COOKING EFFECTS ON FOLATE AND ASCORBIC ACID LEVELS IN SELECTED AFRICAN INDIGENOUS VEGETABLES FROM GITHURAI MARKET, NAIROBI CITY COUNTY, KENYA

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REG. NO: I56/CE/28640/2015

A Research Thesis Submitted in Partial Fulfillment of the Requirements for the Award of the Degree of Master of Science in Applied Analytical Chemistry in the School of Pure and Applied Sciences of Kenyatta University

October/2022

DECLARATION

This thesis is my original work and has not been presented for a degree in any other university or any other award.

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DEDICATION

This thesis is dedicated to my cherished husband Elly Makuba, sons Morgan Makuba and Warren Makuba, and mother Sarah Muthoni who encouraged and inspired me to start and finish my studies successfully.

ACKNOWLEDGMENTS

I am very grateful to the Almighty God for His love, mercy, and grace to me, enabling me to complete this work. I thank my supervisors Prof. Ruth Wanjau and Prof Jane Murungi who have been instrumental in guiding and encouraging me to work hard for the successful completion of this work. I am also grateful to the technician in the Department of Food Nutrition and dietetics, Kenyatta University; John Gachoya for the use of HPLC equipment, and Felix Ondiek for assistance in statistical analysis. My heartfelt appreciation goes to my sisters Christine Washiko and Clare Washiko who assisted in the preparation of the African indigenous vegetables (AIVs) using the recipes provided.

Special thanks to my husband Elly Makuba and our children Morgan Makuba and Warren Makuba for their encouragement, inspiration, and motivation during my studies. I thank my parents Shem Washiko and Sarah Muthoni for their support throughout my learning process.

TABLE OF CONTENT

LIST OF FIGURES

LIST OF TABLES

ABBREVIATIONS AND ACRONYMS

AA	Ascorbic Acid
AIVs	African Indigenous Vegetables
HPLC	High-Pressure Liquid Chromatography
IUPAC	International Union of Pure and Applied Chemistry
MoH	Ministry of Health
MTHF	Methyltetrahydrofolate
NTDs	Neural Tube Defects
RP-HPLC	Reverse Phase High-Pressure Liquid Chromatography
TFA	Total Folate Activity
WHO	World Health Organization

ABSTRACT

Folate (vitamin B_9,5-methyltetrahydrofolate) and ascorbic acid (AA) (vitamin C), play a key role in human health and wellbeing. It is greatly established that AA is beneficial in preventing scurvy while folate helps in the prevention of neural tube defects and congenital malformations. The main sources of these vitamins are fruits and vegetables and especially green leafy vegetables, including the African indigenous vegetables (AIVs). However, these vegetables are consumed after cooking which leads to loss of the vitamins through oxidation, thermal degradation and leaching. Main cooking methods in Kenya include boiling in unspecified amounts of water and discarding the boiling effluents leading to high nutrient loss. There is also the addition of additives such as bicarbonate of soda, lye (traditional salt), milk, cream, sesame and groundnuts paste whose effect on nutrient levels especially folate and AA levels in AIVs are yet to be explained, thus the need for investigation. The study aimed at determining the effect of different cooking methods on the retention of AA and folate in cowpea [*Vigna Unguiculata* (L.) Walp], saget (spider plant) [*Cleome gynandra* (L.)] and pumpkin leaves (*Cucurbita moschata*) as affected by different cooking methods. The vegetables were bought from Githurai market then sorted, prepared and cleaned using tap water then rinsed with distilled water on arrival in the laboratory. Both raw and cooked samples were analyzed. A portion of 100.000 g of the edible portion of vegetable samples including leaves and young shoots, was used in each of the cooking methods. During extraction, mortar and pestle were used to grind 10.000 g of sample then mixed with 50 mL of extraction solution containing; 20 mM $KHCO_3$ (for vitamin B_9 extraction) and 3 % Metaphosphoric acid, MPA, and 8 % acetic acid (for vitamin C extraction). The mixture was then filtered and put in a 100 mL volumetric flask and topped to the mark using the extraction solution. All samples were extracted in triplicates. Folate and AA were determined using high-pressure liquid chromatography with ultra-UV-visible detection, after extraction of the vitamins from raw and cooked samples. Analysis of Variance (ANOVA) was used to determine the difference in nutrient retention by various cooking methods. Significance was imputed at $p<0.05$. The AA and folate levels in raw vegetables ranged from 9.36±0.12 mg/100g to 60.28±0.32 mg/100 and 35.83±0.23 µg/100g to 258.08±0.58 µg/100g respectively. The cooked samples of the vegetables contained folate levels ranging from 15.59±0.19 µg/100g to 258.08±0.58 µg/100g. The AA mean concentration levels in cooked vegetables were found to be ranging from 1.36±0.02 mg/100g to 39.53±0.40 mg/100g. Therefore, it was determined that cooking the vegetable significantly reduced both folate and AA concentration compared to the raw vegetable samples, $p<0.05$. Steaming vegetables resulted in significantly higher retention of vitamins compared to other cooking methods. Significant losses of the vitamins were found in vegetables boiled in lye. Therefore, this study recommended that AIVs should be cooked by steaming which leads to higher retention of both folates and AA. The addition of lye and sodium bicarbonate should be avoided during the cooking of AIVs since they cause significant losses of vitamins. The results will be availed to relevant authorities and also used to sensitize vegetable consumers and cooks.

CHAPTER ONE

INTRODUCTION

1.1 Background information

High number of the population worldwide particularly women and children in resource-poor households are greatly affected by the shortage of essential vitamins and minerals leading to devastating outcomes in public health and social and economic development (Boy *et al*., 2009). The deficiencies of vitamins and minerals contribute highly to the problem of diseases associated with unfavorable functional outcomes like increased susceptibility to infections, stunting, cognitive losses, birth defects, premature mortality and blindness (Boy *et al*., 2009) among others. Nonetheless, drastic vitamin deficiencies remain clinically unacknowledged, yet even minimal deficiencies may have major severe consequences (Griffiths, 2012).

These deficiencies are due to a shortage of access to foods rich in vitamins and minerals caused by poverty and unfavorable climatic conditions (Cheruiyot, 2011). Other factors that have contributed to vitamin deficiencies are overreliance on staple foods and foods that have been highly refined especially in urban areas (Cheruiyot, 2011). To reduce the shortage of vitamins and minerals, many states have resorted to food fortification and supplementation (Cheruiyot, 2011; Wakhanu, 2014) with vital minerals and vitamins. However, this is an expensive resolution for most African states with a larger population being poor and thus cannot afford fortified food products (Wakhanu, 2014). Hence, there is a need to consider alternative sources of vitamins that are less costly (Cheruiyot, 2011; Wakhanu, 2014) that can provide these vitamins.

Vitamin B9 and C are vital in maternal health, and fetal development as well as sustaining the health and well-being of individuals in the population who barely cope during shortages of dietary resources such as those with disabilities and the poor (Halestrap and Scheenstra, 2018). These vitamins are among the vitamins that have been of concern worldwide due to their deficiency outcomes.

A study by (AED, 2006; Mgamb *et al*., 2017) reported folate deficiency of 81.8 % in adolescent and 61.3 % in expectant mothers of which 26.4 % of the pregnant women were from rural areas and the reduction of these deficiencies were reduced by food fortification. Human birth defects are a major concern in public health (Mgamb *et al*., 2017). In the United States, 3 % of birth presents birth defects each year (Crider *et al.*, 2011; CDC-Info, 2020). Among the regular defects are Heart defects and Neural tube defects (NTDs). Spinal bifida and anencephaly are the regular types of NTDs occurring in 1 in 300,000 pregnancies worldwide (Botto *et al.*, 1999; McKillop *et al*., 2002). A study conducted in Kijabe Mission Hospital for a period between 2005-2010 in Kenya indicated that of 6041 surgical records, 93 % were reported as diagnosis of spinal bifida (Githuku *et al*., 2014). Also, 4.6 per 1000 birth in Kenya present NTDs (Muga, 2009) and 800 birth defects were reported in a period of five years, 2013-2017 in Kenya (Wafula, 2017). These are a low estimate of prevalence since studies were conducted on populations visiting specific hospitals only.

Folate deficiencies remain to be prevalent due to reduced consumption of whole grain and green leafy vegetables which are rich in folates (AED, 2006). Intake of adequate amounts of folic acid prior to conception and during pregnancy prevents half the occurrence of NTDs (Butterworth and Bendich, 2004; Botto *et al*., 1999). Houk *et al. (*1992), McKillop *et al*. (2002). Butterworth and

Bendich, (2004) recommended that, to lessen the risk of having a pregnancy affected with spinal bifida or other NTDs all women of accouchement age are to take 0.4 mg (400 µg/400 mcg) of folic acid per day. They should also consume foods rich in folates. In addition to the supplements, one can increase folate ingestion by eating foods rich in folates including those that have been fortified with folates (Australia, 2018).

Furthermore, scurvy is often considered an ailment of the past; however, its manifestation is continually encountered in children with mental or physical disabilities and those with abnormal dietary habits as well as the elderly worldwide (Halestrap and Scheenstra, 2018). Halestrap and Scheenstra (2018) documented its outbreak in a semi-arid region after a period of drought and famine. Despite the massive outbreak, unnecessary investigations and delayed diagnosis put over 65 patients at risk of other diseases affiliated with malnutrition (Halestrap and Scheenstra, 2018). Nonetheless, healing and recovery of the patients were realized after intervention with ascorbic acid (AA) (Halestrap and Scheenstra, 2018). The world health organization (WHO) recommends a daily intake of 40 mg of ascorbic acid per adult person to prevent scurvy (Halestrap and Scheenstra, 2018).

Many foods are rich in folate and AA; good sources include green leafy vegetables, fruits, legumes and some breakfast cereals. Among the green leafy vegetables are the African Indigenous Vegetables (AIVs) that have highly been promoted in terms of production, marketing and consumption (Kebede and Bokelmann, 2017) to enhance food security. In African families, AIVs are considered the cheapest sources of minerals and vitamins but have been underutilized due to limited nutritional information (Everlyn and Chitechi, 2014; Wakhanu, 2014), unpalatability and westernization. These vegetables are characterized by their affordability, availability, being hardy to harsh weather conditions and pathogens hence a good choice for dietary diversification (Habwe *et al*., 2010; Wakhanu, 2014).

Like all foods, nutritional losses occur during the preparation and cooking of vegetables, thus the consumer needs to understand how and why it occurs to limit the losses and enhance the quality of the vegetables (Fabbri and Crosby, 2016). Investigation on the impacts of different domestic cooking methods on folates and AA are limited, with available studies based on industrial processes (McKillop *et al*., 2002) and western vegetables. Furthermore, there is no evidence of the consequence of different cooking techniques on levels of folates and AA in African indigenous vegetables (AIVs) (Wakhanu, 2014).

Like all vitamins B and C 5-MTHF and ascorbic acid are water-soluble (McKillop *et al*., 2002; Fabbri and Crosby, 2016; Arnarson, 2017) and their losses during preparation and cooking are due to thermal decomposition and leaching of the vitamin into cooking water (McKillop *et al*., 2002). Environmental factors such as oxygen content, pH, antioxidant levels, metal ion concentration, duration and product: water ratio also influence folate and AA retention in vegetables (Vicente, 2009; Barrett and Lloyd, 2012). Steaming vegetables does not result in significant losses of folates while boiling significantly reduces the content of both folates and ascorbic acid (McKillop *et al.*, 2002; Habwe *et al*., 2010; Zeng, 2013). Frying vegetables with tomatoes and onions slightly raises vitamin C content compared to raw samples while boiling with traditional salt, lye reduces the vitamin C content (Habwe *et al*., 2010).

In Kenya, cooking methods of AIVs mainly involve boiling in unspecified amounts of water and discarding the boiling effluents leading to high nutrient loss (Wakhanu, 2014). There is also the addition of additives such as bicarbonate of soda, lye (traditional salt), milk, cream, sesame and groundnuts paste whose effect on nutrient levels especially folate levels and bioavailability in AIVs are yet to be explained (Wakhanu, 2014).

1.2 Problem statement and justification

Food fortification, supplementation as well as diversification possess great approval as crucial ways of alleviating food insecurities and vitamin and mineral deficiencies (Cheruiyot, 2011; Wakhanu, 2014). African indigenous vegetables have been characterized as being hardy to adverse weather conditions, cheap thus easily affordable to the poor as well as being resistant to pathogens and calling for less care and observation (Habwe *et al*., 2010; Cheruiyot, 2011; Wakhanu, 2014). For these reasons, AIVs have been endorsed as vital sources of macronutrients, boosters of food security among many African communities and a way of diversifying nutrients and food sources (Habwe *et al*., 2010; Cheruiyot, 2011; Wakhanu, 2014). AIVs are highly consumed in both rural and urban areas in Kenya with the highest consumption being in rural areas (Gido *et al*., 2017). Consumption of AIVs is increased with an increase in the variety of vegetables at retail outlets (Gido *et al*., 2017). However, AIVs remain underutilized due to a shortage of knowledge about them (Wakhanu, 2014). Nonetheless, the nutrient content of the AIVs can be altered by many factors including post-harvesting processes (Cheruiyot, 2011).

One of the factors that affect the nutrient content of AIVs is cooking (Habwe *et al*., 2010). Traditionally, these vegetables are cooked in undefined quantities of water contributing to high nutrient loss (Habwe *et al*., 2010; Wakhanu, 2014). Additives such as fresh and sour milk, bicarbonate of soda, lye (traditional salt) and common salt are also used yet their effects on levels of folate and ascorbic acid in the AIVs are unknown. Nonetheless, some additives are commonly used on some vegetables and avoided in others. Lye is commonly used in cooking the broad-leafed pumpkin as well as the medium-leafed cowpea leaves. In these two vegetables, sour milk is not used. Sour milk is commonly added to spider plant leaves and occasionally in the African nightshade. The selection of additives and recipe varies from place to place and it also differs with the vegetables. There is a need to investigate by quantifying the levels of nutrients with different cooking methods and the use of different additives on AIVs. The effects of these traditional cooking procedures on levels of vital nutrients like folate and AA in AIVs are important in promoting their consumption. The effects of traditional cooking methods on levels of folate and ascorbic acid in selected AIVs such as spider plant (saget), cowpea and pumpkin leaves are necessary for the selection of recipes with the highest folate and ascorbic acid retention. Hence, a recipe with highest retention of the vitamins will be recommended as a preparation procedure in consumption of the vegetables.

1.3 Hypothesis

There is a significant difference in retention of folate and ascorbic acid in *Vigna Unguiculata*, *Cleome gynandra* and *Cucurbita moschata* cooked using different methods.

1.4 Objectives

1.4.1 General objective

To investigate the effect of cooking methods on retention of ascorbic acid and 5-methyltetrahydrofolate in cowpea, spider plant and pumpkin leaves.

1.4.2 Specific objectives

i. To determine the levels of folates and vitamin C in raw cowpea [*Vigna Unguiculata* (L.) Walp], saget (spider plant) [*Cleome gynandra* (L.)] and pumpkin leaves (*Cucurbita moschata*) using High-Pressure Liquid Chromatography.

ii. To determine the effect of different cooking methods on retention of folate and ascorbic acid, AA (vitamin C) levels in cowpea [*Vigna Unguiculata* (L.) Walp], saget (spider plant) [*Cleome gynandra* (L.)] and pumpkin leaves (*Cucurbita moschata*)

1.5 Significance of the study

The investigation brings nutritional information in form of levels of folates and vitamin C in AIVs cooked using different methods. This will advocate for the utilization of the AIVs to increase folate and vitamin C intake to reduce NTDs and prevent scurvy attacks in the Kenyan population. Apart from NTDs, folate deficiencies increase the chances of placenta abruption, premature birth and low birth weight. It also causes infertility, cardiovascular disease decreased cognitive function and autism. Vitamin C deficiency also causes poor immunity, slow healing of wounds, persistent iron deficiency anemia and weak bones. Thus, reducing all these effects is a worthy goal. The information in this study brings into perspective the effect of different cooking methods on folate and vitamin C levels in the selected AIVs for proper decision-making by chefs, nutritionists and consumers to utilize a method with the highest retention thus reducing problems posed by folate and ascorbic acid deficiencies.

