EVALUATION OF THE QUALITY ATTRIBUTES OF SELECTED LOCAL COWPEA ACCESSIONS AND THEIR RESPONSE TO POSTHARVEST TREATMENTS

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DECLARATION

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

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DEDICATION

I dedicate this work to my parents Mr.Kirakou Lochilikou and Mrs. Veronica Kirakou, my wife Doreen Chirchir and my sister Linah Kirakou for their love and support which ensured successful completion of my studies.

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LIST OF ACRONYMS

AFLP	Amplified Fragment Length Polymorphism
ALVs	African leafy vegetables
AOAC	Association of Official Analytical Chemists
ASALs	Arid and Semi-Arid Lands
EPC	Export Promotion Council
HCD	Horticultural Crop Directorate
KALRO	Kenya Agricultural and Livestock Research Organisation
MAP	Modified Atmosphere Packaging

ABSTRACT

African leafy vegetables (ALVs) have a great potential in reducing the gap in nutritional status between low and high income households because of their nutrient densities and affordability. Cowpea is one of the major ALVs produced and consumed widely at the Coast and Western regions of Kenya as a dual purpose crop (grain and leaf). Although local cowpea accessions are preferred by farmers and consumers, there are still many challenges encountered. There is lack of sufficient information on nutrients and micro-nutrient densities and high postharvest losses. High post-harvest losses have also been reported as a result of perishability. The objective of this study was to evaluate and compare the nutritional and sensory attributes of five popular dual purpose local cowpea accessions and an improved variety developed by KALRO. In addition, the effect of blanching, solar drying and modified atmosphere packaging (MAP) on the shelf life and quality attributes of one superior cowpea accession was evaluated.

The cowpeas were planted in The University of Nairobi field station during the short rains from October to December 2014 and long rains from March to May 2015. The cowpea leaves were randomly sampled in the experimental plots. One superior accession was chosen for post-harvest treatments. The treatments were, solar drying without blanching, blanching in pure water and solar drying, blanching in salty water and solar drying and fresh non-blanched leaves as control. The samples were then analysed for proximates, vitamins, minerals, anti-nutrients, sensory characteristics, colour change during processing and packaging, cumulative water loss and wilting in The University of Nairobi and Jomo Kenyatta University of Agriculture and Technology laboratories.

In the first objective on evaluating and comparing nutritional attributes of local cowpea accessions with an improved variety, beta carotene content of M66 which is an improved variety was the lowest at 29.71mg/100g whereas Sura Mbaya had the highest beta carotene content at 36.4mg/100g. On the other hand, M66 had the lowest ascorbic acid content of 192.8 mg/100g whereas Usimpe Mtu Mdogo had the highest ascorbic acid content at 213.1mg/100g in season 1. The iron content of Usimpe Mtu Mdogo was the lowest at 395.9PPM compared to Mnyenze at 1034.3 PPM in season 1.

In the second objective of evaluating the efficacy of post-harvest treatments on the quality of fresh and processed cowpeas, it was found that blanching and dehydration had little effect on most proximate and mineral elements. However, vitamin and total phenolic contents were the most affected. Solar drying without blanching recorded the highest vitamin retention levels at 68.02% for beta carotene and 68.39% for ascorbic acid unlike blanching in pure water and solar drying at 55.58% for beta carotene and 21.08% for ascorbic acid and blanching in salty water and solar drying at 52.78% beta carotene and 20.24% ascorbic acid. In addition, solar drying without blanching recorded the highest retention total phenolic content at 149.91%. Blanching in pure water and solar drying and blanching in salty water and solar drying recorded retention levels of 62.58% and 65.79% of total phenolic content respectively. On the other hand, solar drying without blanching, blanching in pure water and solar drying and blanching in salty water and solar drying recorded a loss of 5.87%, 10.77% and 11.17% of oxalates and 37.22%, 69.98% and 58.7% of nitrates respectively.

In the samples subjected to MAP, the end stage of control, ordinary polythene bag and Extend® bag under room conditions was 1 day, 4 days and 6 days respectively. By the end stage control, ordinary polythene bag and Extend® bag had lost 28.84%, 0.93% and 3.27% cumulative weight for season 1 and 23.84%, 0.89% and 2.31% for season 2 respectively.

