2009; Ismail *et al*., 2013; Schellack *et al*., 2015; Bua and Onang, 2017).

Raw foods such as leafy vegetables deteriorate very fast, in a few days, as a result of biochemical reactions due to the presence of water. The availability of water present in the system is measured by water activity which is affected by changes in the environment such as heat, light, pressure, pH, additions and modifications of particle size by affecting the molecular state of water. A low water activity of less than 0.6 does not permit microbial or enzymatic activity. For bacteria and most yeasts, growth ceases at water activity less than 0.9 while for molds there is no growth at water activity less than 0.7 (Ball, 2005).

Thermal processing of LAIVs for example releases vitamins entrapped in food matrices and thus the vitamin levels vary depending on the kind of vegetable (Hotz and Gibson, 2007). Tumwet *et al*., 2013 reporting on the effect of different cooking methods (fried between 5-10 minutes or boiled for 5 minutes) on the beta-carotene levels of some African indigenous leafy vegetables in Kenya indicated that, both the method of vegetable preparation and duration of cooking had effects on the levels of beta-carotene in *Amaranthus hybridus*, *Gynandropsis gynandra* and *Solanum nigrum*. In the study by Yuan *et al*., (2009), it was shown that other than steaming, the other methods led to loss of nutrients in broccoli. Water soluble vitamins in vegetables are lost during washing and improper storage which may lead to about 25-40 % loss (Ismail *et al.,* 2013). The levels of vitamins in vegetables are determined by among other factors; preparation processes, the species of the vegetable, farming practices used, as well as the age of vegetable (Rodriguez-Amaya and Kimura, 2004; Ahamed *et al.*, 2007), nutrient and phytochemical content (Mathaba, 2017).

A number of analytical methods have been employed in the analysis of beta-carotene and the vitamin B series with reversed phase HPLC being the one most widely used method because of its powerful resolving capability, reproducibility and recovery (Ekinci and Kadakal, 2005; Jedlicka and Klimes, 2005; Hasan *et al.*, 2013; Garai, 2017; Seal and Chaudhuri, 2017). Spectroscopy, another method of analysis that has been widely used, is an accurate and precise detection method for the quantitative analysis of beta-carotene in vegetables and fruits (Arabshahi *et al*., 2007; Zahra *et al*., 2016; Karnjanawipagul *et al*., 2010).

Spider plant (*Cleome gynandra*), cowpeas (*Vigna unguiculata*), vegetable amaranths (*Amaranthus viridis*, vine spinach (*Basella alba*) and vegetable pumpkin (*Cucurbita maxima*) are among the LAIVs species found in Kenya (Opiyo *et al.*, 2015). Production of these vegetables is encouraged due to the fact that they are well adapted to local agro-ecological conditions and grow to maturity within a short time. They are cheap to grow and reliably a source of income for small scale farmers (Croft *et al.*, 2014; Okewole *et al.*, 2018). The leafy African indigenous vegetables are also resistant to pests and adverse environmental conditions and therefore can survive in unfavourable weather conditions (Kunyanga *et al*., 2013). These vegetables are slowly gaining increase in cultivation/production in over 60 % of households in rural and peri-urban parts of Kenya among the Luhya, Luo and Kisii communities (Abukutsa-Onyango, 2007; Kebede and Bokelmann, 2017). They are rich and inexpensive sources of vitamins to millions of people in both developed and developing countries (Kunyanga *et al*., 2013; Bwembya *et al.*, 2014. They are as well high in carbohydrates and fiber content but low in fat content (Ahamed *et al.*, 2013).

However, vegetables are highly perishable due to the high moisture content. They are normally available in plenty during the wet season but remain scarce during the dry season. To preserve them for use during the dry season, vegetables are usually dried by sun, solar or freeze-drying methods after blanching to prohibit enzyme activity and bacteria growth. (Ahamed *et al.*, 2013). Among the advantages of drying vegetables is that preparation, storage and use is easy, they are light, tasty and nutritious (Ahamed *et al.*, 2013). Dried vegetables can be used to prepare food formulations by mixing them in ratios after grinding into powder. The formulations can be used in soups or incorporated in other recipes, a dietary diversification as a food-based approach (James and Matemu, 2018). Kushwaha *et al.,* (2014) reported that consumption of mixed dried green vegetables resulted in a significant increase in serum, retinol in women in India.

Vitamins in vegetables are complexed in the nutrient-matrix, requiring their breakdown to make them bioaccessible for absorption. Bioaccessibility efers to the amount of ingested nutrients that potentially becomes available for absorption in the gastrointestinal tract (Etcheverry *et al*., 2012). Among the factors which affect bioaccessibility are food sources and matrix, processing, interaction with other dietary compounds and physicochemical properties (Yonekura and Nagao, 2007; Failla *et al.,* 2008). Thermal processing, mild heating has been shown to improve bioaccessibility due to the disruption of the cell walls of the plant tissues, dissociation of the nutrient-matrix complexes or transformation into a more active molecular structure (Parada and Aguilera, 2007).

Dietary fiber lowers bioaccessibility, it reduces the rate of release of nutrients by physically entrapping nutrients and enhancing viscosity of gastric fluids thereby restricting the mixing process (Montagne *et al.,* 2003; Palafox-Carlos *et al.,* 2011). The study by Nawiri *et al.,* (2013) on the bioavailability of beta-carotene from sundried cowpeas and amaranth leaves recipes concluded that the indigenous dark green leafy vegetables are effective at increasing serum levels of retinol. Nagao (2014) reported that processing and cooking of vegetables enhances bioavailability of dietary carotenoids through intestinal absorption and metabolism.

Bioavailability refers to the portion of nutrients in food that is available for utilization for normal metabolism or the sum of bioaccessibility and bioactivity (Ferna'ndez-Garci'a *et al*., 2009; Palafox-Carlos *et al*., 2011; Dilworth *et al.*, 2013). It is affected by factors such as digestion, release from the food matrix, absorption by intestinal cells and transport to body cells (Etcheverry *et al.*, 2012). Age, nutrient status, genotype, infections and physiological state such as pregnancy, lactation and obesity are among the other factors that influence bioavailability (Etcheverry *et al.*, 2012).

Bioaccessibility on the other hand is the amount of ingested nutrients that are potentially available for absorption in the gastrointestinal tract. It is dependent on digestion and release from the food matrix (Etcheverry *et al.*, 2012). Bioaccessibility can be determined using either *in vitro* (use of gastrointestinal models) or *in vivo* (use of intervention) method. The *in vivo* methods are known to face ethical constrains and also consume a lot of time and money. *In vitro* methods on the other hand are less expensive and have no ethical constraints. In addition, they are accurate, valid and reproducible and measurements are detailed and sensitive. *In vitro* methods include: solubility, dialyzability, gastrointestinal model and caco-2 models (Etcheverry *et al.*, 2012).

In these methods, an *in vitro* digestion is conducted to stimulate the human digestive system via a two-step digestion process; gastric and intestinal digestion, sometimes a three-step; oral, gastric and intestinal digestion (Etcheverry *et al.*, 2012). The method involves digestion, transformation of food into a form that can be assimilated by the body (Ferna'ndez-Garci'a *et al*., 2009). Bioaccessibility is mainly determined by the digestibility of the food material, the fraction of food components that is transformed into potentially accessible matter through all physical and chemical processes that take place in the ileum (Parada and Aguilera, 2007; Etcheverry *et al*., 2012).

In view of the studies highlighted, a study gap is presented towards assessing the *in vitro* bioaccessibility of BC and the vitamin B series in five LAIVs grown in Kenya.

1.2 Statement of the problem

In developing nations, micronutrient deficiency caused by inadequate intake of one or more vitamins and essential minerals affect over two billion people worldwide and in Kenya, it accounts for almost 40 % of such deaths (Kunyanga *et al.*, 2013). Adequate intake of vitamins and minerals play a key role in reducing the prevalence of disease especially among the vulnerable groups, expectant mothers and children aged five years and below. Vitamin A deficiency for example is the leading cause of preventable childhood blindness and increases the risk of death from common childhood illnesses such as diarrhoea. On the other hand, lack of vitamin B causes beriberi, peripheral neurophatics, pellagra, genital lesions, muscle weakness, asthma, depression, AIDS, multiple sclerosis, tinnitus, low sperm counts, diabetic neuropathy, lack of coordination and death in severe cases (Hasan *et al.*, 2013; Agyemang-Yeboah and Oppong, 2013; Bwembya *et al.*, 2014). Fighting malnutrition and ensuring food security are among the big four agenda of the Kenyan government as well as the seventeen United Nations Development Goals as set by the United Nations Nutritional Security.

Leafy African indigenous vegetables are rich in micronutrients which are useful in preventing and controlling prevalent diseases by ensuring nutrition security. Due to their seasonal variability, plenty during rainy season and absent in dry season, these vegetables can for instance be harvested during rainy season, dried and preserved to be used when absent, and thus availing them throughout the year thereby minimizing post-harvest losses. However, processing of these vegetables in raised temperature such as drying and cooking affect the levels of vitamins. The levels of the vitamins can either increase or decrease due to the extent of release of the vitamin from the food matrix, and the effect of heat, oxygen and light. The levels then obtained will determine the nutritional contribution of the particular vitamin to meet the recommended dietary allowances.

Although the vegetables are rich in vitamins, it is not clear whether heat processing through boiling and frying with oil affects the levels as well as their bioaccessibility and bioavailability. Studies on the amount of nutrients available for absorption and utilization in the human body (bioaccessibility) via both *in vivo* and *in vitro* approaches are important to enhance promotion and utilization of LAIVs. This is supported by Etcheverry *et al.*, (2012) who recommended studies on bioavailability/bioaccessibility of both micro and macronutrients in food. Intervention (*in vivo*) studies with children under the age of five years and people living with HIV and AIDS (Fawlzi, 2003) indicated that serum vitamin A improves substantially with consumption of LAIVs.

The purpose of this study was to evaluate bioaccessibility of beta carotene and vitamin B series in selected LAIVs, formulations and recipes using *in vitro* method. *In vitro* bioaccessibility studies involve use of models for gastric and intestinal digestion which can either be static or dynamic. *In vivo* studies have been done though they have been found to be expensive, have ethical constrains and may not give accurate levels mainly due to compliance issues (Fawlzi, 2003). Bioaccessibility of vitamins is dependent on food matrix, pH and temperature of the food preparation. In temperature treatment, the methods of cooking LAIVs investigated were boiling and boil-frying either as individual vegetables or as a mixture of two different types of vegetables. The vegetables were also dried, ground and processed into formulations that can be consumed as soup or as accompaniment of African dishes.

1.3 Justification of the study

Incidences of nutritional deficiency disorders are on the rise in both rural and urban communities, in many parts of Africa, as a result of the decline in the consumption of African indigenous vegetables (Kachiguma *et al.,* 2015). Minimal efforts have been witnessed towards utilization of LAIV's to curb food security and malnutrition in sub-Saharan Africa (Smith and Eyzaguirre, 2007). Apart from improving food and nutritional security, production of LAIVs as an economic activity would improve standards of living and raise gross domestic product, GDP (Waswa *et al*., 2016). Increased intake of vegetables would help address malnutrition and reduce the risk associated with degeneration diseases such as cancer and cardiovascular diseases (Smith and Eyzaguirre, 2007; Hughes and Keatinge, 2013).

Vitamins in leafy African indigenous vegetables play a key role in the normal metabolic functions of the human body such as growth, function, energy, tissue repair and waste removal (Nkafamiya *et al.,* 2010; Ismail *et al*., 2013). Most of them cannot be synthesized by body tissues of organisms and their deficiency or absence results in specific deficiency diseases, hence essential in the human diet (Ball, 2006; Nkafamiya *et al.,* 2010). Among the factors which may lead to loss of vitamins are light, temperature, soil, water and air (Ismail *et al*., 2013). Investigation of how much beta-carotene and the vitamin B series is available for absorption and utilization in the human body (bioaccessibility) when the vegetables are processed both individually and mixtures (recipes and formulations) is important if nutrition security need to be achieved.

Bioavailability studies have indicated that the vegetables have significant amounts of beta-carotene which gets converted into vitamin A when in the human body. Simulated method by use of a digester has been found to be effective, gives results that are comparable to intervention methods (Mainville *et al.,* 2005; Failla *et al.,* 2008). Simulated bioaccessibility of beta-carotene and vitamin B series in LAIVs and their different formulations are therefore of importance. This will guide on maximum utilization of LAIVs in an effort to minimize vitamin deficiencies among communities and thus be a solution to food security in developing countries.

1.4 Hypothesis

Processing vegetables does not significantly affect bioaccessibility of beta-carotene and vitamin B series from selected leafy African indigenous vegetables.

1.5 Objectives

1.5.1 General objective

To determine the effect of processing on bioaccessibility of beta-carotene and vitamin B series in selected leafy African indigenous vegetables.

1.5.2 Specific objectives

i. To determine the levels of beta-carotene and vitamin B series in fresh and processed *Cleome gynandra*, *Vigna unguiculata*, *Amaranthus viridis*, *Basella alba* and *Cucurbita maxima* vegetables

ii. To determine bioaccessibility of beta-carotene and vitamin B series from fresh and processed *Cleome gynandra*, *Vigna unguiculata*, *Amaranthus viridis Basella alba* and *Cucurbita maxima* vegetables

iii. To determine bioaccessibility of beta-carotene and vitamin B series from recipes prepared from mixtures of *Cleome gynandra*, *Vigna unguiculata*, *Amaranthus viridis*, *Basella alba* and *Cucurbita maxima* vegetables.

iv. To determine bioaccessibility of beta-carotene and vitamin B series from formulations prepared from *Cleome gynandra*, *Vigna unguiculata*, *Amaranthus viridis*, *Basella alba* and *Cucurbita maxima* vegetables.

1.6 Significance of study

The findings provide information on bioaccessible levels of beta-carotene and vitamin B series with processing of LAIVs for nutritional guidance in order to fight malnutrition deficiencies related to vitamin A and B series and further promoting consumption of the vegetables in the right quantities.

1.7 Scope and Limitations

There are a number of available leafy African indigenous vegetables but only five; *Cleome gynandra*, *Vigna unguiculata*, *Amaranthus viridis*, *Basella alba* and *Cucurbita maxima* were considered to represent the wide range of vegetables. The vegetables were processed by boiling, boil-frying and drying. Comparison of duration of cooking was not within the scope and among the drying methods, only oven drying was considered. The source, species and age of the LAIVs were not considered. Further the season was not put into consideration as sampling was only done from an open market in Kisii County, Kenya. For samples that were prepared by boil-frying, the levels of beta-carotene and vitamin B series of the ingredients were not analyzed separately since the main objective was to determine bioaccessibility of the same vitamins in the vegetables as they are commonly consumed in households. Only bioaccessibility of beta-carotene and vitamin B_1, B_2, B_3, B_5, B_6 and B_9 were considered among the nutrients. Among the factors which affect bioaccessibility, interaction with other dietary compounds, physicochemical properties and the rate of absorption/ uptake were not within the scope. For the limitations, the source, variety and age of the vegetable plants were not considered. Seasonality was also not put into consideration.

CHAPTER 2

2 LITERATURE REVIEW

2.1 Malnutrition and Nutrition Security

Malnutrition is a condition that results from poor diet, a diet that lacks proteins, carbohydrates, fats, vitamins and minerals. The effects of malnutrition include bad digestive conditions, severe and repeated infections, fatigue, dizziness and weight loss, and physical and mental disability (WHO, 2002; 2005). However, malnutrition can be reversed through treatment achieved by addressing the underlying conditions and replacing missing nutrients through improved nutrition, supplementation or fortification, (WHO, 2020).

Micronutrient malnutrition currently affects over two billion people worldwide (Bwembya *et al*., 2014). About 10.6 million children aged five years and below are estimated to die annually in the globe among which about 50 % are as a result of malnutrition. The reasons for malnutrition include; soil fertility, climatic conditions compounded by global climatic change affecting food production, poverty and diseases (Younis *et al.*, 2015). Leafy African indigenous vegetables can be used to fight malnutrition because they have high nutritional value and are inexpensive sources of vitamins and other nutrients (Uraku *et al.*, 2015). Bioaccessibility is a dietary strategy that can be utilised for maximum derivation of vitamins from vegetables thereby playing a pivotal role in curbing malnutrition (Veda *et al.*, 2006).

2.2: Leafy African Indigenous Vegetables

Leafy African indigenous vegetables (LAIVs) are a collection of plant species which either grow naturally or are cultivated whose parts (leaves, young shoots and flowers) are consumed (Taruvinga and Nengovhela, 2015). These vegetables include spider plant (*Cleome gynandra*), cowpeas (*Vigna unguiculata*), vegetable amaranths (*Amaranthus viridis)*, vine spinach (*Basella alba*) and vegetable pumpkin (*Cucurbita maxima*), which are inexpensive and readily available, yet are good sources of macro- and micro-nutrients (Kwenin *et al.*, 2011; Croft *et al.*, 2014; Okewole *et al.*, 2018). The vegetables, however, are not fully utilized mainly because of low production, processing, distribution and marketing as well as inadequate nutrition information (Smith and Eyzaguirre, 2007).

Leafy African indigenous vegetables in Kenya, about 210 species, have not been fully utilized. If promoted these vegetables could play a crucial role in food security. The Luhya, Luo and Kisii communities of Western Kenya engage in cultivation of LAIVs (Abukutsa-Onyango, 2007). Kebede and Bokelmann, (2017) reported on vegetables and their production in rural and peri-urban Kenya. According to their report, the highest percentages of amaranth (76.9 %), cowpeas (67.2 %), spider plant (72.6 %) and Ethiopian kale (91.6 %) were recorded in Kisii County. The same county recorded the second highest percentage of African nightshade (44.8 %).

Leafy African indigenous vegetables do well in local agro-ecological conditions, are able to withstand pests and require less capital. Apart from providing a more sustainable alternative source of vegetables compared to exotic ones, they are a source of income for small scale farmers in Kenya (Croft *et al.*, 2014) since they have short production time and investment cost, and are less labour intensive yet the yields are high (Okewole *et al.*, 2018). Increased production of LAIVs would significantly improve food security. Although these vegetables are seasonal, dried and preserved LAIVs would help curb shortage especially during the dry season when production is limited (Opiyo *et al.*, (2015).

The use of LAIVs has declined over the years and is now currently below the recommended levels exposing the population to micronutrient deficiency diseases (Opiyo *et al.,* 2015; Gido *et al.*, 2017). Among the factors which affect the nutritional contribution of vegetables are: nutrient bioavailability, per capita consumption and also nutrient and phytochemical content (Mathaba, 2017). The levels of nutrients in vegetables depend on among other factors species, climatic conditions, farming practices, age of the plant as well as post-harvest handling (Rodriguez-Amaya and Kimura, 2004; Ahamed *et al*., 2007).

Most vegetables are cooked before consumption to make them more palatable, improve their texture and taste (Migliot *et al.,* 2008). The process of cooking vegetables, however, plays a key role in eliminating potential pathogens as well as neutralizing substances which may be poisonous and irritating to the human body (Tumwet *et al.*, 2013). It alters the nutritional value, the difference in retention of cooked vegetables being attributed to the extent of the loss by dripping during cooking (Lee *et al.,* 2018). In their research, Lee *et al.,* 2018 concluded that cooking may cause changes to the content of vitamins, but it depends on the vegetables and the cooking process. Convenience and taste preference are commonly considered when choosing cooking methods than nutrient retention. (Tumwet *et al.,* 2013). Some cooking methods lead to oxidation of antioxidants thus affecting nutrient retention hence bioavailability/bioaccessibility (Shahnaz *et al.*, 2003).

2.3 Vitamins: chemistry and nutritional contribution

2.3.1 Vitamins

Vitamins are a group of organic compounds, important for the normal functioning of the body (Ball, 2006; Nkafamiya *et al.,* 2010; Bwembya *et al.*, 2014). Based on solubility, vitamins can be classified into fat-soluble such as vitamin A, D, E and K and water-soluble including vitamin C and the B series (Ball, 2006). All the vitamins are affected by pH, light, temperature, water and air, leading to their degradation and loss (Ismail *et al*., 2013). The levels of vitamins in vegetables are determined by the soil type, leaching, blanching, vegetable species and postharvest handling as well as processing ((Rodriguez-Amaya and Kimura, 2004; Ahamed *et al*., 2007; Ismail *et al*., 2013).

Most vitamins cannot be synthesized by body tissues of organisms hence obtained from natural food sources such as vegetables and fruits (Ball, 2006; Nkafamiya *et al.,* 2010; Lee *et al.,* 2018). Vitamin A, a fat-soluble vitamin derived from pro-vitamin A carotenoids, is sourced from leafy African indigenous vegetables, tomatoes, carrots, sweet potatoes, pumpkin, liver and fish (Srinivasan, 2009; Carrillo-Lopez *et al.*, 2010). It is also present in non-photosynthetic bacteria, yeasts and fungi where they play a protective role against damage by light and oxygen (Manay and Shadaksharaswamy, 2002; Srinivasan, 2009; Saroj *et al.,* 2012; Bora *et al.,* 2015). Vitamin B series on the other hand is a group of water-soluble vitamins that occur in vegetables, cereals, legumes, meat, milk, sunflower and potatoes among others (Nkafamiya *et al*., 2010; Agyemang-Yeboah and Oppong, 2013).

Though required in very small but appropriate amounts, vitamins play a key role in normal cellular metabolism and normal functioning of the body including growth, energy, tissue repair and waste removal (Ball, 2006; Nkafamiya *et al.,* 2010; Bwembya *et al.,* 2014). Adequate intake of some vitamins for instance prevent anemia and reduce the risk of cancers (Palafox-Carlos *et al*., 2011; Agyemang-Yeboah and Oppong, 2013). Deficit intake on the other side lead to malnutrition while continuous deficiency eventually results in malfunctioning of enzymes (Ismail *et al*., 2013). Poor eating habits, improper absorption of vitamins, alcoholism and emotional stress are among the causes of vitamin deficiency (Nkafamiya *et al.,* 2010).

2.3.2 Beta-carotene

Beta-carotene (BC) (Figure 2.1) is one of the carotenoids synthesized mainly by green vascular plants but not synthesized by animals (Stahl and Sies, 2005; Failla *et al*., 2008; Nagao, 2014). It occurs as carotenoid-protein complexes in chloroplasts or in crystalline form inside chloroplasts (Parada and Aguilera, 2007). Carotenoids, the 40 carbon molecules with multiple conjugate unsaturated carbon-carbon bonds in the *trans*-configuration (Failla *et al*., 2008), are crucial in plants, functioning as light harvesting antenna pigments during photosynthesis (Failla *et al*., 2008; Zahra *et al*., 2016). They are the major red and yellow pigments in many vegetables and fruits, with conjugate polyene structure efficient in absorbing light (Srinivasan, 2009). In the body, BC is enzymatically transformed into retinal and finally into retinol, vitamin A (Zahra *et al.,* 2016).

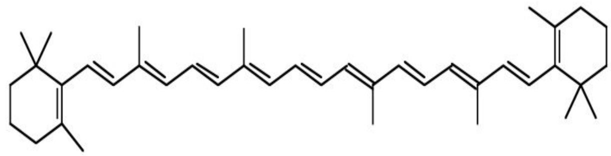

Figure 2. 1: Structure of beta-carotene

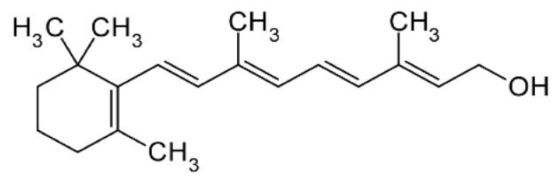

Figure 2. 2: Structure of retinol

Vitamin A is well known for its importance for eye health and good vision. Further, it is useful for metabolism, healthy skin, growth and repair of epithelial cells, and mucous membrane (Zahra *et al.,* 2016). It also plays a major role in growth and repair of lungs, developing teeth, inner ear, sex organs and ducts, gums and cervix among others (Bwembya *et al.,* 2014). Vitamin A also plays a role in the development of embryo and maintenance of adrenal glands and synthesis of hormones such as thyroid hormone, development of the body's immune system, growth and bone formation and also antiviral activity. (Nkafamiya *et al*., 2010; Bwembya *et al.,* 2014).

Severe deficiency of vitamin A leads to permanent blindness and increased severity of pneumonia and diarrhoea. Measles infection is reported to increase the need for vitamin A (Bwembya *et al.,* 2014). Beta-carotene protects the body from free radicals which damage tissues by oxidation, secure immune system, prevents oral cavity and lung cancer, decrease risk of cardiovascular disease and maintenance of epithelial functions (Zahra *et al.*, 2016). However, if too much, BC may cause lung cancer, cataract and other health hazards mainly in smokers and pregnant females (Zahra *et al.*, 2016).

In the chemistry of carotenoids, prolonged cooking and blanching causes thermal destruction (Manay and Shadaksharaswamy, 2002; Srinivasan, 2009). Beta-carotene is fat soluble and readily dissolves in organic solvents such as benzene, petroleum ether and chloroform. It is sensitive to oxidation but stable in oxygen free environment (Srinivasan, 2009). Oxidation, however, takes place in both the presence and absence of enzymes. Among the factors that influence oxidation of BC are heat, light and presence of pro-oxidants and antioxidants (Srinivasan, 2009). Cooking increases the extractability of beta carotene from the food mainly because it softens the plant cell walls and destroys the carotenoid-protein-complexes (denatures the protein). However, cooking can also cause isomerization of beta-carotene from the naturally occurring *trans*-isomers to *cis* form, which are less bioavailable. It also lowers the pro-vitamin A activity. Carotenoids can as well be lost in cooking water and also as a result of long cooking time (Hotz and Gibson, 2007).

LAIVs are rich in micronutrients if proper preparation, processing and preservation methods are considered for nutrient retention, and thus ultimately essential for food and nutrition security (Djuikwo *et al*., 2011; Kunyanga *et al*., 2013; Okpalamma *et al*., 2013; Tumwet *et al*., 2013). In the study by Tumwet *et al*., (2013) where different cooking methods were investigated on the levels of BC in amaranth, the results in mg/100g were: 23.84±14.18 (raw), 46.58±0.61 (boiled for 5 minutes), 36.45±0.66 (fried for 5 minutes) and 35.90±1.49 (fried for 10 minutes); indicating that cooking methods increased the levels.

In the study by Kunyanga *et al*., (2013), the levels of BC in amaranth and pumpkin leaves were 4.29±0.20 mg/100g and 2.66±0.04 mg/100g, respectively. Nyamu, (2013) reported levels of beta-carotene in fresh spider plant, cowpeas, and pumpkin leaves among other vegetables ranging from 0.24 ± 0.01 to 9.61 ± 0.36 mg/100g DW. Cheruiyot, (2011) recorded levels of beta carotene in fresh vegetable amaranth ranging from 35.985 to 49.797 mg/100g DW. Nyamu concluded that indigenous vegetables had enough levels to meet the RDAs while Cheruiyot concluded that the levels differed significantly. Cheptoo *et al.,* (2019) reported levels, mg/100g DW, of beta-carotene in fresh amaranth to be; 47.82 ± 1.32 (young) and 64.35 ± 1.46 (mature). The results from these studies indicate that, the levels of beta-carotene vary depending not only on the type of vegetable but also the maturity stage.

Wakhanu, 2014, reported levels, mg/100 FW, of beta carotene in LAIVs when fresh, boiled for 10 minutes and fried for 10 minutes as spider plant 5.94 ± 1.09 (fresh), 10.07± 0.05 (boiled) 5.16 ± 0.01 (fried), cowpeas 6.24 ± 2.09 (fresh), 10.09 ± 0.01 (boiled), 4.46 ± 0.16 (fried), amaranth 5.02 ± 0.16 (fresh), 4.10 ± 0.02 (boiled), 3.53 ± 0.03 (fried), vine spinach 3.16 ± 1.09 (fresh) 4.75 ± 0.01 (boiled) 1.75 ± 0.01 (fried) and pumpkin leaves 8.16 ± 1.12 (fresh) 11.65 ± 0.01 (boiled) 3.65 ± 0.01 (fried). The observation from the results is that, in all the vegetables, the order of the levels of beta-carotene was; boiled>fresh>fried. In the same study, levels of mixtures of vegetables were recorded including; spider plant + pumpkin leaves 45.33 ± 3.44, amaranth + spider plant + black night shade (56.33 ± 0.33) and cowpeas + jute mallow + slender leaf (54.33 ± 0.33). It was concluded that the vegetables contained beta carotene levels that would meet recommended dietary allowances as set by WHO. Further conclusions were that cooking methods differently affected the levels of beta-carotene, decreased in some while in others they increased and that recipes of mixtures of the vegetables were better than single vegetable recipe.

Okpalamma *et al.*, (2013) and Djuikwo *et al*., (2013) investigated thermal processing of LAIVs and reported that boiling fluted pumpkin in water for 5 minutes increased the levels of BC from 233.41 µg/g in the raw vegetable to 536.2 µg/g (Okpalamma *et al.*, 2013). When both the raw and cooked fluted pumpkin were oven dried in glass trays at 50 ^{o}C for about 48 hours, milled and sieved the beta-carotene (BC) levels were 20.40±0.17, 14.67±1.48, 13.83±0.12, and 18.45±0.06 mg/100g DW in oven dried amaranth, oven dried pumpkin leaves, boiled pumpkin leaves and oven dried cowpeas respectively (Djuikwo *et al*., 2011). Household preparation and processing procedures such as boiling and frying are inevitable as they contribute to liberate the carotenoids from their cellular matrices enabling them to be available for absorption. However, such processes end up lowering the levels due heat treatment and exposure to oxygen.

2.3.3 Vitamin B series

Vitamin B series play key roles in homeostasis and cellular metabolism (Agyemang-Yeboah and Oppong, 2013), for instance in metabolism of food to produce energy, their role as co-enzymes is key (Hasan *et al.*, 2013). The B vitamins also play a crucial role in maintaining healthy skin and muscle tone as well as enhancing immune and nervous function. Promoting health cell division and haematopoietic activity is also a role played by vitamin B (Agyemang-Yeboah and Oppong, 2013). Water soluble vitamins in vegetables and fruits are however lost during washing while improper handling and storage may lead to loss of about 25 % to 40% (Ismail *et al*., 2013). They are also known to extract into water during cooking (Serafini *et al.,* 2002; Bua and Onang, 2017). Deficiency of vitamin B causes diseases such as; beriberi, peripheral neurophatics, pellagra and genital lesions (Hasan *et al.,* 2013). Other symptoms of vitamin B deficiency include; depression, muscle weakness, asthma, low sperm count, AIDS, multiple sclerosis, lack of coordination, tinnitus, diabetic neuropathy and in severe cases death may occur (Akyilmaz *et al.,* 2006; Agyemang-Yeboah and Oppong, 2013).