1.6 Scope and limitations

Although there are many AIVs, this study only looked at three: cowpea [*Vigna Unguiculata* (L.) Walp], saget (spider plant) [*Cleome gynandra* (L.)] and pumpkin leaves (*Cucurbita moschata*). The study focused on investigating folates and vitamin C only among other vitamins. Source, variety and age of the AIVs were not considered. Seasonal variation was also not examined and sampling was only done from one open market, Githurai market, Nairobi Kenya. The soil conditions under which the vegetables were grown were not examined. Folate and ascorbic acid levels in additives used were not determined.

CHAPTER TWO

LITERATURE REVIEW

2.1 Malnutrition

Prolonged drought, food insecurity and famine result in deficiencies of major nutrients particularly in expectant and lactating mothers, children under five years of age, poverty-stricken and individuals in the population who barely cope when there is a shortage of food (Halestrap and Scheenstra, 2018; MoH, 2018). For many years, scientists worked tirelessly to find a simple, cheap and effective public health intervention that reduces the high mortality rates in children as well as keeps them out of wheelchairs (Oakley, 2009). One cheaper way of managing macronutrient deficiencies in Kenya as identified by the ministry of health (MoH) is the fortification of salt, maize flour, wheat flour, vegetable oils and fats (MoH, 2018).

Supplementation is also considered an alternative strategic intervention to macronutrient deficiencies in developing countries (Faber *et al*., 2014). Despite the implementation of these strategies (fortification and supplementation) vitamins and mineral deficiencies remain a concern of the public (Faber *et al*., 2014; MoH, 2018) since the majority of the population are poor and cannot afford fortified foods (Wakhanu, 2014). The most sustainable and reliable strategy to curb vitamin and mineral deficiencies in developing countries as recommended by Faber *et al*. (2014) is diversification and modification of the diet. Some of the commonly deficient vitamins include folates and ascorbic acid (vitamin C) as discussed in the following section.

2.2 Folic acid and folate

Folate refers to the group of chemically related compounds with the folic structure belonging to the B-complex group of vitamins and cannot be synthesized de novo by the body (Greenberg *et al.*, 2011). The metabolic active form of folate found in nature is 5-methyltetrahydrofolate (5-MTHF). Folic acid is the manufactured dietary supplement that is used in food fortification and supplementation, while dietary folate is the naturally occurring nutri,ent found in foods. 5-MTHF is a water-soluble vitamin. The molecular formula of 5-MTHF is $C_{20}H_{25}N_7O_6$ and its IUPAC name is (2*R*)-2-[[4-[(2-amino-5-methyl-4-oxo-3,6,7,8-tetrahydropteridin-6-yl) methylamino]benzoyl]amino]pentanedioic acid (Kwon *et al*., 2008). Folic acid is made up of a pterin moiety (purine and pyrazine fused together) that is linked to the side chain containing *p*-aminobenzoic acid (pteroic acid) and glutamic acid, Figure 2.1. FA exists in lactam and lactim tautomeric forms. The lactam form has a 4-OH moiety on the purine-type ring, Figure 2.1. In naturally occurring folates, the pteridine ring is reduced resulting in either 7,8-dihydrofolate (DHF) or 5,6,7,8-tetrahydrofolate (THF) (Ball, 2006), Figure 2.1.

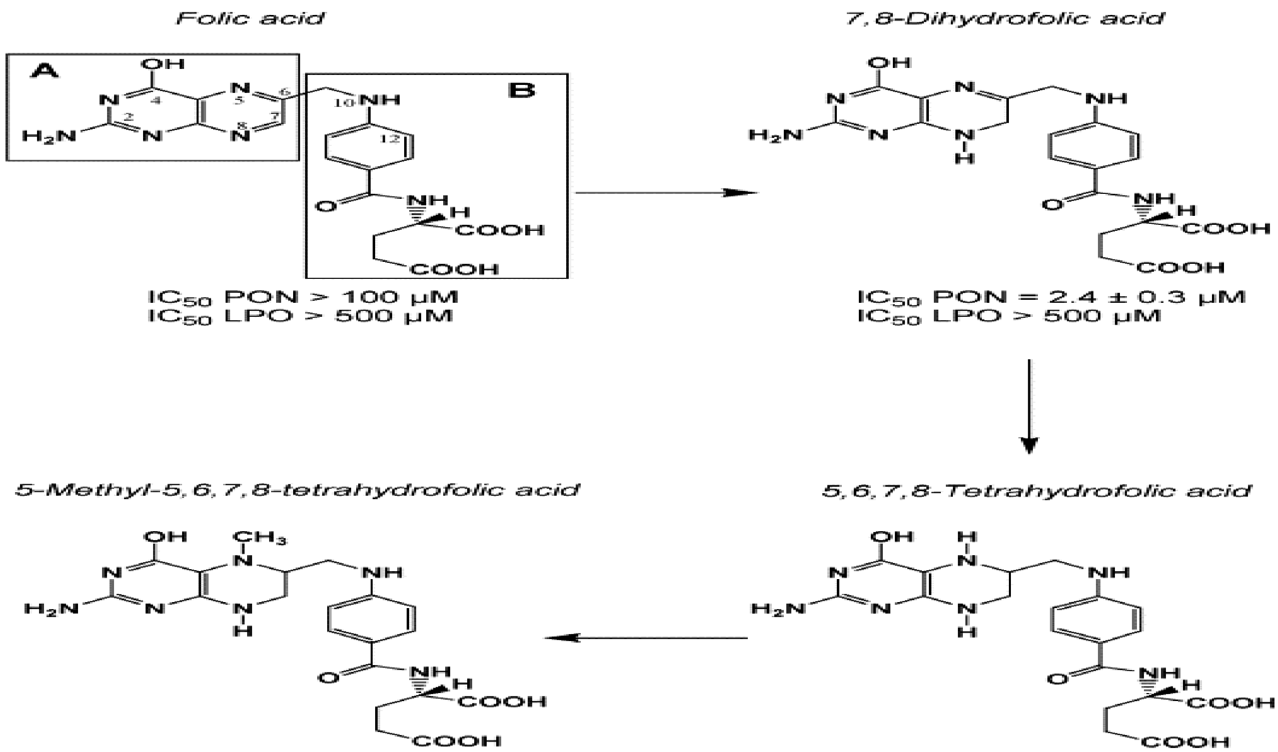

Figure 2.1: Chemical structure of 5-MTHF

. Further substitution of the THF with a covalently bonded one-carbon adduct attached to the nitrogen position 5 or 10 or bridged across leads to the formation of vital intermediates in folate metabolism: 10-formyl-, 5-methyl-, 5-formimino- and 5,10-methyl-THF (Ball, 2006). The most predominant form of folate found in fruits and vegetables is the 5-methyltetrahydrofolate (5-CH_3-H_4folate) (Hefni *et al.*, 2010). Folate is soluble in water and is used in creating more red blood cells, synthesizing DNA and RNA, helping in quick cell division and enhancing growth and preventing hearing loss (Hartridge *et al.*, 1999).

The deficiency of folate can lead to anemia, congenital malformation, a higher risk of developing clinical depression and allergic diseases, challenges with memory and brain function and a high potential long-term risk of lower bone density (Hartridge *et al.*, 1999; Ball, 2006; Soofi *et al.*, 2017). Folate deficiencies can also cause cancer where cell division occurs with misrepaired or unrepaired DNA that has been damaged through folate deficiencies leading to mutations of critical genes like proto-oncogenes or tumor suppressor genes (Ball, 2006). Spinal bifida and anencephaly are regular neural-tube defects, NTDs that are primarily caused by folate deficiency (Botto *et al*., 1999; Oakley, 2009; Crider *et al*., 2011) and their removal is a worthy goal (Oakley, 2009). NTDs affect 300,000 or more children worldwide (Botto *et al*., 1999) and thus remains a concern. Zaganjor et al. (2016) reported that in every 10,000 worldwide, 6.0 % are presented with NTDs. According to (Crider *et al.,* 2011) NTDs occur when the neural tube fails to close early in embryonic development, leading to damage to underlying neural tissues. The defects can be fatal or even cause morbidity depending on the location and severity of the lesion.

Many studies have recommended the intake of folic acid to reduce pregnancies with NTDs by over 50 % (Houk *et al*., 1992; Botto *et al*., 1999; Crider *et al*., 2011; Safi *et al*., 2012; Australia, 2018). According to the studies, women capable of accouchement are recommended to consume 0.4 mg (400 µg/400 mcg) of folic acid per day, in addition to devouring foods rich in folates and those that have been fortified with folic acid. Despite the emphasis on the consumption of folic acid in curbing NTDs, the mechanism(s) by which folic acid limits the risk of NTDs is yet to be done hence open for research (Crider *et al*., 2011).

2.3 Vitamin C and the prevention of scurvy

Vitamin C refers to the group of chemically related compounds that exhibit the biological activity of ascorbic acid. L-ascorbic acid is the principal compound that occurs naturally with vitamin C activity. Its chemical formula is $C_6H_8O_6$ with a molecular weight of 176.1 (Ball, 2006). The

IUPAC name of L-ascorbic acid is (5R)-[(1S)-1,2-dihydroxyethyl]-3,4-dihydroxyfuran-2(5H)-one (Meloni *et al*., 2008). The L-ascorbic acid chemical structure is as shown in Figure 2.2

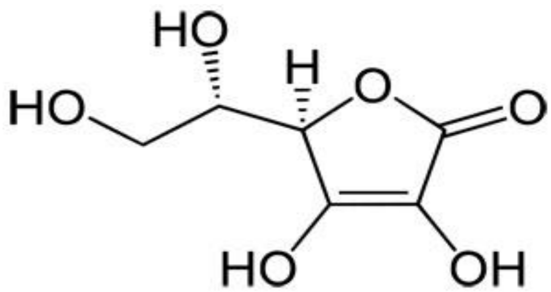

Figure 2.2: Chemical structure of L-ascorbic

One of the diseases associated with dietary deficiency is scurvy. Scuvy results from the deficiency of vitamin C and its symptoms include; swollen and spongy gums, swollen joints, thickening of the epidermis and dependent oedema (Ball, 2006; Halestrap and Scheenstra, 2018; Weise, 1999).

The consideration of scurvy as a historical ailment puts patients at risk since its active occurrence has not been encountered by many doctors thus they may not consider it in their diagnosis (Halestrap and Scheenstra, 2018). Nonetheless, the WHO recommends a daily intake of 40 mg per day per person of vitamin C to prevent scurvy (Halestrap and Scheenstra, 2018). Majorly vitamin C is sourced from a diet containing Fruits and vegetables. L-ascorbic acid is found in citrus fruits and many green leafy vegetables and is also sold as supplements. AA is used in both prevention and treatment of scurvy (Tveden-Nyborg *et al*., 2012). It is a strong reducing agent and a powerful antioxidant useful in fighting bacterial infections, detoxification and formation of collagen in fibrous tissue, teeth, bones, connective tissue, skin, and capillaries (Milanesio *et al*., 1997; Valerica

Georgescu *et al*., 2019). African indigenous vegetables are good sources of 5-MTHF and L-ascorbic acid.

2.4 African Indigenous vegetables and their potential as folate and vitamin C sources

For many years, Africans have been known to consume leaves, young shoots and flowers that grew wildly as vegetables (Abukutsa-Onyango, 2010; Wakhanu, 2014). The vegetables were known to originate within the African continent or had been introduced into the continent long ago and their consumption is considered a cultural heritage (Abukutsa-Onyango, 2014; Wakhanu, 2014) thus the name african indigenous vegetables (AIVs) (Abukutsa-Onyango, 2010). There are over 200 species of AIVs in Kenya, however, urbanization and cultural changes have resulted in the negligence of most of the vegetables (Abukutsa-Onyango *et al*., 2005). The main vegetables that are still in use today are as shown in Table 2.1

Table 2.1: AIVs used in Kenya today

English name
Amaranth
Bitter leaf
Spider plant
Black nightshade
Pumpkin leaves
Cowpea leaves
Local kale
Jute leaves
African vine spinach

(Abukutsa-Onyango *et al*., 2005)

Apart from vitamins, AIVs contain significant levels of minerals like calcium, iron and phosphorous but the advantages and potential of these vegetables remain unexploited (Abukutsa-Onyango, 2010; Wakhanu, 2014) and underutilized due to a shortage of information (Wakhanu, 2014). The contents of vitamins and minerals in AIVs are often higher than in exotic vegetables (Abukutsa-Onyango, 2010; Wakhanu, 2014). AIVs are hardy to harsh environmental conditions, cheap to cultivate and also contain anti-cancer and antioxidants in addition to macronutrients (Wakhanu, 2014). As a result, they are highly recommended nutrient sources to the population in both rural and urban homes in Kenya (Abukutsa-Onyango, 2010).

Some of the AIVs consumed in Kenya include; Amaranths (*Amaranthus species*), saget or spider plant (*Cleome gynandra*), African nightshade (*Solanum species*), cowpeas (*Vigna unguiculata*), jute mallow (*Corchorus olitorius*), pumpkin leaves (*Cucurbita moschata*) (Abukutsa, 2010; Wakhanu, 2014) and many others. Generally, high concentrations of folates and AA are sourced from legumes, green leafy vegetables and some fruits (Van Jaarsveld *et al*., 2014; Benoist, 2015). Delchier *et al*. (2016) found high content in spinach, turnip and cabbage, each having an average of 165, 124 and 66 µg/ 100 g respectively. Other than these exotic vegetables, AIVs have been highly recommended for their high folate content (Benoist, 2015) and their nutritive and mineral values (Wakhanu, 2014). Some of the indigenous vegetables with high folate content are as shown in Table 2.2

Table 2.2: Folate content per 100g edible portion of leaves of raw AIVs

Vegetable	Folate content (µg/100g)
Black nightshade	56
Pigweed	75
Jew's mallow	14
Cowpea	105
Pumpkin	47
Tsamma melon	68
Spider flower	121

(Benoist, 2015)

African indigenous vegetables have also been found to contain vitamin C as shown in Table 2.3

Table 2.3: Ascorbic acid content in mg/g of AIVs

Vegetable	Ascorbic acid content (mg/g)
Slenderleaf	6.4
Amaranth	6
Cowpea	5.7
Nightshade	5.7
Pumpkin leaves	1.7
Spider plant	1.44

(Habwe *et al*., 2010)

2.5 Effect of cooking on folate and vitamin C levels in vegetables

The AIVs are prepared and cooked for consumption to improve texture and flavor. Cooking strongly influences the degradation of folates and ascorbic acid in foods (McKillop *et al*., 2002; Habwe *et al*., 2010) due to thermolysis and draining of the water-soluble vitamin. Several studies conducted by Delchier *et al*. (2016), DeSouza and Eitenmiller (1986) and McKillop *et al*. (2002) on exotic vegetables like spinach and broccoli had the following observations; steaming and microwave heating resulted in no significant loss of folates, boiling leads to a high decrease in the concentration of folate in both vegetables compared to the raw samples of 49 % and 44 % TFA respectively. Water blanching results to lower retention of the folate compared to steam blanching. This is attributed to the leaching of the folate into the blanching effluent. Boiling also reduces significantly the content of vitamin C in vegetables while frying with onions and tomatoes raised the content of the ascorbic acid significantly (Habwe *et al*., 2010). Similarly to these exotic vegetables, cooking also affects the nutrients in AIVs. The process of cooking AIVs varies with the type of vegetable (FAO/Government of Kenya, 2018a) due to the texture and palatability preferences of the different vegetables (Wakhanu, 2014).