The results of the present study indicated that evaluated local cowpea accessions were comparable with each other but slightly superior to the improved variety developed by KALRO. Solar drying without blanching was found to be effective in maintaining the quality attributes of cowpeas. In addition, MAP was found to be effective in preserving quality of fresh cowpea leaves and improving shelf life. Therefore MAP and solar drying without blanching are simple and convenient technologies for preserving cowpea nutrients and improving shelf life.

KEYWORDS: Cowpea accessions; dehydration; MAP; nutritional quality

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CHAPTER ONE

1.0 INTRODUCTION

Agriculture has been the mainstay of Kenya's economy for decades. The sector contributes to nutrition, food security, employment and foreign exchange earnings. According to The Kenya Economic Report (2013), agricultural sector directly accounts for about 26% of Kenya's Gross Domestic Product (GDP) and 27% indirectly through linkages with manufacturing, distribution and other service related sectors. The sector declined from a growth rate of 4.2% in the year 2012 to 2.9% in 2013 partly due to inadequate rainfall received in some growing regions in the country but increased to 3.5% in 2014 (Economic Survey highlights, 2014 and 2015). Agriculture industry accounts for 65% of Kenya's total exports, 18% and 60% of the formal and total employment, respectively. In addition it directly and indirectly supports the livelihood of 80% of the population living in the rural areas (Kenya Economic Report, 2013). According to economic survey (2015), the value of marketed agricultural production declined marginally from Ksh 334.8 billion in 2013 to 333.2 billion in 2014.

The horticulture sector has been a major contributor to the good performance of the agricultural sector. In the year 2012 and 2013, the subsector contributed 26% and 25% respectively to the agricultural growth by value (Economic survey, 2014). By quantity, fresh horticultural produce contributed 205,700T in 2012, 213, 800Tin 2013 and 220,200T in 2014 becoming second after tea (Economic Survey highlights, 2014 and 2015). These statistics should be taken as an estimate to the actual horticultural growth since most of the horticultural commodities such as African leafy vegetables do not reach formal markets or export. In addition, many surveys do not inform of the farm gate prices of vegetables, farm level production quantity and value at the local and informal markets. Considering this situation, the horticultural subsector may be having far much more positive impact to the population than it is estimated.

The horticulture sector is made up of subsectors which include flower and ornamental, fruit and vegetable production. According to HCD report (2014), vegetables occupied the largest production portion at 32% by value, followed by flowers at 30%, fruits at 30%, and nuts at 5%. The major export destination is the European Union where the horticulture industry command about 30% market share. In general, vegetable production in Kenya has been increasing steadily over the past years. In 2012, the quantity of vegetables exported was 66,352 tonnes valued at Ksh.20226 million. In 2013 exports increased to 77172 tonnes valued at Ksh.22923 million and in 2014, exports slightly reduced to 70335T valued at Ksh.18781 million (HCD Annual Report, 2014). Considering the report touched on exports, it means that the production is larger since the consumption in the country is larger compared to the exports. The major producing counties as reflected by exports include Meru, Bungoma, Murang'a, Kiambu and Kirinyaga in that order (HCD Annual Report, 2014).

Vegetable production is widely practised in the country. The development of the sector is due to the readily available markets with a higher marginal return per unit areas compared to cereal crops. Vegetable production has short growth cycles enabling farmers to have two to three seasons in a year. This combination, in addition to emerging health issues has placed vegetable production at a strategic position to expand even more.

Vegetables can be classified as exotic or indigenous depending on their origin, utilization and commercialization. Some exotic vegetables commercialized in Kenya include kales, spinach, snow peas, French beans among others whereas indigenous vegetables include African nightshade, spider plant, cowpeas, amaranth among others. Major export vegetables comprise of exotic and Asian vegetables (EPC, 2014). Current trends have seen the incorporation of value addition strategies to ensure continuous availability and reduction of postharvest losses. Such strategies include canning, freezing, solar drying and/or roasting in addition to pre-packs for fresh produce meant for supermarkets.

Unlike other type of vegetables, trade and consumption of African leafy vegetables had been side-lined to serve the local population especially in the rural areas or among the poor and denied entry to formal markets. However, the HCD report,(2014) indicated that there has been a tremendous increase in production in ALVs in the country. This can be attributed to the awareness created to the population on the health benefits and nutritional superiority of these vegetables (Abukutsa, 2007) and value chain support by non-governmental organisations. As a result, in 2014 the acreage under ALVs increased by 10% and the yields and value rose by 5.6% and 6.2%, respectively (HCD Annual Report, 2014). According to AVRDC (2010), it is estimated that approximately 9000 tonnes of ALVs have been sold to formal and informal markets in the period between 2008 and 2010 in central Kenya only.