2.3.3.1 Thiamin

Thiamin (Figure 2.3), also referred to as aneurin occurs in both plants and animals such as pork beef, chicken, duck, fish, legumes and vegetables. It exists as thiamin diphosphate (TDP) in living cells in combination with enzyme proteins. The other types of thiamin hydrochloride are thiaminmonophosphate (TMP) and thiamin triphosphate (TTP) (Fukawatari and Shibata, 2008; Fukawatari and Shibata, 2009).

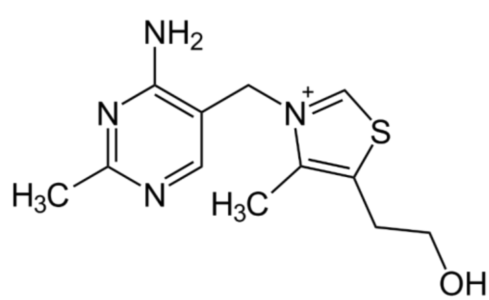

Figure 2. 3: Structure of thiamin

Thiamin plays a role in metabolism of glucose and branched-chain amino acids (Fukawatari and Shibata, 2008; Fukawatari and Shibata, 2009). Beriberi, the deficiency disease caused by lack of vitamin B_1 results in decrease in carbohydrate metabolism (Schellack *et al*., 2015) and occurs in dry (paralytic) and wet (oedematous) forms. It affects milk-fed infants whose mothers are deficient in the vitamin as well as adults whose carbohydrate intakes are high or intake of food containing anti-thiamin factor. Among the symptoms of thiamin deficiency are anorexia, weight loss and decrease in short-term memory, mental changes such as apathy, confusion and irritability, muscle weakness and cardiovascular effects such as an enlarged heart, tiredness, headache and reduced productivity (Schellack *et al*., 2015).

Studies on levels of thiamin in vegetables have reported varying levels in fresh, cooked and dried; Ismail *et al*., (2013) reported levels of thiamin ranging from 0.02 to 0.18 mg/100g in fruits and vegetables while Kunyanga *et al*., (2013) reported thiamin levels in amaranth to be 0.42±0.09 mg/100g and pumpkin leaves 0.08±0.12 mg/100g, and Uraku *et al*., (2015) recorded 9.731±3.250 mg/100g in amaranth dried for two weeks, all indicating that vegetables are good sources of thiamin. Okpalamma *et al*., (2013) recorded 0.08±0.01 mg/100g thiamin in raw fluted pumpkin and 0.04±0.01 mg/100g when cooked in boiling water for five minutes, pointing out that the levels of nutrient retention of flute pumpkin after domestic processing support its inclusion in a daily diet to help overcome vitamin deficiency.

2.3.3.2 Riboflavin

Riboflavin (Figure 2.4) occurs as a yellowish pigment, flavin. The reduced form is colourless and occurs in metabolism along with the oxidised form (Schellack *et al*., 2015). Its main sources are; vegetables, eggs and dairy products.

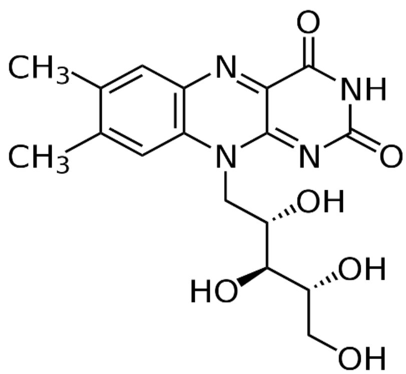

Figure 2. 4: Structure of riboflavin

Riboflavin plays a key role in many metabolic processes, such as in the production of energy in cellular respiration, acting as prosthetic groups of enzymes such as pyruvate dehydrogenase, and taking part in iron, folate metabolism, fats, carbohydrates and proteins (Agyemang-Yeboah and Oppong, 2013; Fukawatari and Shibata, 2008; Schellack *et al*., 2015). Further, riboflavin is an essential dietary component available as free riboflavin, free coenzyme riboflavin-5-phosphate (Flavin mononucleotide) and adenyl derivative of FMN, Flavin adenine dinucleotide, (Fukawatari and Shibata, 2008). The biochemical functions of riboflavin include acting as an oxidizing agent, a precursor of FMN and FAD which function as a co-enzyme and in reduction of isoalloxazine ring-FMNH2 and FADH2 (Schellack *et al*., 2015).

Deficiency of riboflavin is relatively common when dietary intake is insufficient mainly because it is continuously excreted in urine even of healthy individuals (Schellack *et al*., 2015). The deficiency is however accompanied by deficiency of other vitamins. Primary deficiency is caused by poor vitamin sources in one's diet while secondary deficiency is caused by conditions that affect absorption in the intestines, the body not being able to use the vitamins or increase in the excretion of the vitamin from the body (Schellack *et al*., 2015). Among the symptoms of riboflavin deficiency (ariboflavinosis) are inflammation of the membrane on the throat, dermatitis, neurological alterations, glossitis, stomatitis, muscle disorders and anaemia (Fukawatari and Shibata, 2008; Agyemang-Yeboah and Oppong, 2013; Hasan *et al.*, 2013). Other symptoms of vitamin B_2 deficiency include; cracked and red lips, mouth ulcers, cracks at the corners of the mouth (angular cheilitis), dry and scaling skin, fluid in the mucous membranes and iron deficiency. Eyes may become blood shot, itchy, watery and sensitive to bright light (Fukawatari and Shibata, 2008; Schellack *et al*., 2015). In case of severe deficiencies, prolonged febrile illness, malignancy, hyperthyroidism, diabetes mellitus and cardiac disorders febrile illness, malignancy, hyperthyroidism, diabetes mellitus and cardiac disorders (Agyemang-Yeboah and Oppong, 2013).

Kunyanga *et al*., (2013) when studying nutrition evaluation of indigenous food with potential food-based solution to alleviate hunger and malnutrition in Kenya reported that the levels of riboflavin, mg/100g, in amaranth were 0.44±0.03 and pumpkin leaves 0.06±0.01. Ismail *et al*., (2013) determined water soluble vitamins in fruits and vegetables in Sindh Pakistan and recorded levels of riboflavin ranging from 0.016 to

0.2mg/100g; with the level recorded in spinach being 0.1 mg/100g. Uraku *et al*., (2015) recorded the levels of riboflavin in amaranth dried for two weeks to be 7.161±0.521 mg/100g. Okpalamma *et al*., (2013) on the other hand recorded the levels of riboflavin in fluted pumpkin to be 2.07±0.01 mg/100g in raw and 1.02±0.01 mg/100g in cooked (in boiling water for five minutes). Both the raw and cooked flute pumpkin were oven dried in glass trays at 50 ^{o}C for about 48 hours, milled and sieved. The results showed that consumption of 100 g of pumpkin leaves per day would meet the recommended daily allowance of riboflavin in children and adults. The levels of nutrient retention after domestic processing support the inclusion of the vegetable in a daily diet to overcome vitamin deficiency.

2.3.3.3 Niacin

Niacin (Figure 2.5) occurs in vegetables, fruits, meat, poultry, liver and legumes among other sources (Lule *et al.,* 2016).

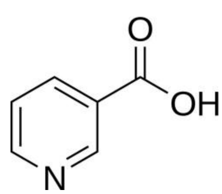

Figure 2. 5: Structure of niacin

Niacin is involved in ATP production, antioxidation, and fatty acid and steroid synthesis. It also plays a role of coenzyme in enzymes such as hydrogenase, repair and synthesis of Deoxyribonucleic acid (DNA) as well as cell differentiation (Fukawatari and Shibata, 2008; Fukawatari and Shibata, 2008). In living cells, niacin exists as cofactor nicotinamide adenine dinucleotide phosphate [NAD (P)]. Vitamin B_3 works in the glycogen stage of the energy cycle.

Pellagra, the niacin deficiency disease is accompanied with symptoms such as aggression, insomnia, weakness, dermatitis, mental confusion and diarrhoea. Pellagra may result in dementia and even death in advanced situations (Fukawatari and Shibata, 2008; Agyemang-Yeboah and Oppong, 2013; Schellack *et al*., 2015). NAD (P) is hydrolyzed to nicotinamide in animal foods and nicotinic acid in plant foods during cooking and processing (Fukawatari and Shibata, 2008). Niacin is the most stable vitamin, resistant to heat and oxidation (Fukawatari and Shibata, 2008).

Kunyanga *et al*., (2013) reported the levels of niacin, mg/100g, in amaranth to be 0.70±0.00 and in pumpkin leaves 0.32±0.01. Ismail *et al*., (2013) carried out a research on determination of water-soluble vitamins in fruits and vegetables in Sindh Pakistan and recorded levels of niacin ranging from 0.01 to 0.1 mg/100g, with spinach having 0.01 mg/100g. Okpalamma *et al*., (2013) on the other hand recorded the levels of niacin in fluted pumpkin to be 2.38±0.01 mg/100g in raw and 0.50±0.00 mg/100g in cooked in boiling water for five minutes.

2.3.3.4 Pantothenic acid

Pantothenic acid (Figure 2.6) is widely distributed in plant and animal tissues such as vegetables, yeast, avocado, whole grain, organ meat and egg yolk (Schellack *et al*., 2015).

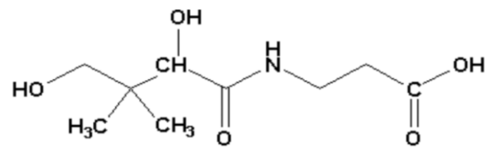

Figure 2. 6: Structure of pantothenic acid

Pantothenic acid is essential for releasing energy from carbohydrates, amino acids and fat, and is also involved in the synthesis of lipids, neurotransmitters, steroid hormones via cholesterol and haemoglobin (Schellack *et al*., 2015). It exists as coenzyme A (CoA) derivatives, acetyl CoA, acyl CoA, acyl-carrier protein (ACP) and 4-phospho-pantethein. Its structure (in which it is found in the diet) is the part of co-enzyme A (CoA) and acyl-carrier protein (ACP) which exist in all tissues. It is hydrolysed to dephospho-CoA and pantetheine in the small intestines and then hydrolysed to pantothenic acid, the absorbable form (Fukawatari and Shibata, 2008; Fukawatari and Shibata, 2009). Fatigue, sleep disturbances, impaired coordination and nausea are among the symptoms of pantothenic acid deficiency (Schellack *et al*., 2015). Cases of pantothenic acid deficiency are however rare since it is widely distributed in foods (Fukawatari and Shibata, 2008; Fukawatari and Shibata, 2009).

2.3.3.5 Pyridoxine

Pyridoxine (Figure 2.7) is one of the water-soluble vitamins whose sources are green leafy vegetables, grains, liver, milk and eggs. The other names for pyridoxine are pyridoxal (PL) and pyridoxamine (PM). The phosphorylated forms are pyridoxine-5-phosphate (PNP), pyridoxal-5-phosphate (PLP) and pyridoxamine-5-phosphate (PMP) (Fukawatari and Shibata, 2008).

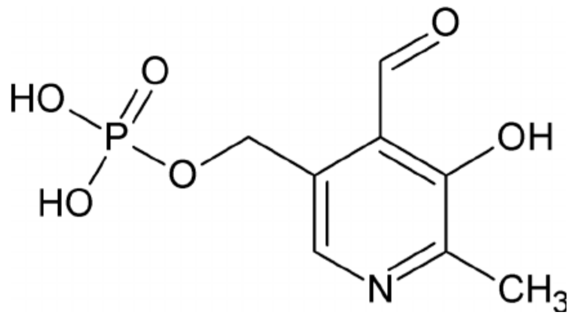

Figure 2 7: Structure of pyridoxine

It takes part in metabolic processes such as metabolism of unsaturated fatty acids and biosynthesis of fat from protein (Agyemang-Yeboah and Oppong, 2013). It also plays a role in nerve transmission, red blood cells and prostaglandins synthesis. In addition, vitamin B_6 is important in cell division and therefore vital in pregnancy and proper function of the immune systems, mucous membrane, skin, red blood cells and brain chemistry (Fukawatari and Shibata, 2008; Agyemang-Yeboah and Oppong, 2013). Vitamin B_6 exists mainly as PLP or PMP combined with enzyme proteins. PLP and PMP dissociate to form absorbable PL and PM. In plants it exists as pyridoxine-5'β- glucoside (PNG) which is absorbed as pyridoxine in humans (Fukawatari and Shibata, 2008). Pyridoxine may occur in protein bound forms which have low availability. The deficiency symptoms include dermatitis, disorders of the blood count, neurological disturbances and autism, linked to a decrease in certain brain neurotransmitters that require vitamin B_6 for normal brain chemistry (Agyemang-Yeboah and Oppong, 2013; Hasan *et al.*, 2013).

Pyridoxine can be decomposed by heat during cooking (Nagao *et al.*, 2014; Parada and Aguilera, 2007). Ismail *et al*., (2013), in their study on determination of water soluble vitamins in fruits and vegetables, recorded levels of pyridoxine ranging from 0.06 to 0.28

mg/100g, that recorded in spinach being 0.26 mg/100g. In their research on determination of some vitamins in three selected African green leafy vegetables, Uraku *et al*., (2015) recorded the levels of pyridoxine in amaranth dried for two weeks to be 25.020±2.667 mg/100g.

2.3.3.6 Folate

Folate (Figure 2.8) occurs in green leafy vegetables, beans, peas, lentils and fruits such as bananas and lemons. Mammals cannot synthesize folate, hence a major dietary requirement (Öhrvik, 2009). Folate naturally occurs combined with glutamate molecules (Fukawatari and Shibata, 2008). In green leafy vegetables folate is covalently bound to macromolecules such as proteins (Parada and Aguilera, 2007).

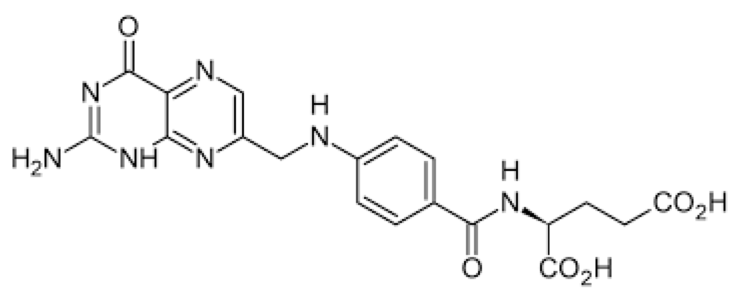

Figure 2. 8: Structure of folate

It is light and heat sensitive, destroyed by oxidation and also lost during cooking. Lack of folate leads to a special kind of anemia called megaloblastic anemia which, in mothers, can lead to fatal neural tube defects and anencephalia (Fukawatari and Shibata, 2008). Consumption of alcohol interferes with the proper metabolism of folate and inactivates circulatory folate (Agyemang-Yeboah and Oppong, 2013). Vitamin activity of folate may be lost by oxidative cleaving resulting in a pterin and para-aminobenzoylglutamate

(PABG) molecule. Oxygen or changes in pH may also cause interconversion of folate found in food (Öhrvik, 2009) while cooking methods which use large quantities of water and involve too much boiling destroy folate. It is of interest to note that people who get higher amounts of folate from their diet or folic acid supplements for 15 years or more have lower risks of colon cancer (Agyemang-Yeboah and Oppong, 2013). Kunyanga *et al.*, (2013) reported the levels of folate, mg/100g, in amaranth to be 0.85±0.02. In their research, Ismail *et al*., (2013) recorded levels of folate in vegetables ranging from 0.016 to 0.19 mg/100g. The level recorded in spinach was 0.12 mg/100g.

2.4 Bioavailability and bioaccessibility of nutrients

Bioavailability refers to the portion of nutrients in food that is available for utilization for normal metabolism or the sum of bioaccessibility and bioactivity (Ferna'ndez-Garci'a *et al.,* 2009; Palafox-Carlos *et al*., 2011; Dilworth *et al.,* 2013). Bioaccessibility on the other hand refers to the amount of ingested nutrients that potentially becomes available for absorption in the gastrointestinal tract (Etcheverry *et al*., 2012). Any nutrient including beta-carotene and vitamin B series can only be available for absorption (enters the blood stream) once it is released from its matrix, that is bioactive compounds have to be released from the food matrix and be modified in the gastrointestinal tract before becoming bioavailable (Parada and Aguilera, 2007).

Bioaccessibility is mainly determined by the digestibility of the food material, the fraction of food components that is transformed into potentially accessible matter through all physical and chemical processes that take place in the ileum (Parada and Aguilera, 2007; Etcheverry *et al*., 2012). Mastication (grinding food into small pieces in the presence of saliva forming boluses that are swallowed) is the first physical transformation of vegetable matrices (Kulp *et al*., 2003; Palafox-Carlos *et al.*, 2011). This decreases the particle size, increasing the surface area for action by digestive enzymes thereby increasing digestion efficiency and absorption (Kulp *et al*., 2003; Palafox-Carlos *et al*., 2011). Apart from mastication, digestibility of food matrices is aided by enzyme action, cooking and pureeing (Kulp *et al*., 2003; Parada and Aguilera, 2007; Palafox-Carlos *et al.*, 2011). In addition to disruption of the cell walls of plant tissues, the processes result in dissociation of the nutrient-matrix complexes or transformation into more active molecular structures (Parada and Aguilera, 2007). Food processing enhances bioavailability by either increasing physicochemical accessibility of micronutrients, such as phylate or increasing the content of compounds that improve bioavailability (Hotz and Gibson, 2007).

Dietary fiber in vegetables decreases bioaccessibility/bioavailability (Gibson, 2007). Fiber contents of vegetables have been reported among them; spider plant, 0.8 % (Lymio *et al.*, 2003), cowpeas, 19.45 % (Enyiukwu *et al.*, 2018), vine spinach, 2.2 % (Vicente *et al.*, 2009), pumpkin leaves, 12.04±0.02 % and amaranth, 8.64 ±0.04 % (Kunyanga *et al.*, 2013). Dietary fiber slows gastric emptying, digestion and absorption of nutrients mainly due to the water retained by pectin that forms viscous solution in the gut (Gibson, 2007). Folate binding proteins for instance decrease bioavailability of folate. The food matrix entraps folate thereby hindering diffusion to the absorptive surface during digestion (Parada and Aguilera, 2007). Entrapment in the food boluses during digestion may also be a cause of incomplete absorption of folates in food (Parada and Aguilera, 2007). Carotenoids have to be released from food matrices at an early stage of digestion.

In vegetables, however, carotenoids are generally difficult to release because of the rigid cell walls (Nagao, 2014). Cooking and processing vegetables destroy food matrices thereby enhancing the release of carotenoids and substantially improving their bioavailability (Nagao, 2014). Released carotenoids are dispersed with the aid of bile which contains bile salts and phosphatidylcholine. They are then solubilized in mixed-micelles, formed through hydrolysis of lipids emulsified in digesta by lipolytic enzymes in pancreatic juice. The mixed micelles comprise of bile acids, cholesterol, lysophosphatidycholine, fatty acid and mono-acylglycerol. It is then taken up into the intestinal epithelia (Nagao, 2014).

Fats and oils enhance bioavailability of dietary carotenoids by increasing bioaccessibility through dispersing carotenoids in the digestive tract (Nagao, 2014). The products of fat digestion (fatty acids, monoglycerides, cholesterol and phospholipids) bile salts solubilize fat soluble vitamins and carotenoids in the intestines (Gibson, 2007). Oil is reported to aid in the absorption of 7 micronutrients (beta carotene, alpha carotene, lutein, lycopene, two forms of vitamin E and vitamin K) in salad vegetables (White *et al.*, 2017). Findings from the study also indicated that the amount of oil added to the vegetables had a proportional relationship with the amount of nutrient absorption, adding twice the amount of salad dressing leads to twice the nutrient absorption. The presence of lysophosphatidylcholine in the micelles increase the uptake of carotenoids while phosphatidylcholine suppress the uptake. Pancreatic phospholipase hydrolyses one of the two fatty ester bonds of phosphatidylcholine derived from bile and food to produce less lipopholic lysophosphatidycholine in the intestinal tract (Nagao, 2014).

Bioavailability/ bioaccessibility of vitamin B series is affected by a number of factors. Thiamin is susceptible to heat, lost when cooking time and length of storage are prolonged. Riboflavin on the other hand is sensitive to light though it remains stable under heat and refrigeration. While niacin has less susceptibility to losses during storage, it withstands reasonable cooking time but leaches into cooking water. Pantothenic acid is quite stable to heat though it is lost in prolonged high temperatures such as boiling and is destroyed by freezing. Pyridoxine is destroyed by heat though it remains stable during storage while folate is easily destroyed by heat and oxygen (Fukawatari and Shibata, 2008).

Pectin decreases bioavailability, while dietary fiber prolongs gastric emptying and retards absorption of nutrients (Palafox-Carlos *et al.*, 2011). Dietary fiber reduces the rate of release of nutrients in the small intestines (Brownlee *et al*., 2006; Palafox-Carlos *et al*., 2011), by physically trapping nutrients within tissues and enhancing viscosity of gastric fluids thereby restricting the mixing process, peristalsis, that promotes transport of enzymes to their substrates, bile salts to unmicellized fat and soluble antioxidants to the gut wall (Montagne *et al*., 2003; Palafox-Carlos *et al*., 2011).

Bioaccessibility of nutrients can be estimated using *in vitro* procedure by measuring the fraction of the nutrient transferred from the food matrix to a supernatant (when centrifugation/decantation is used) or to a micellar phase (when centrifugation/

microfiltration is used) after simulated gastrointestinal digestion (Hedren *et al*., 2002; Granado-lorencio *et al.*, 2007; Alminger *et al*., 2012). Veda *et al.*, (2006) determined bioaccessibility of beta-carotene in vegetables by the method that involves simulated gastrointestinal digestion followed by ultracentrifugation to separate the micellar fraction containing bioaccessible beta carotene and its quantification. The levels of beta-carotene recorded were: 9.15 mg/100g (fenugreek leaves), 8.17 mg/100g (amaranth), 8.14 mg/100g (carrot) and 1.90 mg/100g (pumpkin). On measuring the effect of different cooking methods of processing, pressure cooking increased by 100 % (fenugreek leaves), 48 % (amaranth) and 19 % (carrot). The increase was enormous when stir-frying in the presence of a small quantity of oil was used; 263 % (fenugreek leaves), 192 % (amaranth leaves), 63 % (carrot) and 53 % (pumpkin). In their conclusion, membrane filtration was found to be satisfactory while equilibrium dialysis did not produce satisfactory results. Both methods of heat treatment significantly improved beta carotene bioaccessibility from carrots and amaranth leaves. Higher bioaccessibility was particularly prominent when vegetables were fried. They pointed out that heat processing of vegetables is known to improve bioaccessibility of micronutrient including beta-carotene.

2.5. *In vitro* methods of digestion

In vitro bioaccessibility methods are mainly used to provide knowledge on possible interactions between nutrients and/or food components and the effects of the nature of food matrix, luminal factors (including pH and enzymes) as well as food preparation and processing methods. The use of *in vitro* methods has a number of advantages including, time and money saving, detailed measurements and sensitive, have no ethical constrains and are accurate, valid and reproducible. The *in vitro* digestion models are used to simulate the digestive processes in the gastrointestinal tract in a simplified way. Solubility, dialyzability and gastrointestinal model are the *in vitro* methods available for measuring bioaccessibility of nutrient. *In vitro* digestion stimulates the human digestive system, gastric and intestinal digestion (Etcheverry *et al.*, 2012).

For gastric digestion, pepsin from porcine stomach is added prior to acidification of the samples to pH 2 to stimulate the gastric pH of an adult or to pH 4 to stimulate the gastric pH of an infant. It is necessary to acidify the samples to pH 2 and 4 because pepsin begins to denature itself at pH 5 losing its activity. The samples are adjusted to a pH of between 5.5 and 6.0 then subjected to intestinal digestion. Pancreation consisting of a cocktail of pancreatic enzymes (pancreatic amylase, lipase and ribonuclease) and protease (trypsin and bile salts) is then added (emulsifiers), and finally adjusted to a pH of between 6.5 and 7.0. Sometimes a third digestion step (oral step) is involved in which case it precedes the gastric phase (the digestion by lingual alpha-amylase which is an enzyme that breaks apart the glycosidic bonds of starch molecules, amylase and amylopetin). Once the digestion process has been completed, bioaccessibility can be measured using either solubility, dialyzability or gastrointestinal models (Etcheverry *et al.*, 2012 Minekus *et al*., 2014; Koh and Loh, 2018).

CHAPTER 3

3 MATERIALS AND METHODS

3.1 Research design

The research design was experimental. It involved determination of levels of beta-carotene and vitamin B_1, B_2, B_3 B_5, $B_{6,}$ and B_9 in the fresh, boiled, boiled-fried and dried vegetables, spider plant (*Cleome gynadra*), cowpeas (*Vigna unguiculata*), amaranth (*Amaranthus viridis),* vine spinach *(Basella alba)* and pumpkin leaves *(Cucurbita maxima*). This was followed by determination of the bioaccessibility of the vitamins in both the fresh and heat processed samples (boiled and boiled-fried) after which formulations and recipes of mixtures of the leafy African indigenous vegetables were developed and bioaccessibility of beta-carotene and vitamin B series determined.

3.2 Sampling

Simple random sampling design was used. One kilogram each of the five leafy African indigenous vegetables (LAIVs), spider plant (*Cleome gynadra*), cowpeas (*Vigna unguiculata*), amaranth (*Amaranthus viridis),* vine spinach *(Basella alba)* and pumpkin leaves *(Cucurbita maxima)* were sampled from an open market in Kisii County, Kenya. Only fresh vegetables were sampled, the withered ones were not picked for sampling. The vegetables were immediately sprayed with water to keep them moistened, packed in dark plastic polythene bags to prevent water loss and damage by light and transported within the shortest time possible (within 48 hours) to Department of Food Science and Technology laboratory at Jomo Kenyatta University of Agriculture and Technology (JKUAT) for analysis.

3.3 Reagents and solvents

All the chemicals and reagents used were of analytical grade. KH_2PO_4, HPLC methanol, HPLC water, HCl, standards (beta-carotene, thiamin, riboflavin, niacin, pantothenic acid, pyridoxine and folic acid) and the enzymes (pepsin (porcine), pancreation (porcine), lipase and bile salts) were obtained from Sigma-Aldrich,, acetone, petroleum ether, and Sep-Pak C18 (500 mg) cartridges were obtained from Sigma-Aldrich, Germany.

3.4 Cleaning of apparatus

Plastic containers and glassware were washed with liquid detergent and warm water then rinsed with tap water. They were soaked overnight in 10 % analytical grade nitric acid and then rinsed with distilled water. The glassware was dried in an oven at 105 ^oC for 24 hours.

3.5 Instrumentation

HPLC was Shimadzu model with a PDA detector and an auto sampler. Monitoring was done at wavelengths; 234 (B_1), 266 (B_2), 261 (B_3), 204 (B_5), 324 (B_6) and 282 (B_9). UV-VIS spectrophotometer was Shimadzu model UV-1601 PC, Kyoto Japan, wavelength 440 nm.

3.5. Preparation of standard solutions for calibration curves

Calibration curves for each vitamin were drawn from a series of standards.

3.5.1 Beta-carotene

The stock solution of beta-carotene was prepared by dissolving 0.100 g of beta-carotene in n-hexane and making it up to 100 ml. Working standards were freshly prepared by diluting the stock solution with n-hexane to appropriate concentrations ranging 0-10 ppm. Absorbance of the standard solutions (0-10 ppm) at a wavelength of 440 nm was used to prepare calibration curve.

3.5.2 Vitamin B series

The stock solutions of vitamin B_1, B_2, B_3, B_5, B_6 and B_9 were prepared by dissolving 0.01 g of each standard in 50 ml of 0.1 M HCl in 100 ml volumetric flask and diluted to the mark. Following appropriate dilutions using 0.1M HCl, serial standards were prepared in the following ranges in ppm; B_1 (0-10), B_2 (0-10), B_3 (0-8), B_5 (0-8), B_6 (0-8) and B_9 (0-8) then filtered using 0.45 µm. A calibration line was obtained by plotting the peak area values as a function of the concentration of vitamin.

3.6 Sample preparation

Each of the types of vegetables was washed under tap water, rinsed with distilled water and flapped to remove water. Pumpkin leaves (*Cucurbita maxima)* which had broad leaves was cut into small pieces after washing. The vegetables were then further prepared for analysis as either boiled, boiled-fried or dried on one hand and recipes of mixtures of the vegetables and formulations on the other.

3.6.1 Preparation of processed vegetables

Eighty grams (80 g) of each vegetable variety was weighed and divided into 2 portions, 40 g to be analyzed fresh and the remainder boiled. About 80 g of the vegetables were boiled in 200 ml of distilled water for 10 minutes at 100 ^{o}C and then cooled to room temperature. To prepare the boiled-fried samples, 40 ml of vegetable oil was heated (100 ^{o}C) in a cooking pan, 5 g of onion added and fried to golden brown before adding 10 g of tomato and 40 g of the boiled vegetable (salt was added to taste). The vegetables were fried for 10 minutes then cooled to room temperature. All the fresh, boiled and boiled-fried samples were placed in zip locked bags, frozen for 5 hours at -20 ^{o}C and then freeze dried at -50 ^{o}C for 72 hours. The freeze-dried samples were then wrapped in aluminium foil to keep away light and then kept in a refrigerator at 4 ^{o}C until analyzed. For the dried samples, 40 g of each fresh vegetable was weighed and dried in an oven at 50 ^{o}C for 24 hours, ground into fine powder and kept in amber bottles until analyzed.

3.6.2 Preparation of mixed vegetable recipes for determination of levels of beta-carotene and vitamin B series

Two types of vegetables (60 g each) were weighed and used for every mixture. A total of three mixtures were obtained; spider plant + amaranth (SP+A), cowpeas + amaranth (C+A) and vine spinach + pumpkin leaves (VS + PL). Each vegetable mixture was divided into 2 portions, 40 g to be analyzed fresh and the remainder boiled. About 80 g of the mixture of vegetables were boiled in 200 ml of distilled water for 10 minutes at 100 ^{o}C and then cooled to room temperature (recipe 1). To prepare the boiled-fried samples , 40 ml of vegetable oil was heated (100 ^{o}C) in a cooking pan, 5 g of onion added and fried to golden brown before adding 10 g of tomato and 40g of the boiled mixture of vegetable (salt was added to taste). The vegetables were fried for 10 minutes then cooled to room temperature (recipe 2). All the fresh, boiled and boiled-fried samples were placed in zip locked bags, frozen for 5 hours at -20 ^{o}C and then freeze dried at -50 ^{o}C for 72 hours. The freeze-dried samples were then wrapped in aluminium foil to keep away light and then kept in a refrigerator at 4 ^{o}C until analyzed.