2.6 Cooking methods for AIVs

In most African countries, vegetables are considered an accompanying dish for starchy staples like maize paste and porridge made from cereals (Shackleton *et al*., 2009). The preparation methods vary from one place to another. Nonetheless, pots are more commonly used in most rural areas than pans for a better simmering effect (Abukutsa-Onyango *et al*., 2005). There is also variation in the cooking method in urban and rural areas (Shackleton *et al*., 2009). Lye is a common additive used in cooking AIVs in rural Kenya while bicarbonate of soda is commonly used in urban areas.

In Senegal, cooking AIVs involves mixing the vegetables with staples, fish or meat and cooking the mixture over a prolonged period (Shackleton *et al*., 2009). The most common cooking method employed in Kenya and Tanzania is cooking the vegetables by boiling in an unspecified amount of water for about 40 minutes and then discarding the boiling eluent (Abukutsa-Onyango *et al*., 2005; Shackleton *et al*., 2009; Wakhanu, 2014). Boiling, steaming and frying are the most common methods used in cooking vegetables (Shackleton *et al*., 2009).

There is also the addition of ingredients in cooking AIVs. The use of the ingredients varies from one place to another depending on their availability (Shackleton *et al*., 2009). In coastal Tanzania, coconut milk is used while the use of groundnuts is common in semi-arid central Tanzania (Shackleton *et al*., 2009). Commonly used additives for cooking AIVs in Kenya include ghee, cream, milk, simsim sauce or groundnut sauce (Abukutsa-Onyango *et al*., 2005). The rationale behind the use of additives is to improve vegetable palatability as well as enhance their nutrient level (Abukutsa-Onyango *et al*., 2005). There is therefore a need to assess the effect of different cooking methods on nutrient levels in AIVs.

2.7 Techniques of analyzing folates and ascorbic acid

The most often used methods for folate determination are microbiological assay and High-Pressure Liquid Chromatography (HPLC) assay (Holasová *et al.*,2008). While analysis of AA is commonly done by chemical analysis involving reducing dichlorophenolindophenol (Martin *et al*., 2016). This study employed the use of reversed-phase HPLC as described by Watada (1982), Holasová *et al*. (2008) and Abdullah (2016).

2.7.1 High-pressure liquid chromatography (HPLC) and its working principle

Introduction of HPLC lead to increased sample analysis in scientific research. Classical column chromatography relied on gravity to pass samples and mobile phases through a column for separation. Some of the merits of HPLC analysis include high speed analysis with reduced solvent consumption and low level of detection and quantification. There is also high separation efficiencies with lowest column back pressure and separation over wide ranges of temperature.

Separation of sample components in HPLC occurs when the sample and liquid mobile phase are injected and forced by high pressure through a liquid stationary phase coated on small particles in a packed column (Ahuja and Jespersen, 2008). In the case of water-soluble vitamins, the separation of sample components is based on their polarities (Sibley and Porter, 2010). The block diagram for HPLC is shown in figure 2.3.

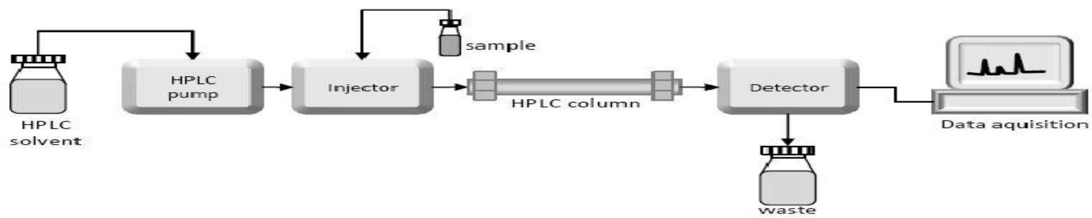

Figure 2.3: Schematic diagram of HPLC

On reaching an inline UV-visible spectrometer detector, the analyte components are identified and quantified by analyzing the difference in the amount of light absorbed by the analyte in comparison to incident light as explained by Beer-Lambert law (Clark, 2020). The law states that absorbance is proportional to the concentration of the substance.

A = *€ b c*

A = absorbance

€ = molar absorptivity

b = optical path length in centimeters

c = concentration

A wavelength of maximum absorption for the analyte is selected when determining the concentration of the analyte. Several known concentrations of a standard solution of the analyte are measured to obtain a calibration curve; usually a straight line if the Beer-Lambert law holds.

The curve is then used to determine the unknown concentration of the analyte (Ahuja and Jespersen, 2008). Nonetheless, at higher concentrations (>0.01 $molL^{-1}$) Beer's law is invalid due to interactions of the analyte molecules (LibreText, 2020). There are also instrument and chemical deviations that limit Beer's law. The validity of Beer's law is confirmed by the closeness of data points to the line of best fit, this shows direct proportionality between absorbance and concentration (LibreText, 2020).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Research design

The research was an experimental design where levels of folate (Vitamin B_9) and ascorbic acid, AA (Vitamin C) were analyzed in triplicates from formulated recipes and then compared to the raw vegetable samples obtained from selected AIVs from Githurai market in Nairobi County Kenya.

3.2 Samples and sample preparation

Vegetable samples: Saget (*Cleome gynandra*), Cowpeas (*Vigna unguiculata*), and pumpkin (*Cucurbita moschata*) were bought at the local Githurai market between December 2019 and February 2020. The market was selected due to its proximity to Kenyatta University; thus, the vegetables would be purchased while still fresh and transported quickly to the laboratory for analysis. The samples were purchased on the morning of the experiment day from ten different vendors and mixed to uniformity. A total of 6.000 Kg of each type of vegetable was purchased on a given morning. Each vegetable type was purchased twice on different days for analysis of each vitamin. The samples were placed in dark plastic buckets, sealed and transported to the laboratory. On arrival in the laboratory, the fresh and healthy vegetables were mixed and washed immediately under tap water then rinsed with distilled water and excessive water dripped off. All procedures were carried out carefully without much exposure to light. Raw samples were extracted and analyzed while the samples for cooking were cooked and then extracted as explained in the extraction procedure.

3.3 Chemicals and reagents

The HPLC grade reagents; acetonitrile and HPLC water used in flashing the equipment were obtained from Merck Germany. Vitamin standards (5-methyltetrahydrofolate, 5MTHF and L-ascorbic acid), potassium hydrogen carbonate, Metaphosphoric acid (MPA) and acetic acid were of analytical grade from Sigma-Aldrich, Germany. Distilled water was used in the experimental procedures.

3.4 Preparation of extraction solution

3.4.1 Ascorbic acid

Acetic acid, 8 % and MPA 3 % were prepared by dissolving about 15.000 g MPA in 40 mL acetic acid and 200 mL distilled water in a 500 mL beaker and pouring the solution into a 500 mL volumetric flask. The beaker was rinsed thrice with distilled water and the contents were poured into the volumetric flask. To get it to the 500 mL mark, distilled water was added.

3.4.2 Folate

In a 500 mL beaker, 2.002 g of $KHCO_3$ was dissolved in 200ml distilled water before being put into a 1000 mL volumetric flask. The beaker was rinsed thrice with distilled water and the contents were poured into the volumetric flask. To get it to the 1000 mL mark with distilled water added. The resulting solution was 20 mM $KHCO_3$.

3.5 Preparation of standards

An amount of 100.000 mg of vitamin C (L-ascorbic acid) standard and 100.000 mg of folate standards (5-methyltetrahydrofolate, 5MTHF) were weighed into 100 mL beakers and dissolved with 35 mL of the extraction solutions. The solutions were transferred into 1000 mL volumetric flasks and the beakers were rinsed thrice using extraction solutions the contents were poured into the volumetric flasks which were topped up with the extraction solutions to the 1000 mL marks. The concentrations of the resulting stock solutions were equal to 100 ppm. Standards of different concentrations ranging from 5 to 25 ppm and 10 to 100 ppm for AA and folate respectively were prepared by diluting the stock solutions using extraction solution. Each standard was prepared separately since the analysis of the vitamins was also done separately and used for the preparation of calibration curves.

3.6 Preparation of lye (traditional salt)

After harvesting beans, the beans seeds were gotten rid of and then pods were smoldered on a hot pan. A 200.000 g of the ash collected was put in a container with holes at the bottom. Distilled water (500 mL) was poured through the ash a little at a time and filtrate (lye) was collected underneath. This was done according to the modified method described by Wakhanu (2014).

3.7 Cooking

A portion of 100.000 g of the edible portion of vegetable samples including leaves and young shoots, were used in each of the cooking methods according to modified recipes described by FAO/Government of Kenya (2018a). Modifications include the use of additives like lye, sour milk and sodium bicarbonate then cooking each sample for 30 minutes. Blanching and steaming were done for 2 and 5 minutes respectively. Each cooking was done three times.

3.7.1 Blanching and steaming

After dripping off the washing water, 100.000 g of the raw vegetables were cooked. The vegetables were blanched by immersing in boiling water for 2 minutes while steaming was done for 5 minutes. The cooked samples were then immersed in ice-cold water immediately to halt the cooking process.

3.7.2 Boiling in plain water

Boiling was done by putting 100.000 g of raw vegetables in 600 mL of water and boiling for 30 minutes. Decantation was then done to discard the boiling water.

3.7.3 Boiling with lye and sodium bicarbonate

A 25 mL aliquot of lye and 3.000 g of sodium bicarbonate (baking powder) were added separately. The lye and sodium bicarbonate were added to 600 mL of water and then 100.000 g of raw vegetable samples were added and then boiled for 30 minutes. The same amount of lye and sodium bicarbonate was used in other cooking procedures which involved boiling in the additives for 20 minutes, discarding the water then adding milk and cooking for 10 minutes. Some samples were boiled in the additives for 20 minutes, the solution discarded and then fried with a mixture of oil, onions and tomatoes and cook for 10 minutes.

3.7.4 Boling then frying

The vegetable samples that were boiled and then fried were cooked in two continuous processes involving boiling for 20 minutes in 600 mL of water containing 25 mL lye or 3.000 g sodium bicarbonate separately, discarding the boiling water and then frying for 10 minutes. In one case, 100.000 g of vegetables were fried in oil only while in the other case, a mixture of oil, onions and tomatoes was used. During frying with oil, onion and tomatoes, the quantity used for each was 5 mL, 10.000 g and 15.000 g respectively.

3.7.5 Boling then adding milk

Vegetable samples were boiled for 20 minutes in 600 mL water containing 25 mL lye or 3.000 g sodium bicarbonate separately then boiling water was discarded followed by adding 60 mL fresh milk and left to cook for further 10 minutes. A portion of 100.000g of raw vegetables was cooked thrice.

3.7.6 Direct frying

Some 100.000 g raw samples were fried directly without boiling using 5 mL oil only and cooked for 30 minutes. Other samples were fried in a mixture of 5 mL oil, 10.000 g onions and 15.000 g tomatoes then left to cook for 30 minutes. An amount of 10 mL water was added a little at a time during cooking while stirring to avoid scorching.

Each cooking procedure was done three times and after every cooking procedure, rapid cooling was done by spreading the cooked sample on an aluminium foil and floating it in ice-cold water followed by immediate extraction of both 5-MTHF and ascorbic acid. An amount of 10.000 g of each cooked sample was used during extraction. Since the cooking procedure was done three times and for every sample that was cooked, the vitamin of interest was extracted thrice for each analysis.

3.8 Extraction of vitamin

Vitamin B_9 was extracted according to the modified procedure described by Thermo Fischer Scientific (2017) and McKillop *et al.* (2002) while Vitamin C was extracted according to the procedure described by Cheruiyot (2011), Ismail and Fun (2003) and Dodson *et al*. (1992). According to the procedures, mortar and pestle were used to grind 10.000 g of sample then mixed with 50 mL extraction solution containing; 20 mM $KHCO_3$ (for vitamin B_9 extraction) and 3 % Metaphosphoric acid, MPA, and 8 % acetic acid (for vitamin C extraction). The mixture was then placed in a conical flask wrapped with aluminium foil and agitated at 100 rpm using an orbital shaker for 15 minutes at room temperature. The extract was then filtered using Whatman filter paper No. 42 and transferred to a 100 mL volumetric flask and made to the mark with extraction solution. Further filtration was done by passing through a 0.45 µl filter membrane followed by immediate analysis to determine the concentration of vitamins. All samples were extracted in triplicates.

3.9 Identification and quantification of vitamins

Reverse-phase high-pressure liquid chromatography (RP-HPLC Shimadzu 20A Kyoto, Japan) consists of a column (model CTO-10AS VP), a degasser (model DGU-20A5R), an LC pump (model LC-20AD), a UV-Visible diode array detector (model SPD_20A) and an autosampler (model SIL-20AHT) was used in identification and quantification of Vitamin C and folates respectively. The samples were separated with a reverse-phase C_{18} column (Phenomenex C_{18}, 250 x 46mm, 5µm particle size, Luna 5u). Figure 3.1 shows the HPLC equipment used in this study.

Figure 3.1: HPLC equipment used in the study

(Photo taken during analysis in Kenyatta University- Department of food and nutrition)

Sample separations for AA and folate were done isocratically using 8 % MPA as the mobile phase for AA at a flow rate of 1.2 mL/min as described by Cheruiyot (2011) and 25 mM phosphate buffer (pH 3.6) flowing at 0.5 mL/min as the mobile phase for folates as described by Thermo Fischer Scientific, (2017). Detection of AA and folate was done at 254 nm and 280 nm respectively using a UV/Vis detector (Shimadzu UFCL SPD-20A). 20 µL of standards and samples were injected into the column after filtering with a 0.45 µL filter membrane.

3.10 Method verification

3.10.1 Linearity

The quantification of the method used was based on an external standard method. Multilevel calibration curves were used where n=5 for folate and n=6 for ascorbic acid. The standards were prepared as follows: An amount of 100.000 mg of (L-ascorbic acid) standard and 100.000 mg of 5-MTHF were weighed into 100 mL beakers and dissolved with 35 mL of the extraction solutions. The solutions were transferred into 1000 mL volumetric flasks and the beakers were rinsed thrice using extraction solutions and the contents were poured into the volumetric flasks which were topped up with the extraction solutions to the 1000 mL marks. The concentrations of the resulting stock solutions were equal to 100 ppm. Standards of different concentrations ranging from 5 to 25 ppm and 10 to 100 ppm for AA and folate respectively were prepared by diluting the stock solutions using extraction solution and peak area was plotted against concentration and regression analysis was used to fit lines to the data then used for the preparation of calibration curves.

3.10.2 Selectivity

Purity and authenticity of the AA peak in the samples were assessed first by confirming the co-migration of the analyte peaks in the samples with authentic analyte standards.

3.10.3 Precision

Precision was measured in terms of repeatability, which was determined by a sufficient number of aliquots of a homogeneous sample and its % RSD was calculated.

3.10.4 Limit of Detection (LoD)

The limit of detection (LoD) was defined as the lowest analyte concentration yielding a signal-to-noise (S/N) ratio of 3:1. It was calculated using the expressions LoD = 3 s/a; where s is the standard deviation of the blank signal and a is the slope (or angular coefficient) of the calibration curve.

3.10.5 Limit of quantification (LoQ)

The limit of quantification (LOQ) was defined as the lowest analyte concentration yielding a signal-to-noise (S/N) ratio of 10:1. It was calculated using the expressions LOQ = 10 s/a; where s is the standard deviation of the blank signal and a is the slope of the calibration curve.

3.11 Data analysis

Composition of nutrients in vegetable samples was determined in triplicates and means were computed. The obtained data was analyzed using Statistical Product and Service Solutions software (version 20). Analysis of Variance (ANOVA) was used to the determine difference in nutrient retention by various cooking methods. Significance was imputed at p values of less than 0.05.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Chapter overview

The results obtained in this study are elaborated in this chapter.