Table 1: Performance of African Leafy vegetables 2012-2014

	2012			**2013**			**2014**		
Crop	**Area (Ha)**	**Quantity (Ton)**	**Value Kshs (million)**	**Area (Ha)**	**Quantity (Ton)**	**Value Kshs (million)**	**Area (Ha)**	**Quantity (Ton)**	**Value Kshs (million)**
African Nightshade	2,820	18,945	505	3,018	29,796	561	3,376	25,435	763
Spider plant	2,273	20,134	455.1	2,239	20,912	529.1	2,435	16,752	640.5
Cowpeas	25,544	69,940	910	23,195	55,223	764	24,431	65,096	622
Jute mallow	1,708	7,919	215	2,096	10,269	251.1	1,832	9,290	284.5
Leaf Amaranth	1,035	9,913	208.3	1,187	12,208	227.7	1,586	17,001	195.6
Pumpkin leaves	797	3,948	107.8	877	4,552	119.4	921	4,602	129.2
Rattle pod	286	1,984	43.2	370	2,780	58.2	533	5,100	119.1
Grain Amaranth	525	3,951	85.3	445	1,856	63.3	389	2,057	70
Total	**34,988**	**136,734**	**2,530**	**33,427**	**137,596**	**2,574**	**35,503**	**145,333**	**2,824**

Source: HCD report (2014).

Kenya, like many other tropical countries, is endowed with a great diversity of ALVs. These vegetables have a great social and economic importance for the local communities therefore making them part and parcel of their culture. The priority species grown and marketed in Kenya include African nightshades *(Solanum* spp*)*, amaranth *(Amaranthus* spp.*)*, spider plant *(Cleome gynandra)*, cowpeas *(Vigna unguiculata)*, Ethiopian kale (*Brassica carinata*), 'mitoo' *(Crotalaria ochroleuca* and *C. brevidens))*, 'kahuhura' *(Cucurbita ficifolia)*, jute plant *(Corchorus olitorius)* and pumpkin leaves (*Cucurbita maxima and C. moschata).* (Irungu *et al.*, 2007). Among the ALVs, African nightshade accounted for 27% of market value followed by Spider plant and Cowpeas at 23% and 22% respectively in 2014 (HCD report, 2014). However, in terms of quantity produced, cowpea outperformed all other ALVs from 2012 to 2014 as shown in Table 1.

Cowpea (*Vigna unguiculata*) is an important grain legume in tropical and subtropical regions where a shortage of animal protein sources is often experienced (Tshovhote *et al*., 2003). Although a lot of emphasis has been put on the grain crop, the high potential in the vegetable has not been fully exploited (Abukutsa, 2003).

1.1.Problem statement

Cowpea leaves has been viewed as a woman's crop and therefore it has received little attention from stakeholders (Abukutsa, 2003).Nutritional information and quality among the cultivated cowpea varieties is scanty (Muchoki *et al.,* 2007). However, the situation is worse among local cowpea accessions although they are preferred by farmers because of superior taste and palatability compared to improved varieties such as KVU, K-80 and M66 (KARI, 2010). The information available on the nutritional quality of cowpea leaves has been restricted to improved varieties and some few local accessions (Mamiro *et al.,* 2011). However, the nutritional information available for the few lines studied has recorded a very large variability. For instance Mamiro *et al.,* (2011) indicated that cowpeas crude protein ranges from 18 to 25%, Okonya and Maass (2014) found the protein content to be between 29.4 to 34.3% whereas, Ono *et al.,* (1996) recorded as high as 43% crude protein content. On the other hand, ascorbic acid levels reported range of between 33.5mg/100g to 308 mg/100g (Muchoki *et al*., 2007; Ahenkora *et al.,* 1998; Njoroge *et al.,* 2015).

The potential of cowpea leaves has not been maximized due to post-harvest handling limitations (Affognona *et al.,* 2014). It is estimated that post-harvest losses contribute to about 50% of total losses in the cowpea value chain (Masarirambi *et al.,* 2010). The high losses can be attributed to lack of proper post-harvest knowledge, high perishability, poor processing practises and inefficient or high cost of post-harvest technologies. The situation is worsened during the periods of glut where production of these vegetables exceeds market demand.