3.6.3 Preparation of formulations

Formulations were prepared from the mixtures of vegetables: spider plant + amaranth (SP+A) – formulation 1 (FMLN1), cowpeas + amaranth (C+A) – formulation 2 (FMLN2) and pumpkin leaves + vine spinach (PL+ VS) – formulation 3 (FMLN3). For each mixture, 20 g of each vegetable was taken, the mixture dried in an oven at 50 ^{o}C for 24 hours, ground into fine powder. Forty (40) ml of vegetable oil was heated (100 ^{o}C) in a cooking pan, 5 g of onion added and fried to golden brown before adding 10 g of tomato. 200 ml of distilled water was added allowed to boil after which the powdered mixture of vegetable was added, stirred and allowed to cook for five minutes. Salt was then added to taste.

3.7 Method validation

The accuracy of UV-Vis and HPLC were investigated by spiking samples with a known amount of standards. Triplicate analysis was performed in each case. The concentration of unspiked and spiked sample was determined and the results are given in Table 4.1.

3.7.1 Extraction and measurement of beta-carotene

Extraction of BC was performed according to Rodriquez-Amaya and Kimura (2004). Two grams of each sample were weighed in triplicates and extracted with 10 mL acetone by grinding in a mortar using a pestle. The extract was transferred to 100 mL volumetric flask then residue further re-extracted with 10 mL acetone. This was repeated until the residue no longer gave orange color to acetone (4 times). The extracts were added to the contents of the 100 mL volumetric flask and topped up to the mark with acetone. Exactly 25 mL of the extract was evaporated to dryness using rotary evaporator, residue dissolved in about 1 mL petroleum ether. The solution was introduced into a chromatographic column that was packed with cotton wool and silica gel (70-230 mesh ASTM) and eluted with 10 mL petroleum ether. The eluate was collected in a 25 ml volumetric flask, topped to the mark with petroleum ether and the absorbance was determined at 440 nm in a UV-Vis spectrophotometer (Shimadzu model UV-1601 PC, Kyoto, Japan) and plotted against their corresponding standard concentrations. This was done according to the Beer-Lambert's law.

3.7.2 Extraction and measurement of vitamin B

Extraction of vitamin B series was performed according to Ekinci and Kadakal, (2005). To 5 g of each vegetable sample, 20 mL of deionized water was added and the mixture homogenized at medium speed for 1 minute before centrifuging for 10 minutes at 14 × 103 *g* (Sigma, Bioblock Scientific 2-16). A sample (10 mL) of the supernatant was then loaded to a Sep Pak C18 cartridge flushed with 10 mL methanol and 10 mL water adjusted to pH 4.2. It was then eluted with 5 mL acidified water, pH 4.2 (prepared by adding a 0.005 M HCl) followed 10 mL methanol at a flow rate of 1 mL/min. The eluent was collected in a bottle and evaporated to dryness using a rotary evaporator then reconstituted using 25 mL mobile phase, 0.1 mol/L KH_2PO_4 (pH 7) and methanol (90:10). Before HPLC analysis, all samples were filtered through 0.45 µm pore size (FP 30/45 CA-S filters, Schleicher and Schuell, Darmstadt, Germany) at 7 bar max. 20 µL of each sample solution was injected into the HPLC column by an auto sampler. Freshly prepared standards (section 3.5) of the vitamins were run before the samples. Analysis was done simultaneously for thiamin (B_1), niacin (B_3), pantothenic acid (B_5), pyridoxine (B_6) and folate (B_9) while riboflavin (B_2) was analyzed separately using the mobile phase. All samples were analyzed in triplicates.

The column elute was monitored with a PDA at 234 nm for thiamin, 266 nm for riboflavin, 261 nm for niacin, 204 nm for pantothenic acid 324 nm for pyridoxine and 282 nm for folic acid. The mobile phase was filtered through a 0.45 µm membrane and degassed by sonication before use. The flow- rate was 0.7 mL/min and the column was operated at room temperature (25 ^{o}C). Chromatographic peak data were integrated up to 39 minutes. Identification of compounds was achieved by comparing their retention times and UV spectra with those of the standards. Calibration curves were plotted for each vitamin and the concentrations of the vitamins were calculated from integrated areas of the sample and the corresponding standards.

3.8 Simulated gastrointestinal digestion

Simulated *in vitro* digestion method was performed according to Veda *et al.*, (2006). The process involved the gastric phase and intestinal phase. For each sample (individual vegetables fresh, individual vegetables boiled, individual vegetables boiled-fried, recipes of mixtures of vegetables and formulations), 2 g, was subjected to simulated gastric digestion at pH 2.0 in the presence of pepsin at 37 ^{o}C (16 g in 100 ml 0.1M HCl) for 2 hours, followed by simulated intestinal digestion in the presence of pancreatin-bile extract mixture (4 g porcine pancreatin and 25 g bile extract (porcine) in 1000 ml of 0.1 M $NaHCO_3$ pH 7.5) at 37 ^{o}C for 2 hours. The micellar fraction containing the bioaccessible vitamin was separated by ultracentrifugation at 70 000 x g for 120 minutes using a Beck-man L7- 65 ultracentrifuge. The micellar fraction was measured for bioaccessible beta-carotene (using UV/VIS spectrophotometer) and vitamin B series (using HPLC).

3.9 Data analysis

Data was analyzed by SPSS 18 for windows version 24 employing one-way ANOVA to compare the mean levels and percentage bioaccessibility of vitamins (BC, thiamin, riboflavin, niacin, pantothenic acid, pyridoxine and folate) with mean separations by standard error (Sawyer and Beebe, 2007). P-values <0.05 were considered significantly different, (SNK, $\alpha=0.05$). The levels of beta-carotene and vitamin B were reported in mg/100g. Bioaccessibility of each vitamin was calculated by dividing the bioaccessible fraction (mg /100g) by total vitamin content (mg/100g) then multiplying by one hundred.

CHAPTER 4

4 RESULTS AND DISCUSSIONS

4.1 Introduction

The chapter presents and discusses results for analysis of beta-carotene (BC), and the vitamin B series (B_{1}-thiamin, B_{2}-riboflavin, B_{3}-niacin, B_{5}-pantothenic acid, B_{6}-pyridoxine and B_{9}-folate) in five selected leafy African indigenous vegetables (LAIVs) namely, spider plant (*Cleome gynandra)*, cowpeas leaves (*Vigna unguiculata)*, amaranth (*Amaranthus viridis*, vine spinach (*Basella alba)* and pumpkin leaves (*Cucurbita maxima).* The bioaccessibility of these compounds in the vegetables when fresh, boiled and fried, as well as in recipes of mixtures of the vegetables (spider plant + amaranth, cowpeas + amaranth and vine spinach + pumpkin leaves) and formulations is also reported. The levels beta-carotene and vitamin B series are reported in mg/100g (DW).

4.2 Method validation

4.2.1 Regression Analysis

Calibration curves were plotted for each vitamin and Figure 4.1 represents the calibration curve for folate, the other curves are in the appendix (Figures 1-6).

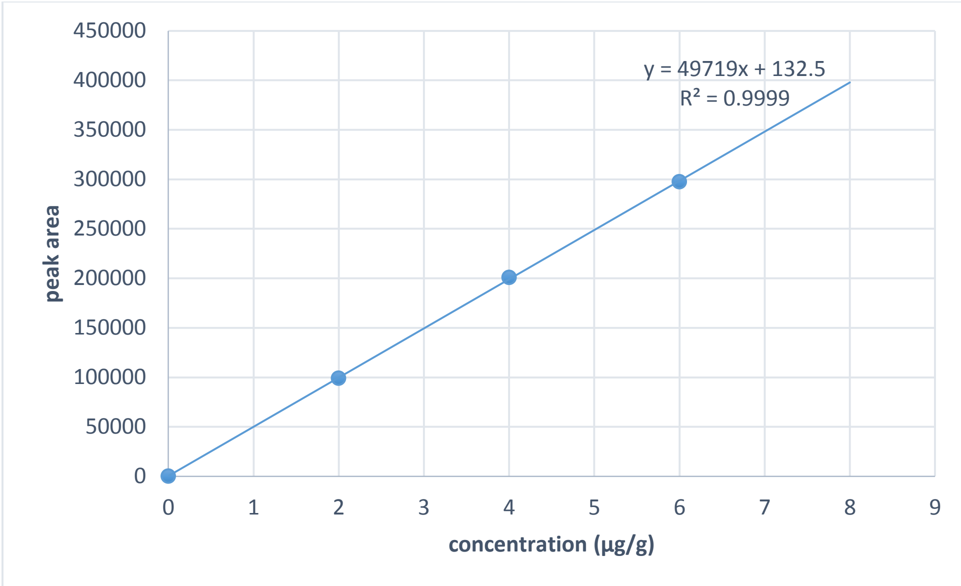

Figure 4. 1: Calibration curve for series of folate standard

The LoDs, regression equations and the correlation coefficients are presented in Table 4.1.

Table 4. 1: Method validation parameters of beta-carotene and the vitamin B series

Vitamin	LoD (ppm)	Correlation coefficient	Regression equation	% recovery
Beta-carotene	0.02	0.9998	y=0.1965x-0.0043	98.96±0.15
Thiamine	0.12	0.9783	y=36499x-2346.8	101.27±1.27
Riboflavin	0.04	0.9998	y=49440x-496.57	99.13±0.47
Niacin	0.08	1	y=9441x-72.8	100.37±0.38
Pantothenic acid	0.10	0.9736	y=2203.8x+547.67	100.18±0.16
Pyridoxine	0.03	0.9960	y=14982x-2931.8	99.74±1.38
Folate	0.03	0.9990	y=49747x+132.5	100.35±0.35

The LoDs ranged from 0.02 to 0.12 and this is comparable to that reported by Cheruiyot, 2011 (0.03 to 0.17). The correlation coefficient values were above 0.9736, indicating at least 97.36 % relationship of absorbance against concentration, thus implying linearity (Jedlicka and Klimes, 2004). The percentage recoveries were between 98.96±0.15 and

101.27 % implying that the methods had a conventionally acceptable precision and accuracy (Taylor *et al*., 2006).

4.2.2 HPLC separation of vitamin B series

The column used was C-18, detector-PDA at 25 ^{o}C and 1 mL/min flow rate. The mobile phase was 0.1 mol/L KH_2PO_4 and methanol in the ratio 90:10 at pH 7. The wavelengths were; B_1-234 nm, B_2-266 nm, B_3-261 nm, B_5-204 nm, B_6-324 nm and B_9-288 nm. The chromatographic peak areas were integrated up to 39 minutes. The chromatogram for folate is given in Figure 4.2, the other chromatograms are given in the appendix (Figures 7-13).

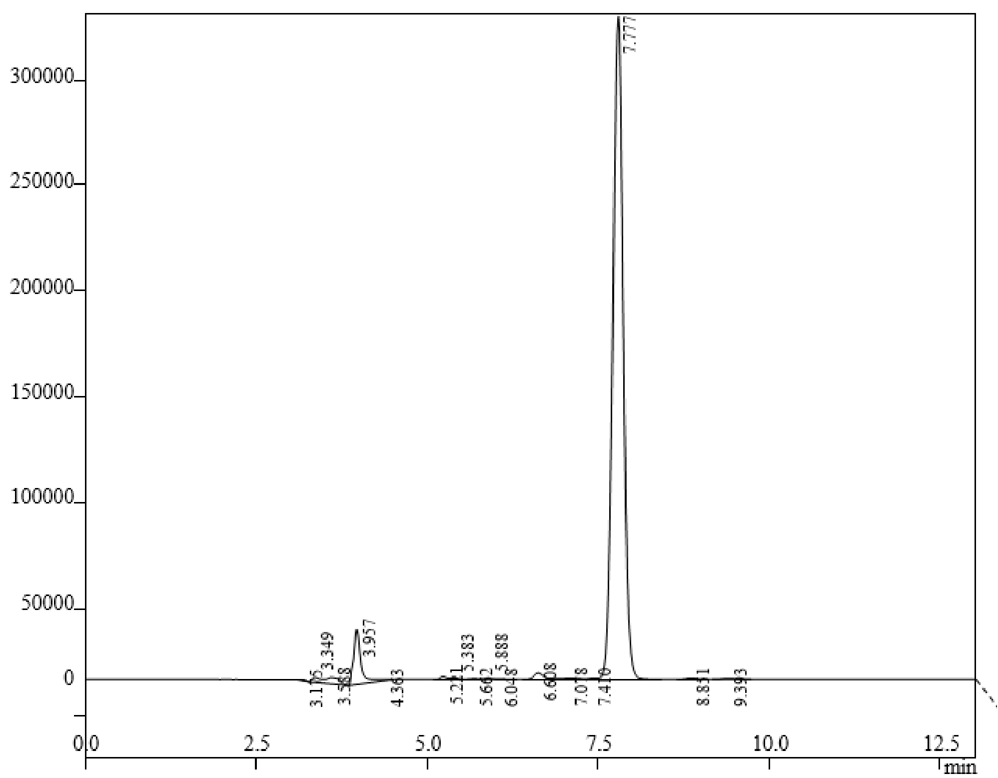

Figure 4. 2: Chromatogram for vitamin B_9 standard

4.3: Levels of beta-carotene and vitamin B series in fresh and processed LAIVs

The mean levels of beta-carotene (BC), thiamin (B_1), riboflavin (B_2), niacin (B_3), pantothenic acid (B_5), pyridoxine (B_6) and folate (B_9) in fresh and processed spider plant, cowpeas, amaranth, vine spinach and pumpkin leaves are presented in Table 4.2.

Table 4. 2: Mean levels of beta-carotene and vitamin B series in fresh and processed LAIVs

		Mean levels of beta-carotene and vitamin B series, mean ± SD mg/100g DW, n=3						
LAIVs	**Fresh/ Processed**	**BC**	**B_1**	**B_2**	**B_3**	**B_5**	**B_6**	**B_9**
Spider plant	Fresh	27.79±1.01^{b}	3.12±0.06^{a}	127.64±9.70^{b}	107.70±1.80^{c}	9.98±0.06^{d}	5.88±0.03^{c}	0.73±0.01^{d}
	Boiled	42.43±3.98^{c}	5.48±0.11^{b}	73.19±5.70^{a}	125.13±1.55^{d}	5.45±0.16^{b}	6.96±0.01^{d}	0.42±0.01^{c}
	Boiled-fried	10.36±1.20^{a}	16.41±1.00^{c}	ND	40.16±1.40^{a}	2.92±0.11^{a}	2.59±0.39^{b}	0.33±0.01^{b}
	Dried	8.34±0.58^{a}	2.26±0.08^{a}	ND	87.52±2.54^{b}	6.82±0.27^{c}	1.31±0.43^{a}	0.20±0.00^{a}
	p-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Cowpeas	Fresh	23.06±0.51^{c}	2.90±0.09^{b}	60.83±1.58^{b}	24.51±1.22^{b}	9.45±0.20^{a}	3.60±0.04^{a}	20.68±0.12^{c}
	Boiled	25.63±0.51^{d}	2.44±0.10^{a}	1.30±0.05^{a}	95.66±2.23^{c}	24.56±0.50^{b}	14.72±0.51^{b}	0.36±0.01^{a}
	Boiled-fried	7.51±0.46^{b}	ND	1.69±0.02^{a}	ND	33.56±0.90^{c}	16.20±0.06^{c}	7.54±0.02^{b}
	Dried	4.47±1.06^{a}	ND	ND	10.67±0.99^{a}	ND	3.37±0.34^{a}	19.39±1.60^{c}
	p-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Amaranth	Fresh	29.99±3.80^{b}	3.76±0.11^{a}	79.73±5.41^{c}	10.36±0.87^{b}	11.18±0.24^{b}	83.10±0.92^{d}	1.70±0.02^{a}
	Boiled	42.63±2.36^{c}	8.63±0.05^{c}	6.03±0.18^{a}	ND	5.12±0.70^{a}	21.18±0.94^{b}	8.86±0.12^{c}
	Boiled-fried	7.38±0.70^{a}	4.60±0.58^{b}	6.31±0.38^{a}	ND	46.69±1.50^{c}	25.18±0.40^{c}	3.71±0.01^{b}
	Dried	5.47±0.39^{a}	ND	23.91±1.45^{b}	8.81±0.66^{a}	ND	2.49±0.13^{a}	ND
	p-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Vine spinach	Fresh	8.57±0.50^{b}	0.83±0.01^{b}	174.16±3.50^{d}	23.25±2.12^{b}	13.52±0.51^{c}	5.97±0.10^{b}	3.71±0.09^{a}
	Boiled	14.22±2.30^{c}	1.07±0.04^{c}	1.35±0.03^{a}	127.14±2.79^{d}	13.46±0.66^{c}	18.68±0.30^{c}	ND
	Boiled-fried	6.09±0.47^{a}	ND	18.45±0.50^{b}	36.61±2.20^{c}	4.49±0.07^{a}	2.85±0.06^{a}	ND
	Dried	7.72±0.28^{ab}	0.78±0.01^{a}	48.93±3.68^{c}	14.66±0.28^{a}	7.96±0.09^{b}	ND	ND
	p-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Pumpkin leaves	Fresh	20.14±2.26^{b}	4.56±0.04^{c}	35.44±0.72^{c}	40.40±3.49^{b}	14.00±0.15^{a}	8.62±0.10^{c}	2.46±0.03^{c}
	Boiled	25.70±0.92^{c}	3.72±0.06^{b}	0.86±0.04^{a}	43.92±2.62^{b}	20.75±1.51^{b}	0.63±0.01^{a}	1.35±0.03^{b}
	Boiled-fried	5.40±0.41^{a}	10.26±0.80^{d}	ND	26.49±2.10^{a}	45.84±2.00^{c}	7.24±0.48^{b}	3.47±0.20^{d}
	Dried	7.08±0.33^{a}	3.71±0.01^{a}	13.34±1.01b	ND	ND	ND	0.98±0.11^{a}
	p-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Mean values followed by the same letters (superscript) within the same column of individual vegetables are not significantly different (SNK, α=0.05)
ND- Not Detected

4.3.1 Beta-carotene (BC)

The fresh vegetables contained mean levels (mg/100g) of BC in the range of: 8.57±0.50 (vine spinach) to 29.99±3.80 (amaranth). Both lower and higher levels of BC have been reported in LAIVs in several studies (Veda *et al.*, 2006; Cheruiyot, 2011; Kunyanga *et al.*, 2013; Nyamu, 2013; Wakhanu, 2014; Cheptoo *et al.*, 2019). Levels as low as 0.24 ± 0.01 mg/100g, of BC have been documented for fresh indigenous vegetables, in particular in amaranth and pumpkin leaves (Nyamu, 2013). Other studies have reported higher levels than those found in this study, such as 64.35 mg/100g, of BC in vegetable amaranth (Cheptoo *et al.*, 2019). Several factors, such as soil factors, climate, the age of the crop and its variety, management practices, and postharvest handling and storage are known to contribute to the levels of nutrients in fresh plants (Rodriguez-Amaya and Kimura, 2004; Ahamed *et al*., 2007).

Processing of the vegetables resulted in the mean levels ranging as follows: boiled vegetables 14.22±2.30 (vine spinach) to 42.63±3.80 (spider plant), boiled-fried vegetables 5.40±0.41 (pumpkin leaves) to 10.36±1.27 (spider plant) and dried vegetables 4.47±1.06 (cowpeas) to 8.34±0.58 (spider plant). It was observed that processing of the vegetables had effects that resulted to both significant ($p<0.001$) increase and decrease on the nutrient BC. The increases recorded ranged between 11.14 % and 65.93 % while reductions ranged between 9.92 % and 81.76 % (Appendix Table A 1, Appendix). There were variations in the trend of the carotenoid increase and decrease between individual vegetables and methods of processing them. Boiling, for example resulted in an increase of the levels of BC in all the vegetables while boil-frying and drying both had decrease of the levels indicating that thermal processing in the presence of oxygen and light breaks down BC (Rodriguez-Amaya and Kimura, 2004; Bernhardt and Schlich, 2006; Ahamed *et al.,* 2007). However, thermal processing is inevitable as it increases the surface area and interactions of hydrolytic enzymes and emulsifiers during digestion (Veda *et al.,* 2006; Srinivasan, 2009; Nagao, 2014). Boiling releases BC from the food matrix by breaking the cell wall and dissociating carotene-food matrix complexes, thus explaining the significant increase between the boiled and fresh vegetables.

4.3.2 Thiamin (B_1)

The mean levels (mg/100g) of thiamin (B_1) in fresh vegetables were found to range from 0.78±0.01 (vine spinach) to 4.56±0.04 (pumpkin leaves). Studies have reported thiamin levels in LAIVs (Ismail *et al.,* 2013; Kunyanga *et al.,* 2013; Okpalamma *et al.,* 2013; Uraku *et al.,* 2015). While Uraku *et al.,* (2015) reported levels comparable to those recorded in this study, 9.731±3.250 mg/100g, Kunyanga *et al.,* (2013) reported lower levels, 0.42±0.09 mg/100g. The variation in the levels of the nutrient in vegetables are not only determined by the variety but also the age, farm management practices, soil, climate, handling of the crop after harvesting as well as storage conditions (Rodriguez-Amaya and Kimura, 2004; Ahamed *et al*., 2007).

The resultant mean levels on processing the vegetables had the following ranges; boiled 1.07±0.04 (vine spinach) to 5.48±0.11 mg/100g (spider plant), boiled-fried 4.61±0.58 (amaranth) to 16.41±1.09 mg/100g (spider plant) and dried 0.78±0.01 (vine spinach) to 3.71±0.01 mg/100g (pumpkin leaves). The vitamin was below the detection limit of 0.12 ppm in boiled-fried and dried cowpeas, dried amaranth and boiled-fried vine spinach. It was observed that processing of the vegetables caused significant ($p<0.001$) increase or decrease of thiamin. While the increases recorded ranged between 22.34 % and 425.96 %, the range for the reductions was between 6.02 % and 100.00 %.

Boiling and boil-frying led to increases of thiamin in spider plant (75.64 %, 425.96 %) and amaranth (129.52 %, 22.34 %) vegetables. While vine spinach had the boiling process leading to an increase (28.92 %) in the vitamin, boil-frying showed no detectable amounts. Similarly, the levels of vitamin B_1 in cowpeas (15.86 %) and pumpkin leaves (18.42 %) reduced with boiling but increased with boil-frying in spider plant, amaranth and pumpkin leaves. Levels after boil-frying reduced to undetectable levels in cowpeas and vine spinach. The reduction in the levels can be explained based on the fact that thiamin is very sensitive to heat and oxidation, while the increase is mainly due to the vitamin being readily released from its protein matrix during cooking (Hotz and Gibson 2007).

4.3.3 Riboflavin (B_2)

The mean levels (mg/100g) of riboflavin (B_2) contained in the fresh vegetables ranged from 35.44±0.73 (pumpkin leaves) to 174.16±3.57 (vine spinach). Lower levels of riboflavin than those reported in this study have been documented 0.44 ± 0.03 mg/100g (Kunyanga *et al.*, 2013) Variations are explained by the fact that, the levels of nutrients in vegetables not only depend on the variety and age but also the soil, climate as well as how the vegetables were handled in the farm and even after harvesting (Ahamed *et al.*, 2007).

On processing, the resultant mean levels in the vegetables were found to be; boiled 0.86±0.04 (pumpkin leaves) to 73.19±5.70 (spider plant), boiled-fried 1.69±0.02 (cowpeas) to 18.45±0.56 (vine spinach) and dried 13.34±1.02 (pumpkin leaves) to 48.93±3.68 (vine spinach). The vitamin was not detected in boiled-fried and dried spinach as well as in dried cowpeas and pumpkin leaves. Okpalamma *et al.,* (2013) reported levels of riboflavin in fluted pumpkin boiled in water for five minutes to be 1.02±0.01 mg/100g while Uraku *et al.,* (2015) documented 7.161±0.521 mg/100g in dried amaranth. These levels were lower than those reported in this study and the variation may be attributed to loss during washing, leaching into cooking water during boiling, storage that may lead to loss of about 25 % to 40 % if not properly done (Ismail *et al.*, 2013).

It was observed that all the methods of processing the vegetables led to significant reduction ($p<0.001$) of the vitamin and in some cases to levels that were not detectable. The reductions are substantial, ranging between 42.66 % and 100.00 %. This reduction or loss of riboflavin when vegetables were thermally processed is attributed to the vitamin being sensitive to heat and light on one hand (Schellack *et al.,* 2015) and being water soluble on the other (Ismail *et al.,* 2013).

4.3.4 Niacin (B_3)

The mean levels (mg/100g) of niacin (B_3) contained in the fresh vegetables ranged from 10.36±0.88 (amaranth) to 107.70±1.86 (spider plant). Studies have reported lower levels than those recorded in this study 0.01 mg/100g (Ismail *et al.,* 2013; Okpalamma *et al.,* 2013), 0.70±0.01 in amaranth and 0.32±0.01 in pumpkin leaves (Kunyanga *et al.*, 2013).

The variation in the levels of the vitamin may be attributed to the nutrient content (Mathaba, 2017) as well as the age of the crop, variety, management practices, postharvest handling and storage among others (Rodriguez-Amaya and Kimura, 2004; Ahamed *et al*., 2007).

The processed vegetables had mean levels whose ranges were; boiled 43.92±2.62 (pumpkin leaves) to 127.14± 2.79 (vine spinach), boiled-fried 26.49±2.17 (pumpkin leaves) to 40.16±1.46 (spider plant) and dried 8.81±0.66 (amaranth) to 87.52±2.54 (spider plant). Niacin was not detected in boiled-fried cowpeas, boiled and boiled-fried amaranth and dried pumpkin leaves. Levels as low as 0.50±0.00 mg/100g have been documented in fluted pumpkin boiled in water for five minutes Okpalamma *et al.*, 2013.The levels of niacin differed significantly ($p<0.001$) with processing of the vegetable. The increases recorded ranged between 8.71 % and 446.84 % when fresh vegetables were processed while for reductions, the range was between 14.96 % and 100.00 % (Appendix Table A 1).

It was observed that boiling increased the levels of niacin in some vegetables while the same process caused a reduction in others. Both boiling and boil-frying led to increases of niacin in vine spinach (446.84 %, 57.46 %), unlike in spider plant, cowpeas and pumpkin leaves where boiling led to an increase (16.18 %, 290.29 % and 8.47 % respectively) in the vitamin while boil-frying reduced to 62.71 % (spider plant) and 34.43 % (pumpkin leaves), Appendix Table A 1. Cowpeas leaves and amaranth however, showed no detectable amounts on processing. The observation made is supported by the fact that niacin is very sensitive to light (Schellack *et al.,* 2015) and easily extracts into the cooking water (Serafini *et al.*, 2002).

4.3.5 Pantothenic acid (B_5)

The mean levels of pantothenic acid (B_5) in the fresh vegetables (mg/100g) ranged from 9.45±0.20 (cowpeas) to 14.00±0.15 (pumpkin leaves). The resultant mean levels on processing had the following ranges; boiled vegetable 5.12±0.70 (spider plant) to 24.56±0.50 mg/100g (cowpeas), boiled-fried vegetables 2.92±0.11 (spider plant) to 46.69 ±1.55 mg/100g (amaranth) and dried vegetables 6.82±0.27 (spider plant) to 7.96±0.09 mg/100g (vine spinach). The vitamin was not detected in dried cowpeas, amaranth and pumpkin leaves.

Processing of the vegetables either increased or a decreased the levels of the nutrient significantly ($p<0.001$), with the increase ranging between 48.21 % and 317.63 % while reductions ranged between 0.44 % and 100.00 %. There were variations in the trend of the vitamin between individual vegetables and methods of processing them. Boiling for instance increased the levels of pantothenic acid in some vegetables while in others a reduction was observed. Boiling and boil-frying led to increase of the vitamin in cowpeas (159.89 %, 225.13 % respectively) and pumpkin leaves (48.21 %, 227.43 % respectively).

Boil-frying process increased the level of the vitamin (317.63 %) while boiling reduced 54.20 %. The dried cowpeas, amaranth and pumpkin leaves however showed no detectable amounts, while the spider plant recorded a reduction in the levels of the vitamin in boiled, boiled-fried and dried (45.39 %, 70.74% and 31.66 % respectively) and vine spinach (0.44 %, 66.79 % and 41.12 % respectively). The increase in the levels is mainly due to the fact that pantothenic acid is readily released from its protein matrix during cooking (Hotz and Gibson 2007; Nagao *et al.*, 2014) and the reduction is attributed to the sensitivity of pantothenic acid to heat, can be decomposed by heat during cooking (Hotz and Gibson, 2007; Fukawatari and Shibata, 2008; Ismail *et al.*, 2013).

4.3.6 Pyridoxine (B_6)

The mean levels (mg/100g) of pyridoxine (B_6) in the fresh vegetables were in the range of 3.60±0.04 (cowpeas) to 83.09±0.92 (amaranth). While levels as low as 0.06 mg/100g, have however been documented (Ismail *et al.*, 2013), Uraku *et al.*, (2015) reported comparable levels of pyridoxine, 25.020 ± 2.667 mg/100g. Apart from nutrient content, the variation of the levels may be due loss during washing, handling both in the farm and after harvesting as well as storage among other factors (Ahamed *et al.*, 2007; Mathaba, 2017).

The levels in the processed vegetables were found to range from 0.63±0.01 (pumpkin leaves) to 21.18±0.94 (amaranth) in the boiled, 2.59±0.39 (spider plant) to 25.18±0.42 (amaranth) in the boiled-fried and 1.31±0.43 (spider plant) to 3.37±0.31 (cowpeas) in the dried. The vitamin was however not detected in dried vine spinach and pumpkin leaves. A study by Uraku *et al.*, 2015 documented levels of pyridoxine in vegetable amaranth dried for two weeks, 25.020±2.667 mg/100g. The levels are higher than those reported in this study and this may be due to the fact that pyridoxine can be decomposed by heat during cooking (Parada and Aguilera, 2007; Nagao *et al.*, 2014).