4.2 Method verification

4.2.1 Linearity

Analytical curves were constructed using folate and AA concentrations between 5 and 25 mg/ L and 10 -100 mg/ L respectively, prepared from a stock 100 mg/ L solution and the results are presented in **Figures 4.1** and **4.2** respectively.

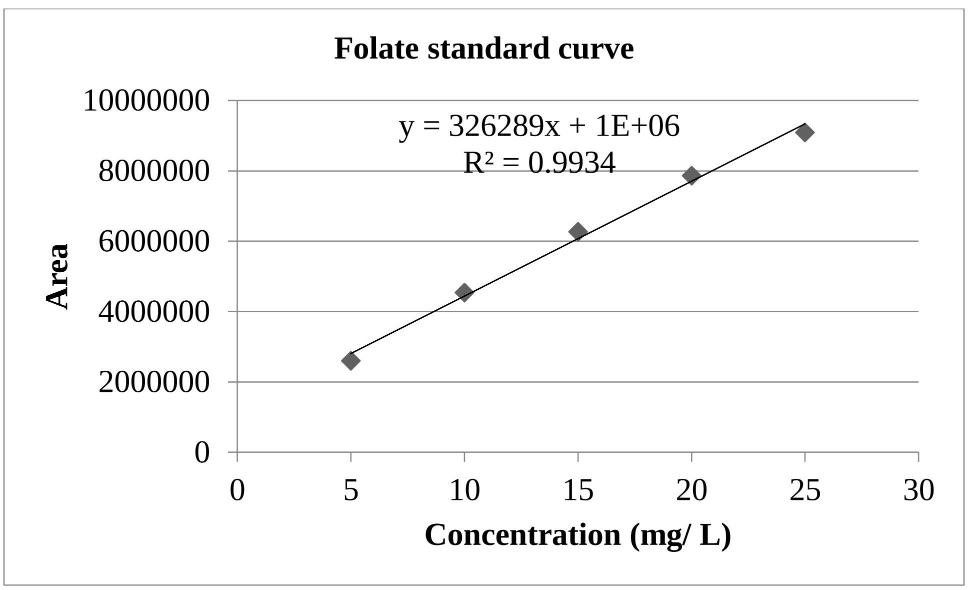

Figure 4.1: Calibration curve for folate determination

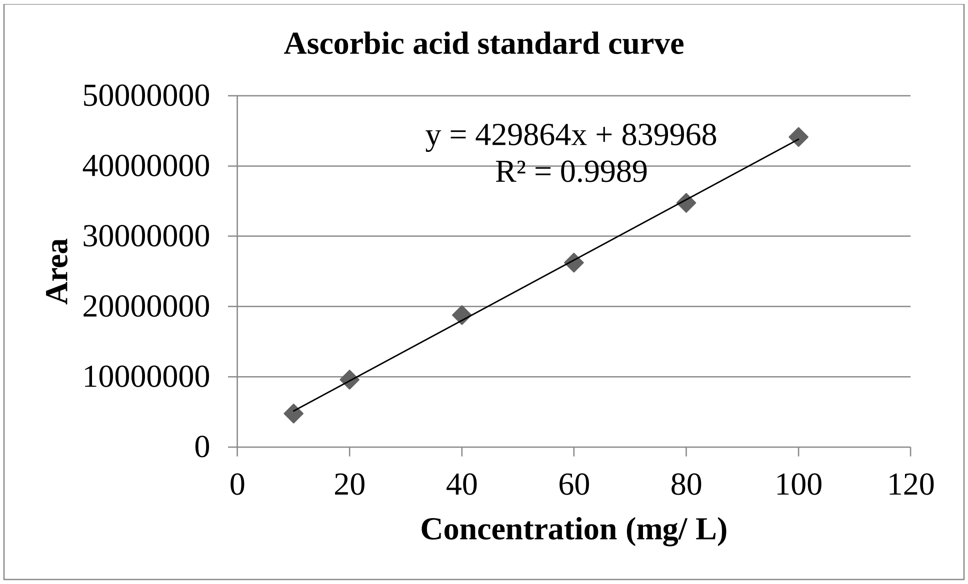

Figure 4.2: Calibration curve for ascorbic acid determination

From figures 4.1 and 4.2, it was observed that both folate and AA methods showed excellent linearity in the concentration ranges analyzed for each. The coefficient of determination r^2 for folic acid and ascorbic acid was 0.9934 and 0.9989, respectively which was higher than the 0.9 threshold for a good linearity curve.

4.2.2 Selectivity

Sample standard and extracts for both folate and ascorbic acid were run through the HPLC-UV equipment to obtain chromatograms and the results were as seen in **figures 4.3, 4.4, 4.5 and 4.6**

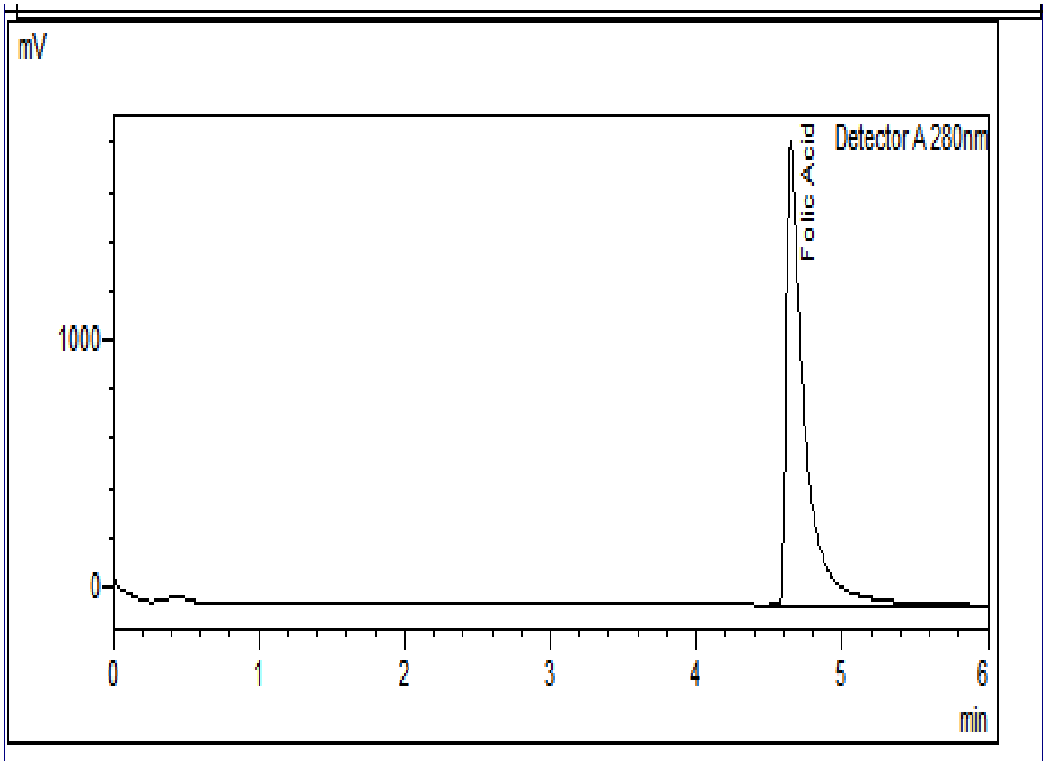

Figure 4.3: HPLC Chromatogram showing peak for folic acid standard

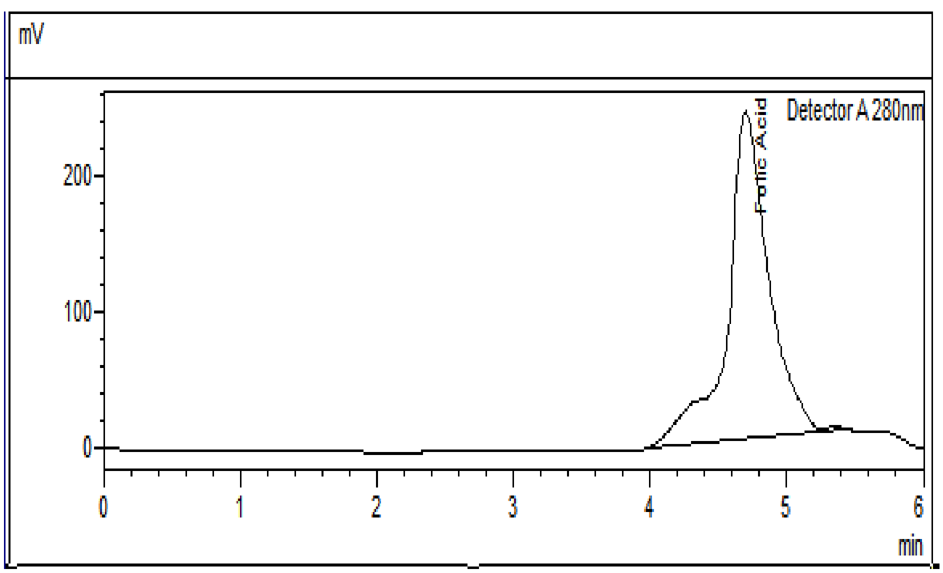

Figure 4.4: HPLC Chromatogram showing peak for folic acid extract

From Figures 4.3 and 4.4, the chromatograms for folate standard and extract show similar retention time (of about 4.75 min) at similar absorbance of 280nm.

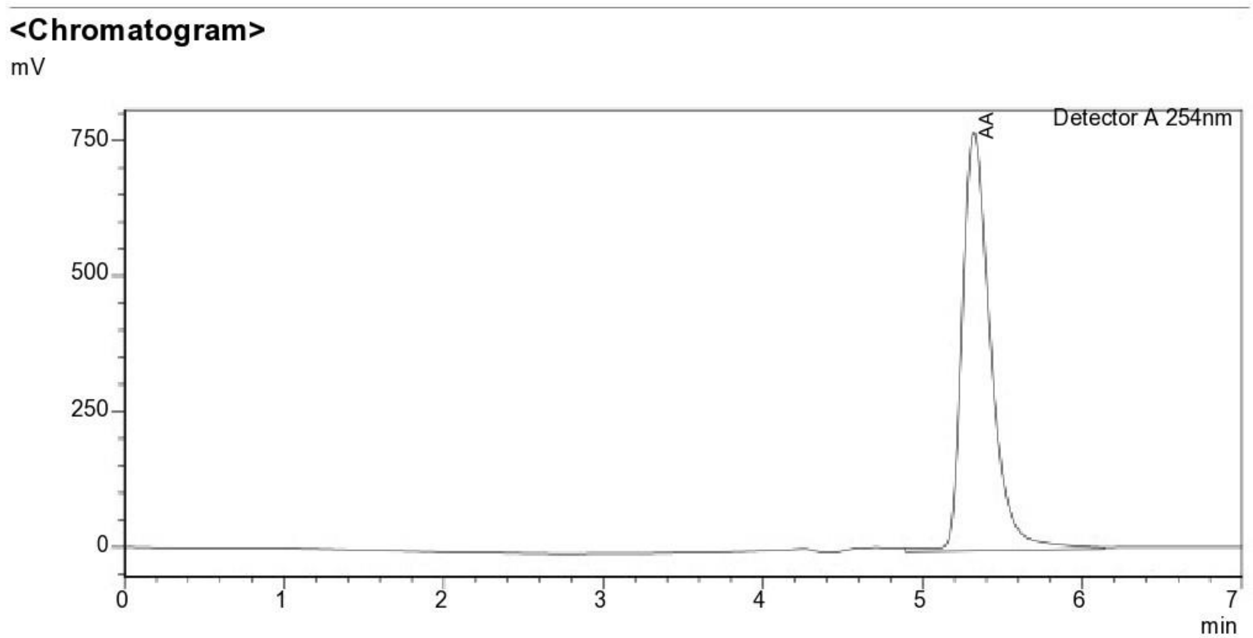

Figure 4.5: HPLC Chromatogram showing peak for ascorbic acid standard

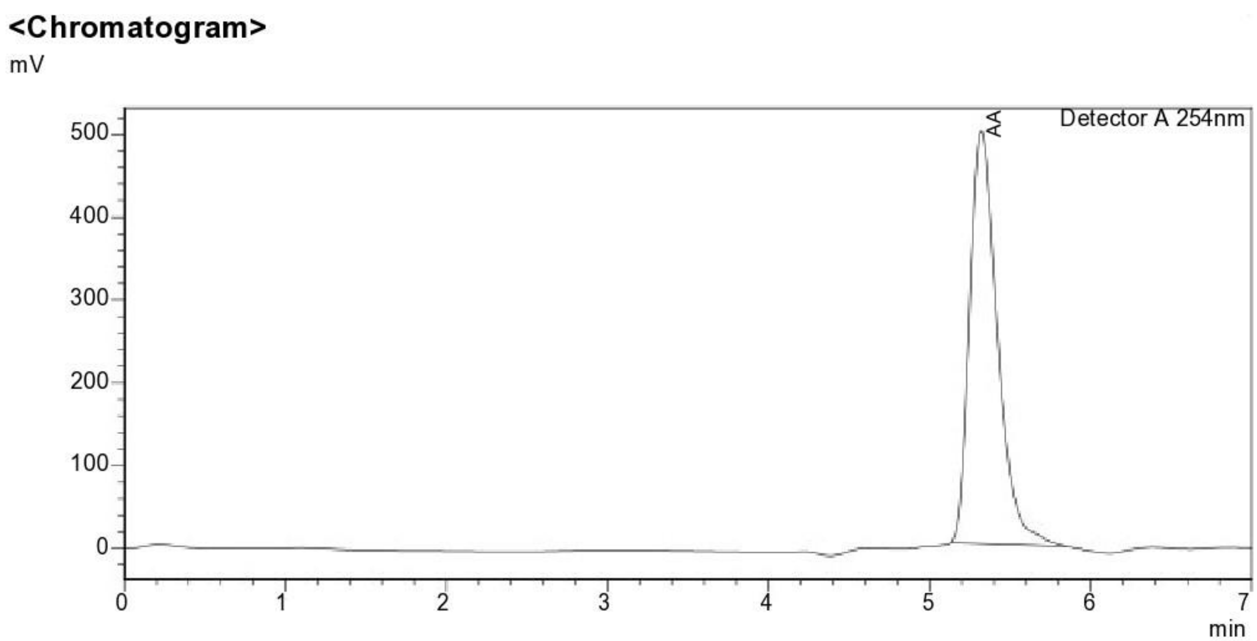

Figure 4.6: HPLC Chromatogram showing peak for ascorbic acid extract

From figures 4.5 and 4.6, the chromatograms for ascorbic acid standards and extracts also showed similarity in retention time (of about 5.32 min) at similar absorbance of 254 nm.