1.2.Justification of the study

Local cowpea accessions have important significance to farmers although their nutritional quality has not extensively assessed (KARI, 2010). Nutritional profiling of superior local cowpea accessions study will compliment or add new information that will help in sensitizing the entire population to enhance utilization of cowpea vegetable. Ilelaboye *et al.,* (2013) indicated that adequate nutritional information on ALVs will be useful for nutritional education to the public especially the vulnerable groups as a means to improving their nutritional status. The information will also enable further improvement of the local accessions which are thought to be adapted to wide range of climatic conditions (D'Andrea *et al.*, 2007).

The high post-harvest losses on cowpea vegetable have led to led to decreased availability of the vegetable in households and markets (Shiundu and Oniang'o, 2007). The existing technologies to reduce post-harvest have been inefficient or expensive to the resource constrained farmer. Such technologies include sun drying, fermentation, charcoal cooling and refrigeration (Muchoki *et al.*, 2007). However, very few technologies that suit small scale farmers been evaluated. Low cost methods of improving and lengthening shelf life such as modified atmosphere packaging and solar drying have been proposed (Chavasit *et al.*, 2002) but have not been tested for ALVs. The reduction in post-harvest losses will be improved nutritional and food security and income.

1.3.General objective

To evaluate the nutritional quality attributes of local cowpea accessions and reduce loss of the quality attributes to improve the shelf life and enhance availability of quality vegetables and improve food security.

1.3.1. Specific objectives

1. To compare the nutritional quality attributes of selected superior local leafy cowpeas accessions with an improved variety.
2. To evaluate the effect of blanching, solar drying and modified atmosphere packaging on the quality attributes and shelf life of cowpeas leaves.

1.3.2. Null hypotheses

1. Selected superior local cowpea accessions have similar nutritional content compared to the improved variety.
2. Blanching, solar drying and modified atmosphere packaging does not preserve quality and does not improve the shelf life of cowpea leaves.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Nutrition status in Kenya

Malnutrition is one of the major concerns in Kenya and many other developing countries (Mibei *et al.*, 2011). Children being weaned, pregnant and lactating mothers are the most affected. The occurrence of nutrient-deficiency diseases such as night blindness, scurvy and rickets are common in rural areas and informal settlements in Kenya (Mibei *et al.*, 2011). It is estimated that 35% of children under the age of five years are stunted, 4% are severely underweight, 6% are wasted and one in ten infants are born with a low birth weight in Kenya (UNICEF, 2009).

It is noted that protein-energy malnutrition of infants is one of the major nutritional problems in the world (Oyeleke *et al.,* 1985). This can be attributed to the lack of proper constituted weaning foods, the provision of weaning foods with inadequate protein content, and to the use of foods too low in energy density to satisfy the needs of the growing infant. Nutritional deficiencies of iron and zinc are also widespread in developing countries, where staple diets are frequently plant-based and consumption of meat and other animal-based food products is low due to prohibitively high price (Smith and Eyzaguirre, 2007). One of the contributing factors to malnutrition is the poor quality of food that is consumed (Senga *et al.*, 2013). Owing to the rich nutrient content of ALVs, (Abukutsa, 2007) consumption of the right amount of these vegetables can improve the status of malnutrition.

2.2African leafy vegetables (ALVs) in Kenya

Kenya just like any other African country is endowed with a great diversity of African leafy vegetables (ALVs). The priority species marketed in Kenya include African nightshades *(Solanum* spp*),* amaranth *(Amaranthus* spp.*),* spider plant *(Cleome gynandra),* cowpeas *(Vigna unguiculata)*, Ethiopian kale (*Brassica carinata*), 'mitoo' *(Crotalaria ochroleuca* and *C. brevidens)),* 'kahuhura' *(Cucurbita ficifolia),* jute plant *(Corchorus olitorius)* and pumpkin leaves (*Cucurbita maxima and C. moschata)* (Irungu *et al*., 2007). Some of the vegetables grow naturally in the wild and not domesticated. They includestinging nettle (*Urtica massaica*) and vine spinach (*Basella alba*) which are consumed in western Kenya and parts of Rift Valley (Tumwet *et al*., 2014). The degree of importance and preference of these vegetables varies with the various regions and ethnic groups in Kenya. For instance, cowpeas, slenderleaf, spiderplant, African kale and African nightshades are popular in Nyanza and Western region (Abukutsa *et al*., 2005).