Both significant ($p<0.001$) increases and decreases were observed on processing the vegetables. The increases recorded ranged between 18.37 % and 350.00 % when fresh vegetables were processed while for reductions, the range was between 6.39 % and 100.00 % (Appendix Table 1). While both boiling and boil-frying led to increase of the vitamin in cowpeas (308.89 %, 350.00 %), boiling increased the levels in spider plant and vine spinach (18.37 % and 212.90 % respectively) and boil-frying reduced the levels of the same vegetables to 55.95 % and 52.26 % respectively (Appendix Table1). This is because pyridoxine is decomposed by heat during cooking (Nagao *et al.,* 2014; Parada and Aguilera, 2007). All the methods of processing reduced the levels of the vitamin in boiled, boiled-fried and dried amaranth (74.51 %, 69.70 % and 97.00 % respectively) and in boiled and boiled-fried pumpkin leaves (92.69 % and 16.01 % respectively), Appendix Table1. The dried pumpkin leaves showed no detectable amounts.

4.3.7 Folate (B_9)

The fresh vegetables recorded mean levels (mg/100g) of folate (B_9) that ranged from 0.74±0.01 (spider plant) to 20.68±0.12 (cowpeas). Studies on determination of folate in LAIVs have reported both lower and comparable levels (Ismail *et al.,* 2013; Kunyanga *et al.,* 2013). Ismail *et al.,* (2013) reported levels as low as 0.12 mg/100g, while Kunyanga *et al.,* (2013) reported levels, 0.85 ± 0.02 mg/100g, that were comparable to those recorded in this study. The variation in levels may be attributed to interconversion caused by oxygen or changes in pH. Activity of folate may also be lost by oxidative cleaving (Öhrvik, 2009). The other factor that may have contributed to the effect on the levels of the vitamin is the vitamin content in the vegetables (Mathaba, 2017).

The processed vegetables had mean levels that ranged from; boiled 0.36±0.01 (cowpeas) to 8.86±0.12 (amaranth), boiled-fried 0.33±0.01 (spider plant) to 7.54±0.20 (cowpeas) and dried 0.20±0.00 (spider plant) to 19.39±1.60 (cowpeas). Folate was not detected in fried amaranth as well as boiled, boiled-fried and dried vine spinach. Significant ($p<0.001$) increase or decrease on the levels of the vitamin was observed as a result of processing the vegetables. The increases recorded ranged between 41.06 and 421.18 % while the reductions were found to range between 6.24 and 100.00 %. Variations were observed in the trend of the vitamin between individual vegetables and methods of processing. While both boiling and boil-frying led to an increase of the vitamin in amaranth (421.18 %, 118.24 %), for pumpkin leaves only the boil-frying process led to an increase (41.06 %). The vitamin reduced (45.12 %) for the samples of pumpkin leaves that were boiled.

It was noted that all the methods of processing the vegetables led to reductions in the levels of the vitamin in boiled, boiled-fried and dried spider plant (49.32 %, 54.79 % and 72.60 %) and cowpeas (98.26 %, 63.52 % and 6.24 %), Appendix Table 1. The dried amaranth and all the methods of processing in vine spinach showed no detectable amounts. These observations are supported by the fact that folate is light and heat sensitive, destroyed by oxidation and also lost during cooking (Fukawatari and Shibata, 2008). Vitamin activity of folate may be lost by oxidative cleaving resulting in a pterin and para-aminobenzoylglutamate (PABG) molecule. Folates found in food are prone to interconversion caused by oxygen or changes in pH (Öhrvik, 2009). Cooking methods which involve excessive boiling and use of large amounts of water lead to loss of folate (Agyemang-Yeboah and Oppong, 2013).

4.4: Mean bioaccessible levels of beta-carotene and vitamin B series in LAIVs

The mean bioaccessible levels of beta-carotene and the vitamin B series in fresh, boiled and boiled-fried LAIVs are presented in Table 4.3.

Table 4. 3: Mean bioaccessible levels of beta-carotene and vitamin B series in fresh and processed LAIVs

		Mean bioaccessible levels of beta-carotene and vitamin B series, Mean ± SD mg/100g, DW, n=3						
LAIVs	Fresh/ Processed	BC	B_1	B_2	B_3	B_5	B_6	B_9
Spider plant	Fresh	27.02±1.00[b]	0.89±0.10[a]	126.75±9.77[b]	105.62±1.86[b]	0.43±0.02[a]	5.24±0.03[b]	0.73±0.0[c]
	Boiled	33.48±3.79[c]	0.39±0.06[a]	72.48±5.65[a]	125.13±1.55[c]	2.04±0.26[b]	1.21±0.25[a]	0.23±0.01[b]
	Boiled-fried	5.40±1.32[a]	16.13±1.09[b]	ND	38.70±1.55[a]	0.60±0.12[a]	1.15±0.37[a]	0.19±0.01[a]
	p-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Cowpeas	Fresh	18.82±0.49[b]	2.49±0.09[b]	60.83±1.58[b]	24.51±1.22[a]	1.18±0.21[a]	2.12±0.11[a]	20.68±0.12[c]
	Boiled	21.61±0.47[c]	0.42±0.04[a]	1.30±0.05[a]	95.66±2.22[b]	0.99±0.56[a]	16.50±3.14[b]	0.24±0.01[a]
	Boiled-fried	2.33±0.21[a]	ND	1.60±0.03[a]	ND	15.64±0.99[b]	47.23±0.86[c]	7.54±0.20[b]
	p-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Amaranth	Fresh	21.47±0.42[b]	0.77±0.04[a]	79.73±5.41[b]	6.93±0.64[a]	12.72±1.82[a]	75.23±1.39[c]	3.71±0.14[b]
	Boiled	39.35±2.37[c]	1.99±0.01[b]	3.70±0.25[a]	ND	10.36±0.64[a]	16.33±0.88[a]	8.86±0.12[c]
	Boiled-fried	1.13±0.12[a]	3.67±0.15[c]	2.73±0.29[a]	ND	15.64±0.99[b]	24.16±3.14[b]	3.66±0.12[a]
	p-value	<0.001	<0.001	<0.001	<0.001	<0.006	<0.006	<0.006
Vine spinach	Fresh	5.26±0.47[b]	0.27±0.01[b]	174.12±3.57[c]	6.93±0.64[a]	2.82±0.60[b]	5.00±0.30[b]	3.17±0.10[a]
	Boiled	9.97±0.03[c]	0.19±0.00[a]	1.35±0.03[a]	127.14±7.79[c]	1.50±0.17[a]	17.50±0.33[c]	ND
	Boiled-fried	1.40±0.14[a]	ND	18.17±0.55[b]	36.22±2.29[b]	0.64±0.17[a]	2.71±0.06[a]	ND
	p-value	<0.001	<0.001	<0.001	<0.001	<0.004	<0.004	<0.004
Pumpkin leaves	Fresh	19.04±2.25[b]	3.06±0.09[b]	34.67±0.72[b]	40.40±3.49[b]	0.93±0.38[a]	1.58±0.01[b]	2.24±0.04[b]
	Boiled	20.76±0.92[b]	2.04±0.18[a]	0.19±0.01[a]	37.27±4.71[b]	4.75±2.35[b]	0.63±0.01[a]	0.95±0.03[a]
	Boiled-fried	1.26±0.16[a]	9.74±0.84[c]	ND	24.87±2.13[a]	25.51±2.79[c]	3.86±0.05[c]	2.98±0.30[c]
	p-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	

Mean values followed by the same letters of the same vegetable within the same column are not significantly different (SNK, α=0.05).

4.4.1 Beta-carotene (BC)

The mean bioaccessible levels (mg/100g) of BC in fresh vegetables was found to be 5.26±0.47 (vine spinach) to 27.02±1.00 (spider plant). The processed vegetables recorded mean bioaccessible levels ranging from 9.97±0.03 (vine spinach) to 39.35±3.70 (amaranth) in the boiled and 1.13±0.12 (amaranth) to 5.40±1.32 (spider plant) in the boiled-fried. It was observed that processing of the vegetables resulted in both significant ($p<0.001$) increase and decrease on bioaccessible levels of BC (Table 4.3). There was a general trend on the effect of processing the vegetables in which boiling resulted in significant ($p<0.001$) increase in the bioaccessible levels of the carotenoid while a decrease was observed when the vegetables were processed by boil-frying. Boiling releases BC from the food matrix by breaking the cell wall and dissociating carotene-food matrix complexes, explaining the significant increases (Rodriguez-Amaya and Kimura, 2004; Bernhardt and Schlich, 2006; Ahamed *et al.,* 2007).

4.4.2 Thiamin (B_1)

The mean bioaccessible levels (mg/100g) of thiamin (B_1) in fresh vegetables were found to range from 0.27±0.01 (vine spinach) to 3.06±0.09 (pumpkin leaves). When processed, the mean levels of the vegetables were; in the boiled 0.19±0.00 (vine spinach) to 2.4±0.18 (cowpeas) and in the boil-fried 3.67±0.15 (amaranth) to 16.13±1.09 (spider plant). The vitamin was not detected in boil- fried cowpeas and vine spinach. Processing of the vegetables both significantly ($p<0.001$) increased and decreased the mean bioaccessible levels (Table 4.3). Thiamin is very sensitive to heat and oxidation, leading to the reduction in the bioaccessible levels (Hotz and Gibson, 2007; Ismail *et al.,* 2013;

Terefe *et al.*, 2014; Bua and Onang, 2017). Vitamin B are also lost by cooking methods that use water (Ismail *et al.*, 2013) due to the vitamin being readily released from its protein matrix during cooking (Hotz and Gibson, 2007).

4.4.3 Riboflavin (B_2)

The bioaccessible mean levels (mg/100g) of riboflavin (B_2) in fresh vegetables ranged between 34.67±0.72 (pumpkin leaves) and 174.52±3.57 (vine spinach), in the boiled from 0.19±0.01 (pumpkin leaves) to 72.48 ±5.65 (spider plant) and in the boil-fried from 1.60 ±0.02 (cowpeas) to 18.17 ±0.55 (vine spinach). It was observed that the mean bioaccessible levels of riboflavin in all the vegetables decreased as a result of both the boiling and boil-frying processes, boil-frying process reducing the vitamin in spider plant and pumpkin leaves to a point of no detection (Table 4.3). Although riboflavin is known to be stable even when heat processed such as normal cooking of foods in the absence of light (Schellack *et al*., 2015), the vitamin is lost by leaching when cooked by methods that use water (Ismail *et al.*, 2013), the cooking method commonly used.

4.4.4 Niacin (B_3)

The bioaccessible mean levels (mg/100g) of niacin (B_3) in fresh vegetables ranged from 6.93±0.64 (vine spinach) to 105.62±1.86 (spider plant). When processed, the bioaccessible mean levels in the vegetables were found to range from; in the boiled 7.27±4.71 (pumpkin leaves) to 127.14±7.79 (vine spinach) and in the boiled-fried 24.87±2.13 (pumpkin leaves) to 38.70±1.55 (spider plant). The vitamin was not detected in boiled-fried cowpeas as well as boiled and boiled-fried amaranth. When the vegetables were processed, both significant ($p<0.001$) increase and decrease on the mean bioaccessible levels of the vitamin were observed (Table 4.3). The increase in bioaccessible levels of niacin may be attributed to its release from the food matrix (Hotz and Gibson, 2007). The other factor that contributes to increase in the levels of niacin is hydrolysis of nicotinamide adenine dinucleotide phosphate, NAD (P), to nicotinic acid during cooking (Fukawatari and Shibata, 2008).

There were variations in the trend of the vitamin between individual vegetables and the two methods of processing by boiling and boil-frying that were employed. Boiling decreased the levels of thiamin in spider plant while the same process caused an increase in vine spinach and pumpkin leaves. The vitamin was reduced to no detection levels in vegetable amaranth. When the vegetables were boiled-fried, the vitamin increased in spider plant, vine spinach and pumpkin leaves and levels reduced to undetectable levels in cowpeas and amaranth (Table 4.3).

4.4.5 Pantothenic acid (B_5)

The bioaccessible mean levels (mg/100g) of pantothenic acid (B_5) in fresh vegetables ranged from 0.43±0.02 (spider plant) to 12.72±1.82 (amaranth). When processed, the mean levels of the vitamin in the boiled vegetables varied from 0.99±0.56 (cowpeas) to 10.36±0.64 (amaranth) and 0.60±0.12 (spider plant) to 25.51±2.79 (pumpkin leaves) in the boiled-fried vegetables. Significant ($p<0.001$) differences were observed in the mean bioaccessible levels of the vitamin on processing the vegetables (Table 4.3). The two methods of processing employed, boiling and boil-frying, decreased the bioaccessible levels of thiamin in spider plant and vine spinach while the same processes caused an increase in cowpeas, amaranth and pumpkin leaves (Table 4.3). The difference in the alteration of matrices of the vegetables explains the variation in the bioaccessible levels (Nagao, 2014). The decrease in the bioaccessible levels may be due to the fact that the vitamin is destroyed during cooking (Bua and Onang, 2017).

4.4.6 Pyridoxine (B_6)

The bioaccessible mean levels (mg/100g) of pyridoxine (B_6) in fresh vegetables were found to vary from 1.58±0.01 (pumpkin leaves) to 75.23±1.39 (amaranth). The levels in the processed vegetables were; in the boiled 0.63±0.01 (pumpkin leaves) to 17.50±0.33 (vine spinach) and in the boiled-fried 1.15±0.37 (spider plant) to 47.23±0.85 (cowpeas). Both significant ($p<0.001$) increase and decrease in the mean bioaccessible levels of pyridoxine were observed when the vegetables were processed (Table 4.3). Boiling increased the mean bioaccessible levels of pyridoxine in spider plant, cowpeas, vine spinach and pumpkin leaves while the same process caused a decrease in amaranth and pumpkin (Table 4.3). Boil-frying the vegetables increased the levels of the vitamin in cowpeas and pumpkin leaves while it caused a decrease in spider plant, amaranth and vine spinach (Table 4.3). Heat processing resulted in both increase and decrease in bioaccessible levels. While cooking releases pyridoxine from the food matrix during cooking (Hotz and Gibson, 2007), some may be destroyed during the process (Bua and Onang, 2017). Further, the variation in bioaccessible levels may be attributed to the protein bound form of pyridoxine which has low bioaccessibility (Hotz and Gibson, 2007).

4.4.7 Folate (B_9)

In the fresh vegetables, the bioaccessible mean levels (mg/100g) of folate (B_9) were found to range from 0.73±0.01 (spider plant) to 20.68±0.12 (cowpeas). The levels in the processed vegetables were; in the boiled 0.23±0.01 (spider plant) to 8.86±0.12 (amaranth) and in the boiled-fried 0.19±0.01 (spider plant) to 7.54±0.20 (cowpeas). Both significant ($p<0.001$) increase and decrease in the bioaccessible levels of folate were observed (Table 4.3). Folate is light sensitive, destroyed by oxidation and lost during cooking (Fukawatari and Shibata, 2008). Folates in food are also prone to interconversion caused by oxygen and changes in PH (Öhrvid, 2009).

4.5: Mean percentage bioaccessibility of beta-carotene and vitamin B series in LAIVs

The mean percentage bioaccessibility of beta-carotene and vitamin B series in the fresh and processed LAIVs are presented in Table 4.4 and a comparison shown in Figures 4.3 - 4.9 for the different vegetables.

Table 4. 4: Mean percentage bioaccessibility of beta-carotene and vitamin B series in fresh and processed LAIVs

		Mean Percentage bioaccessibility of beta-carotene and vitamin B series, Mean ± SD mg/100g DW, n=3						
LAIVs	Fresh/processed	BC	B_1	B_2	B_3	B_5	B_6	B_9
Spider plant	Fresh	97.23±0.06[c]	28.61±2.56[b]	99.30±0.07[a]	98.07±0.03[b]	4.38±0.20[a]	89.10±0.29[c]	100.00±0.00[b]
	Boiled	78.80±1.86[b]	7.08±0.96[a]	99.03±0.05[c]	100.00±0.00[c]	37.35±3.68[c]	12.30±3.37[a]	55.04±1.28[a]
	Boiled-fried	48.19±0.82[a]	98.27±0.13[c]	ND	96.34±0.40[a]	22.89±0.07[b]	39.78±4.74[b]	55.80±2.01[a]
	p-value	≤0.001	≤0.001	≤0.005	≤0.001	≤0.001	≤0.001	≤0.001
Cowpeas	Fresh	81.60±0.36[b]	85.88±0.69[b]	100.00±0.00[b]	100.00±0.00[a]	11.63±1.35[b]	61.88±0.37[a]	100.00±0.00[b]
	Boiled	84.31±0.27[b]	17.20±1.09[a]	100.00±0.00[b]	100.00±0.00[a]	5.18±0.87[a]	100.00±0.00[b]	66.87±0.67[a]
	Boiled-fried	31.02±3.09[a]	ND	94.74±0.90a	ND	46.57±1.66[c]	98.74±0.02[b]	100.00±0.00[b]
	p-value	≤0.001	≤0.001	≤0.001	≤0.001	≤0.001	≤0.001	≤0.001
Amaranth	Fresh	77.22±0.05[b]	20.62±1.40[a]	100.00±0.0[c]	63.25±5.13[a]	34.53±0.77[b]	90.63±0.83[b]	61.80±2.81[a]
	Boiled	92.28±0.46[c]	23.00±0.14[b]	61.41±1.53[a]	ND	18.15±0.92[a]	75.21±0.99[a]	100.00±0.00[c]
	Boiled-fried	16.97±0.02[a]	85.55±1.08[c]	43.17±2.20[b]	ND	46.57±1.65[c]	95.83±0.85[c]	98.72±0.15[b]
	p-value	≤0.001	≤0.001	≤0.001	≤0.001	≤0.001	≤0.001	≤0.001
Vine spinach	Fresh	61.36±1.87[b]	32.31±1.53[b]	99.98±0.00[b]	98.50±0.08[a]	22.94±0.56[c]	83.66±3.81[a]	100.00±0.00[a]
	Boiled	65.67±5.52[b]	18.09±0.59[a]	100.00±0.00[c]	100.00±0.0o[c]	9.12±0.48[a]	93.67±0.37[b]	ND
	Boiled-fried	23.15±2.82[a]	ND	98.49±0.05[a]	98.94±1.35[b]	14.23±3.69[b]	94.86±0.14[b]	ND
	p-value	≤0.001	≤0.001	≤0.001	≤0.001	≤0.001	≤0.001	≤0.001
Pumpkin leaves	Fresh	94.48±0.57[c]	67.22±1.55[b]	97.82±0.05[b]	100.00±0.00[b]	5.17±0.93[a]	59.45±0.23[a]	90.87±0.33[c]
	Boiled	80.75±0.69[b]	54.94±4.59[a]	22.28±1.55[a]	100.00±0.00[b]	16.97±0.48[b]	100.00±0.00[b]	70.81±0.55[a]
	Boiled-fried	23.33±2.89[a]	95.02±0.38[c]	ND	93.85±0.00[a]	55.58±4.13[c]	100.00±0.00[b]	85.79±1.57[b]
	p-value	≤0.001	≤0.001	≤0.001	≤0.001	≤0.001	≤0.001	≤0.001

Mean values of the same vegetable within the same column followed by the same letters are not significantly different (SNK, α=0.05)

4.5.1 Beta-carotene (BC)

The percentage bioaccessibility of BC varied from 61.36±1.87 % (vine spinach) to 97.23±0.06 % (spider plant) in the fresh vegetables, 65.67±5.53 % (vine spinach) to 92.28±0.46 % (amaranth) in the boiled vegetables and 16.97±0.02 % (amaranth) to 48.19±0.82 % (spider plant) in the boiled fried vegetables (Table 4.4). The variations in percentage bioaccessibility of beta carotene are shown in the figure 4.3. The increases in percentage bioaccessibility recorded ranged from 3 % to 15 % while reductions were found to range from 14 % to 71 % (Appendix Table A 2).

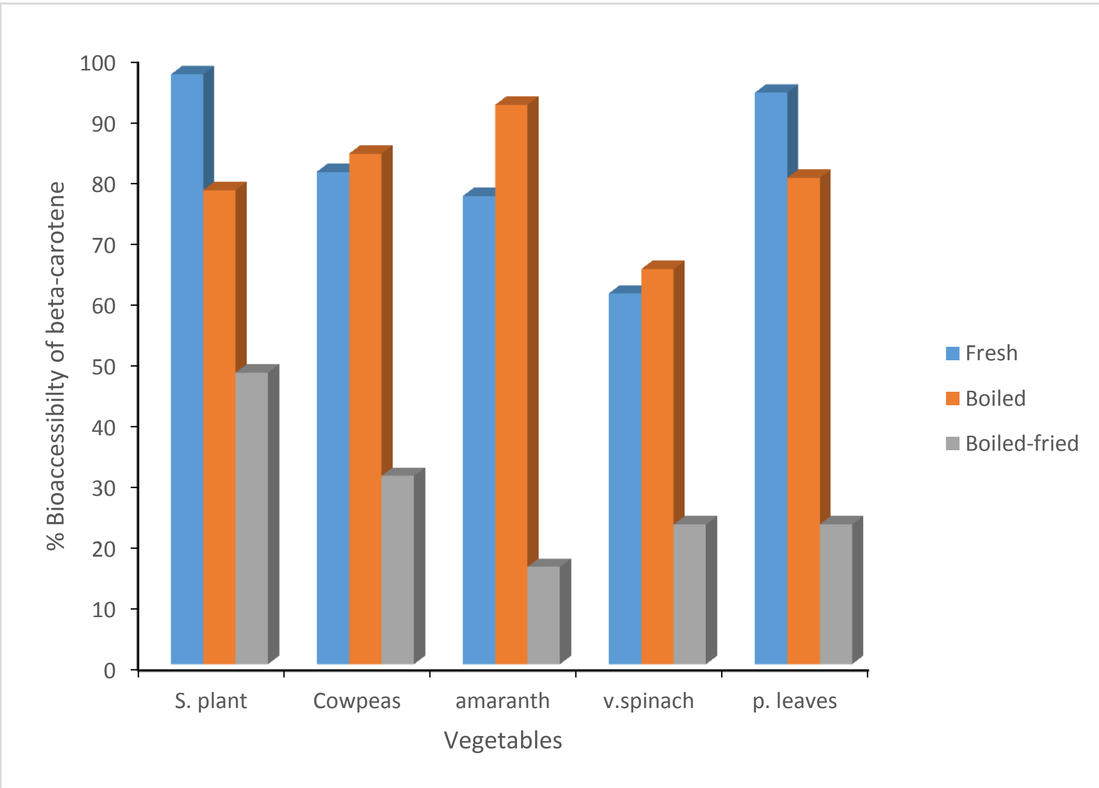

Figure 4. 3: Percentage bioaccessibility of beta-carotene in fresh and processed vegetables

The percentage bioaccessibility of BC after boil-frying the vegetables was spider plant (48.19±0.82 %), cowpeas (31.02±3.09 %), amaranth (16.97±0.02 %), vine spinach (23.15±2.82 %) and pumpkin leaves (23.33±2.89 %), a decrease observed (Table 4.4).

Heat processing generally improves bioaccessibility of BC as a result of disruption of the matrix thereby enhancing its availability for absorption (Hotz and Gibson, 2007; Veda *et al.,* 2006; Srinivasan, 2009; Nagao, 2014. Further, the difference in bioaccessibility of BC could have been influenced by the plant cell wall (which has to be broken to release BC) made up of cellulose and pectin, the dietary fiber (Koh and Loh, 2018). Improved bioaccessibility as a result of thermal processing has also been reported by Koh and Loh, (2018) in pumpkin 10.56±0.44 % (raw), 11.42±0.63 % (boiled) 68.86±0.86 % (deep fried) and butternut squash 1.65±0.04 % (fresh), 2.03±0.15 % (boiled), 22.32±2.12 % (deep fried). Veda *et al.,* (2006) observed a contradicting trend in their study. On analyzing the vegetables amaranth, carrot, fenugreek leaves and pumpkin, an increase in percentage bioaccessibility of BC as a result of frying was observed. The vegetables were stir-fried in the presence of a small quantity of oil recording an increase of 263 % (fenugreek), 192 % (amaranth leaves), 63 % (carrot) and 53 % (pumpkin leaves)

The microstructure of vegetables influences bioaccessibility of beta-carotene (Svelander, 2011), resulting in variations. Pectin, a soluble fiber, affect lipid absorption as it can bind with fatty acids, cholesterol and bile acid. This could result in the formation of fatty acid-fiber complex thus losing the ability to form micelles (Koh and Loh, 2018). Aschoff *et al*., (2014) for instance recorded an increased bioaccessibility with diminished fiber content in the sample. In this study, spider plant recorded higher bioaccessibility, 97 % in the fresh and 48 % in the fried, compared to vegetables cowpeas, amaranth, vine spinach and pumpkin leaves (Figure 4.3) which may be attributed to its low fiber content, 0.8 % as reported by Lymio *et al.,* 2003. The variations in bioaccessibility may be attributed to the differences in fiber content; cowpeas, 19.45 % (Enyiukwu *et al.*, 2018), vine spinach 2.2 % (Vicente *et al.*, 2009), pumpkin leaves, 12.04±0.02 % and amaranth, 8.64 ±0.04 % (Kunyanga *et al.*, 2013). Different varieties of vegetables exhibit differences in the alteration of the matrices as a result of heat processing (Veda *et al.,* 2006), particle size reduction and duration of heat treatment are among the variations during processing which are likely to affect bioaccessibility of beta carotene (Koh and Loh, 2018).

4.5.2 Thiamin (B_1)

The percentage bioaccessibility of vitamin B_1 ranged from, in fresh vegetables 20.62±1.40 % (amaranth) to 85.88±0.69 % (cowpeas), in boiled vegetables 7.08±0.96 % (spider plant) to 54.94±4.59 % (pumpkin leaves) and in boiled-fried vegetables 85.55±0.96 % (amaranth) to 98.27±0.13 % (spider plant). The results of bioaccessibility of vitamin B_1 are summarized in Table 4.4. The variations in percentage bioaccessibility are shown in figures 4.4. The increases in percent bioaccessibility recorded ranged from 3 % to 70 % while reductions ranged 13 % to 70 % (Appendix Table A 2)

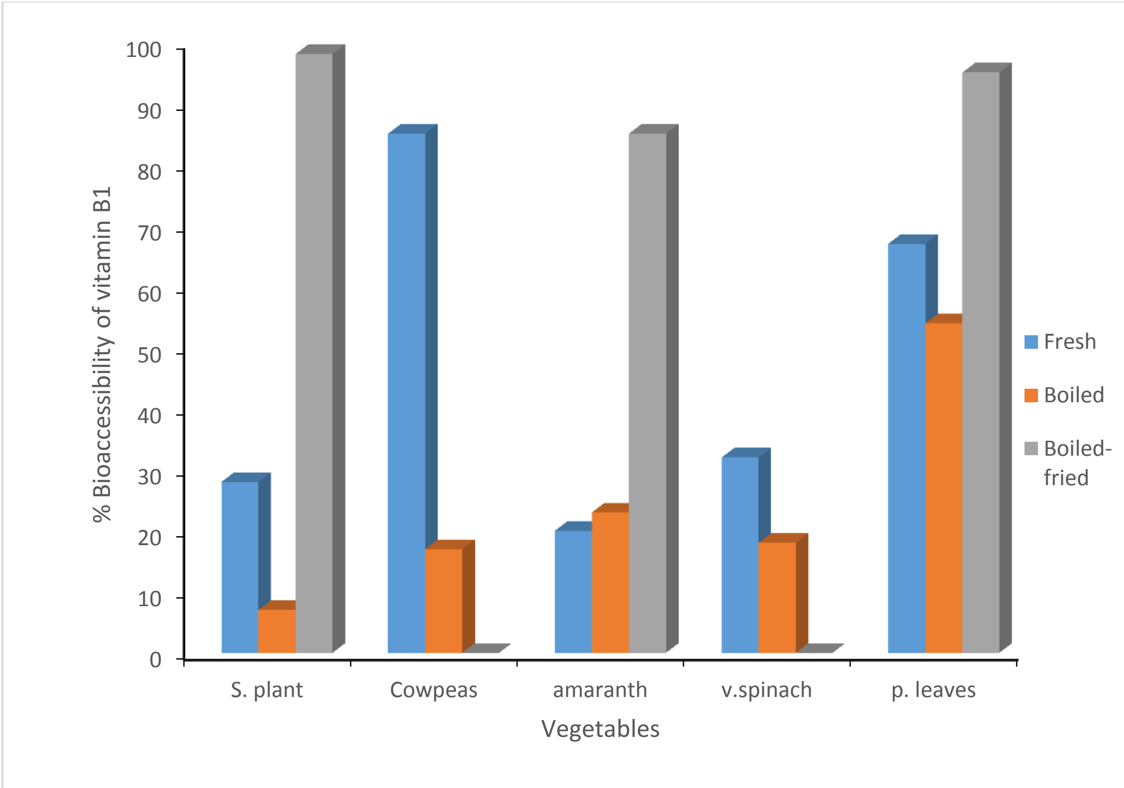

Figure 4. 4: Percentage bioaccessibility of vitamin B_1 in fresh and processed vegetables

Variations were observed in the trend of the vitamin between vegetables and the methods of processing. Boiling decreased bioaccessibility of thiamin in four of the vegetables; spider plant, cowpeas, vine spinach and pumpkin leaves while the same process caused an increase in amaranth (figure 4.4). When the vegetables were boiled-fried, bioaccessibility of the vitamin increased in spider plant, amaranth and pumpkin leaves (figure 4.4). Heat processing leads to increase in bioaccessibility of thiamin due to the disruption of the cell wall, dissociation of the nutrient–matrix complexes or transformation into a more active molecular structure (Parada and Aguilera, 2007) and thus increasing digestibility of the nutrients in the vegetables (Parada and Aguilera, 2007; Etcheverry *et al*, 2012).

4.5.3 Riboflavin (B_2)

The percentage bioaccessibility of vitamin B_2 in the fresh vegetables ranged from 97.82±0.04 % (pumpkin leaves) to 100.00±0.00 % (cowpeas and amaranth), in the boiled 22.28±1.55 % (pumpkin leaves and cowpeas) to 100.00±0.00 % (cowpeas) and in the boiled-fried 43.17±2.09 % (amaranth) to 98.49±0.54 % (vine spinach), Table 4.4. While the reductions in percentage bioaccessibility ranged between 1 % and 75 %, an increase of 1 % was observed when the vegetable vine spinach was boiled. The percent bioaccessibility, however, remained the same when vegetables spider plant and cowpeas were boiled (Appendix Table A 2).