4.2.3 Precision

The results suggest good reliability of the method used for both folic acid and ascorbic acid, since the relative standard deviations for the peak area obtained for each method were below the limit of 25 % acceptable threshold: The % RSD folic acid and ascorbic acid determination were 0.7998 % and 4.64 % respectively.

a) Folic acid standard curve

Descriptive Statistics

	N	Mean	Std. Deviation
Concentration	5	15.0000	.790569
Peak	5	6070057.0000	258807.328194
RF	5	429464.4400	3403.194125
Valid N (listwise)	5		

$$\boldsymbol{Response\ factor(RF)} = \frac{\boldsymbol{Peak}}{\boldsymbol{concentration}}$$

$$\%\ RSD = \frac{SD}{Mean\ RF} \times 100$$

$$\%\ RSD = \frac{3403.194125}{429464.4400} \times 100$$

$$= 0.7998$$

b) Ascorbic acid standard curve

Descriptive Statistics

	N	Mean	Std. Deviation
Concentration	6	51.66666667	3.4880749227
Peak	6	23049632.33333334	1500221.8549891620
RF	6	456709.81208333	21172.827019683
Valid N (listwise)	6		

$$\%RSD = \frac{21172.827019683}{456709.81208333} \text{ x } 100 = 4.64$$

4.2.4 Limit of Detection (LoD)

The limit of detection (LOD) was defined as the lowest analyte concentration yielding a signal-to-noise (S/N) ratio of 3:1. It was calculated using the expressions LOD = 3 *s/a*; where *s* is the standard deviation of the blank signal and *a* is the slope (or angular coefficient) of the calibration curve.

a. Folic acid

$$LOD = 3 \times \frac{3403.194125}{326289}$$

= 0.0313 mg/L= 3.13 µg/100g

b. Ascorbic acid

$$LOD = 3 \times \frac{21172.827019683}{429864.4400}$$

= 0.15 mg/L= 0.015 mg/100g

4.2.5 Limit of quantification (LoQ)

The limit of quantification (LOQ) was defined as the lowest analyte concentration yielding a signal-to-noise (S/N) ratio of 10:1. It was calculated using the expressions LOQ = 10 *s/a*; where *s* is the standard deviation of the blank signal and *a* is the slope of the calibration curve.

a. Folic acid analysis

$$LOQ \quad = 10 \times \frac{3403.194}{326289}$$

= 0.1043 mg/L = 10.43 µg100/g

b. Ascorbic acid analysis

$$LOQ \quad = 10 \times \frac{21172.8270196}{429864}$$

= 0.49 mg/L = 0.049 mg/100g

The lowest concentration of folate in the analyzed samples was 15.59 µg/100g while that of ascorbic acid was 1.36 mg/100g. The results show that both LOD and the LOQ fell below the lowest concentrations of the analytes in the samples analyzed. The methods were thus able to detect and adequately quantify the test analytes (folic acid and ascorbic acid) in the samples.

4.3 Levels of folate in raw and cooked cowpea (*Vigna Unguiculata*) and pumpkin leaves (*Cucurbita moschata*)

The mean concentration of folate in raw and cooked cowpea and pumpkin leaves and their percentage retentions are revealed in Table 4.1.

Table 4.1: Mean concentrations of folate and its retention in pumpkin and cowpea leaves subjected to various cooking treatments

Sample Treatment	Pumpkin Leaves		Cowpea Leaves	
	Concentration (μg/100g)+SE (n=3)	% retention	Concentration (μg/100g)+SE (n=3)	% retention
RL	35.83+0.23[a]	100.00	91.74+0.59[a]	100.00
STL	27.46+0.06[b]	76.64	65.13+0.01[b]	71.00
BL	26.94+0.03[b]	75.19	64.50+0.14[c]	70.31
R-Fro+t	25.76+0.078[c]	71.90	61.54+0.31[d]	67.08
R-Fr	24.291+0.19[d]	67.79	60.35+0.02[e]	65.79
BoBcb+M	23.33+0.12[e]	65.12	56.419+0.01[f]	61.50
BoLye+M	23.04+0.02[e]	64.32	55.47+0.06[g]	60.46
BoBcb-Fro+t	22.46+0.18[f]	62.69	54.37+0.35[h]	59.27
BoLye-Fro+t	22.15+0.15[f]	61.82	53.85+0.00[h]	58.70
BoBcb-Fr	19.38+0.27[g]	54.08	50.91+0.24[i]	55.49
BoL	17.95+0.18[h]	50.11	49.14+0.05[j]	53.56
BoLye-Fr	16.86+0.19[i]	47.05	45.56+0.06[k]	49.66
BoBcb	16.09+0.09[j]	44.91	41.28+0.04[l]	45.00
BoLye	15.59+0.19[k]	43.50	40.71+0.01[l]	44.38
Mean square	89.50, 0.08		488.93, 0.14	
df	13, 28		13, 28	
F-value	1151.748		3515.635	
P-value	<0.05		<0.05	

Key

- **RL** ∞ Raw leaves; **STL** ∞ Steamed Leaves; **BL** ∞ Blanched Leaves; **R-Fro+t** ∞ Raw Leaves Fried with onions and tomatoes; **R-Fr** ∞ Raw Leaves Fried with oil only; **BoBcb+M** ∞ Leaves Boiled in Bicarbonate and Milk added; **BoLye+M** ∞ Leaves Boiled in Lye and Milk added; **BoBcb-Fro+t** ∞ Leaves Boiled in Bicarbonate and Fried with onions and tomatoes; **BoLye-Fro+t** ∞ Leaves Boiled in Lye and Fried with onions and tomatoes; **BoBcb-Fr** ∞ Leaves Boiled in Bicarbonate and Fried with oil only; **BoL** ∞ Boiled Leaves; **BoLye-Fr** ∞ Leaves Boiled in Lye and Fried with oil only; **BoBcb** ∞ Leaves Boiled with Bicarbonate; **BoLye** ∞ Leaves Boiled with Lye
- Concentration values with superscripts of different letters in the same column are significantly different at p=0.05

From Table 4.1, the levels of folate in pumpkin leaves ranged from 15.59±0.19 µg/100g to 35.83±0.23 µg/100g. Raw pumpkin leaves had the highest concentration while the samples boiled in lye had the lowest concentration. Cooking the vegetable resulted in a significant loss of folate compared to the raw samples ($p<0.05$). The mean concentration of folate in cooked pumpkin leaves varied from 15.59±0.19 µg/100g to 27.46±0.06 µg/100g with steamed samples retaining the highest mean concentration of folate at 76.64 % and the lowest retention being found in samples boiled in lye at 43.50 % (as shown in Figure 4.7) of the folate present in raw vegetables. Table 4.1 shows that blanching also led to significant retention of folate at a mean concentration of 26.94±0.03 µg/100g.

Boiling pumpkin leaves in lye and sodium bicarbonate and boiling in lye then frying with oil only, resulted in a significant reduction of folate concentration than boiling the vegetables in water. Nevertheless, boiling the vegetable in water significantly reduced folate concentrations than frying raw vegetables directly, which resulted in 67.79 % retention of the folate as shown in Figure 4.7. Boiling also caused a significant reduction of folate in pumpkin leaves compared to boiling it in sodium bicarbonate or lye and then adding milk. The two cooking methods (boiling in sodium bicarbonate then adding milk and boiling in lye then adding milk) caused retention of 65.12 % and 64.32 % respectively, Figure 4.7. There was also a significant loss during boiling in water than boiling in the sodium bicarbonate or lye then frying with onions and tomatoes or boiling in sodium bicarbonate and then frying in oil only. Lye significantly reduced folate than sodium bicarbonate when used in cooking pumpkin leaves.

Table 4.1, also shows that the levels of folate in cowpea leaves extended from 40.71±0.01 µg/100g to 91.74±0.59 µg/100g. Raw pumpkin leaves had the highest concentration while the samples boiled in lye had the lowest concentration. Cooking the vegetable resulted in a significant loss of folate compared to the raw samples ($p<0.05$). The mean concentration of folate in cooked cowpea leaves varied from 40.71±0.01 µg/100g to 65.13±0.01 µg/100g with steamed samples retaining the highest mean concentration of folate at 71.00 % and the lowest retention being found in samples boiled in lye at 44.38 % (as shown in Figure 4.8) of the folate present in raw vegetables. Table 4.1 shows that blanching also led to significant retention of folate at a mean concentration of 64.50±0.14 µg/100g. Boiling cowpea leaves in lye and sodium bicarbonate and boiling in lye then frying with oil only, resulted in a significant reduction of folate concentration than boiling the vegetables in water. Nevertheless, boiling the vegetable in water significantly reduced folate concentrations than frying raw vegetables directly, which resulted in 65.79 % retention of the folate as shown in Figure 4.8.

Boiling also caused a significant reduction of folate in cowpea leaves compared to boiling it in sodium bicarbonate or lye and then adding milk. The two cooking methods (boiling in sodium bicarbonate then adding milk and boiling in lye then adding milk) caused retention of 61.50 % and 60.46 % respectively, Figure 4.8. There was also a significant loss during boiling in water than boiling in the sodium bicarbonate or lye then frying with onions and tomatoes or boiling in sodium bicarbonate and then frying in oil only. Lye significantly reduced folate than sodium bicarbonate when used in cooking *Vigna Unguiculata* leaves.

This study found that folate concentrations in pumpkin and cowpea leaves were 35.83±0.23 µg/100 and 91.74±0.59 µg/100g respectively, which were not far from the findings by FAO/Government of Kenya (2018b) of 36 µg/100g and 95 µg/100g of edible portions of the vegetable. Therefore, consumption of these vegetables with staple foods in Kenya should be encouraged. The results showed a significant reduction of folate in the vegetables during different cooking methods which was in agreement with the findings of McKillop *et al.* (2002) who attributed the loss in western vegetables (spinach and broccoli) to thermal degradation, oxidation and leaching of the vitamin from vegetables into the cooking water. Our present results showed that steaming followed by blanching did not cause statistically significant loss of folate but rather resulted in higher retention compared to other cooking methods. These findings on pumpkin and cowpea leaves (AIVs) were in agreement with findings in the analysis of western vegetables like spinach, broccoli and brassica (DeSouza and Eitenmiller, 1986; McKillop *et al.*, 2002; Lucia *et al.*, 2014). These studies determined 58 % retention in spinach and 91 % in broccoli, each cooked by steam blanching. Vital to note is that these cooking methods (steaming and blanching) had shorter cooking times compared to those used both in current and previous studies.

There was a significant decrease of folate in boiled pumpkin and cowpea leaves due to leaching of the vitamin into cooking water. DeSouza and Eitenmiller (1986) reported that water blanching caused a higher loss of folate than steam blanching in spinach and broccoli. McKillop *et al.* (2002) reported that boiling spinach and broccoli led to more folate loss than steaming the vegetable while Delchier *et al.* (2016) stated that soaking spinach, turnip, cabbage, and broccoli caused more folates to be leached into the water than that retained in the vegetables. Thus, folate in AIVs is leached into the water just like in western vegetables.

Furthermore, the addition of lye and bicarbonate of soda during boiling caused a further loss of folate in *Cucurbita moschata* and *Vigna unguiculate.* The two additives are characterized by high alkalinity (Sodium bicarbonate has a pH of 8.1 (Haug, 2010) while lye has a pH of 10.8 (Bergeson, 2014)) which destroys cellulose in vitamin cells thus increasing the instability of 5-methyltetrahydrofolate (Ball, 2006). At temperatures above 40 ^{0}C the stability of 5-methyltetrahydrofolate is highly reduced (Gazzali *et al*., 2018). The combination of the high cooking temperature and high alkalinity from lye and sodium bicarbonate resulted in the significant loss of folate in cowpea leaves. This also explains the significant retention observed when the raw vegetables were fried directly either with oil only or in addition of onions and tomatoes during frying compared to boiling and cooking with lye and sodium bicarbonate. Nonetheless, a minimal amount of water was used during the two frying procedures to prevent scorching hence reducing the loss of folate through leaching (DeSouza and Eitenmiller, 1986; McKillop *et al.*, 2002; Ball, 2006; Lucia *et al.*, 2014). Preparation of lye involved burning bean pods at a high temperature of above 300 ^{0}C (Wotton *et al.*, 2012) in presence of oxygen. This results in thermal degradation of 5-MTHF since it is denatured at temperatures above 40 ^{0}C (Gazzali *et al*., 2018) and is also lost through oxidation (Delchier *et al.*, 2014). Therefore, this led to the assumption that the contribution of folate by lye when used as an additive was negligible. On the other hand, no folate was added by sodium bicarbonate into the cooking vegetables since it does not contain folate as an ingredient.

Boiling the vegetables in water only resulted in a significant loss of folate than cooking *Cucurbita moschata* and *Vigna unguiculate* with addition of milk and a mixture of oil, onions and tomatoes even after boiling with lye and sodium bicarbonate. This was attributed to addition of the vitamin into the mixture by the additives. Onions and tomatoes contain significant amounts of folate, 25 µg/100g and 15 µg/100g respectively. Milk also contains 6 µg/100g of folate (George, 2001; FAO/Government of Kenya, 2018b). Thus, cooking vegetables with these additives resulted in significant retention of folate, compared to boiling, which was attributed to the addition of the vitamin into the vegetables by the additives. Nonetheless, addition of milk in the AIV after cooking with lye or sodium bicarbonate resulted in a significantly higher retention of folate compared to the addition of onions and tomatoes. Besides, when the vegetables were boiled in lye and sodium bicarbonate, folate retention was significantly low. According to Ball (2006) thermal stability of 5-methyltetrahydrofolate is greatly increased in presence of iron (II), ascorbate and the protein casein, all present in milk. They reduce dissolved oxygen, hence, reducing the loss of the vitamin through oxidation. The added folate from onions and tomatoes also undergoes more oxidation compared to that added by milk which is more stable due to presence of iron (II), ascorbate and the protein casein, all present in milk.

4.4 Levels of folate in raw and cooked saget (*Cleome gynandra*)

The mean concentrations of folate in raw and cooked saget and their percentage retention are expressed in Table 4.2.

Table 4.2: Mean concentrations of folate and its retention in saget leaves subjected to various cooking treatments

	Sample	**Concentration (µg/100g)±SE(n=3)**	**% retention**
1	Raw	325.47±3.03[a]	100.00
2	Steamed	258.08±0.58[b]	79.30
3	Blanched	252.29±0.71[c]	77.52
4	Raw-fried (onions+tomatoes)	241.28±0.77[d]	74.13
5	Boiled with sour milk added	230.90±0.78[e]	70.94
6	Boiled with fresh milk added	226.57±0.29[f]	69.61
7	Raw-fried (oil only)	205.56±0.75[g]	63.16
8	Boiled then fried (onions+tomatoes)	192.48±0.75[h]	59.14
9	Boiled then fried (oil only)	174.27±0.37[i]	53.54
10	Boiled	166.54±0.32[j]	51.17
	Mean square	6535.43, 3.80	
	df	9, 20	
	F-value	1720.176	
	P-value	<0.05	

Note: Concentration values with superscripts of different letters are significantly different at $p<0.05$

Raw saget had a mean concentration of 325.47±3.03 µg/100g of folate, a value that was in agreement with the findings of FAO/Government of Kenya, (2018b) which reported concentrations of 346 µg/100. The mean concentration of folate in saget was determined to be significantly higher than in cowpea and pumpkin leaves ($p<0.05$) thus saget should be considered as a good alternative source for folates and its consumption with staple foods should be encouraged. Different cooking methods resulted in significant differences in the mean concentration of folates in saget ($p<0.05$).

The mean concentration of folate in cooked saget ranged from 166.54±0.32 µg/ 100g to 258.08±0.58 µg/ 100g. These results showed a significant reduction of folate in cooked saget just like in cowpea and pumpkin leaves as already explained. Similarly, the present study showed that steaming and blanching saget did not cause a statistically significant loss of folate compared to boiling. Steaming led to a retention of 79.30 % while blanching caused retention of 77.52 % of the vitamin as shown in Figure 4.3. Boiling led to the highest loss of the vitamin in cooked saget due to leaching of folates into boiling water (DeSouza and Eitenmiller, 1986; McKillop *et al.*, 2002; Lucia *et al.*, 2014). Nonetheless, boiling saget and then frying reduced folate concentration significantly more than frying raw saget directly. This was due to leaching of the folates into boiling water unlike in direct frying where a minimal amount of water is added to prevent scorching (DeSouza and Eitenmiller, 1986) as it was the case for cowpea and pumpkin leaves.

Adding milk while cooking saget significantly reduced the loss of folate than boiling. This was attributed to the presence of iron (ii), ascorbate and the protein casein present in milk, which reduce dissolve oxygen hence reducing folate loss due to oxidation. Folate is also added to the vegetable from milk while cooking with it as an additive since milk contains 6 µg/100g of folate (FAO/Government of Kenya, 2018b; George, 2001). Nonetheless, sour milk led to significant retention of folate than fresh milk. Figure 4.9 shows retention of 70.94 % and 69.61 % of folate for sour and fresh milk respectively, $p<0.05$. This was attributed to an increase in folate during fermentation which was in agreement with the studies by DeMan (1999) which stated that natural folate levels increase during fermentation of milk and milk products. During fermentation, there is synthesis of natural folate by lactic acid bacteria (Mahara *et al*., 2019). Therefore, in addition to iron (ii), ascorbate and the protein casein present that reduces oxidation of 5-MTHF, sour milk contributes more folate to the vegetables than fresh milk.