African leafy vegetables have been grown and utilized traditionally by many African communities and are believed to possess several nutritional advantages and potentials that have not been fully exploited (Schippers, 2000). They have been found to be important source of adequate amounts of vitamins and minerals (Abukutsa, 2007). Since high proportions of ALVs are consumed in the household level, they contribute to nutrition security (Hutchinson *et al.*, 2016).In addition, the excess sold contributes to household incomes (Mwaura *et al*., 2013).

In recent years, ALVs have gained commercial importance as a result of the growth in awareness and marketing (Irungu *et al*., 2007). As a result, trade in these vegetables have been conducted in more formal marketing channels, especially in urban areas. African leafy vegetables have therefore provided empowerment opportunities to women and other resource constrained groups through their role in subsistence and commercial production. Abukutsa *et al.,* (2005) indicated that, although growing of ALVs still follows traditional farming system such as using organic sources of manure that are available on-farms, intervention to exploit the potential for commercialization exist. Therefore, to realize full potential, technical support for ALV farming is important (Mwaura *et al*., 2013). The suggested support include seed supply systems, value chain intervention, postharvest management, promotion and marketing and awareness campaigns.

2.3Botany and centre of origin of cowpeas

Cowpea is a widely grown crop in the semi-arid and sub-humid zones of Africa and Asia (Padolosi and Ng, 1997). West and Central Africa particularly dominate world production (Lambot, 2002). It is primarily a savannah species, highly adapted to marginal environments and depleted field conditions where other crops do not perform well (D'Andrea *et al.,* 2007).

Cowpea [*Vigna unguicalata* (L.)Walp.] is a diploid species (2n=2x=22) belonging to the section catiang, subspecies *unguiculata*, genus *Vigna*, tribe *Phaseoleae* and the family *Fabaceae* (Ng and Marechal, 1985). Studies using amplified fragment length polymorphism analysis (AFLP) have concluded that the two most likely areas of early domestication of cowpea are tropical West or north-eastern Africa (Coulibaly *et al.,* 2002). Specifically, cowpea has been found to be native to Africa, West Africa, Nigeria being the primary centre of diversity (Ng and Marechal, 1985). South-Eastern Africa is however reported as the centre of diversity of the wild *Vigna* species (Ng and Marechal, 1985). Studies have further shown that India appears to be a secondary centre of diversity since significant genetic variability occurs on the subcontinent (Pant *et al*., 1982). The study also indicated that there is likelihood that the crop was first introduced to India during the Neolithic period**.**

2.4 Cowpea morphology

Cowpea is an herbaceous warm-season annual plant that shares common characteristics such as appearance with common bean except that their leaves are generally darker green, shinier, and less pubescent (Timko *et al.,* 2007). In addition, they are more robust, with better developed root systems, thicker stems and branches (Timko *et al.,* 2007). Cowpea growth habit can be erect, semi-erect, prostrate or climbing depending on the genotype. Ecological factors also have shown to greatly influence the morphology and physiology of the crop. Emergence is epigeal just like common bean and lupin (Timko *et al.,* 2007). Cowpea primarily is self-pollinating, but with outcrossing rates of as high as 5%. Most cowpea genotypes are photoperiod sensitive (Craufurd *et al.,* 1997) and according to Wien and Summerfield (1980), they are generally quantitative short day plants with a varying critical day length but generally lies close to 13.5 hours. Photoperiod has tremendous effect on vegetative development, phenology and reproductive development. For instance, flowering is delayed but not prevented by photoperiods longer than critical value (Nuhu and Mukhtar, 2013). Cowpea genotypes usually adapt to their diverse environment through plasticity in phenology and morphology conditions affected by temperature and photoperiod (Wien and Summerfield, 1980).