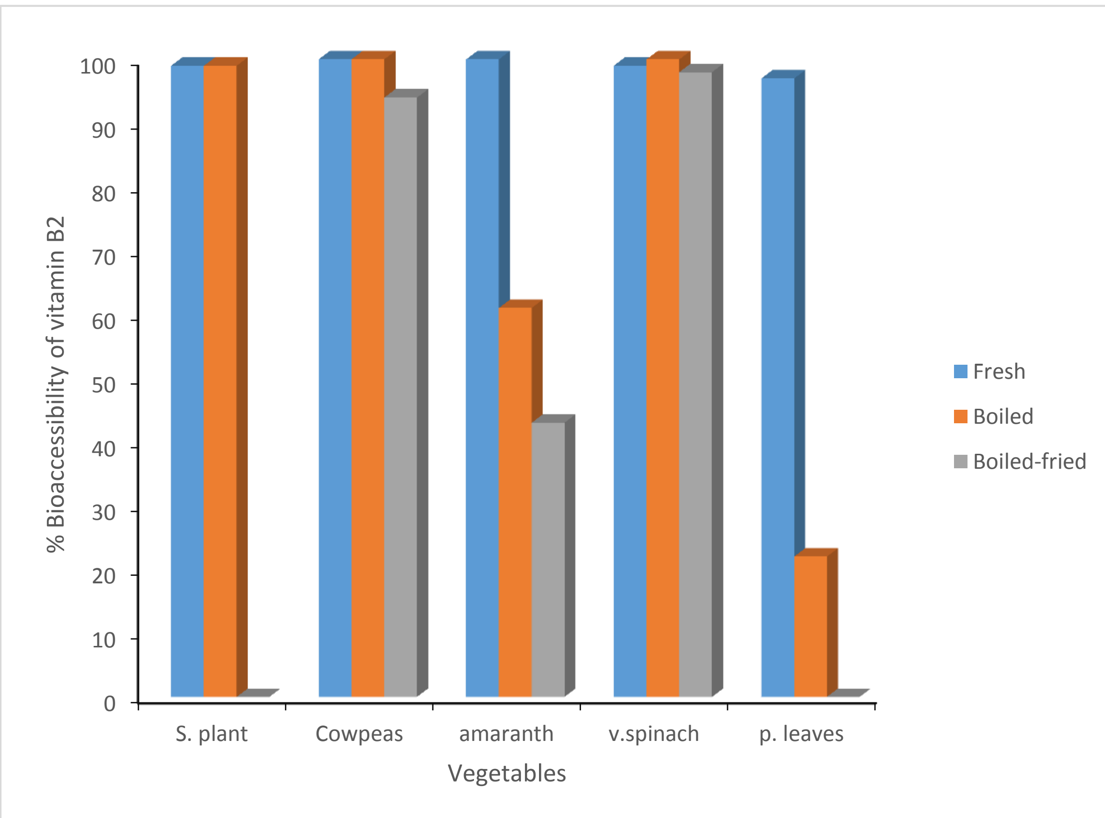

Figure 4. 5: Percentage bioaccessibility of vitamin B_2 in fresh and processed vegetables

There was no particular trend in bioaccessibility of the vitamin from the vegetables. While boiling resulted to an increase in bioaccessibility from vine spinach, the same process resulted to a decrease from amaranth and pumpkin leaves while there was no change in bioaccessibility from spider plant and amaranth when the vegetables were boiled (Figure 4.5). The variations may be due to the fact that different vegetables have different matrices which differently contribute to the mean levels and bioaccessible levels of the vitamin directly influencing bioaccessibility (Nagao, 2014).

4.5.4 Niacin (B_3)

The percentage bioaccessibility of vitamin B_3 in fresh vegetables varied from 98.07±0.03 % (amaranth) to 100.00±0.00 % (cowpeas and pumpkin leaves). All the boiled vegetables recorded 100.00±0.00 % bioaccessibility while in boiled-fried vegetables the percentage bioaccessibility varied from 93.85±0.00 % (pumpkin leaves) to 98.94±1.35 % (vine spinach), Table 4.4. The increases in percentage bioaccessibility ranged between 1 % and 71 % while a decrease of 7 % was detected when pumpkin leaves were boiled-fried. No change in % bioaccessibility was observed when cowpeas and pumpkin leaves were boiled (Appendix Table A 2).

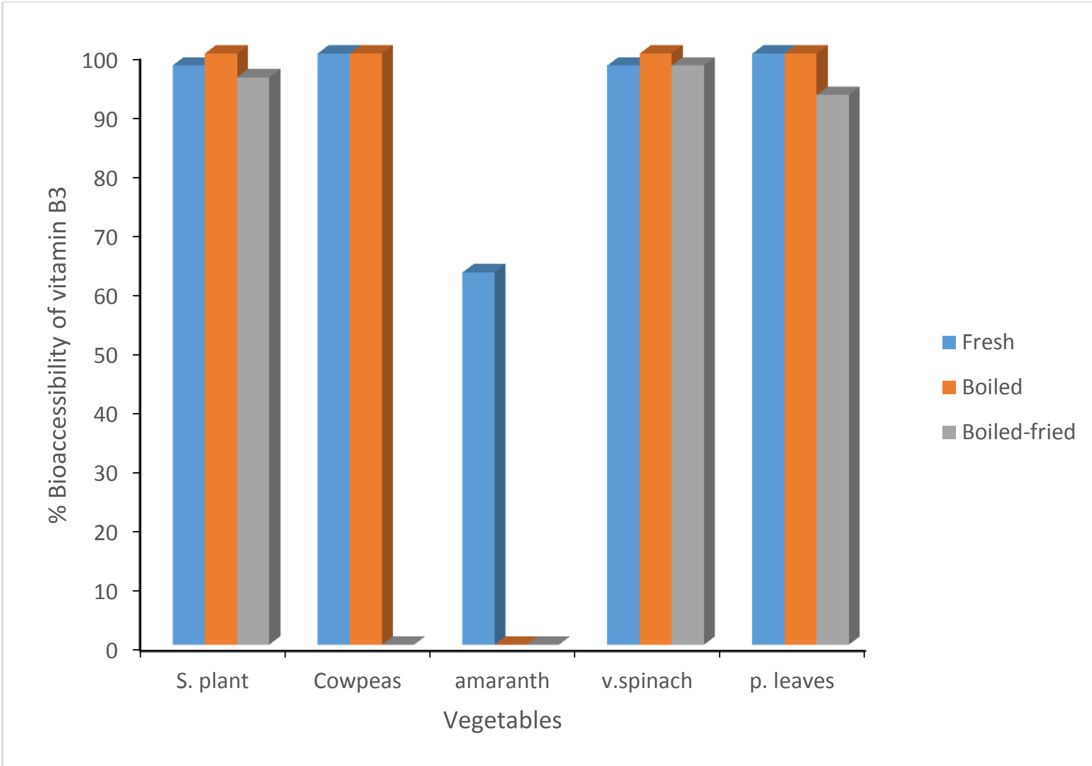

Figure 4. 6: Percentage bioaccessibility of vitamin B_3 in fresh and processed vegetables

No particular trend was observed in the bioaccessibility of the vitamin. While boiling increased bioaccessibility in spider plant and vine spinach, no change was observed in cowpeas and pumpkin leaves (Figure 4.6). The variations in bioaccessibility may be attributed to the food matrix (Hotz and Gibson, 2007; Parada and Aguilera, 2007) as well as the digestibility of the vegetables (Parada and Aguilera, 2007). The chemistry of the vitamin is also a contributing factor to the observation made. Niacin is known to be very sensitive to light (Schellack *et al.*, 2015) and easily extracts into the cooking water being a water soluble vitamin (Serafini *et al.*, 2002).

4.5.5 Pantothenic acid (B_5)

It is clear from Table 4.4 that the percentage bioaccessibility of vitamin B_5 varied from 4.38± 0.20 % (spider plant) to 34.52±0.77 % (amaranth) in fresh vegetables, 5.18±0.87 % (cowpeas) to 37.35±3.68 % (spider plant) in the boiled and 14.23±3.69 % (vine spinach) to 55.58±4.13 % (pumpkin leaves) in the boiled-fried, Table 4.4. The increases recorded ranged between 11% and 50 % while reductions ranged between 6 % and 16 % (Appendix Table A 2).

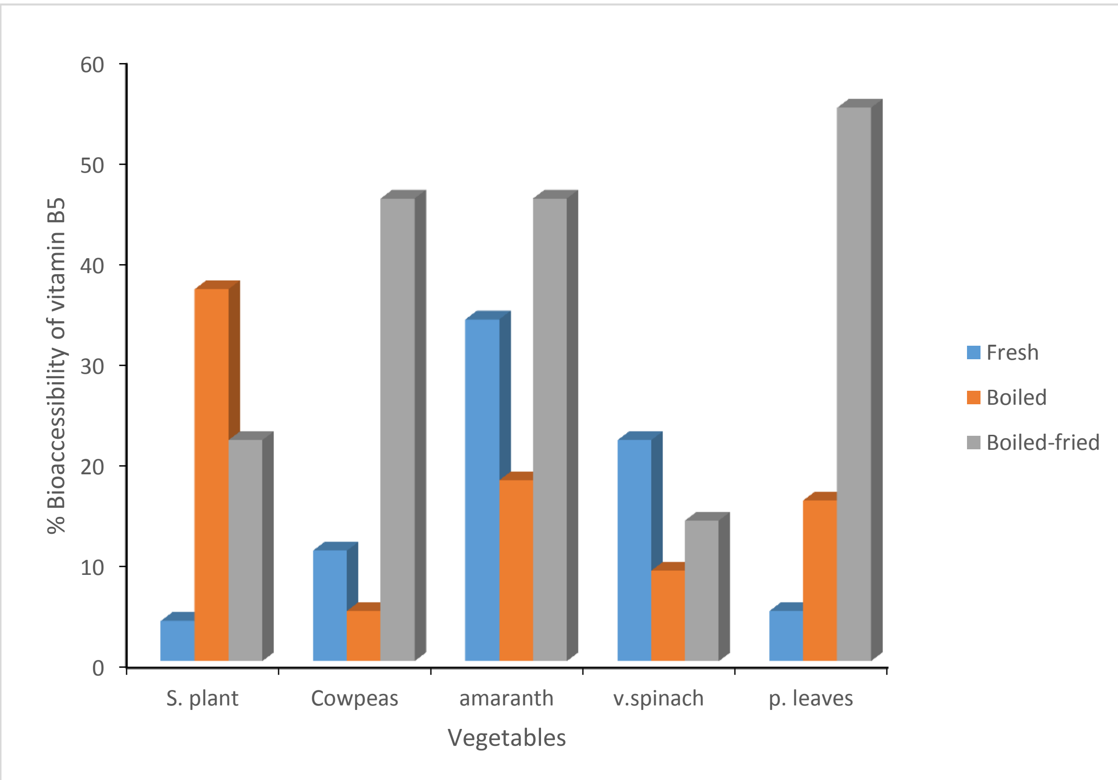

Figure 4. 7: Percentage bioaccessibility of vitamin B_5 in fresh and processed vegetables

The variations in percentage bioaccessibility are shown in figure 4.7. The bioaccessibility observed may be attributed to pectin in the dietary fiber which may have decreased bioaccessibility of vitamin (Brownlee *et al.*, 2006; Parafox-Carlos *et al*., 2011). The difference in digestibility of the vegetables is the other factor attributed to the variation (Parada and Aguilera, 2007; Etcheverry *et al.*, 2012) as well as the alteration of matrices of the vegetables (Nagao, 2014).

4.5.6 Pyridoxine (B_6)

It is clear from Table 4.4 that the percentage bioaccessibility of pyridoxine varied from 59.45±0.22 % (pumpkin leaves) to 90.63±0.83 % (amaranth) in the fresh vegetables, 12.30±3.3 % (spider plant) to 100.00.0.00 % (cowpeas and pumpkin leaves) in the boiled vegetables and 39.78±4.74 % (spider plant) to 98.74±0.02 % (cowpeas) in the boiled-fried vegetables. Increases recorded ranged between 5 % and 82 % while reductions ranged between 15% and 77 % (Appendix Table A 2).

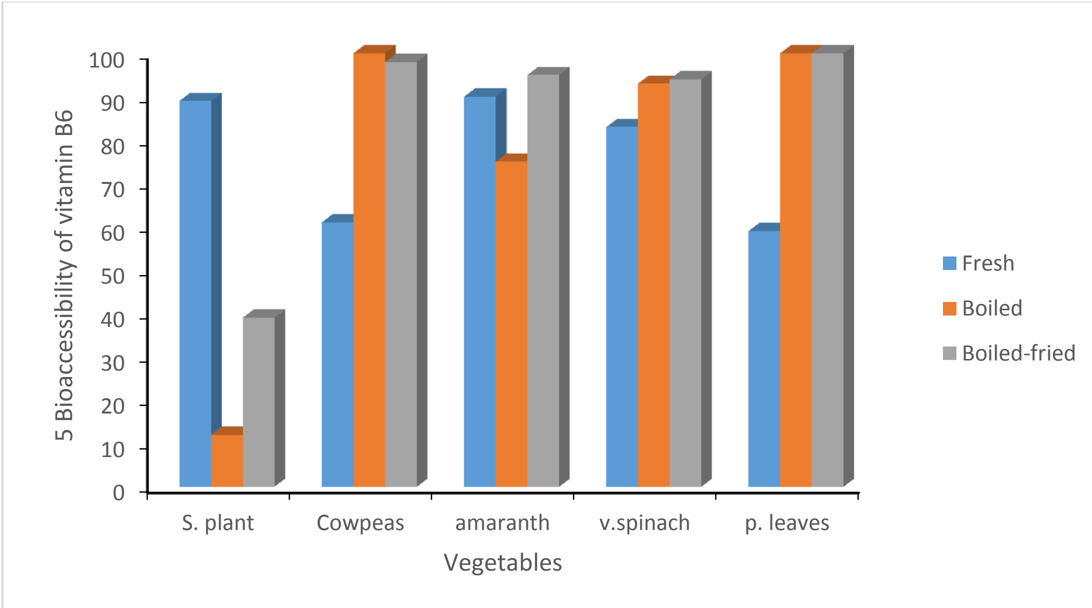

Figure 4. 8: Percentage bioaccessibility of vitamin B_6 in fresh and processed vegetables

The variations in percentage bioaccessibility are shown in figure 4.8. No particular trend observed and the variations in percentage bioaccessibility may be due to the different matrices in the vegetables (Nagao, 2014). Cooking releases pyridoxine from the food matrix during (Hotz and Gibson, 2007) while some may be destroyed during the process (Bua and Onang, 2017). Further, the protein bound form of pyridoxine has low bioaccessibility (Hotz and Gibson, 2007).

4.5.7 Folate (B_9)

The percentage bioaccessibility of folate ranged from 61.80± 2.81 % (amaranth) to 100.00±0.00 % (spider plant, cowpeas and vine spinach) in the fresh vegetables, 55.04±1.28 % (spider plant) to 100.00±0.00 % (amaranth) in the boiled vegetables and

55.80±0.01 % (spider plant) to 100.00±0.00 % (cowpeas) in the boiled fried vegetables, Table 4.4. The increases recorded ranged between 37 % and 45 % while reductions ranged between 5 % and 34 % (Appendix Table A 2). No change in percentage bioaccessibility was observed when vegetable cowpeas was boiled-fried (Appendix Table A 2). The variations in percentage bioaccessibility are shown in figure 4.8.

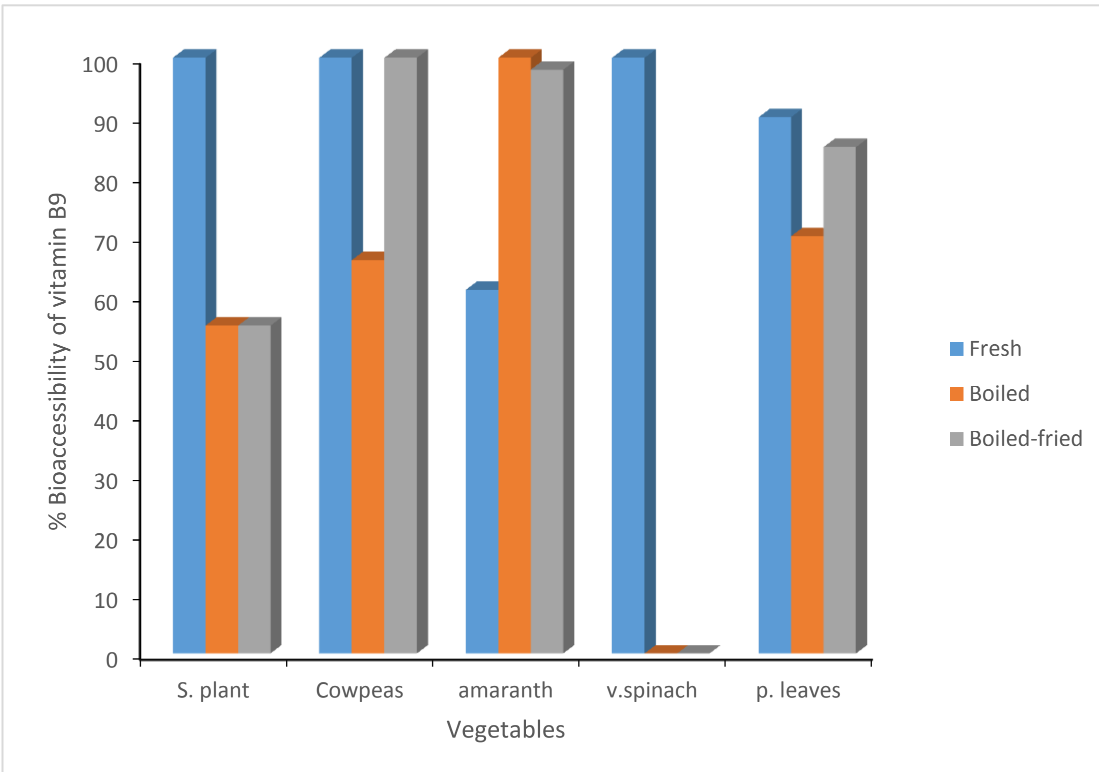

Figure 4. 9: Percentage bioaccessibility of vitamin B_9 in fresh and processed vegetables

Apart from different matrices in the vegetables (Nagao, 2014) the variation in bioaccessibility may be attributed to the sources of the vegetables, the methods of processing as well as physicochemical properties of the vitamin (Yonekura and Nagao, 2007; Failla *et al.*, 2008). Further, the digestibility of the vegetables differ depending on the fiber content (Parada and Aguilera, 2007; Etcheverry *et al*., 2012). Dietary fiber lowers bioaccessibility by physically entrapping nutrients and enhancing viscosity of gastric fluids thereby restricting the mixing process (Montagne *et al.,* 2003; Palafox-Carlos *et al.,* 2011).

4.6: Mean levels of beta-carotene and vitamin B series in recipes of LAIVs

The mean levels of beta-carotene, vitamin B_1, B_2, B_3, B_5, B_6 and B_9 in recipes of LAIVs mixtures; spider plant + amaranth (SP+A), cowpeas + amaranth (C+A) and pumpkin leaves + vine spinach (PL+VS) are presented in Table 4.5

Table 4. 5: Mean levels of beta-carotene and vitamin B series in recipes of LAIVs

		Mean levels of beta-carotene and vitamin B series, Mean ± SD mg/100g DW, n=3						
LAIVs	Recipes	BC	B_1	B_2	B_3	B_5	B_6	B_9
SP+A	UNC	20.07±0.03[b]	3.51±0.18[a]	100.50±0.17[c]	54.99±6.68[b]	8.89±0.73[c]	90.08±1.68[c]	1.23±0.06[a]
	RCP1	40.76±0.03[c]	6.41±0.26[b]	33.69±3.20[b]	60.72±0.85[b]	5.90±0.21[b]	56.29±2.22[a]	4.87±0.04[c]
	RCP2	3.69±0.02[a]	8.79±0.12[c]	3.02±0.08[a]	19.97±0.01[a]	2.60±0.13[a]	69.93±0.95[b]	2.03±0.08[b]
	p-value	≤0.001	≤0.001	≤0.001	≤0.001	≤0.001	≤0.049	≤0.001
C+A	UNC	24.16±0.52[b]	3.34±0.24[b]	71.12±0.81[b]	72.77±4.50[b]	90.35±4.03[c]	74.15±1.47[a]	11.54±0.55[b]
	RCP1	33.40±0.90[c]	3.61±0.30[b]	3.37±0.81[b]	40.91±3.74[a]	13.15±0.63[a]	81.64±0.39[b]	5.04±0.22[a]
	RCP2	6.73±0.02[a]	2.29±0.14[a]	4.10±0.03[a]	ND	30.86±0.10[b]	92.54±0.84[c]	4.54±2.20[a]
	p-value	≤0.001	≤0.001	≤0.001	≤0.001	≤0.001	≤0.001	≤0.001
VS+PL	UNC	12.62±0.03[b]	1.96±0.04[a]	99.98±0.77[c]	30.10±1.53[a]	12.08±0.32[a]	68.37±1.47[a]	11.54±0.55[c]
	RCP1	18.07±0.77[c]	2.44±0.09[b]	1.19±0.06[a]	63.37±5.76[b]	16.93±1.87[b]	90.07±2.23[b]	0.69±0.02[a]
	RCP2	10.25±0.58[a]	4.66±0.39[c]	9.46±0.25[b]	31.21±1.12[a]	25.59±1.41[c]	92.18±0.63[c]	1.77±0.11[b]
	p-value	≤0.001	≤0.001	≤0.001	≤0.070	≤0.001	≤0.001	≤0.001

Mean values of the same vegetable within the same column followed by the same letters are not significantly different (SNK, α=0.05)

The recipes were prepared from mixtures of two vegetables each; spider plant + amaranth (SP + A), cowpeas + amaranth (C + A) and vine spinach + pumpkin leaves (VS + PL). The vegetables were mixed such that one vegetable had higher fiber content while the other had lower. The mixtures of vegetables were analyzed uncooked (UNC), boiled (RCP1) and boiled-fried (RCP2).

4.6.1 Uncooked vegetables

The uncooked mixtures of vegetables contained mean levels (mg/100g) of beta-carotene (BC) in the range of 12.62±0.03 (VS+PL) to 24.16±0.52 (C+A), thiamin (B_1) that ranged from 1.96±0.04 (VS+PL) to 3.51±0.18 (SP+A), riboflavin in the range of 71.12±0.81 (C+A) to 100.50±0.17 (SP+A), niacin (B_3) in the range of 30.10±1.53 (VS+PL) to 72.77±4.50 (C+A), pantothenic acid (B_5) in the range of 8.89±0.73 (SP+A) to 90.35±4.03 (C+A), pyridoxine (B_6) in the range of 68.37±1.47 (VS+PL) to 90.08±1.68 (SP+A) folate (B_9) in the range of 1.23±0.06 (SP+A) to 11.54±0.55 (VS+PL), Table 4.5.

There was no particular trend in the levels of the analyzed nutrients in the individual vegetables and the mixtures of vegetables used in the preparation of recipes. For example, both spider plant and amaranth recorded higher mean levels (mg/100g, DW) of BC individually, 27.79±1.01 and 29.99±0.51 respectively (Table 4.2), than in the mixture SP+A, 20.07±0.03 (Table 4.5). While cowpeas recorded lower mean levels, 23.06±0.51, as an individual vegetable, amaranth recorded higher levels, 29.99±0.51 (Table 4.2), than what was recorded in the mixture C+A, 24.16±0.52 (Table 4.5). Vine spinach on the other hand recorded lower mean levels individually, 8.57±0.50 while amaranth recorded higher levels, 20.14±2.26 (Table 4.2), than what was recorded when the two vegetables were mixed (VS+PL), 12.62±0.03 (Table 4.5). The variations observed may be attributed to the differences in fiber content in the vegetables (Aschoff *et al.*, 2014).

4.6.2 Recipe 1 (boiled)

In RCP1 the mean levels of BC ranged from 18.07±0.77 (VS+PL) to 40.76±1.66 (SP+A), thiamin 2.44±0.09 (VS+PL) to 6.41±0.26 (SP+A), riboflavin 1.19±0.06 (VS+PL) to 33.69±3.20 (SP+A), niacin 40.91±3.74 (C+A) to 63.37±5.76 (VS+PL), pantothenic acid 5.90±0.21 (SP+A) to 16.93±1.87 (VS+PL), pyridoxine 56.29±2.22 (SP+A) to 90.07±2.23 (VS+PL) and folate 0.69±0.02 (VS+PL) to 5.04±0.22 (C+A), Table 4.5.

A significant ($p<0.001$) increase in the nutrient BC ranging from 8.08 % to 103.09 % was observed on preparing RCP1 (Appendix Table A 3), the levels of BC in all the vegetable mixtures. Preparation of RCP1 releases BC from the food matrix by breaking the cell wall and dissociating carotene-food matrix complexes explaining the significant increase between RCP1 and uncooked vegetables. The preparation of recipes involved heating which breaks the plant cell wall releasing BC from the food matrix thus explaining the increase (Hotz and Gibson, 2007). Increases in thiamin levels ranged between 8. % and 82.91 % were observed on preparation of RCP1 (Appendix Table A 3). The increases may be attributed to heating in the process of cooking which disrupts the cell walls of the vegetable tissues, dissociates the nutrient-matrix complexes or transforms the vitamin into more active molecular structures (Parada and Aguilera, 2007). For riboflavin, significant ($p<0.001$) reductions ranging from 66.48 % to 98.81 % were observed in

RCP1 (Appendix Table A 3). Among the factors that contribute to the reduction of the levels of the vitamin is extraction into the cooking fluid, being water soluble (Bua and Onago, 2017). Although riboflavin is stable even when heat processed and during normal cooking, some is lost due to the effect of light (Schellack *et al*., 2015).

It was observed that the levels of niacin in RCP1 increased by 10.42 % (SP+A) and 110.53 % (VS+PL). In the mixture C+A, however, the levels reduced by 43.78 % (Appendix Table A 3). Apart from niacin being very sensitive to light (Schellack *et al.*, 2015), it extracts into water during cooking (Serafini *et al.,* 2002; Bua and Onang, 2017). The release of the vitamin from the food matrix during the preparation of RCP1 explains the increases in its levels (Hotz and Gibson, 2007). It was observed that preparing RCP1 of the vegetables had both effects that resulted to significant ($p<0.001$) increase or decrease on the pantothenic acid. The increase was recorded in VS+PL (40.15 %) while the decreases were recorded in SP+A (33.63 %) and C+A (85.45 %), Appendix Table A 3. While the increase is mainly due to the fact that pantothenic acid is readily released from its protein matrix during cooking (Hotz and Gibson 2007; Nagao *et al.,* 2014) the reduction is attributed to the sensitivity of pantothenic acid to heat, it gets decomposed by heat during cooking (Hotz and Gibson, 2007; Fukawatari and Shibata, 2008; Ismail *et al.*, 2013).

It was noted that, both significant ($p<0.001$) increase and decrease in levels of the pyridoxine were observed in RCP1. On preparation of RCP1, the levels of pyridoxine increased in C+A (10.10 %) and VS+PL (34.74 %) while the levels decreased in SP+A (37.15 %), Appendix Table A 3. This observations may be due to the fact the preparation of the recipes led to the release of the vitamin from the plant matrix (Hotz and Gibson, 2007). Apart from pyridoxine getting destroyed during cooking, it extracts into the cooking water (Bua and Onang, 2007; Schellack *et al.,* 2015). The levels of the folate in SP+A increased by 295.93 % when RCP1 was prepared while in C+A and VS+PL, the levels decreased by 56.32 and 93.94 % (Appendix Table A 3). While the vitamin extracts into the cooking liquid, water (Bua and Onang, 2007), it may also get lost during cooking (Bua and Onang, 2017; Agyemang-Yeboah and Oppong, 2013). The observations are further supported by the fact that folate is sensitive to light (Fukawatari and Shibata, 2008) and its activity may be lost by oxidative cleaving resulting in a pterin and para-aminobenzoylglutamate (PABG) molecule. The presence of oxygen or changes in pH may also lead to interconversion of folate (Öhrvik, 2009).

4.6.3 Recipe 2 (boiled-fried)

In RCP2 the mean levels of BC were found to range from 3.63±0.02 (SP+A) to 10.23±0.57 (VS+PL), thiamin 2.29±0.14 (C+A) to 8.79±0.12 (SP+A), riboflavin 3.02±0.08 (SP+A) to 9.46±0.23 (VS+PL), niacin 19.97±1.01 (SP+A) to 31.21±1.12 (VS+PL), pantothenic acid 2.60±0.13 (SP+A) to 30.86±0.10 (C+A), pyridoxine 69.93±0.95 (SP+A) to 92.54±0.84 (C+A) and folate 1.77±0.11 (VS+PL) to 4.54±2.20 (C+A), Table 4.5.

A decrease in levels of BC was observed in all the mixtures of vegetables that ranged from 18.78 % to 81.61 % (Appendix Table A 3) The decrease in the levels of BC with preparing RCP2 may be attributed to BC's biological activity in the presence of oxygen and light (Rodriguez-Amaya and Kimura, 2004; Bernhardt and Schlich, 2006; Ahamed *et al.*, 2007). The decrease may be due to destruction by oxidation during cooking as well as isomerization of BC from the naturally occurring *trans*-isomer to *cis* form which are less bio accessible (Bernhardt and Schlich, 2006).

It was observed that on preparing RCP2, the mean levels of thiamin in the mixtures of vegetables increased S.P+A (156.13 %) and VS+PL (137.76 %), Appendix Table A 3. The increases are mainly due to the vitamin being readily released from its protein matrix during cooking (Hotz and Gibson 2007). In the mixture of vegetables VS +PL (RCP2), however, thiamin levels reduced (31.44 %). This is explained by the fact that thiamin is very sensitive to heat and oxidation. Reductions of the riboflavin ranging from 90.54 % to 97.00 % were observed in RCP2 (Appendix Table A 3). Although this can be explained by the fact that the vitamin extracts into the cooking water (Bua and Onago, 2017) it may as well be lost due to the effect of light (Schellack *et al*., 2015).