4.5 Levels of ascorbic acid in raw and cooked cowpea (*Vigna Unguiculata*) and pumpkin leaves (*Cucurbita moschata*)

The mean concentration of ascorbic acid, AA in raw and cooked cowpea and pumpkin leaves and their percentage retention are depicted in Table 4.3.

Table 4.3: Mean concentrations of ascorbic acid and its retention in pumpkin and cowpea leaves subjected to various cooking treatments

Sample	Pumpkin leaves		Cowpea leaves	
	Concentration (mg/100g)±SE (n=3)	**% retention**	**Concentration (mg/100g)±SE (n=3)**	**% retention**
RL	9.36±0.12[a]	100.00	45.52±0.65[a]	100.00
STL	5.66±0.04[b]	60.45	24.18±0.05[b]	53.13
BL	5.26±0.03[c]	56.23	22.98±0.24[bc]	49.75
R-Fro+t	4.72±0.03[d]	50.45	21.84±0.25[c]	45.05
BoL	4.15±0.00[e]	44.28	20.62±0.25[d]	44.65
R-Fr	3.95±0.03[f]	42.15	20.06±0.54[de]	44.07
BoBcb+M	3.54±0.04[g]	37.78	19.15±0.21[e]	43.24
BoLye+M	3.43±0.02[g]	36.62	16.35±0.28[e]	35.93
BoBcb-Fro+t	3.14±0.07[h]	33.59	15.54±0.29[f]	34.15
BoLye-Fro+t	2.74±0.05[i]	29.29	14.06±0.04[g]	30.88
BoBcb-Fr	2.08±0.04[j]	22.20	13.13±0.18[h]	28.84
BoLye-Fr	1.76±0.06[k]	18.82	12.37±0.22[i]	27.18
BoBcb	1.64±0.02[k]	17.51	11.06±0.15[j]	24.31
BoLye	1.36±0.02[l]	14.47	10.72±0.06[j]	23.55
Mean square	13.16, 0.01		231.28	
df	13, 28		13, 28	
F-value	1814.161		1273.081	
P-value	<0.05		<0.05	

Key

- **RL** ∞ Raw leaves; **STL** ∞ Steamed Leaves; **BL** ∞ Blanched Leaves; **R-Fro+t** ∞ Raw Leaves Fried with onions and tomatoes; **R-Fr** ∞ Raw Leaves Fried with oil only; **BoBcb+M** ∞ Leaves Boiled in Bicarbonate and Milk added; **BoLye+M** ∞ Leaves Boiled in Lye and Milk added; **BoBcb-Fro+t** ∞ Leaves Boiled in Bicarbonate and Fried with onions and tomatoes; **BoLye-Fro+t** ∞ Leaves Boiled in Lye and Fried with onions and tomatoes; **BoBcb-Fr** ∞ Leaves Boiled in Bicarbonate and Fried with oil only; **BoL** ∞ Boiled Leaves; **BoLye-Fr** ∞ Leaves Boiled in Lye and Fried with oil only; **BoBcb** ∞ Leaves Boiled with Bicarbonate; **BoLye** ∞ Leaves Boiled with Lye
- At $p<0.05$, concentration values with superscripts of different letters in the same column differ significantly

From Table 4.3, the levels of AA in pumpkin leaves ranged from 1.36±0.02 mg/100g to 9.36±0.12 mg/100g. Raw pumpkin leaves had the highest concentration while the ones boiled in lye had the lowest concentration. Cooking pumpkin leaves resulted in a significant reduction of AA compared to raw vegetables ($p<0.05$). The mean concentrations of AA in cooked pumpkin leaves ranged from 1.36±0.02 mg/100g to 5.66±0.04 mg/100g with steamed vegetables retaining the highest percentage, 60.45 % of AA initially present in raw vegetables. The samples boiled in lye had a retention of 14.47 % as shown in Figure 4.10. Blanching also led to significant retention of AA compared to boiling, having retained 56.23 % of the vitamin.

From Figure 4.10, frying the raw vegetables with a combination of oil, onions and tomatoes led to significantly less reduction of AA with a retention of 42.15 % compared to boiling. Moreover, frying the raw vegetables in oil only led to significant loss of AA than boiling the vegetables. Use of additives like lye, sodium bicarbonate and milk resulted in significant loss of AA compared to boiling the vegetable in water only. Boiling the vegetable in lye caused significant loss of AA compared to the use of bicarbonate of soda. Onions and tomatoes resulted in greater reduction of AA than milk.

Table 4.3 shows that the levels of AA in cowpea leaves ranged from 10.72+0.06 mg/100g to 45.52±0.65 mg/100g with raw vegetables having the highest while vegetables boiled in lye had the lowest concentration. The mean concentration of AA in *Vigna unguiculata* was significantly altered by cooking ($p<0.05$). The AA concentration in cooked cowpea leaves ranged from 10.72±0.06 mg/100g to 24.18±0.05 mg/100g. Steaming led to the highest retention of 53.13% while boiling in lye caused the lowest retention of 23.55% as illustrated in Figure 4.11. Blanching retained significant amounts of AA compared to boiling in water only, having retained 49.75 % of AA that was initially present in the raw sample, Figure 4.11. Boiling *Vigna unguiculata* resulted in significant loss of the vitamin with a retention of 44.65 % at a mean concentration of 20.62±0.25 mg/100g than frying the raw vegetables with onions and tomatoes which retained 45.05 % of AA initially in raw sample at a mean concentration of 21.84±0.25 mg/100g. Moreover, frying the raw vegetables in oil only led to significant loss of AA than boiling the vegetables. Furthermore, boiling the vegetables with additives like milk, lye and sodium bicarbonate resulted in adverse reduction of ascorbic acid in *Vigna unguiculate* than boiling in water only.

There was significant reduction of AA in vegetables cooked using lye than when sodium bicarbonate was used. Cooking *Vigna unguiculate* with onions and tomatoes resulted in greater reduction than milk. Sample boiled in sodium bicarbonate and lye with addition of milk had a mean concentration of AA at 19.15±0.21 mg/100g and 16.35±0.28 mg/100g respectively. From Table 4.3, cowpea leaves boiled in sodium bicarbonate and lye and then fried with a mixture of oil, onions and tomatoes had mean concentrations of AA at 15.54±0.29 mg/100g and 14.06±0.04 mg/100g respectively.

The present study reported a concentration of 9.36±0.12 mg/100g and 45.52±0.65 mg/100g of AA in pumpkin and cowpea leaves respectively, which was in agreement with levels reported by FAO/Government of Kenya (2018b) of 12.3 mg/100g and 50 mg/100g respectively. The high concentrations of the vitamin are the reason why consumption of the AIVs should be encouraged. The significant reduction of AA in pumpkin and cowpea leaves with cooking was attributed to thermal degradation, leaching and oxidation of the vitamin (Ball, 2006; Wakhanu, 2014) during the cooking process. A study by Madhavi *et al*. (2017) also reported that heating increases the destruction of ascorbic acid by oxidation.

Steaming and blanching the vegetables led to significant retention of AA in both cowpea and pumpkin leaves compared to boiling. From Figure 4.10, steamed pumpkin leaves retained 60.45 %, blanched ones had 56.23 % while boiling retained 44.28 % of AA initially present in raw leaves. Steaming caused a retention of 53.13 %, blanching 49.75 % while boiling 44.65 % of AA in cowpea leaves (Figure 4.11). The findings were comparable to the report by Mellova *et al.* (1996) which showed that vitamin C retention in spinach (western vegetable) decreased with increased water activity since AA is highly soluble in water at 33 g/100 mL at 25 ^{o}C (Ball, 2006). The combination of heating and leaching causes significant loss of AA in vegetables (Madhavi *et al*., 2017).

There was significantly less reduction of AA when the vegetables were fried directly with a mixture of oil, onions and tomatoes than boiling. This was due to reduced water content for leaching of the vitamins (Mellova *et al*., 1996) and addition of AA from onions and tomatoes. According to Janos *et al*. (2007) tomatoes and onions contain 10-15 mg/100g and 10-201 mg/100g AA of the edible portions respectively. Vital to note is that when the vegetable was fried in oil only, there was a significantly higher loss of AA than boiling. The higher reduction of AA in fried vegetables than in boiled vegetables agrees with a study by Wakhanu (2014) which attributed it to higher oxidation during frying than boiling. As per the previous study, frying employs a very small amount of water to avoid scorching, thus, food is exposed to air. The contact of food with oxygen leads to higher oxidation of vitamins resulting in lower concentrations of AA. On the hand, during boiling, the vegetables are cooked at a temperature of about 100^0C while completely immersed in water. The enzymes are denatured and oxygen initially present in water is quickly used up. Diffusion of air into boiling water is negligible, resulting in reduced oxidation of vitamins thus the higher retention of AA compared to frying. The cooking oil used in the current study was sunflower oil which also contains iron and copper, 0.0094 mg/100g and 0.0002 mg/100g respectively (De Leonardis *et al*., 2000). The addition of these transition metal ions to the AIVs during cooking increases the loss of AA due to catalytic oxidation of AA leading to formation of metal-oxygen-ascorbate complexes (Ball, 2006). The fried pumpkin leaves had a concentration of 3.95+0.03 mg/100g while fried cowpea leave had a concentration of 20.06+0.54 mg/100g of AA. Boiled pumpkin and cowpea leaves had mean concentrations of 4.15+0.00 mg/100g and 20.62+0.25 mg/100g respectively.

Cooking *Vigna unguiculate* and *Cucurbita moschata* using lye and sodium bicarbonate reduces AA significantly compared to boiling. Chirambo and Senga, (2004) also reported a decrease in AA mean concentration in some vegetables in Southern Malawi after cooking with bicarbonate of soda and *chidulo* (extract from ash obtained from plant materials). According to Habwe and Walingo (2011), there occurs an increase in amounts of ionizable iron and copper when cooking vegetables with lye. In the presence of these ions, ascorbic acid undergoes catalytic oxidation to form a metal-oxygen-ascorbate complex (Ball, 2006). In addition to the ions, alkaline conditions (due to lye and sodium bicarbonate) denature the cell's cellulose structure, exposing AA to thermal degradation (Chirambo and Senga, 2004). Thus, cooking the AIVs using lye and sodium bicarbonate significantly reduces AA concentrations. Just like folates, AA is also lost through oxidation and thermal degradation during the preparation of lye. At temperatures above 45 ^{0}C, AA undergoes thermal degradation and oxidation (Ejoh *et al*., 2005) thus the addition of AA from lye to cowpea leaves during cooking was assumed to be negligible. Sodium bicarbonate does not contain AA among the ingredients used in its preparation hence, does not add the vitamin to the cooking vegetables.

Furthermore, when the vegetables were boiled in lye and milk as well as sodium bicarbonate and milk, there was a significant reduction of AA concentration compared to boiling. Similarly, the samples cooked in lye and sodium bicarbonate and then fried with either oil only or a mixture of oil, onions and tomatoes contained significantly lower AA content than the boiled ones. Milk contains 14 mg/100g AA while onions and tomatoes contain 13 mg/100g and 25 mg/100g respectively. However, when these additives were added to the vegetables, the AA levels were significantly lowered. These findings were comparable to (Wakhanu, 2014) who attributed these findings to an increase in extractable ions of iron and copper from the additives, (milk, lye, tomatoes and oil) leading to oxidation of ascorbic acid and rapid thermal degradation of AA, thus reducing the levels. Cooking the cowpea leaves with lye/sodium bicarbonate and adding the additives did, however, lower AA content in the vegetable than boiling in water only. This is because the alkalinity of lye and sodium bicarbonate caused rapid thermal degradation of AA (Ball, 2006).

4.6 Levels of ascorbic acid in raw and cooked saget (*Cleome gynandra*)

The mean concentration of ascorbic acid, AA in raw and cooked saget and their percentage retention are expressed in Table 4.4.

Table 4.4: Mean concentrations of ascorbic acid and its retention in saget leaves subjected to various cooking treatments

	Sample	**Concentration (mg/100g)±SE (n=3)**	**% retention**
1	Raw	60.28±0.32[a]	100.00
2	Steamed	39.53±0.40[b]	65.57
3	Blanched	37.98±0.43[c]	62.99
4	Raw-fried (onions+tomatoes)	34.67±0.62[d]	57.50
5	Boiled	30.56±0.11[e]	50.70
6	Boiled then fresh milk added	28.92±0.61[f]	47.97
7	Boiled then fried (onions+tomatoes)	28.17±0.34[f]	46.73
8	Raw-fried (oil only)	24.65±0.37[g]	40.88
9	Boiled, sour milk added	23.92±0.14[g]	39.69
10	Boiled then fried (oil only)	22.26±0.19[h]	36.92
	Mean square	375.977, 0.824	
	df	9, 20	
	F-value	456.475	
	P-value	<0.05	

Note: Concentration values with superscripts of different letters are significantly different at $p<0.05$

From Table 4.4, the mean concentration of AA in *Cleome gynandra* varied from 22.26±0.19 mg/100g to 60.28±0.32 mg/100g. Raw saget was determined to have the highest mean concentration of 60.28±0.32 mg/100g of AA, a value that was in agreement with the findings of FAO/Government of Kenya, (2018b) which reported concentrations of 64 mg/100g. Cooking *Cleome gynandra* resulted in a significant loss of ascorbic acid like in cowpea and pumpkin leaves compared to the raw samples ($p<0.05$). The loss was attributed to thermal degradation, leaching and oxidation of AA (Ball, 2006; Madhavi *et al*., 2017; Wakhanu, 2014) during cooking. The mean concentration of AA in cooked saget leaves ranged from 22.26±0.19 mg/100g to 39.53±0.40 mg/100g with the highest being reported in the steamed sample having retained 65.57 % of AA initially present in the raw sample and the lowest retention of 36.92 % was found in samples boiled then fried in oil only as shown in Figure 4.12. Blanching and frying the raw saget with a mixture of oil, onions and tomatoes led to significant retention of AA than boiling.

From Figure 4.12, blanching retained 62.99 % while frying the raw saget with oil, onions and tomatoes retained 57. 50 % of AA compared to boiling which retained 50.70 %. However, Figure 4.12 shows that boiling led to significant retention of AA in saget than when the vegetables were boiled then fresh or sour milk added (47.97 % and 39.69 % respectively) as well as boiled then fried with oil only (40.88 %) or boiled then fried with a mixture of oil, onions and tomatoes (46.73 %). Frying the raw vegetables with oil only also led to a significant loss of AA than boiling.

The significant retention of AA in *Cleome gynandra* after steaming and blanching (65.57 % and 62.99 % respectively) compared to boiling (50.70 %), $p<0.05$ as reported in this study was a similar finding to a study by Mellova *et al.* (1996) which reported that the two cooking methods led to significant retention of AA in spinach (western vegetable) compared to other cooking methods used in the study and this was attributed to decreased water activity in steaming and blanching since AA is highly soluble in water, 33 g/100mL at 25 ^{o}C (Ball, 2006). Thus, the loss of AA through leaching in AIVs is comparable to western vegetables. Nonetheless, it is vital to note the two cooking methods also used shorter cooking time compared to other cooking methods used in both studies.