2.5Uses of cowpea

Cowpea is a versatile crop with a wide range of uses. Young cowpea leaves are consumed as an accompaniment of a stable starch food in at least 18 countries in Africa and 7 countries in Asia and the Pacific (Ahenkora *et al.,* 1998). The leaves are boiled as with most traditional preparation systems, boiled and fried or fried only (Imungi and Potter, 1983). Cowpea grain can be boiled and fried then eaten as an accompaniment or in a mixture with other starch based grains such as maize. In addition, the grain can be ground to obtain flour used in food/feed fortification, generation of oil and manufacture of cakes (FAO, 1997). The addition of cowpea to plantain based snacks has been documented to increase their protein content considerably (Etsey *et al.*, 2007). Oyeleke *et al.*, (1985) indicated that cowpea grain can be used to improve popular Nigerian weaning foods among breastfeeding children which included a mixture of sorghum *(Sorghum bicolor)* with a limited amount of dried-milk powder.

2.6 Ecological growth requirements of cowpeas

Cowpeas and many other African indigenous vegetables are domesticated at almost all the agro-ecological zones. They are grown from the sea level to regions above 1800 meters above the sea level. In general, indigenous vegetables are well adapted to harsh climatic conditions and disease infestation and are easier to grow in comparison to their exotic counterparts (Abukutsa, 2010). This can be attributed to the existing wide genetic base as a result of low selection pressure in addition to the early maturity intervals.

2.6.1 Temperatures

The importance of temperature in the crop production has been studied for a long time. The rate of growth and other metabolic reactions highly depend on temperatures. However, the effects of high temperatures and drought are confounding therefore strategies to mitigate one can apply to the other. The best vegetative growth of cowpea is possible with temperatures varying from 21 to 33°C, with higher temperatures causing earlier flowering and flower abscission which results to poor pod set (Dugje *et al.*, 2009). Night temperatures above 17°C can cause flower abscission in some cultivars during flowering (Dugje *et al.*, 2009).

2.6.2 Rainfall

Cowpeas naturally have some tolerance to droughts, a factor which has been improved by the development of early maturity varieties which avoids drought for the purposes of seed production (Dugje *et al.*, 2009). Cowpeas perform well in areas with rainfall range is between 500-1200 mm/year. The development of extra-early and early maturing cowpea varieties has seen the crop thriving in areas getting rainfall is less than 500 mm/year (Dugje *et al.*, 2009). Proper development of cowpea is hampered by excessive rainfall during vegetative growth. Hailstones are detrimental during the flowering period since the crop will lose its flowers leading to 100% yield loss. Supplemental irrigation during the dry seasons is necessary to ensure that seed production is optimized in addition to maximizing other physiological processes leading to improved productivity.

2.6.3 Edaphic factors and fertilizer application of ALVs

Cowpeas and other ALVs quantitatively respond very well to added organic and inorganic nutrients. However the crop can thrive to produce protein-rich vegetative material as well as seed (Dugje *et al.,* 2009).The application of NPK fertilizer in small quantities to cowpea has been shown to be beneficial however, the response is genotype dependent (Abayomi *et al,* 2008). Application of animal manures have been shown to significantly increase the number of leaves, caused greater nodulation, produced higher number of pods per plant, yielded maximum number of seeds per pod, greater 100-seed weight, higher yields and increased, seed quality compared to chemical and non-fertilizer treatments (Shahardeen and Seran, 2013).

Cowpeas can be planted in soils that vary from sandy to clayey, but productivity is restricted by water logged soils. Neutral soil pH is optimal for cowpea production however, they can grow in acid soils where aluminium toxicity occurs (Dugje *et al.*, 2009). In addition, the crop is also highly adapted to marginal environments and depleted field conditions where other crops do not perform well (D'Andrea *et al.*, 2007).

2.6.4 Light intensity

Small holder farmers mostly practise intercropping when growing cowpeas and other ALVs because of limited parcels of land. Intercropping directly affects light intensity to any shorter crop in the intercrop. Varied results have been obtained as a result of intercropping cowpea with various crops. In a study, it was found that corn intercropped with cowpea was far more effective than mono-crop corn to produce higher dry matter yield and roughage for silage with better quality (Geren *et al.*, 2008). The situation works for maize because of nitrogen fixation but with reduction in the productivity of the cowpea. Obadoni *et al.,* (2009) indicated that dry matter yield of cowpea varieties significantly decreased with increasing weed densities. Other parameters affected by impaired light intensities are days to flowering, number of pods and yield.