While it was noted that preparation of RCP2 led to an increase in the levels of niacin in VS+PL (3.69 %), in the mixtures SP+A and C+A the levels decrease (63.68 % and 100.00 % respectively), Appendix Table A 3. During the preparation of RCP2 the vitamin is released from the food matrix increasing its levels (Hotz and Gibson, 2007). The difference in matrices of the vegetables explain the variation in the mean levels between the individual vegetables (Table 4.2) and the mixture of vegetables (Table 4.5). Preparation of RCP2 resulted to both significant ($p<0.001$) increase and decrease in the levels of pantothenic acid. The increase was recorded in VS+PL (109.35 %) while the decreases were recorded in SP+A (70.75 %) and C+A (65.84 %), Appendix Table A 3.

Pantothenic acid is readily released from its protein matrix during cooking explaining the increase (Hotz and Gibson 2007) the reduction is attributed to decomposition by heat during cooking (Hotz and Gibson, 2007; Fukawatari and Shibata, 2008; Ismail *et al.*, 2013).

It was noted that, both significant ($p<0.001$) increase and decrease in levels of the pyridoxine were observed in RCP2. On preparation of RCP2, the levels of pyridoxine increased in C+A (24.80 %) and VS+PL (34.82 %) while the levels decreased in SP+A (22.36 %), Appendix Table A 3. The increases may be due to the fact the cooking breaks the food matrix releasing the vitamin (Hotz and Gibson, 2007), while the reduction may be due to destruction during cooking (Bua and Onang, 2007; Schellack *et al.*, 2015). It was observed that preparing RCP2 led to increase of the folate in S.P+A (65.04 %). For V+A and VS+PL, the decreases were 60.66 % and 84.66 % respectively (Appendix Table A 3). This may be explained by the fact that folate gets lost during cooking (Bua and Onang, 2017; Agyemang-Yeboah and Oppong, 2013). Folate is also sensitive to light (Fukawatari and Shibata, 2008) and the presence of oxygen as well as changes in pH may lead to interconversion of folate (Öhrvik, 2009).

4.7 Mean bioaccessible levels of beta-carotene and vitamin B series in recipes

The mean bioaccessible levels of beta-carotene, vitamin B_1, B_2, B_3, B_5, B_6 and B_9 of recipes of LAIVs; SP+A, C+A and PL+VS are presented in Table 4.6.

Table 4. 6: Mean bioaccessible levels of beta-carotene and vitamin B series in recipes of LAIVs

Mean bioaccessible levels of beta-carotene and vitamin B series Mean ± SD mg/100g DW, n=3								
LAIVs	Recipe	BC	B_1	B_2	B_3	B_5	B_6	B_9
S.P+A	UNC	16.54±0.05[b]	1.39±0.21[a]	99.43±0.15[c]	42.43±6.99[c]	2.18±0.55[b]	83.38±1.49[b]	1.03±0.14[a]
	RCP1	34.85±0.03[c]	1.46±0.19[b]	28.38±2.20[b]	30.65±1.86[b]	2.05±0.05[b]	49.44±1.12[a]	4.36±0.55[c]
	RCP2	0.93±0.04[a]	4.58±0.12[c]	0.58±0.07[a]	9.21±0.91[a]	0.15±0.03[a]	46.03±1.17[a]	1.81±0.25[b]
	p-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.007	0.001
C+A	UNC	18.87±0.43[b]	1.56±0.24[b]	2.38±0.37[a]	57.69±4.81[b]	17.73±0.41[c]	6.53±0.89[a]	9.23±0.61[b]
	RCP1	29.72±0.76[c]	0.72±0.15[a]	2.75±0.02[b]	17.50±2.91[a]	0.63±0.10[a]	68.40±0.80[b]	4.27±0.21[a]
	RCP2	1.38±0.09[a]	1.09±0.20[a]	2.92±0.04[b]	ND	8.87±0.48[b]	70.56±0.46[c]	4.02±2.13[a]
	p-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
V.S+P.L	UNC	9.40±0.02[b]	0.39±0.29[a]	98.63±0.33[c]	24.40±1.39[a]	0.86±0.67[a]	57.15±1.71[a]	9.23±0.61[b]
	RCP1	12.49±0.77[c]	0.82±0.22[a]	0.74±0.04[a]	63.37±5.76[b]	3.59±0.70[b]	76.73±1.05[b]	0.22±0.01[a]
	RCP2	1.82±0.79[a]	2.38±0.30[b]	4.99±0.23[b]	28.89±1.18[a]	8.65±1.55[c]	75.28±0.75[b]	0.93±0.11[a]
	p-value	≤0.001	≤0.001	≤0.001	≤0.070	≤0.001	≤0.001	≤0.001

Mean values of the same vegetable within the same column followed by the same letters are not significantly different (SNK, α=0.05).

Key: UNC=Uncooked, RCP1=Recipe 1 and RCP2=Recipe 2

Recipe 1 - boiled only

Recipe 2 - boiled and fried

4.7.1 Uncooked vegetables

The mean bioaccessible levels (mg/100g) of BC were found to range from 9.40±0.02 (VS+PL) to 18.87±0.43 (C+A), thiamin (B_1) 0.39±0.29 (VS+PL) to 1.56±0.24 (C+A), riboflavin (B_2) were; in UNC 2.38±0.37 (C+A) to 99.43±0.15 (SP+A), niacin (B_3) 24.40±1.39 (VS+PL) to 57.69±4.81 (C+A), pantothenic acid (B_5) 0.86±0.67 (VS+PL) to 17.73±0.41 (C+A), pyridoxine (B_6) 6.53±0.89 (C+A) to 83.38±1.49 (SP+A) and folate (B_9) 1.03±0.14 (SP+A) to 9.23±0.61 (C+A and VS+PL), Table 4.6.

There was no particular trend observed in the bioaccessible mean levels of BC and vitamin B series in the mixtures of vegetables (Table 4.6). The variation may be attributed to the difference in the food matrix of the vegetables (Hotz and Gibson, 2007) as well as the chemistry of the vitamin. Vitamin B series for example are water soluble and may be lost even when vegetables are washed (Ismail *et al.*, 2013). Others like riboflavin and folate are known to be light sensitive (Hotz and Gibson, 2007). The difference in the microstructure of different vegetables (Parada and Aguilera, 2007), digestibility which differs depending on the type of vegetable (Parada and Aguilera, 2007; Etcheverry *et al*., 2012) as well as variation in dietary fiber (Aschoff *et al.*, 2014) contributes to the variation.

4.7.2 Recipe 1

The mean bioaccessible levels of BC ranged from 12.49±0.77 (VS+PL) to 34.85±1.41 (SP+A), thiamin (B_1) 0.72±0.15 (C+A) to 1.46±0.19 (SP+A), riboflavin (B_2) 1 0.74±0.04 (VS+PL) to 28.38±2.20 (SP+A), niacin 17.50±2.91 (C+A) to 63.37±5.76 (VS+PL), Pantothenic acid 0.63±0.10 (C+A) to 3.57±0.70 (VS+PL), Pyridoxine 49.44±1.12

(SP+A) to 68.40±0.80 (C+A) and folate 0.22±0.01 (VS+PL) to 4.36±0.55 (SP+A), Table 4.6.

The mean bioaccessible levels of BC in all the mixtures of vegetables increased on preparing RCP1. This may be attributed to the release of BC from the food matrix (Hotz and Gibson, 2007). Preparation of RCP1 decreased the bioaccessible mean levels of thiamin in C+A while the same process caused an increase in SP+A and VS+PL (Table 4.6). Vitamin B are also lost by cooking methods that use water (Ismail *et al.*, 2013). The increases are mainly due to the vitamin being readily released from its protein matrix during cooking (Hotz and Gibson, 2007). For riboflavin, mean bioaccessible levels decreased in all the mixtures of vegetables (Table 4.6). Although riboflavin is known to be stable during normal cooking of vegetables, it is lost in the presence of light (Schellack *et al*., 2015) and when cooked by methods that use water by leaching (Ismail *et al.*, 2013). The ease with which vegetables are digested mainly contribute to the variation in bioaccessibility of the vitamin (Parada and Aguilera *et al*., 2007; Etcheverry *et al*., 2012).

Preparation of RCP1 decreased the mean bioaccessible levels of niacin in SP+A and C+A while the same process caused an increase in VS+PL (Table 4.6). The increase in the mean bioaccessible levels of niacin is explained by the fact that it is released from the food matrix during recipe preparation as it involves heat processing (Hotz and Gibson, 2007) as well as hydrolysis of nicotinamide adenine dinucleotide phosphate, NAD (P), to nicotinic acid during cooking (Fukawatari and Shibata, 2008). On preparing of RCP1 the mean bioaccessible levels of pantothenic acid decreased in SP+A and C+A while the same processes caused an increase in VS+PL (Table 4.6). The variation is attributed to the difference in the alteration of matrices of the vegetables (Nagao, 2014).

The mean bioaccessible levels of pyridoxine in SP+A decreased when RCP1 was prepared. The same process, however, caused an increase in C+A and VS+PL (Table 4.6). While preparing RCP1 releases pyridoxine from the food matrix, which differs depending on the vegetable, during cooking (Hotz and Gibson, 2007), the vitamin may be destroyed during the process (Bua and Onang, 2017), pyridoxine is decomposed by heat during cooking (Uraku *et al.,* 2015). Recipe 1 (RCP1) recorded decreased mean bioaccessible levels of folate in C+A and VS+PL while the same processes caused an increase in SP+A (Table 4.6). The variations are supported by the fact that folate is destroyed by oxidation, light sensitive and lost during cooking (Fukawatari and Shibata, 2008). Folates in food may also undergo interconversion due to the presence of oxygen as well as changes in pH (Öhrvid, 2009).

4.7.3 Recipe 2

The mean bioaccessible levels of beta-carotene were found to range from 0.93±0.04 SP+A) to 1.82±0.79 (VS+ PL), thiamin (B_1)1.09±0.20 (C+A) to 4.58±0.12 (SP+A), riboflavin (B2) (B_2) 0.58±0.07 (SP+A) to 4.99±0.23 (VS+PL), niacin (B_3) 9.21±0.91 (SP+A) to 28.89±1.18 (VS+PL), Pantothenic acid (B_5) 0.15±00.03 (SP+A) to 8.87±0.48 (C+A), Pyridoxine (B_6) 46.03±1.17 (SP+A) to 75.28±0.75 (VS+PL) and folate (B_9) 0.93±0.11 (VS+PL) to 4.02±2.13 (C+A), Table 4.6.

When RCP2 was prepared, thiamin increased in SP+A and VS+PL while it reduced in C+A (Table 4.6). Thiamin is known to be very sensitive to heat and oxidation (Hotz and Gibson, 2007; Ismail *et al.*, 2013; Terefe *et al.*, 2014; Bua and Onang, 2017). On preparing RCP2, the mean bioaccessible levels of riboflavin in all the mixtures of vegetables reduced (Table 4.6). Riboflavin is known to be lost in the presence of light (Schellack *et al.*, 2015) and when cooked by methods that use water by leaching (Ismail *et al.*, 2013). The variation in bioaccessibility of the vitamin is also influenced by the ease with which the vegetables are digested (Parada and Aguilera *et al.*, 2007; Etcheverry *et al.*, 2012).

When RCP2 was prepared, the mean bioaccessible levels of niacin increased in VS+PL while a reduction was observed in SP+A. In C+A the vitamin was reduced to no detection (Table 4.6). The increases in the mean bioaccessible levels are attributed to the release of niacin from the food matrix during recipe preparation (Hotz and Gibson, 2007). Hydrolysis of nicotinamide adenine dinucleotide phosphate, NAD (P), to nicotinic acid during cooking (Fukawatari and Shibata, 2008), further increases the mean bioaccessible levels. Preparation of RCP2 decreased the mean bioaccessible levels of pantothenic acid in SP+A and C+A while the same processes caused an increase in VS+PL (Table 4.6). It is known that processing of food alters the food matrix (Nagao, 2014). The difference in the alteration of matrices of the vegetables explains the variation in the bioaccessible levels (Nagao, 2014).

The mean bioaccessible levels of pyridoxine in SP+A decreased on preparing RCP2. The same process, however, caused an increase in C+A and VS+PL (Table 4.6). Preparing recipes resulted in both increase and decrease in bioaccessibility. The process of preparing RCP2 releases pyridoxine from the food matrix, the release is different depending on the type of vegetable (Hotz and Gibson, 2007). The vitamin may be destroyed during the process (Bua and Onang, 2017), pyridoxine is decomposed by heat during cooking (Uraku *et al.*, 2015). Recipe 2 recorded decreased mean bioaccessible levels of folate in C+A and VS+PL while the same processes caused an increase in SP+A (Table 4.6). The variations are attributed to the destruction of folate by oxidation, light sensitivity and loss during cooking (Fukawatari and Shibata, 2008). It is also known that folates in food are prone to interconversion in the presence of oxygen as well as changes in pH (Öhrvid, 2009).

4.8: Mean percentage bioaccessibility of beta-carotene and vitamin B series in recipes

The mean percentage bioaccessibility of beta-carotene, vitamin B_1, B_2, B_3, B_5, B_6 and B_9 of recipes of LAIVs; SP+A, C+A and PL+VS are presented in Table 4.7 and shown in figures 4.10 to 4.12.

Table 4. 7: Mean percentage bioaccessibility of BC and vitamin B in recipes of mixtures of LAIVs

Percentage bioaccessibility of beta-carotene and vitamin B series in Mean ± SD mg/100g DW, n=3								
LAIVs	Recipes	BC	B_1	B_2	B_3	B_5	B_6	B_9
S.P+A	UNC	82.40±0.26[b]	39.58±3.94[b]	98.94±0.02[c]	97.88±3.61[b]	24.34±4.08[b]	92.56±0.08[b]	78.75±0.55[a]
	RCP1	85.48±0.05[c]	23.66±1.95[a]	82.91±2.59[b]	50.47±2.56[a]	34.86±0.47[c]	88.81±3.76[b]	79.29±1.19[a]
	RCP2	25.30±1.05[a]	88.22±1.49[c]	19.07±0.97[a]	46.06±2.36[a]	5.57±1.18[a]	65.81±1.14[a]	78.25±0.39[a]
	p-value	≤0.001	≤0.001	≤0.001	≤0.001	≤0.001	≤0.049	≤0.337
C+A	UNC	78.13±0.11[b]	46.70±4.48[b]	4.41±0.24[a]	79.22±1.40[b]	19.67±1.33[b]	8.80±0.10[a]	79.89±1.49[a]
	RCP1	88.97±0.22[c]	19.97±3.57[a]	77.09±4.24[c]	42.64±3.82[a]	4.77±0.53[a]	83.81±0.61[c]	84.70±0.67[a]
	RCP2	20.57±1.34[a]	47.52±6.09[b]	70.76±0.24[b]	ND	28.79±1.47[c]	76.25±0.20[b]	86.33±7.24[a]
	p-value	≤0.001	≤0.001	≤0.001	≤0.001	≤0.001	≤0.001	≤0.239
V.S+P.L	UNC	74.56±0.05[b]	8.98±2.40[a]	98.30±0.47[c]	81.05±0.54[a]	7.05±0.57[a]	83.58±0.19[b]	79.89±1.48[c]
	RCP1	69.09±1.29[b]	26.86±4.70[b]	62.35±1.04[b]	100.00±0.00[c]	21.123±2.08[b]	85.21±0.97b[b]	32.21±2.53[a]
	RCP2	17.45±6.47[a]	51.15±2.73[c]	52.18±1.12[a]	92.57±0.62[b]	33.82±1.69[c]	81.67±0.73[a]	52.17±3.05[b]
	p-value	≤0.001	≤0.001	≤0.001	≤0.070	≤0.001	≤0.001	≤0.001

Mean values of the same vegetable within the same column followed by the same letters are not significantly different (SNK, α=0.05).

Key: UNC=Uncooked, RCP1=Recipe 1 and RCP2=Recipe 2

4.8.1 Uncooked vegetables

Percentage bioaccessibility of BC was found to range from 74.56±0.05 % V.S+P.L) to 82.40±0.26 % (SP+A), thiamin 8.98±2.40 % (VS+PL) to 46.70±4.48 % (C+A), riboflavin 4.41±0.24 % (C+A) to 98.94±0.02 % (SP+A), niacin 79.22±1.40 % (C+A) to 97.88±3.61 % (SP+A), pantothenic acid 7.05±0.57 % (VS+PL) to 24.34±4.08 % (SP+A), pyridoxine, 8.80±0.10 % (C+A) to 92.56±0.08 % (SP+A) and folate 78.75±0.55 (C+A) to 79.89±0.49 (VS+PL), Table 4.7. The comparisons of percentage bioaccessibility of beta carotene and vitamin B series in the mixtures of vegetables are shown in figure 4.10.

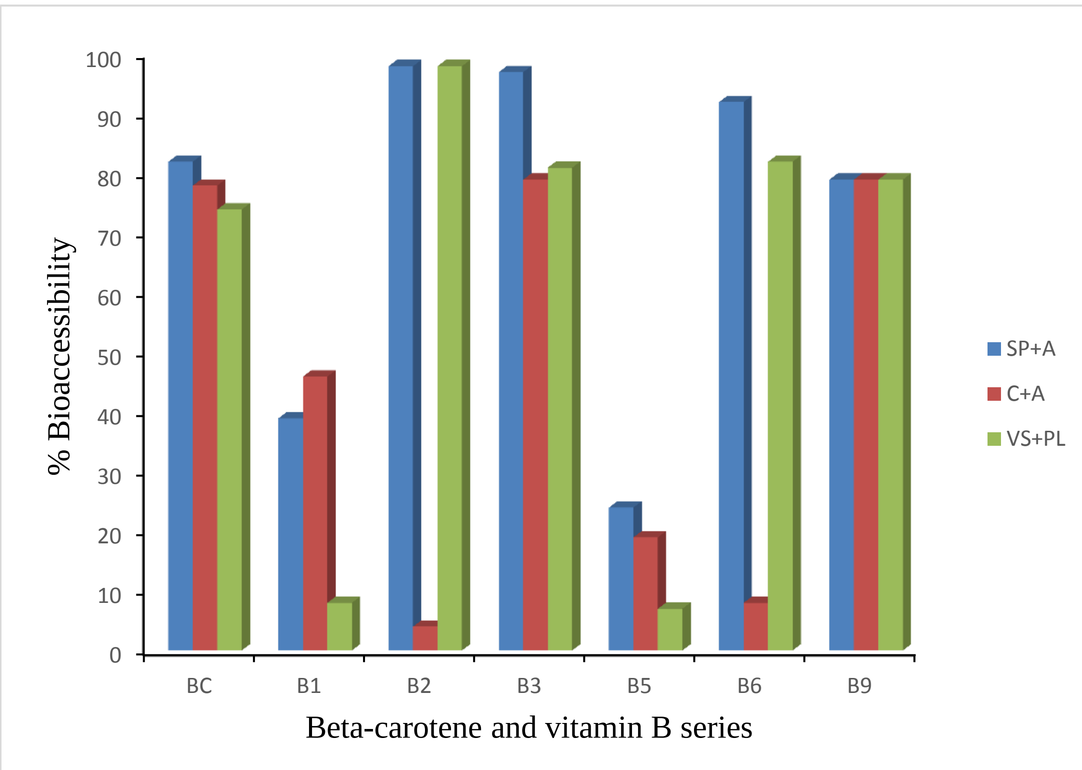

Figure 4. 10: percentage bioaccessibility of beta-carotene and vitamin B series from uncooked vegetables

No particular trend in percentage bioaccessibility of beta-carotene and vitamin B series was observed among the mixtures of vegetables analyzed. Variations were noted which may be due to the fact that food matrices differ in different vegetables (Hotz and Gibson, 2007). The variation in dietary fiber as well affects bioaccessibility, improved bioaccesibility has been reported with diminished fiber content (Aschoff *et al.*, 2014). This is further supported by the fact that there is a variation in the digestibility of the vegetables depending on the type (Parada and Aguilera, 2007; Etcheverry *et al*., 2012). The other determinant is the nature of the vitamins. The B vitamins for instance are water soluble and easily lost during washing the vegetables lowering the levels eventually available for absorption (Ismail *et al.,* 2013).

4.8.2 Recipe 1

Percentage bioaccessibility of BC ranged from 69.09±1.29 % V.S+P.L) to 88.97±0.22 % (C+A), thiamin (B_1) 19.97±3.57 % (C+A) to 26.86±4.70 % (VS+PL), riboflavin (B_2) 62.35±1.04 % (VS+PL) to 82.91±2.59 % (SP+A), niacin (B_3) 42.64±3.82 % (C+A) to 100.00±0.00 % (VS+PL), pantothenic acid (B_5) 4.77±0.53 % (C+A) to 34.86±0.47 % (SP+A), pyridoxine B_6) 83.81±0.61 % (C+A) to 88.81±3.76 % (SP+A) and folate (B_9) 32.21±2.53 (VS+PL) to 84.70±0.67 (C+A), Table 4.7. The comparisons of percentage bioaccessibility of beta-carotene and vitamin B series in the mixtures of vegetables are shown in figure 4.11.

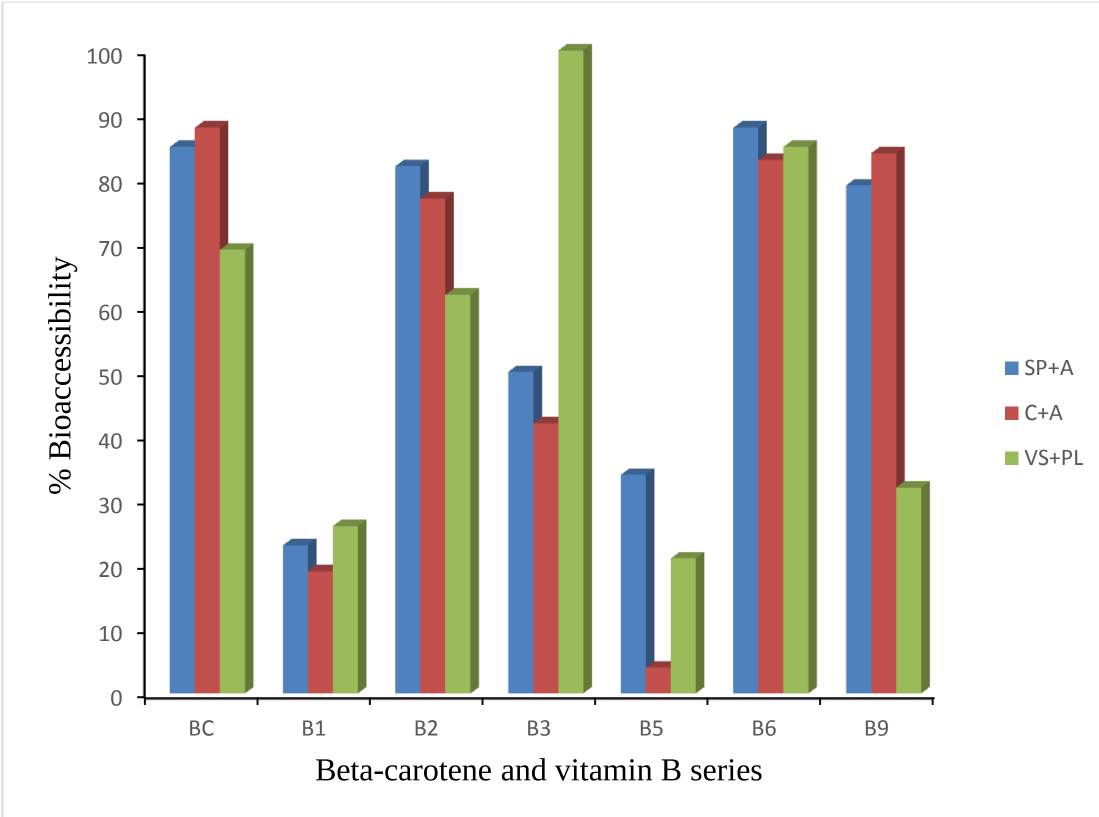

Figure 4.11: percentage bioaccessibility of beta-carotene and vitamin B series from recipe1

Preparation of RCP1 involved heat processing which is known to improve bioaccessibility of BC. This it does by disrupting the food matrix which enhances availability of the nutrient for absorption (Hotz and Gibson, 2007; Veda *et al.*, 2006; Srinivasan, 2009; Nagao, 2014). The variations in percent bioaccessibility are shown in figure 4.11, the increases were 3 % (SP+A) to 10 % (C+A) while the reduction was found to be from 57 % (VS+PL), Appendix Table A 4.

An increase in percentage bioaccessibility of thiamin was observed in VS+PL (18 %) while in SP+A and C+A, reductions were observed 16 % to 30 % respectively. (Appendix Table A 4). Among the factors that lead to increase in bioaccessibility of thiamin is thermal processing due to the disruption of the cell wall, dissociation of the nutrient–matrix complexes or transformation into a more active molecular structure (Parada and Aguilera, 2007), digestibility of the nutrients in the vegetables (Parada and Aguilera, 2007: Etcheverry *et al*, 2012) and thiamin antagonists which reduce absorption of thiamin (Terefe *et al.*, 2014).

In the mixture of vegetables C+A, percentage bioaccessibility of riboflavin increased by 73 % while in SP+A and VS+PL it decreased by 16 % and 36 % respectively. For niacin percent bioaccessibility in VS+PL increased by 19 % while in SP+A and C+A reductions of 27 % and 30 % respectively were observed (Appendix Table A 4). Preparation of recipes involves heating which improves bioaccessibility of the vitamin (Hotz and Gibson, 2007). While variation in percentage bioaccessibility may be attributed to digestibility of the vegetables (Parada and Aguilera, 2007; Etcheverry *et al*., 2012), alteration of matrices of various vegetables is also a contributing factor (Nagao, 2014).

An increase in percentage bioaccesibility of the pantothenic acid was recorded in VS+PL (14 %) while in SP+A and C+A, decreases were recorded of 10 % and 15 % respectively (Appendix Table A 4). Different vegetables have different digestibilities leading to variations in bioaccessibility (Brownlee *et al.,* 2006; Parada and Aguilera, 2007; Parafox-Carlos *et al*., 2011; Etcheverry *et al*., 2012. It is known that bioaccesibility decreases as a result of pectin retaining water that forms viscous solution in the digestive tract (Gibson, 2007). Leaching also decreases bioaccessibility since the vitamin is water soluble (Ismail *et al.*, 2013).

It was observed that processing of the vegetables had effects that resulted to increases in percentage bioaccessibility in C+A (75 %) and VS+PL (2 %) while a decrease was observed in SP+A (4 %), Appendix Table A 4. Preparing recipes resulted in both increase and decrease in bioaccessibility. The release of pyridoxine from the food matrix during cooking (Hotz and Gibson, 2007), the destruction of the vitamin during the process (Bua and Onang, 2017) as pyridoxine is decomposed by heat during cooking (Uraku *et al.*, 2015) explains the variation in bioaccessibility. Further, the variation may be attributed to the protein bound form of pyridoxine which has low bio accessibility (Hotz and Gibson, 2007).

Increases in percentage bioaccessibility were recorded in SP+A (1 %) and C+A (5 %) while a decrease was observed in VS+PL (47 %), Appendix Table A 4. Variation in bioaccessibility may be attributed to the digestibility of the vegetables (Parada and Aguilera, 2007; Etcheverry *et al.*, 2012). Further, during digestion folate may be entrapped resulting in incomplete digestion of thereby decreasing bioaccessibility ((Parada and Aguilera, 2007). Another factor that lowers folate bioaccessibility is dietary fiber (Öhrvik, 2009), there is also variation in fiber content of different vegetables.

4.8.3 Recipe 2

From Table 4.7 and figure 4.12, the percentage bioaccessibility of BC was found to range from 17.45±6.47 % (V.S+P.L) to 25.30±1.05 % (SP+A), thiamin (B_1) 47.52±6.09 % (C+A) to 88.22±1.49 % (SP+A), riboflavin (B_2) 19.07±0.97 % (SP+A) to 70.76±0.24

% (C+A), niacin (B_3) 46.06±2.36 % (SP+A) to 92.57±0.62 % (VS+PL), pantothenic acid (B_5) 5.57±1.18 % (SP+A) to 33.82±1.69 % (VS+PL), pyridoxine (B_6) 65.81±1.14 % (SP+A) to 81.67±0.73 % (VS+PL) and folate (B_9) 52.17±3.05 (VS+PL) to 86.33±7.24 (C+A), Table 4.7. The comparisons of percentage bioaccessibility of beta-carotene and vitamin B series in the mixtures of vegetables are shown in figure 4.12.

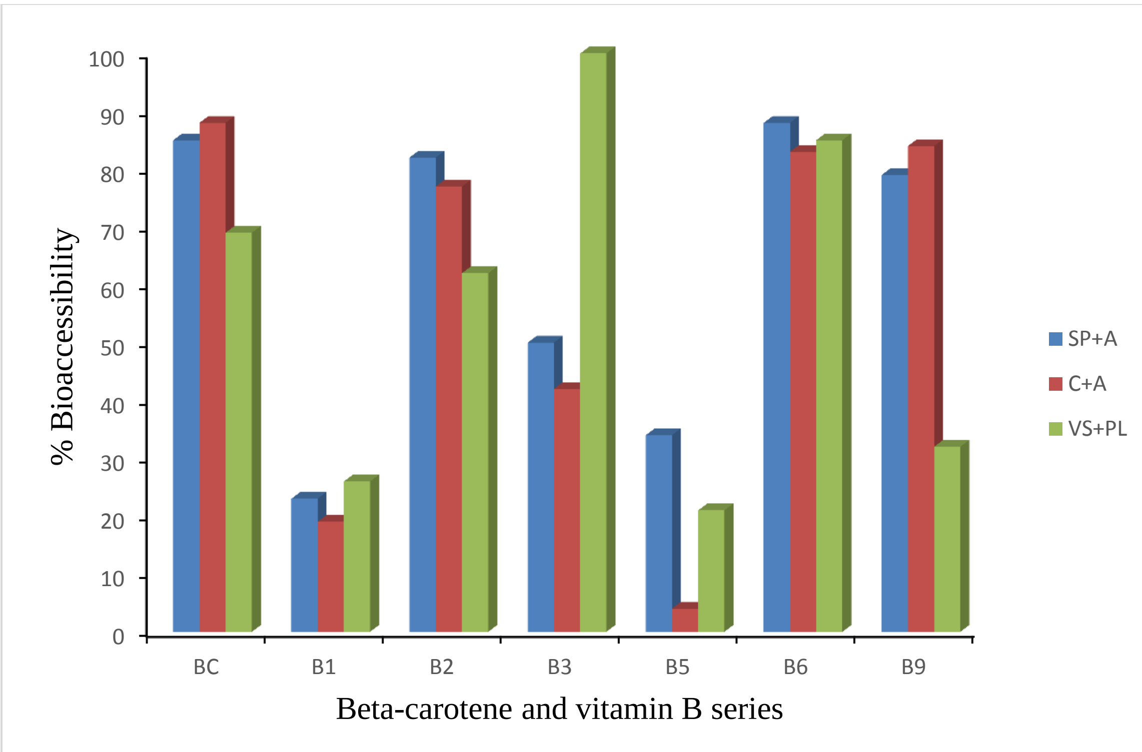

Figure 4.12: percentage bioaccessibility of beta-carotene and vitamin B series from recipe2

A decrease in percentage bioaccessibility of BC was observed in SP+A (57 %), C+A (58 %) and VS+PL (57 %). The observation may be attributed to variations in the dietary fiber in the vegetables (Koh and Loh, 2018). Increases in percentage bioaccessibility of thiamin were observed in all the mixtures of vegetables; SP+A (49 %), C+A (1 %) VS+PL (43 %) while in SP+ and C+A, reductions were observed 16 % to 30 %

respectively (Appendix Table A 4). Preparation of RCP2 involves thermal processing which disrupts the cell wall, dissociating the nutrient–matrix complexes hence increasing bioaccessibility (Parada and Aguilera, 2007). The other factor that influence bioaccessibility of the vitamin is the digestibility of the vegetables (Parada and Aguilera, 2007: Etcheverry *et al*, 2012).