There was significantly less reduction of AA when saget was fried directly with a combined mixture of oil, onions and tomatoes than boiling. The lesser reduction was also ascribed to reduced leaching since water content was reduced (Mellova *et al*., 1996) in the frying procedure compared to the boiling procedure. It was also linked to the addition of AA from onions and tomatoes since tomatoes contain 0.22 to 0.48 mg/g of AA (Abushita *et al.*, 1997). However, frying *Cleome gynandra* with oil only led to a significant loss of AA than boiling. This loss was attributed to a high rate of oxidation since the vegetables were exposed to air unlike in boiling where they are completely immersed in water. After oxygen in boiling water is used, the dissolving of more oxygen from air is negligible hence oxidation is reduced (Wakhanu, 2014). In addition to oxidation, the cooking oil used in the current study was sunflower oil which also contains iron and copper, 0.0094 mg/100g and 0.0002 mg/100g respectively (De Leonardis *et al*., 2000). The addition of these transition metals ions to the AIV during cooking increases the loss of AA due to catalytic oxidation of AA leading to formation of metal-oxygen-ascorbate complexes (Ball, 2006).

Boiling saget in water only caused significant retention of AA than Boiling the vegetable in water then adding milk or boiling then frying with oil, onions and tomatoes. Ascorbic acid was first leached into the boiling eluent which was discarded then the vegetable samples were further cooked with the additives. The additives used (milk, onions and tomatoes) are known to contain significant levels of AA (14 mg/100g, 13 mg/100g and 25 mg/100g respectively (FAO/Government of Kenya, 2018b)) however the loss of AA in saget was attributed to the presence of extractable ions of copper and iron in the additives. In presence of the ions and heat, AA undergoes rapid oxidation causing its loss in the vegetables during cooking (Habwe Florence *et al*., 2010; Wakhanu, 2014).Thus the AA was lost due to leaching, rapid oxidation and thermal degradation. Vital to note also, is that sour milk resulted in a statistically significant loss of AA than fresh milk which is attributed to the lower levels of AA in sour milk than in fresh milk (DeMan, 1999).

CHAPTER FIVE

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

The mean levels of folate and ascorbic acid in different AIVs are different. In the case of the vegetables that were studied, the highest concentrations of the vitamins are found in saget, followed by cowpea leaves then pumpkin leaves. It was concluded that cooking reduces folate and AA concentration in AIVs. Cooking the vegetables in large amounts of water reduces vitamins concentrations significantly than when vegetables are cooked in processes involving minimal amounts of water such as steaming. There is also reduced loss of vitamins when the cooking process involves a minimal time of contact between the vegetables and water such as blanching than when there is more contact time like in boiling. Additives such as lye and bicarbonate of soda also reduce the concentration of the vitamins. Additives like milk, onions and tomatoes add folate to AIVs when used in cooking the vegetables while boiling in plain water reduces the folate. However, AA is significantly lost in the process than when the vegetables are just boiled. Addition of sour milk in cooking AIVs results in significant retention of folate than the addition of fresh milk. However, sour milk causes a significant loss of AA than fresh milk.

5.2 Recommendations

5.2.1 Recommendations from the study

Consumption of Saget (*Cleome gynandra*), Cowpeas (*Vigna unguiculata*), and pumpkin (*Cucurbita moschata*) should be encouraged since they contain high levels of both folate and ascorbic acid. The method that should be used for cooking AIVs is steaming since it leads to significant retention of vitamins. Additives such as lye and bicarbonate of soda should be avoided while cooking AIVs since they cause significant losses of vitamins. Milk, onions and tomatoes can be added to AIVs while cooking if folate is the vitamin of focus. However, these additives should be avoided if AA is to be retained. Sour milk should be encouraged for use than fresh milk for more folate.

5.2.2 Recommendations for further studies

The bioavailability study of folate and ascorbic acid with different cooking methods needs to be conducted *in vitro*. This will provide necessary information necessary for proper decision-making by chefs, nutritionists and consumers in selecting cooking methods that lead to maximum utilization of the vitamins to reduce problems posed by folate and ascorbic acid deficiencies.

Further analysis on the contribution level of vitamins by additives needs to be investigated to quantify the number of vitamins added to the vegetables during cooking. There is also a need to determine the level of the vitamins in mixed vegetable recipes. The levels of AA and B9 need to be determined in samples that have been cooked and stored to determine the effect of storage with time, on the retention of the vitamins.

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APPENDICES

Appendix I: Publication

African Journal of Pure & Applied Sciences

ISSN: 2710-0758

DOI 10.33886/ajpas. v1i2.187

Journal homepage: http://journal.ku.ac.ke/index.php/AJPAS

Research Article Open Access

Effect of cooking methods on levels of folates and ascorbic acid in cowpea leaves (Vigna unguiculata L. Walp) from Nairobi, Kenya

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Received: 18 July 2020 / Accepted: 30 November 2020 / Published online: 29th January 2021

ABSTRACT

Folate (vitamin B_9,5-methyltetrahydrofolate) and ascorbic acid (AA) (vitamin C) play a key role in human health andwell-being. It is greatly established that AA is beneficial inpreventing scurvy while folate helps in the prevention of neuraltube defects and congenital malformations. The main sources of these vitamins are fruits and vegetables especially green leafy vegetables including the African Indigenous Vegetables (AIVs). However, these vegetables are consumed after cooking, which leads to loss of the vitamins through oxidation, thermal degradation and leaching. The study aimed at determining the effect of different cooking methods on the retention of AA and 5-methyltetrahydrofolate (5-MTHF) in cowpea leaves (*Vigna unguiculata* L. Walp) as affected by different cooking methods. 5-MTHF and AA were determined using high-pressure liquid chromatography with ultra-UV-visibledetection, HPLC-UV after extraction of the vitamins from raw and cooked samples. Analysis of Variance (ANOVA) was used to determine the difference in nutrient retention by various cooking methods. Significance was imputed at $p<0.05$. Raw *V. unguiculata* contained 45.516±0.649mg/100g AA and 91.736±0.586 µg/100g folate. The cooked samples of the vegetable contained folate ranging from 40.713±0.081 to 65.128±0.007 µg/100g and AA ranging from 10.719±0.063 to 24.181±0.051 mg/100g of the edible portion of the vegetable.

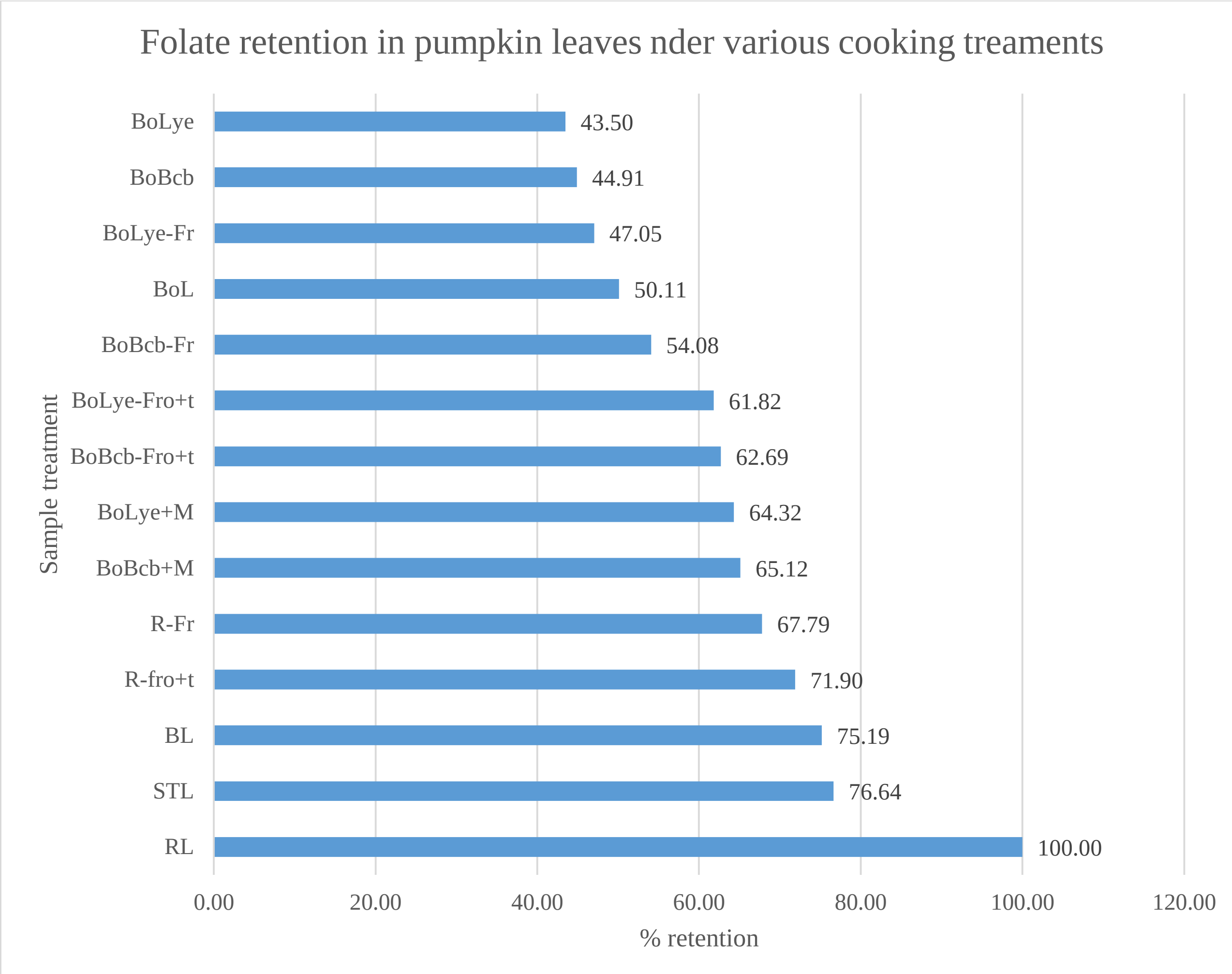
Cooking the vegetable significantly reduced both folate and AA concentration compared to the raw vegetable samples, $p<0.05$. Fryingraw vegetables with onions and tomatoes was found to retain significant folate and AA than boiling the vegetables, $p<0.05$. Addition of milk resulted in significant retentionof folate and significant loss of AA than boiling, $p<0.05$. Both lye and sodium bicarbonate significantly reduced folate and AA concentration than boiling, with lye causing a significant reduction of both vitamins than sodium bicarbonate $p<0.05$. It is concluded that cooking reducesfolate and AA concentration in cowpea (*V. unguiculata* L.Walp). Additive such as lye and bicarbonate also reducethe concentration of the vitamins. Cooking cowpea leaves with the addition of milk and frying with onion and tomatoes retains more folate than boiling. However, AA issignificantly lost in the process than when the vegetable isjust boiled in plain water.

Keywords: Folate, ascorbic acid, cooking method,retention, cowpea.

Citation: Mumbi, J., Wanjau, R., and Murungi, J. (2021). Effects of cooking methods on levels of folate and ascorbic acid in cowpea leaves (*Vigna unguiculata* L. Walp) from Nairobi, Kenya. *African journal of Pure and Applied Sciences*, *2*(1), 53-60.

Appendix II: Figures for retention of vitamins in AIVs

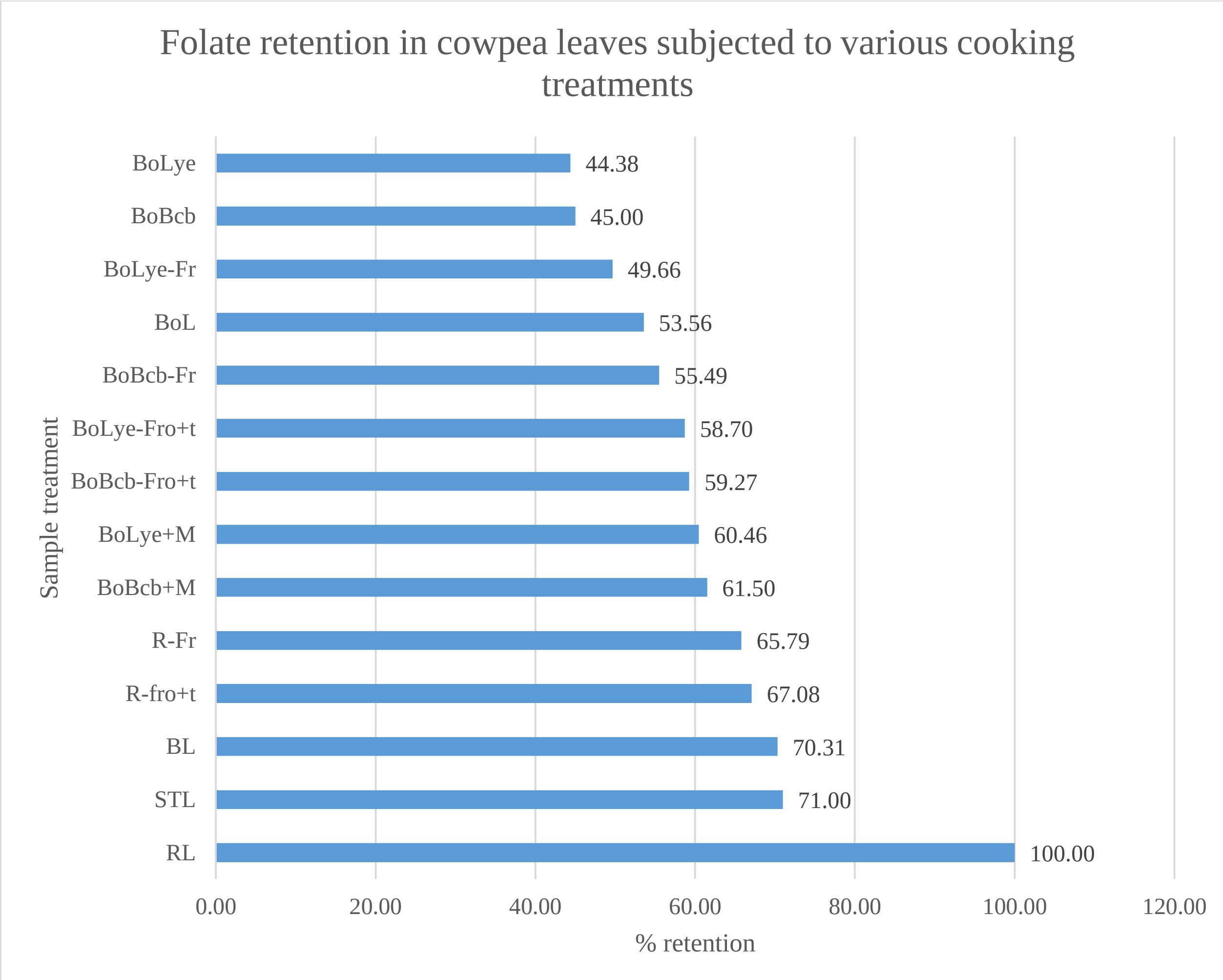
Figure 4.7: Retention of folate in pumpkin leaves subjected to various cooking treatments



Key

- **RL** ∞ Raw leaves; **STL** ∞ Steamed Leaves; **BL** ∞ Blanched Leaves; **R-Fro+t** ∞ Raw Leaves Fried with onions and tomatoes; **R-Fr** ∞ Raw Leaves Fried with oil only; **BoBcb+M** ∞ Leaves Boiled in Bicarbonate and Milk added; **BoLye+M** ∞ Leaves Boiled in Lye and Milk added; **BoBcb-Fro+t** ∞ Leaves Boiled in Bicarbonate and Fried with onions and tomatoes; **BoLye-Fro+t** ∞ Leaves Boiled in Lye and Fried with onions and tomatoes; **BoBcb-Fr** ∞ Leaves Boiled in Bicarbonate and Fried with oil only; **BoL** ∞ Boiled Leaves; **BoLye-Fr** ∞ Leaves Boiled in Lye and Fried with oil only; **BoBcb** ∞ Leaves Boiled with Bicarbonate; **BoLye** ∞ Leaves Boiled with Lye