2.7 Constraints of production and utilization of cowpeas

Growth and development of ALV production has been limited by several policy, production and postharvest factors. According to Abukutsa (2010), there has been a continuous tendency of neglect from consumers, breeders and educated population for the development and consumption of these vegetables due to the introduction of exotic and more palatable vegetables. Although there have been increased campaigns on the value of these vegetables, there is still inadequate knowledge on nutrition and therapeutic properties.

There has been inadequate high quality seed to the industry as seeds are relied from untrained farmer seeds. The few sold by formal seed providers are not pure and more than often contains a mixture of genotypes and weeds. In addition, there is lack of enough technical and utilization packages. The available technologies have shown low levels of adoption partly because of socioeconomic aspects of the people.

Lack of awareness on postharvest technologies has hindered the expansion of the subsector to meet the demand of the vast market. The produce tends to have a very short shelf life coupled with poor post-harvest handling techniques. The occurrence of peaks and troughs in production in addition to poor marketing strategies has led the industry to suffer devastating losses.

Production of most food crops such as grains, legumes and vegetables are largely dependent on the seasonal and inter-annual change in rainfall (Awotoye *et al.*, 2010). It is documented that the response of cowpea yield to climate change vary from one geographical location to the other (Ajetomobi *et al.,* 2010) giving an indication that other factors such as soil and biological systems play an important role in climate change response. Awotoye *et al.*, (2010) also indicated that a number factors both climatic and non-climatic interact which confounds the effects of rainfall amount and the crop yields. In general, it is indicated that there is a negative correlation between climate change and cowpea productivity (Ajetomobi *et al.,* 2010).

2.8Nutritional composition of ALVs

African leafy vegetables have provided food and nutritional security to various communities in Africa. This has led them to acquire a significant portion of the traditional diets especially to resource constrained households (Grubben and Denton, 2004; Schippers, 2000). Nair et *al.*, (2013) and Aja *et al.*, (2010) indicated that ALVs are essential in supplying the body with minerals, vitamins and certain hormone precursors, proteins and calories. It has been indicated that majority of ALVs are nutritionally superior to exotic vegetables (Nnamani *et al.,* 2009; Rachel, 2015). The factors that make them popular among resource constrained population include their superiority in health benefits and affordability (Abukutsa, 2003). However, in the recent years, ALVs have gained momentum among urban middle class and rich due to their nutritional and health benefits (Chweya and Eyzaguirre, 1999).

2.8.1 Anti-oxidative activities of ALVs

Vitamins are key component on ALVs which are important in maintaining the body defence system hence prevention of diseases (Abukutsa, 2003). Ascorbic acid is a potent antioxidant that facilitates the transport and uptake of non-heme iron at the mucosa in addition to reduction of folic acid intermediates and the synthesis of cortisol (Bender, 2009). Its deficiency includes fragility to blood capillaries, gum decay and scurvy (Bender, 2009).Vitamin A is important for normal vision, gene expression, growth and immune function through its ability in maintaining and enhancing epithelial cell functions (Lukaski, 2004). Vitamin B complex helps in conversion of carbohydrates to glucose for energy production. They are also important for healthy skin, liver, eyes and hair in addition to enhancing proper functioning of the nervous system (Bender, 2009). On the other hand, vitamin E is a powerful antioxidant which helps to protect cells from damage by free radicals such as hydrogen peroxides in addition to formation and normal function of red blood cell and muscles (Lukaksi, 2004).

African leafy vegetable are rich in vitamins such as beta carotenes, vitamin B and C (Adéoti *et al.,* 2012). Nnamani *et al.,* (2007) indicated that ALVs are important food, which are highly beneficial for the maintenance of healthy body and prevention of diseases. The components which form the protective agents include carotenes, ascorbic acid, riboflavin, folic acid (Nnamani *et al.,* 2007). On the other hand, George, (2003)stated that ALVs represent an unquestionable natural pharmacy of minerals, vitamins and phytochemicals. A study on cowpea leaves indicated that ascorbic acid and beta carotene content was 308 mg/100g and 33mg/100g (Muchoki *at al.,* 2007) while Njoroge *et al.,* (2015) reported ascorbic acid levels of 91.0 mg/100g and beta carotene of 6.5mg/100g. On the other hand, Chikwendu *et al.,* (2014) found ascorbic acid content at 59.24mg/100g and beta carotene at 9.10mg/100g.