While an increase in percentage bioaccessibility of riboflavin was recorded in the mixture of vegetables C+A (66 %), decreases were observed in SP+A (79 %) and VS+PL (46 %), Appendix Table A 4. The variations may be attributed to the difference in digestibility of the vegetables (Parada and Aguilera, 2007: Etcheverry *et al*, 2012). Riboflavin is also known to be light sensitive (Schellack *et al*., 2015), lost by leaching during cooking, being water soluble (Ismail *et al.,* 2013).

Percentage bioaccessibility of niacin in VS+PL increased by 11 % while a reduction of 31 % was observed in SP+A (Appendix Table A 4) Processing that involve heating improves bioaccessibility of the vitamin (Hotz and Gibson, 2007). While variation in percentage bioaccessibility may be attributed to digestibility of the vegetables (Parada and Aguilera, 2007; Etcheverry *et al*., 2012), alteration of matrices of various vegetables is also a contributing factor (Nagao, 2014).

Increases in percentage bioaccesibility of the pantothenic acid were recorded in C+A (9 %) and VS+PL (26 %) while a decrease was observed in SP+A (29 %), Appendix Table A 4. The vegetables also have different digestibilities as well as pectin in the dietary fiber which decreases bioaccessibility of the vitamin (Brownlee *et al.,* 2006; Parada and Aguilera, 2007; Parafox-Carlos *et al*., 2011; Etcheverry *et al*., 2012. It is known that pectin retains water that forms viscous solution in the digestive tract decreasing bioaccessibility (Gibson, 2007). The vitamin being water soluble, leaching is a contributing factor to decreases in bioaccessibility (Ismail *et al.,* 2013).

It was observed that percentage bioaccessibility of pyridoxine increased in C+A (68 %) while a decreases were observed in SP+A (27 %) and VS+PL (2 %), Appendix Table A 4. The variation in bioaccessibility is attributed to the release of pyridoxine from the food matrix (Hotz and Gibson, 2007) as well as the destruction of the vitamin during the process (Bua and Onang, 2017) since pyridoxine is decomposed by heat during cooking (Uraku *et al.,* 2015). The variation may further be attributed to the protein bound form of pyridoxine which has low bio accessibility (Hotz and Gibson, 2007).

An increase in percentage bioaccessibility was recorded in C+A (7 %), a decrease in VS+PL (27) while in SP+A, no change was observed Appendix Table A 4. The variations in bioaccessibility observed may be attributed to the digestibility of the vegetables (Parada and Aguilera, 2007; Etcheverry *et al*., 2012) as well as entrapment of folate during digestion resulting in incomplete digestion thereby decreasing bioaccessibility ((Parada and Aguilera, 2007). Dietary fiber also lowers folate bioaccessibility, different vegetables having different fiber content (Öhrvik, 2009.

4.9: Mean levels, bioaccessible levels, and percentage bioaccessibility of beta-carotene and vitamin B series in LAIVs formulations

The mean levels, bioaccessible levels and percentage bioaccessibility of beta-carotene, vitamin B_1, B_2, B_3, B_5, B_6 and B_9 in LAIVs; formulations are presented in Table 4.8 and shown in figures 4.13.

Table 4. 8: Mean levels, bioaccessible levels, and percentage bioaccessibility of beta-carotene and vitamin B series in LAIVs formulations

Mean levels, bioaccessible levels and percentage bioaccessibility of beta-carotene and vitamin B series in LAIVs formulations Mean±SD mg/100g DW, n=3				
Pro-vitamin/Vitamin	Formulation	Level present	Bio accessible levels	% bio accessibility
Beta carotene	FMLN1	17.05±0.21[c]	12.02±0.26[c]	70.45±0.78[c]
	FMLN2	1.35±1.13[a]	0.66±0.11[a]	48.28±3.66[a]
	FMLN3	2.76±0.07[b]	1.58±0.05[b]	59.19±0.48[b]
	p-value	≤0.001	≤0.001	≤0.001
Thiamine	FMLN1	2.43±0.72[b]	1.37±0.06[b]	56.33±0.76[c]
	FMLN2	2.69±0.19[c]	0.17±0.02[a]	6.28±0.21[a]
	FMLN3	1.68±0.02[a]	0.16±0.00[a]	9.51±0.11[b]
	p-value	≤0.001	≤0.001	≤0.001
Riboflavin	FMLN1	18.26±0.45[c]	10.95±0.41[b]	60.15±0.99[b]
	FMLN2	6.75±0.10[b]	1.17±0.07[a]	17.28±0.02[a]
	FMLN3	1.55±0.02[a]	1.37±0.02[a]	88.28±0.26[c]
	p-value	≤0.001	≤0.001	≤0.001
Niacin	FMLN1	7.28±0.45[a]	5.94±0.58[a]	81.56±2.08[ab]
	FMLN2	10.04±0.32[c]	7.75±0.26[b]	97.20±2.01[a]
	FMLN3	8.49±0.81[b]	7.04±0.88a[b]	82.70±2.83[b]
	p-value	≤0.003	≤0.029	≤0.061
Pantothenic acid	FMLN1	1.75±0.12[a]	0.65±0.05[a]	37.43±1.16[b]
	FMLN2	8.26±0.56[b]	1.24±0.14[a]	14.96±0.76[a]
	FMLN3	8.04±0.82[b]	7.14±0.88[b]	88.60±1.96[c]
	p-value	≤0.001	≤0.001	≤0.001
Pyridoxine	FMLN1	7.36±0.13[b]	6.10±0.26[c]	82.88±1.07[b]
	FMLN2	10.57±0.22[c]	0.93±0.03[a]	9.31±0.29[a]
	FMLN3	3.09±0.15[a]	2.65±0.12[b]	85.64±0.72[c]
	p-value	≤0.001	≤0.001	≤0.001
Folate	FMLN1	0.13±0.01[a]	0.10±0.01[a]	82.68±1.37[a]
	FMLN2	0.39±0.51a[b]	0.37±0.50[a]	81.46±13.57[a]
	FMLN3	0.73±0.01[b]	0.66±0.00[a]	89.79±0.33[a]
	p-value	≤0.110	≤0.144	≤0.429

FMLN1- SP+A

FMLN2- C+A

FMLN3-VS+PL

Mean values of the same vegetable within the same column followed by the same letters are not significantly different (SNK,α=0.05)

4.9.1 Beta-carotene

The mean levels (mg/100g) of BC in the formulations were found to range from; 1.35±0.13 (FMLN2) to 17.05±0.21 (FMLN1), bioaccessible mean levels from 0.66±0.11 (FMLN2) to 12.02±0.26 (FMLN1) and percentage bioaccessibility from 48.28±3.66 % (FMLN2) to 70.45±0.78 % (FMLN1). FMLN1 recorded higher levels of beta-carotene than FMLN2 and FMLN3 (Table 4.8). The comparisons of percentage bioaccessibility of beta-carotene and vitamin B series in the mixtures of vegetables are shown in figure 4.13.

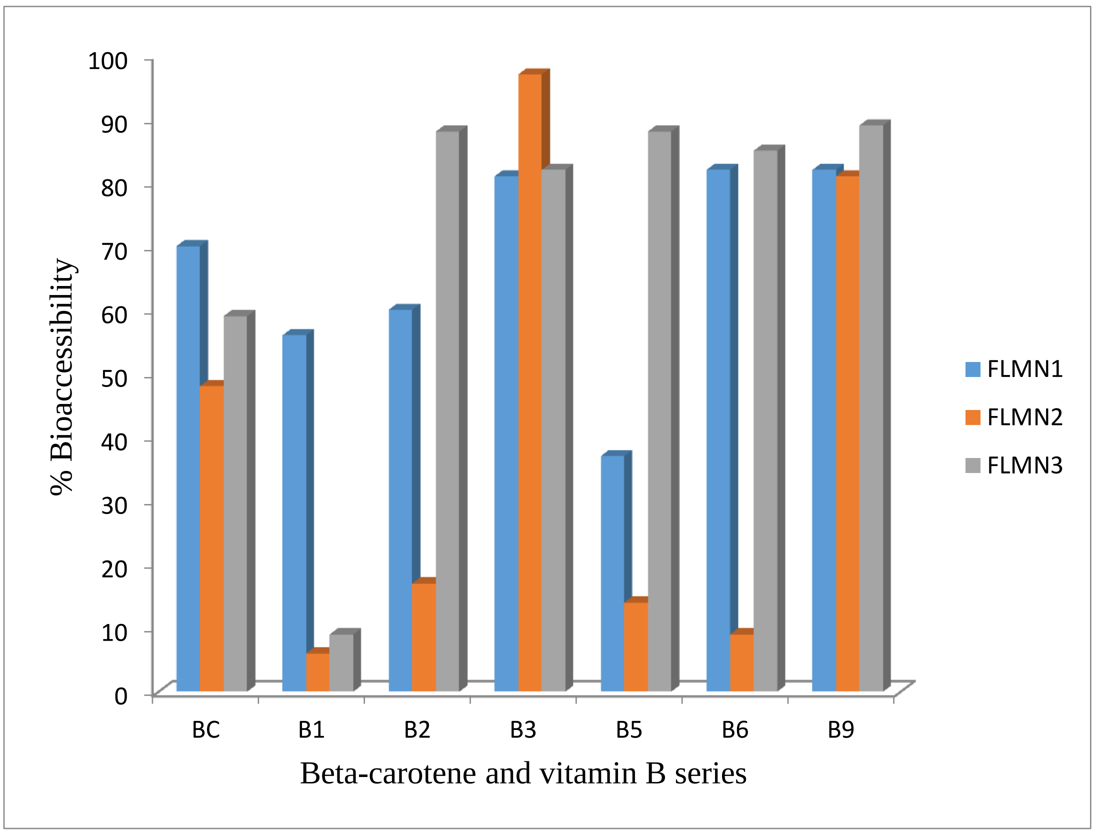

Figure 4. 13: percentage bioaccessibility of beta-carotene and vitamin B series from LAIVs formulations

The variations in percentage bioaccessibility observed may be attributed to the difference in the alteration of matrices of the different vegetables used to prepare the formulations (Nagao, 2014). The food matrices in different vegetables determine the ease with which nutrients are released, structures such as the cell walls have to be broken (Parada and Aguilera, 2007). The higher bioaccessible levels and percentage bioaccessibility recorded in FMLN1 may be due to the difference in dietary fiber content, cowpeas and pumpkin leaves may have contributed higher fiber as compared to amaranth, spider plant and vine spinach which have lower fiber content. Dietary fiber decreases bioaccessibility of beta carotene by binding bile acids and inhibiting solubilization of beta carotene (Gibson, 2007; Nagao, 2014). Dietary fiber traps beta-carotene inside the fiber matrix in the chyme reducing its availability for absorption. It further increases the viscosity slowing down enzymatic activity which results in reduced release of beta-carotene (Palafox-carlos *et al.*, 2011).

4.9.2 Vitamin B series

The mean levels (mg/100g) of thiamin (B_1) in the formulations ranged from 1.687±0.02 (FMLN3) to 2.69±0.19 (FMLN2), bioaccessible levels 0.16±0.00 (FMLN3) to 1.37±0.06 % (FMLN1) and percentage bioaccessibility 6.28±0.21 % (FMLN2) to 56.32±0.76 % (FMLN1). For riboflavin (B_2) the ranges were; mean levels 18.26±0.45 (FMLN1) to 1.55±0.02 (FMLN3), bioaccessible levels 17±0.007 (FMLN2) to 10.95±0.41 (FMLN1) and percentage bioaccessibility 17.28±0.02 % (FMLN2) to 88.28±0.26 % (FMLN3) while for niacin (B_3) the mean levels ranged from 7.28±0.45 (FMLN1) to 10.04±0.32 (FMLN2), bioaccessible levels from 5.94±0.58 (FMLN1) to 7.75±0.26 (FMLN2) and percentage bioaccessibility from 81.56±2.08 % (FMLN1) to 97.20±2.01 % (FMLN2), Table 4.8.

Pantothenic acid (B_5) recorded mean levels that ranged from 1.75±0.12 (FMLN1) to 8.26±0.56 (FMLN2), bioaccessible levels 0.65±0.05 (FMLN1) to 7.14±0.88 (FMLN3) and percentage bioaccessibility 14.96±0.76 % (FMLN2) to 88.60±1.96 % (FMLN3). Pyridoxine (B_6) in the formulations was found to have mean levels ranging from 3.09±0.15 (FMLN3) to 10.57±0.22 (FMLN2), bioaccessible levels 0.93±0.03 (FMLN2) to 6.10±0.26 (FMLN1) and percentage bioaccessibility 9.31±0.29 % (FMLN2) to 85.64±0.72 % (FMLN3) while folate (B_9) recorded mean levels that ranged from 0.13±0.01 (FMLN1) to 0.73±0.01 (FMLN3), bioaccessible levels 0.10±0.01 (FMLN1) to 0.66±0.00 (FMLN3) and percentage bioaccessibility 81.46±13.57 % (FMLN2) to 89.79±0.33 % (FMLN3), Table 4.8.

It was observed that the formulations had variations in the mean levels, bioaccessible levels and percentage bioaccessibility of the vitamins. This may be attributed to the differences in digestibility of the vegetables (Parada and Aguilera, 2007; Etcheverry *et al*., 2012), as well as the alteration of matrices of different vegetables (Nagao, 2014). Mild heating during food processing disrupts the cell walls of the plant tissues and also dissociates the nutrient-matrix complexes releasing the nutrient that lead to increase in the levels (Parada and Aguilera, 2007). The B vitamins especially thiamin and riboflavin are heat-labile and therefore destroyed by processes that involve heating thereby reducing the levels (Gibson, 2005). There was no particular trend in the bio accessible levels and percentage bioaccessibility of the vitamin B series from the formulations as both increases and decreases were observed (Table 4.7). Significant differences ($p<0.001$) in the mean levels, mean bioaccessible levels and percentage bioaccessibility of thiamin, riboflavin, niacin, pantothenic acid and pyridoxine were observed between all the formulations. For folate, however, no significant differences were observed in the mean levels ($p<0.110$), mean bio accessible levels ($p<0.144$) and mean percentage bioaccessibility ($p<0.429$) between the formulations.

Apart from vitamin B_5 which recorded 37 %, all the vitamins recorded percentage bioaccessibility of more than 50 % from FMLN1. From FMLN3, all the vitamins recorded % bioaccessibility of more than 50 % apart from vitamin B1 which recorded 9 %. Vitamin B_1, B_2, B_5 and B_6 recorded less than 20 % bioaccessibility from FMLN2 while vitamin B_3 and B_9 recorded above 80 % from all the formulations (Figure 4.13). The high bioaccessibility observed may be explained by the fact that the B vitamins including thiamin, niacin, pyridoxine and folate are released from their complexes by heating, enhancing bioaccessibility (Gibson, 2005).

CHAPTER 5

5 CONLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

With respect to the results obtained from this study, the following conclusions were made:-

i. Both the fresh and processed *Cleome gynandra, Vigna unguiculata, Amaranthus viridis*, *Basella alba* and *Cucurbita maxima* vegetables contained significant levels of beta-carotene and vitamin B series (B_1, B_2, B_3, B_5, B_6 and B_9).

ii. The levels of beta-carotene and vitamin B series (B_1, B_2, B_3, B_5, B_6 and B_9) in the vegetables *Cleome gynandra*, *Vigna unguiculata*, *Amaranthus viridis, Basella alba* and *Cucurbita maxima* were sufficient and bioaccessible.

iii. Recipes of mixtures of the vegetables; *Cleome gynandra, Vigna unguiculata, Amaranthus viridis*, *Basella alba* and *Cucurbita maxima* contain levels of beta-carotene and vitamin B series (B_1, B_2, B_3, B_5, B_6 and B_9) that are bioaccessible.

iv. The levels of beta-carotene and vitamin B series (B_1, B_2, B_3, B_5, B_6 and B_9) from formulations prepared from mixtures of the vegetables; *Cleome gynandra, Vigna unguiculata*, *Amaranthus viridis*, *Basella alba* and *Cucurbita maxima* vegetables are adequate and bioaccessible though some vitamins; B_1, B_2 and B_5 have significantly lower bioaccessibility.

5.2 Recommendations from the study

The following recommendations were made from the study;

i. Promote consumption of fresh, processed and recipes of mixtures of *Cleome gynandra, Vigna unguiculata, Amaranthus viridis, Basella alba* and *Cucurbita maxima* vegetables to address food security and malnutrition cases (for all ages) that relate to deficiency of beta-carotene and vitamin B series (B_1, B_2, B_3, B_5, B_6 and B_9). This is supported by the fact that the vegetables contain adequate and bioaccessible levels of the vitamins.

ii. The formulations prepared from mixture of the vegetables; *Cleome gynandra, Vigna unguiculata, Amaranthus viridis, Basella alba* and *Cucurbita maxima* vegetables are recommended for consumption for bioaccessible levels of BC and B_3, B_6 and B_9).

5.3 Recommendations for further studies

The recommendations made for further studies;

i. *In vivo* bioaccessibility studies of vitamin B series from fresh and processed LAIVs

ii. Bioaccessibility studies (in vitro & *in vivo*) for other micro and macronutrients in LAIVs