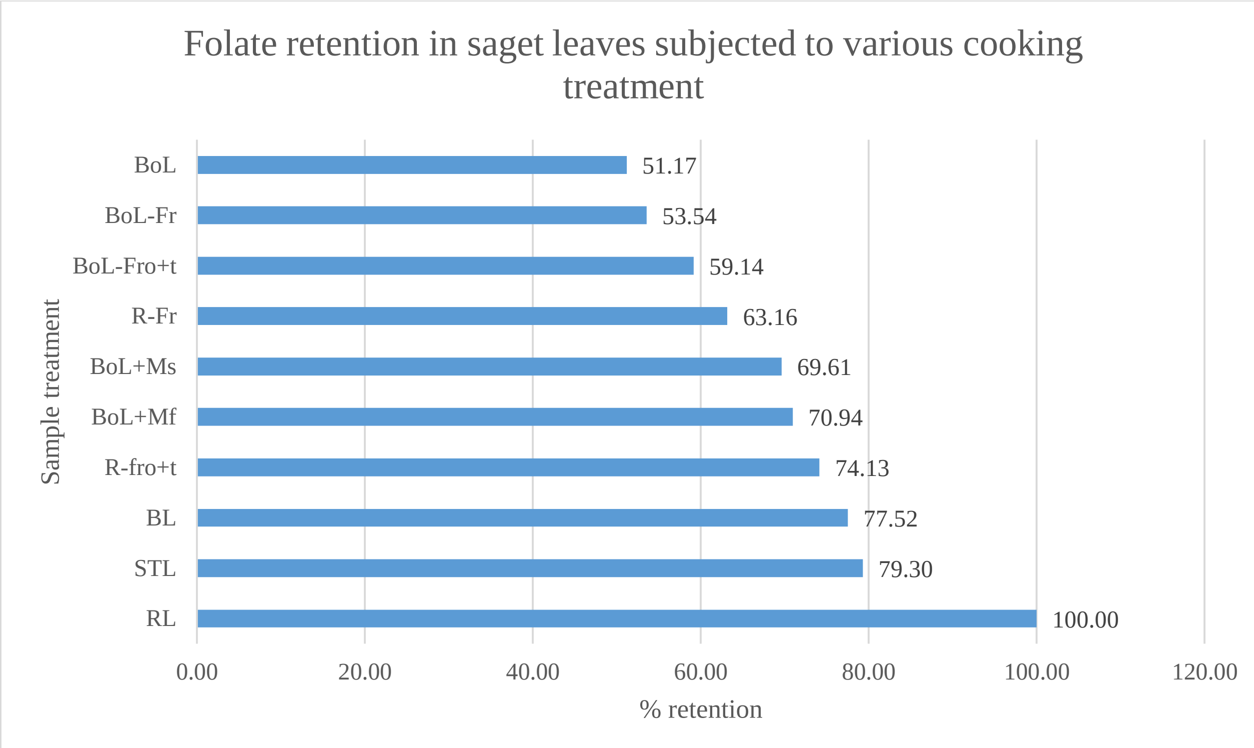
Figure 4.8: Retention of folate in cowpea leaves subjected to various cooking treatments



Key

RL ∞ Raw leaves; **STL** ∞ Steamed Leaves; **BL** ∞ Blanched Leaves; **R-Fro+t** ∞ Raw Leaves Fried with onions and tomatoes; **R-Fr** ∞ Raw Leaves Fried with oil only; **BoBcb+M** ∞ Leaves Boiled in Bicarbonate and Milk added; **BoLye+M** ∞ Leaves Boiled in Lye and Milk added; **BoBcb-Fro+t** ∞ Leaves Boiled in Bicarbonate and Fried with onions and tomatoes; **BoLye-Fro+t** ∞ Leaves Boiled in Lye and Fried with onions and tomatoes; **BoBcb-Fr** ∞ Leaves Boiled in Bicarbonate and Fried with oil only; **BoL** ∞ Boiled Leaves; **BoLye-Fr** ∞ Leaves Boiled in Lye and Fried with oil only; **BoBcb** ∞ Leaves Boiled with Bicarbonate; **BoLye** ∞ Leaves Boiled with Lye

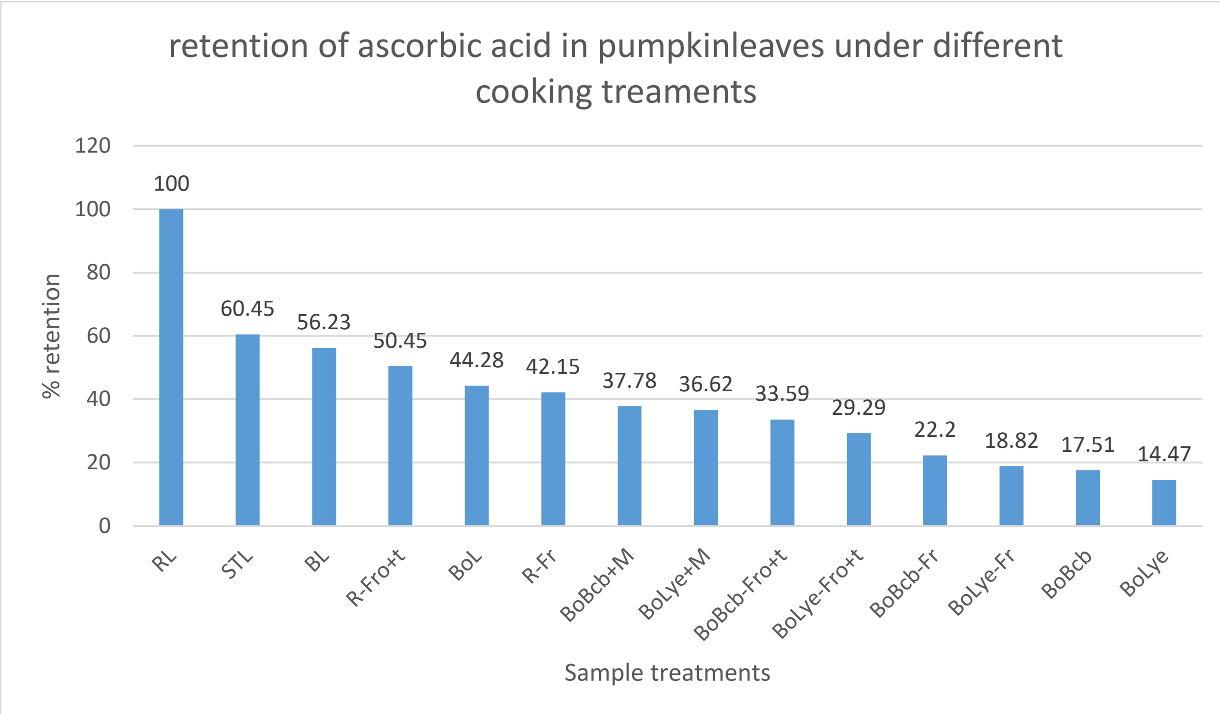
Figure 4.9: Retention of folate in saget subjected to various cooking treatments



Key

RL ∞ Raw leaves; **STL** ∞ Steamed Leaves; **BL** ∞ Blanched Leaves; **R-Fro+t** ∞ Raw Leaves Fried with onions and tomatoes; **R-Fr** ∞ Raw Leaves Fried with oil only; **BoL+Mf** ∞ Leaves Boiled and added Fresh Milk; **BoL+Ms** ∞ Leaves Boiled and added Sour Milk; **BoL-Fro+t** ∞ Leaves Boiled and Fried with onions and tomatoes; **BoL-Fr** ∞ Leaves Boiled and Fried with oil only; **BoL** ∞ Boiled Leaves

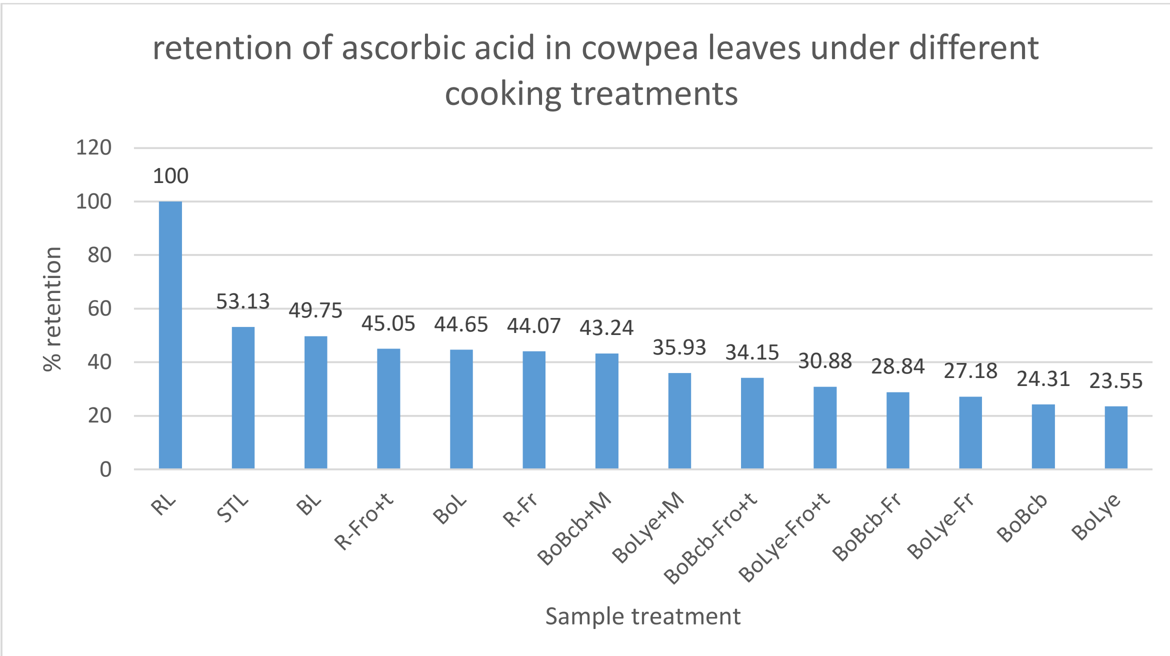
Figure 4.10: Retention of ascorbic acid in pumpkin leaves subjected to various cooking treatments



Key

- **RL** ∞ Raw leaves; **STL** ∞ Steamed Leaves; **BL** ∞ Blanched Leaves; **R-Fro+t** ∞ Raw Leaves Fried with onions and tomatoes; **R-Fr** ∞ Raw Leaves Fried with oil only; **BoBcb+M** ∞ Leaves Boiled in Bicarbonate and Milk added; **BoLye+M** ∞ Leaves Boiled in Lye and Milk added; **BoBcb-Fro+t** ∞ Leaves Boiled in Bicarbonate and Fried with onions and tomatoes; **BoLye-Fro+t** ∞ Leaves Boiled in Lye and Fried with onions and tomatoes; **BoBcb-Fr** ∞ Leaves Boiled in Bicarbonate and Fried with oil only; **BoL** ∞ Boiled Leaves; **BoLye-Fr** ∞ Leaves Boiled in Lye and Fried with oil only; **BoBcb** ∞ Leaves Boiled with Bicarbonate; **BoLye** ∞ Leaves Boiled with Lye

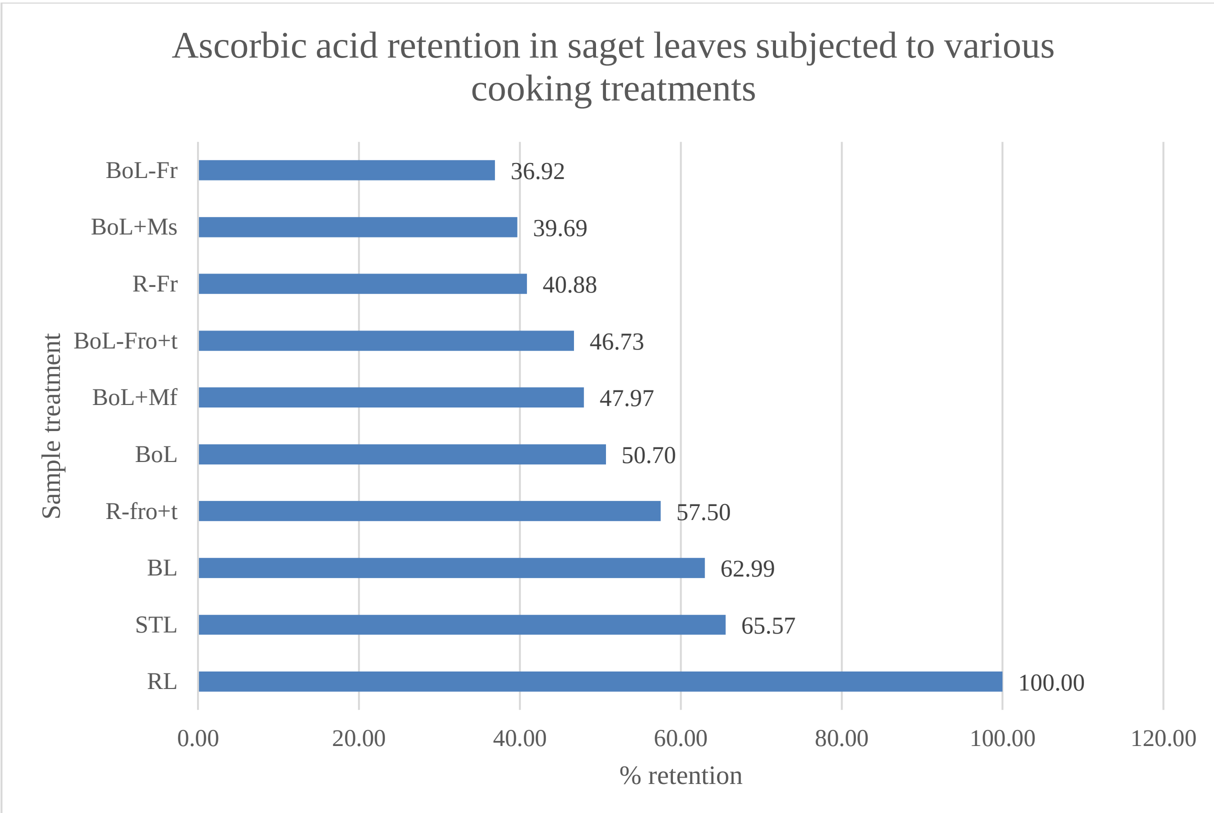
Figure 4.11: Retention of ascorbic acid in cowpea leaves subjected to various cooking treatments



Key

RL ∞ Raw leaves; **STL** ∞ Steamed Leaves; **BL** ∞ Blanched Leaves; **R-Fro+t** ∞ Raw Leaves Fried with onions and tomatoes; **R-Fr** ∞ Raw Leaves Fried with oil only; **BoBcb+M** ∞ Leaves Boiled in Bicarbonate and Milk added; **BoLye+M** ∞ Leaves Boiled in Lye and Milk added; **BoBcb-Fro+t** ∞ Leaves Boiled in Bicarbonate and Fried with onions and tomatoes; **BoLye-Fro+t** ∞ Leaves Boiled in Lye and Fried with onions and tomatoes; **BoBcb-Fr** ∞ Leaves Boiled in Bicarbonate and Fried with oil only; **BoL** ∞ Boiled Leaves; **BoLye-Fr** ∞ Leaves Boiled in Lye and Fried with oil only; **BoBcb** ∞ Leaves Boiled with Bicarbonate; **BoLye** ∞ Leaves Boiled with Lye

Figure 4.12: Retention of ascorbic acid in saget (*Cleome gynandra*) subjected to various cooking treatments



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- **RL** ∞ Raw leaves; **STL** ∞ Steamed Leaves; **BL** ∞ Blanched Leaves; **R-Fro+t** ∞ Raw Leaves Fried with onions and tomatoes; **R-Fr** ∞ Raw Leaves Fried with oil only; **BoL+Mf** ∞ Leaves Boiled and added Fresh Milk; **BoL+Ms** ∞ Leaves Boiled and added Sour Milk; **BoL-Fro+t** ∞ Leaves Boiled and Fried with onions and tomatoes; **BoL-Fr** ∞ Leaves Boiled and Fried with oil only; **BoL** ∞ Boiled Leaves

Appendix III: Research Permit from NACOSTI

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