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APPENDIX I

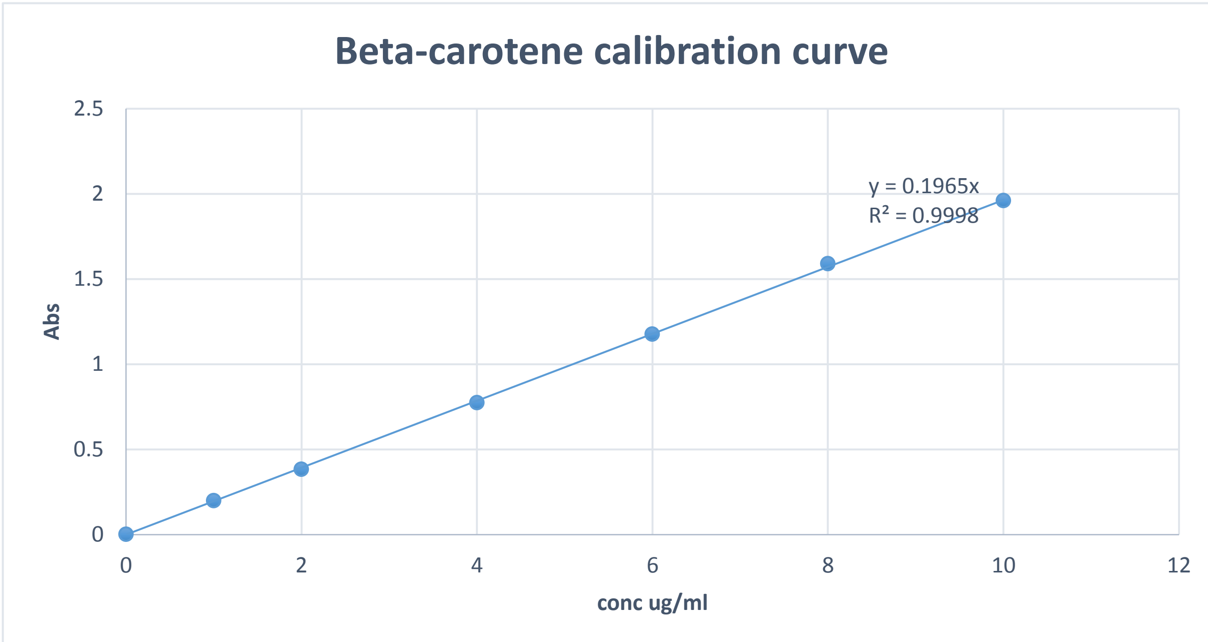

Figure A 1: Calibration curve for series of beta-carotene standards

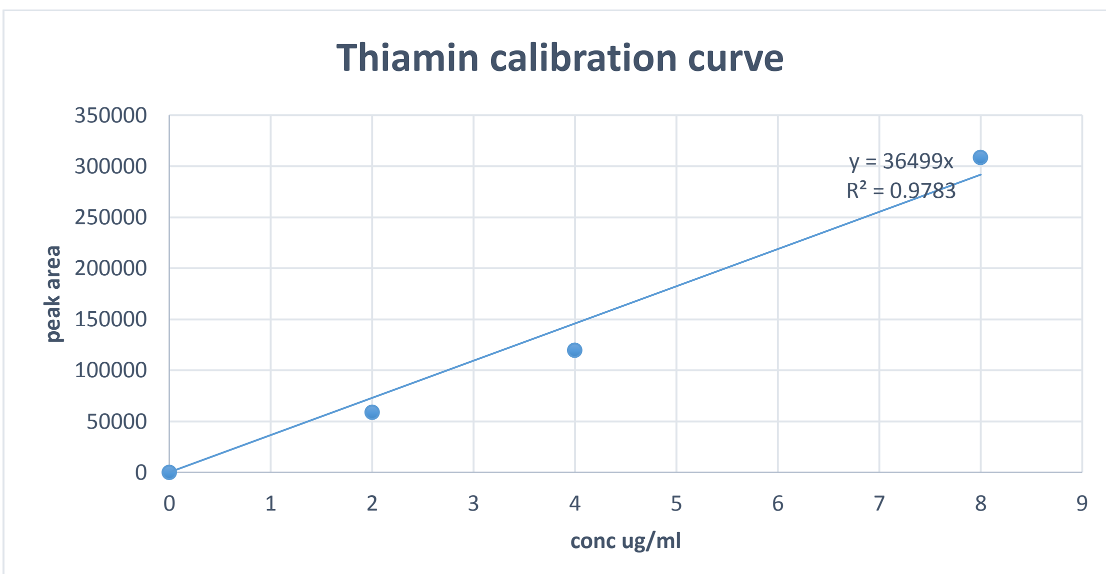

Figure A 2: Calibration curve for series of thiamin standards

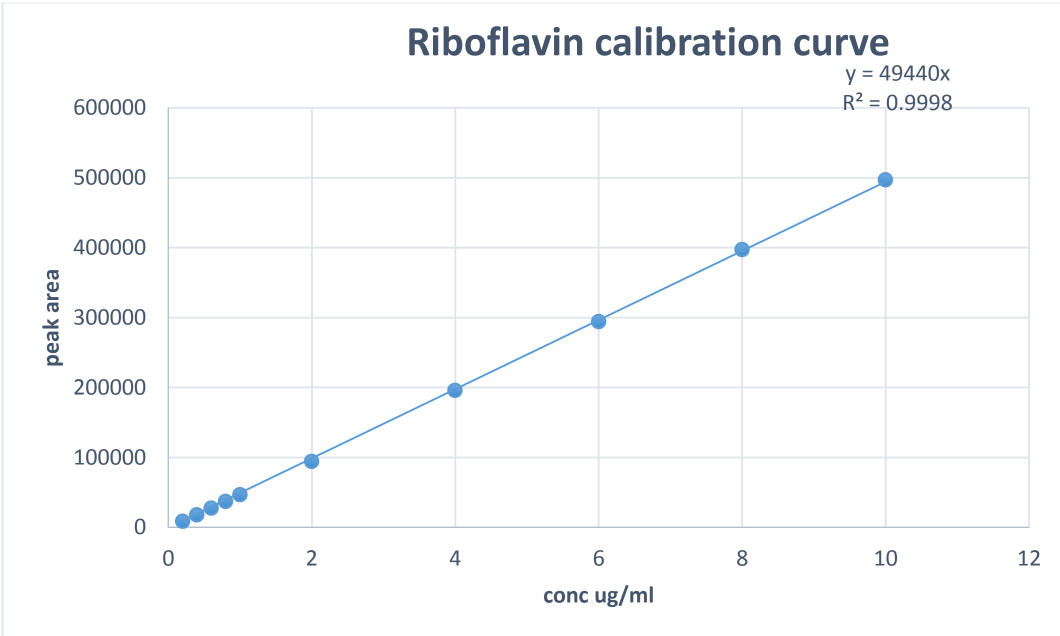

Figure A 3: Calibration curve for series of riboflavin standards

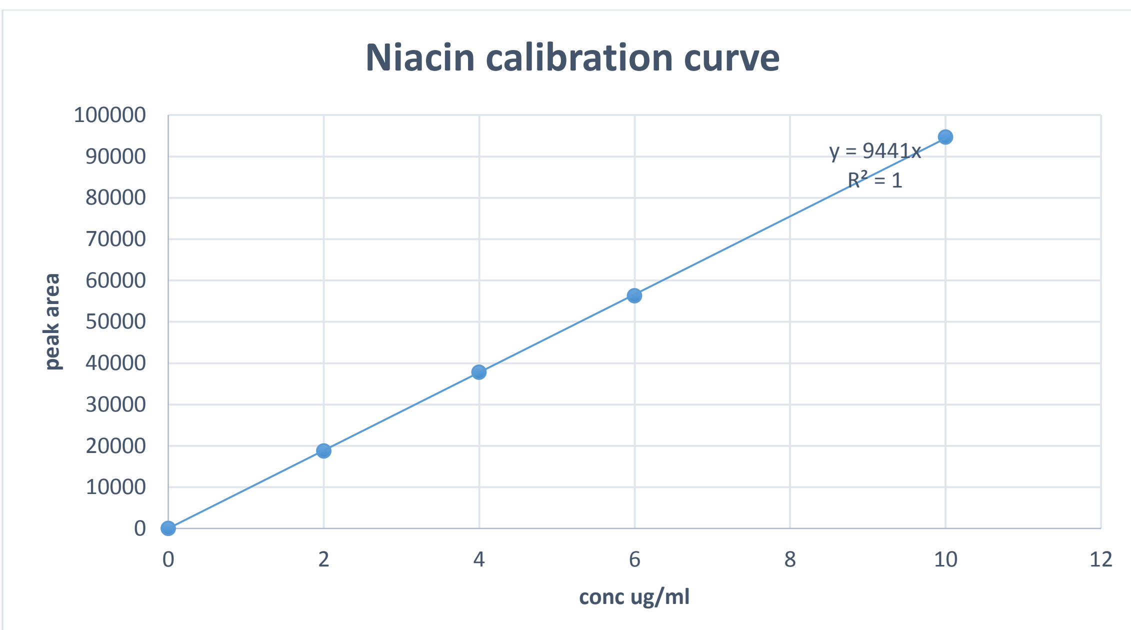

Figure A 4: Calibration curve for series of niacin standards

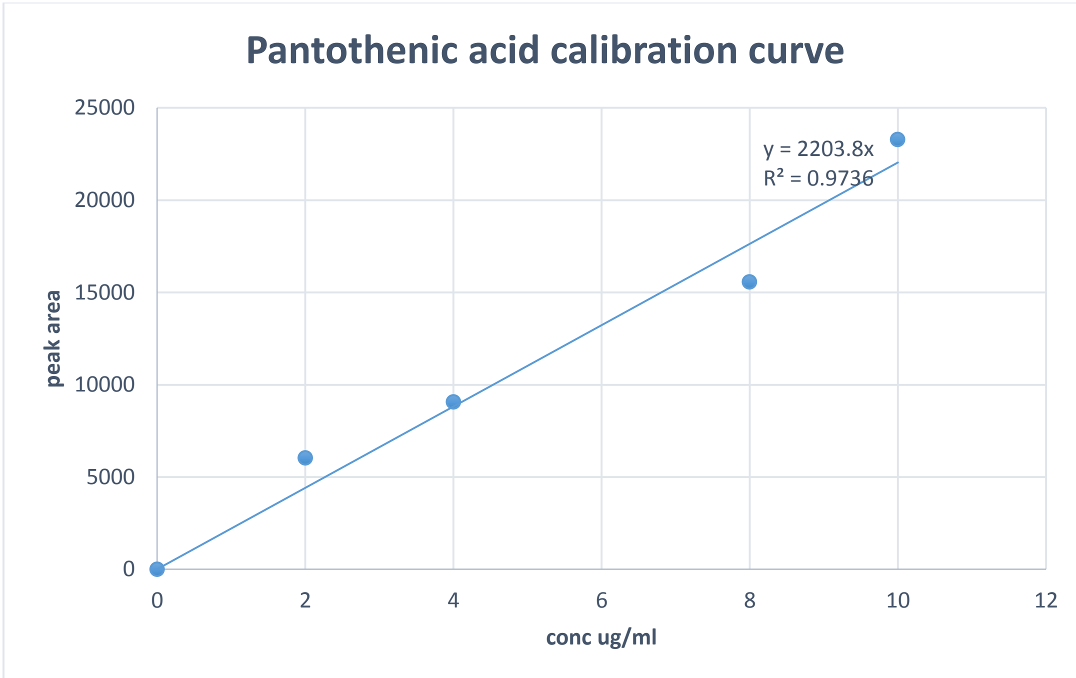

Figure A 5: Calibration curve for series of pantothenic acid standards

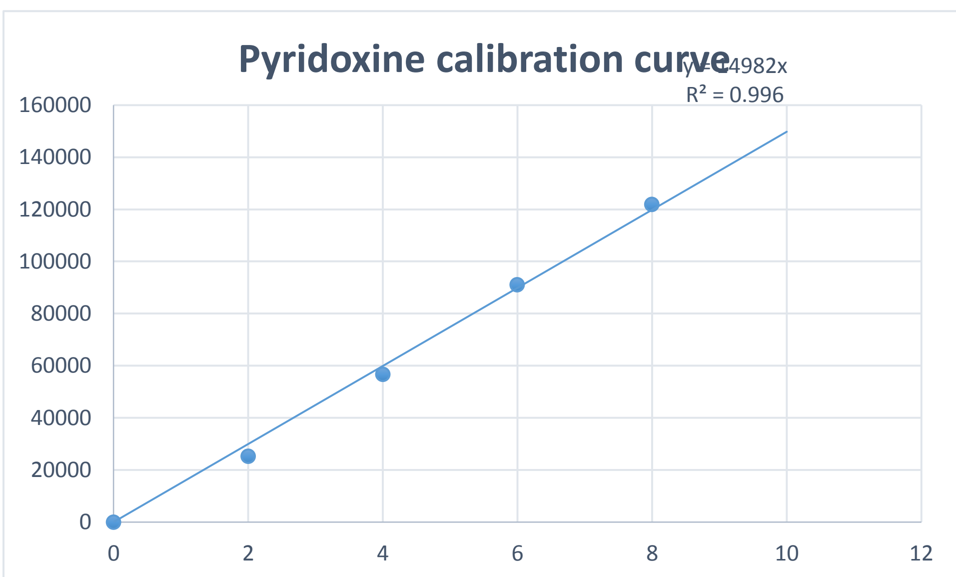

Figure A 6: Calibration curve for series of pyridoxine standards

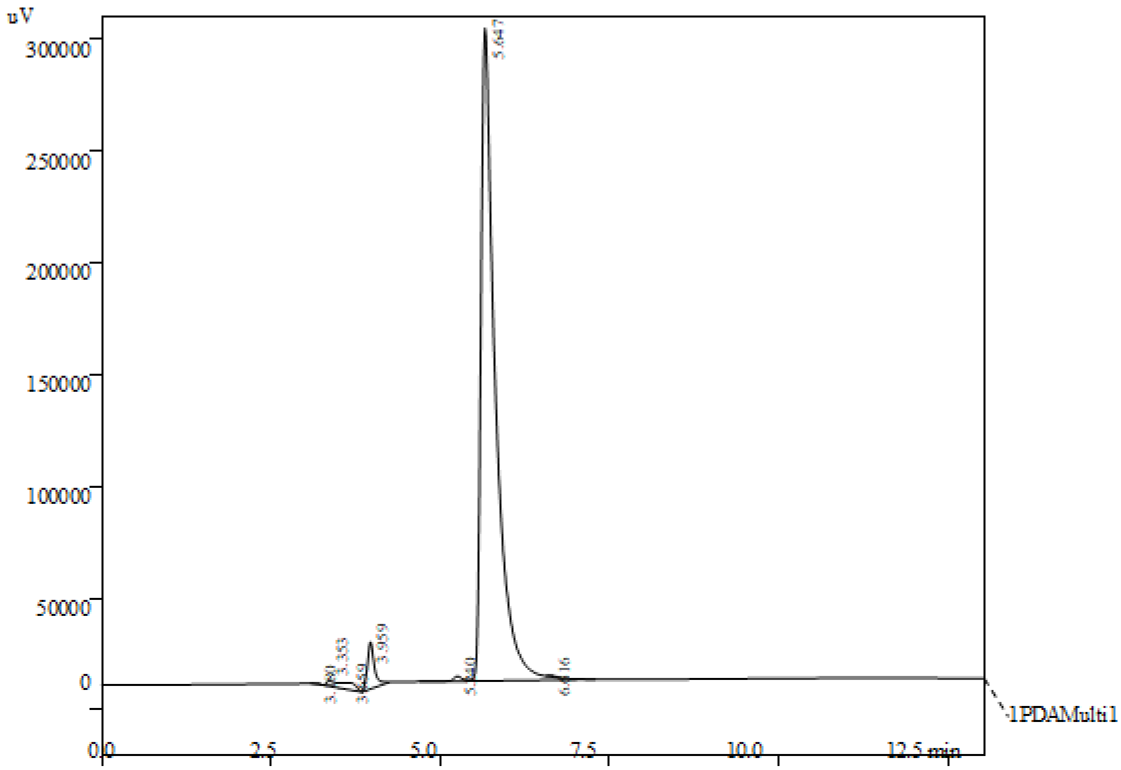

Figure A 7: Chromatogram for vitamin B_1 standard

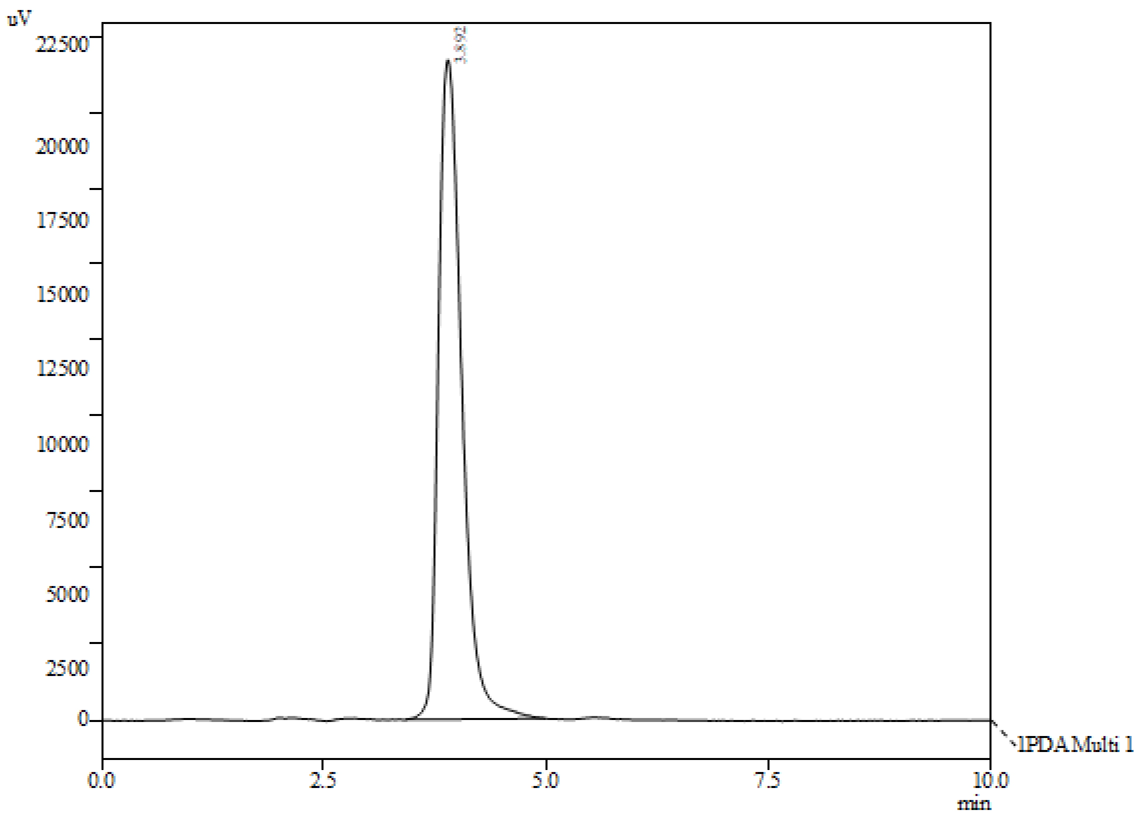

Figure A 8: Chromatogram for vitamin B_2 standard

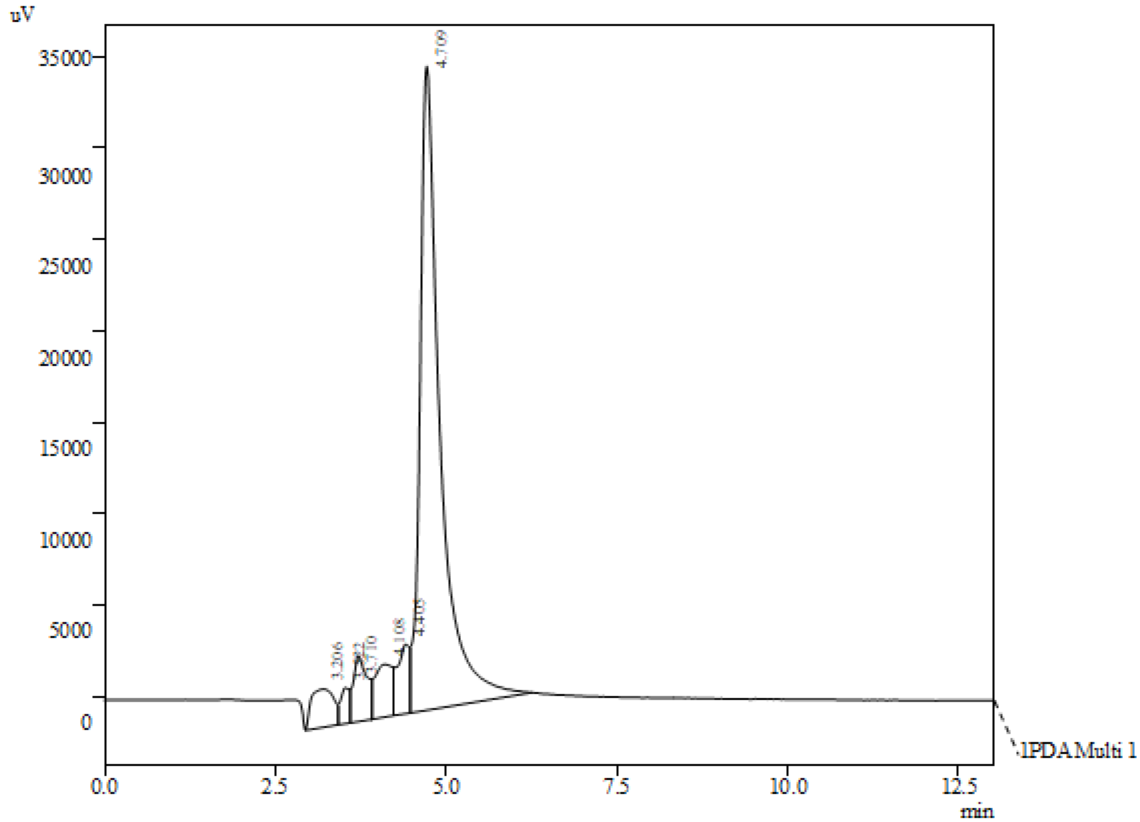

Figure A 9: Chromatogram of vitamin B_3 standard

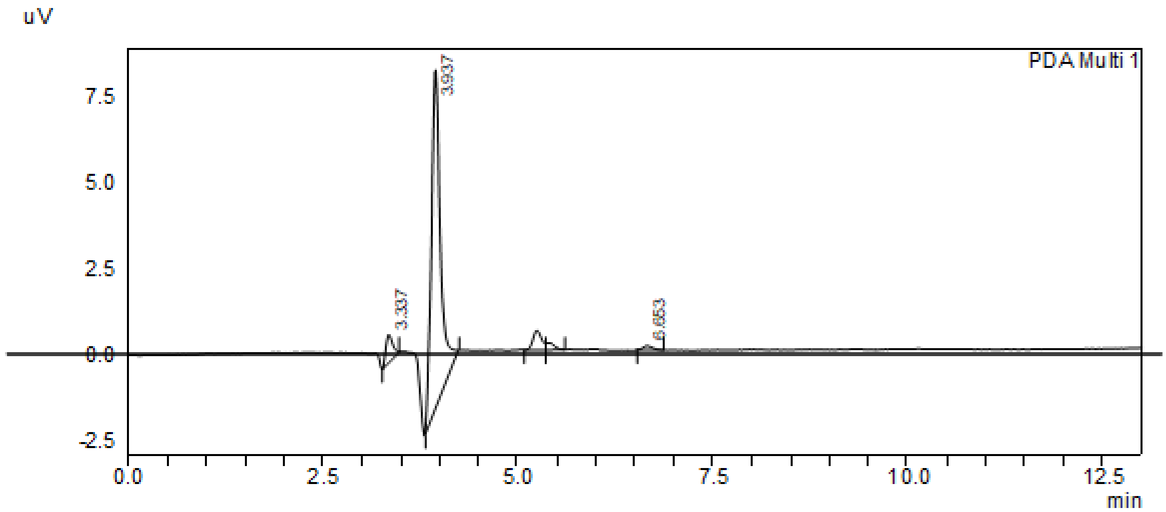

Figure A 10: Chromatogram of vitamin B_5 standard

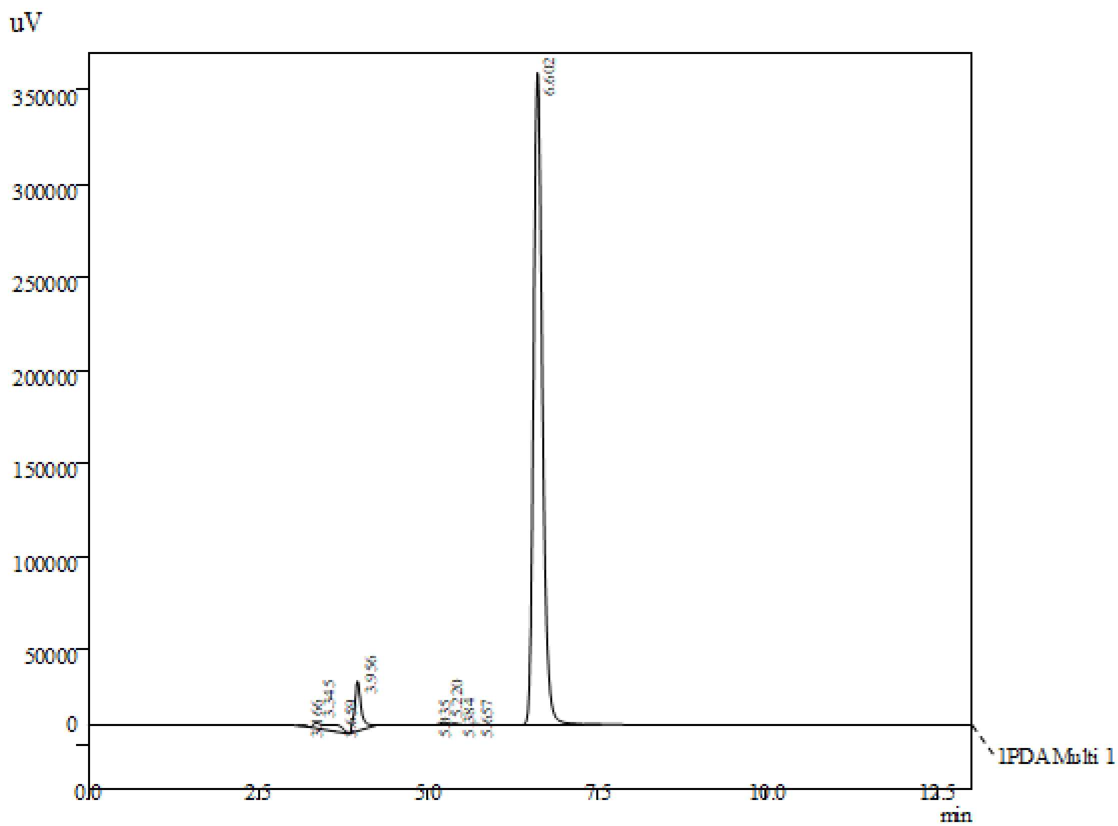

Figure A 11: Chromatogram of vitamin B_6 standard

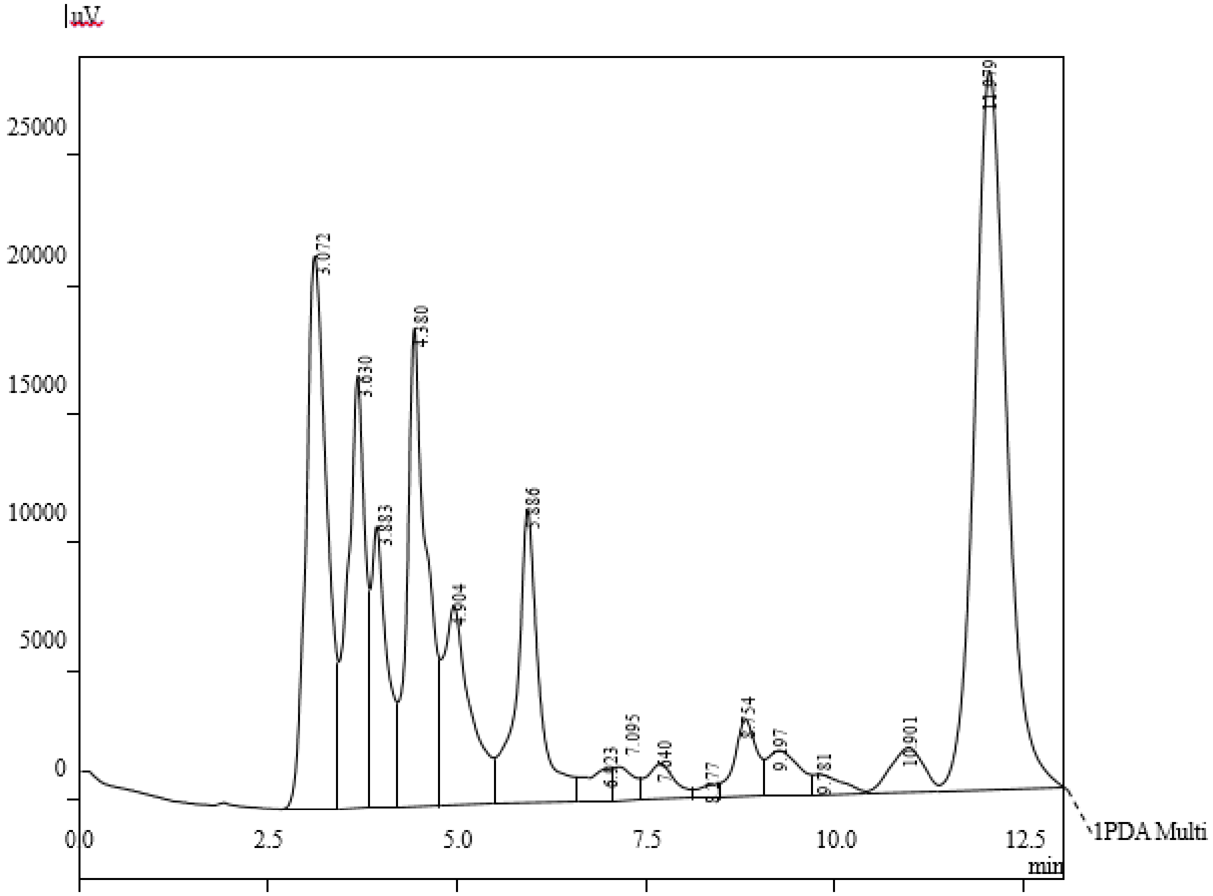

Figure A 12: Chromatogram vitamin B_1, B_3, B_5, B_6 and B_9 data

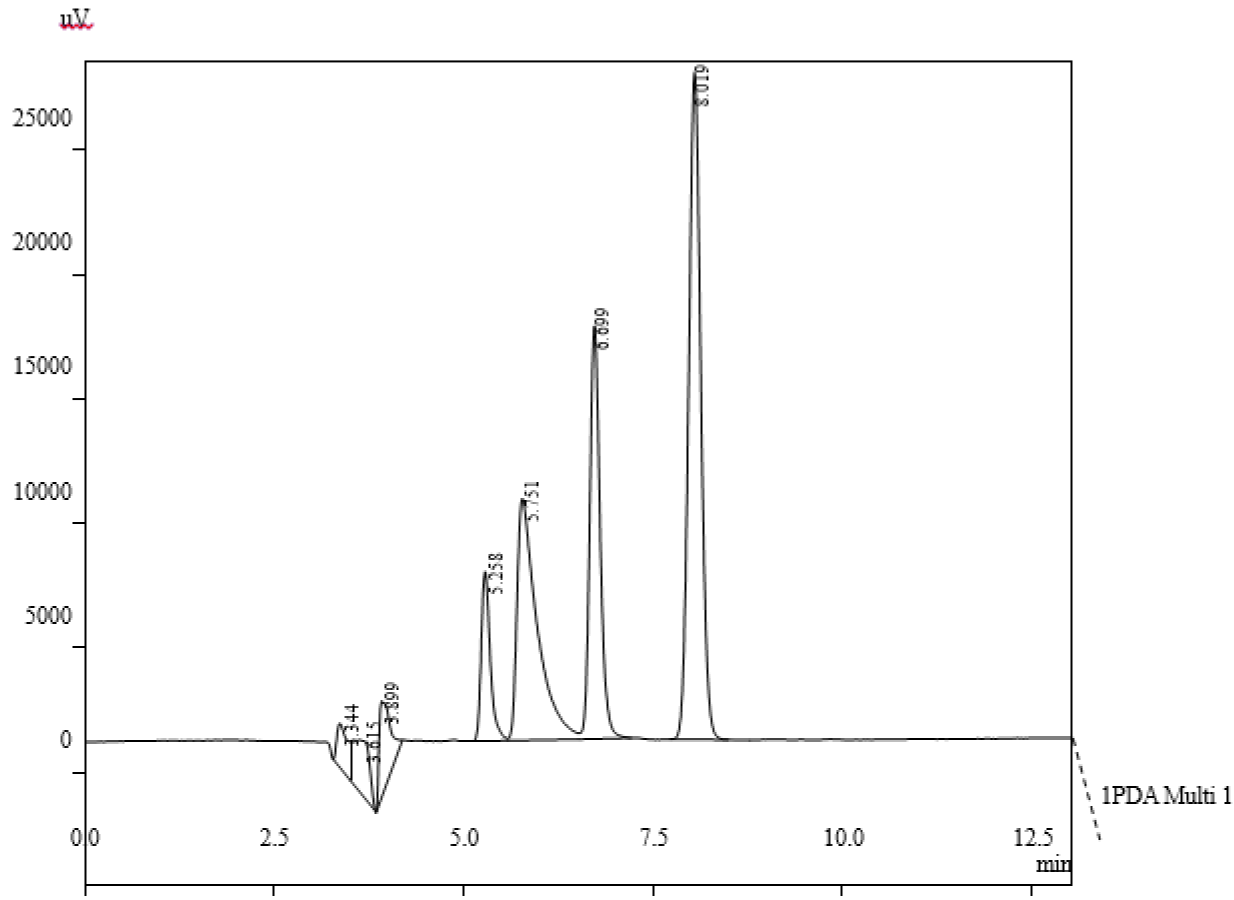

Figure A 13: Chromatogram vitamin B_1, B_3, B_5, B_6 and B_9 standards

APPENDIX II

Table A 1: Percentage change in levels of beta-carotene and vitamin B series with vegetable processing

		Percentage change in levels of beta-carotene and vitamin B series						
Vegetable	Process	BC	B_1	B_2	B_3	B_5	B_6	B_9
Spider Plant	Boiled	+52.68	+75.64	-42.66	+16.18	-45.39	+18.37	-49.32
	Fried	-62.72	+425.96	-100.00	-62.71	-70.74	-55.95	-54.79
	Dried	-69.98	-27.56	-100.00	-18.74	-31.66	-77.72	-72.60
Cowpeas	Boiled	+11.14	-15.86	-97.86	+290.29	+159.89	+308.89	-98.26
	Fried	-67.43	-100.00	-97.22	-100.00	+225.13	+350.00	-63.53
	Dried	-80.61	-100.00	-100.00	-56.47	-100.00	-6.39	-6.24
Amaranth	Boiled	+42.15	+129.52	-92.44	-100.00	-54.20	-74.51	+421.18
	Fried	-75.39	+22.34	-92.09	-100.00	+317.63	-69.70	+118.24
	Dried	-81.76	-100.00	-70.01	-14.96	-100.00	-97.00	-100.00
Vine Spinach	Boiled	+65.93	+28.92	-99.22	+446.84	-0.44	+212.90	-100.00
	Fried	-28.94	-100.00	-89.40	+57.46	-66.79	-52.26	-100.00
	Dried	-9.92	-6.02	-71.92	-36.95	-41.12	-100.00	-100.00
Pumpkin Leaves	Boiled	+27.61	-18.42	-97.57	+8.71	+48.21	-92.69	-45.12
	Fried	-73.19	+125.00	-100.00	-34.43	+227.43	-16.01	+41.06
	Dried	-64.85	-18.64	-62.36	-100.00	-100.00	-100.00	-60.16

Table A 2: Percentage change in bioaccessibility of beta-carotene and vitamin B series with vegetable processing

		Percentage change in bioaccessibility of beta-carotene and vitamin B series						
Vegetable	Process	BC	B_1	B_2	B_3	B_5	B_6	B_9
Spider Plant	Boiled	-19	-21	No change	+2	+33	-77	+45
	Fried	-49	+70	ND	+1	+18	-50	+45
Cowpeas	Boiled	+3	-70	No change	No change	-6	+39	-34
	Fried	-50	ND	-16	ND	+35	+37	No change
Amaranth	Boiled	+15	+3	-39	ND	-16	-15	+39
	Fried	-76	+65	-57	ND	+12	+5	+37
Vine Spinach	Boiled	+4	-14	+1	+71	-13	+10	ND
	Fried	-38	ND	-1	+70	-8	+11	ND
Pumpkin Leaves	Boiled	-14	-13	-75	No change	+11	+82	-20
	Fried	-71	+28	ND	-7	+50	+35	-5

Table A 3: Percentage change in levels of beta-carotene and vitamin B series with recipe preparation

		Percentage change in levels of beta-carotene and vitamin B series						
Vegetable	Recipe	BC	B_1	B_2	B_3	B_5	B_6	B_9
SP+A	RCP1	+103.08	+82.91	-66.48	+10.42	-33.63	-37.51	+295.93
	RCP2	-81.61	+156.13	-97.00	-63.48	-70.75	-22.36	+65.04
C+A	RCP1	+8.08	+8.08	-95.20	-43.78	-85.45	+10.10	-56.32
	RCP2	-72.14	-31.44	-94.24	-100.00	-65.84	+24.80	-60.65
VS+PL	RCP1	+43.19	+24.49	-98.81	+110.53	+40.15	+34.74	-93.94
	RCP2	-18.78	+137.76	-90.54	+3.69	+109.35	+34.82	-84.66

Table A 4: Percentage change in bioaccessibility of beta-carotene and vitamin B series with recipe preparation

Percentage change in vitamin bioaccessibility of beta-carotene and vitamin B series								
Vegetable	Recipe	BC	B_1	B_2	B_3	B_5	B_6	B_9
SP+A	RCP1	+3	-16	-16	-27	-10	-4	+1
	RCP2	-57	+49	-79	-31	-29	-27	No change
C+A	RCP1	+10	-30	+73	-30	-15	+75	+5
	RCP2	-58	+1	+66	ND	+9	+68	+7
VS+PL	RCP1	-5	+18	-36	+19	+14	+2	-47
	RCP2	-57	+43	-46	+11	+26	-2	-27

APPENDIX III: Publication 1

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In vitro Bioaccessibility of Beta Carotene from Thermally Processed Leafy African Indigenous Vegetables

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Abstract

Beta carotene (BC), a pro-vitamin A carotenoid found in leafy African indigenous vegetables (LAIVs) and fruits, plays important biological roles towards protection against cardiovascular diseases, cancer and many others. Lack of vitamin A is a major challenge in many developing countries where its source is mainly vegetables. The carotenoid exists as a complex in different food matrices and has to be released from the food matrix for it to be bioaccessible. Different processing procedures affect bioacessibility, boiling and boil-frying being the main thermal processes used by many households in developing countries. The study assessed the bioaccessibility of BC in thermally processed (boiled and boiled-fried) spider plant, cowpeas, amaranth, vine spinach and pumpkin leaves using an *in vitro* method. After extraction and separation using column chromatography the levels of BC were determined using UV-Vis spectrophotometry. A static gastrointestinal digestion procedure was used to obtain bioaccessible levels of BC. The respective percentage bioaccessibility (%) of BC from spider plant, cowpeas, amaranth, vine spinach and pumpkin leaves were as follows: fresh 97.23±0.01, 81.60±0.36, 77.22±0.05, 61.36±1.87 and 94.48±0.57, boiled 78.80±1.86, 84.31±0.27, 92.28±0.46, 65.67±5.53 and 80.75±0.69, and boiled-fried [48.19±0.82, 31.02±3.09, 16.97±0.02, 23.15±2.82 and 23.33±2.89 respectively. Boiled LAIVs had higher percentage bioaccessibility than boiled-fried due to the effect of longer period of exposure to heat during processing. The knowledge on bioaccessibility of BC from the LAIVs reported in this study would play a key role in encouraging their consumption thus contributing to food security as well as curbing malnutrition.

Keywords: *In vitro* bioaccessibility; beta carotene; Leafy African Indigenous vegetables; thermal processing

APPENDIX III: Publication I1

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Research Article

In Vitro Bioaccessibility of the Vitamin B Series from Thermally Processed Leafy African Indigenous Vegetables

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Thermal processing of leafy African indigenous vegetables (LAIVs), which are rich in nutrients, especially vitamin B series affects the levels and bioaccessibility of the vitamins. This study investigated the bioaccessibility of vitamin B series in fresh and thermally processed LAIVs. Five commonly consumed indigenous vegetables, *Cleome gynadra, Vigna unguilata, Amaranthus viridis, Basella alba,* and *Cucurbita maxima,* were processed by boiling and/or frying, treated to *in vitro* gastrointestinal digestion procedure, and levels of vitamin B series determined before and after treatment. The vitamin B series in fresh LAIVs ranged from 0.73 ± 0.01 mg/100 g (B_9; spider plant) to 174.16 ± 3.50 mg/100 g (B_2; vine spinach) and had both significant increase (ranging from +8.71% to +446.84%) and decrease (ranging from −0.44% to −100.00%) with thermal processing ($p < 0.001$). The *in vitro* digestion resulted in a significant increase ($p < 0.001$) of vitamins ranging from 5.18% (B_5; boiled cowpeas) to 100% (B_2, B_3, and B_6 in several processed vegetables). Where detected, the bioaccessible levels of vitamin B series in fresh, processed, and *in vitro* digested samples were sufficient to meet the Recommended Dietary Allowances (RDA) of children and adults. These findings support the promotion of a nutritional approach to malnutrition resulting from vitamin B series deficiency.