Abukutsa, (2010) indicated that ALVs contain non-nutrient compounds, preferably known as phytochemicals that provide health benefits. One of the most important groups of phytochemicals in ALVs is phenolics and flavonoids. Oboh and Akindahunsi, (2004) stated that phenolics have potent anti-oxidative activity. However, the antioxidant activities of phenolics in different vegetables markedly vary due to the differences in the phenolic compound structures primarily related to their hydroxylation and methylation patterns (Meyer *et al.,* 1998). A survey conducted by Tumwet *et al.,* (2014) gives an indication that most people in Kenya treasure the medicinal properties of ALVs. It was suggested that the vegetables are consumed not only for good nutrition but also for their association with various medicinal and immune boosting claims. Adéoti *et al.*, (2012) indicated that ALVs have been traditionally used as medicine to treat several ailments because of the numerous phytochemicals they inherently possess. Some of the health claims included anti-aging, ensuring smooth skin and boosting blood (Tumwet *et al.*, 2014). African leafy vegetable such as *Celosia argentea* L. possess mitotic inhibitory activities which can be used in the development of drugs to prevent the uncontrolled proliferation of cancer cells. In other cases, boiled amaranth leaves and roots have been traditionally used as laxative, diuretic, anti-diabetic, antipyretic, anti-snake venom, anti-leprotic, anti-gonorrheal, expectorant, to relieve breathing in acute bronchitis, anti-inflammatory, immuno-modulatory activity, anti-androgenic activity and anti-helmintic properties (Alegbejo, 2013). In general, regular consumption of vegetables is recommended for better health and management of chronic diseases such as cardiovascular complications, diabetes and cancer (Thompson, 1993).

2.8.2 Minerals constituents of ALVs

Most ALVs are rich sources of minerals like calcium, iron and phosphorous (Nnamani *et al.*, 2007). Calcium is a constituent of bones and teeth and regulates nerve and muscle function. Potassium is the principal cation in intracellular fluid and functions in acid base balance, regulation of osmotic pressure, muscle contraction and Na^+/K^+ ATPase (Murray *et al.*, 2000).Iron, on the other hand is essential in red blood cell formation (Chandra, 1990). Its deficiency results in decrease in red blood cells hence anaemia. Too little iron in the diet could cause poor absorption of iron by the body which can result to low blood pressure. Vegetable cowpea diet like many other ALVs can by far alleviate the level of iron in the body hence proper iron based metabolic functions are realized (Abukutsa, 2003). Zinc is an important trace element in human nutrition and fulfils many biochemical functions in human metabolism (Chandra, 1990). It is the activation factor of several enzymes such as carboanhydrase, alkaline phosphatase and other enzymes in nucleic acid synthesis. It stabilizes the structure of RNA, DNA and the ribosomes, and influences hormone metabolism (Institute of Medicine, 2001). Zinc deficiency leads to several disorders such as growth retardation, diarrhoea and interferences of cerebral functions (Institute of Medicine, 2001). Manganese is necessary for proper function of the reproductive organs. In addition, it promotes growth and development, cell function, bone growth, healthy immune system and healthy nerves (Minerals Education Coalition, 2013).

Njoroge *et al.,* (2015) found cowpea leaf iron and calcium content to be 10.8mg/100g and 174mg/100g respectively whereas Muchoki *et al.,* (2007) indicated that the content is 64.4mg/100g and1736mg/100g respectively. Okonya and Maass (2014) on the other hand found iron content of several cowpea accessions to range from 17.6 mg/100g to 38.7 mg/100g. Chikwendu *et al.,* (2014) found that iron, zinc, calcium, phosphorus contents of cowpea leaves were 77.29, 12.91, 39.87 and 383.20 respectively in mg/100g. It has been reported that the proportion of micro and macro nutrients is significant for both developed lines and local varieties of cowpea in Tanzania (Mamiro *et al.,* 2011). Therefore, research has shown that the leaves of cowpea can substantially contribute to the dietary intake of calcium, iron and zinc which are constantly required for proper development and functioning of the body. It has been noted that the potassium content in many ALVs is good in the control of diuretic and hypertensive complications, because it lowers arterial blood pressure (George, 2003). An ALV such as *Corchorus olitorius* is usually recommended for pregnant women and nursing mothers in Nigeria due to its richness in iron (Taiga *et al.,* 2008).

2.8.3 Other macro-nutrients

African leafy vegetable are rich in fibres, carbohydrates and proteins (Adéoti et *al.,* 2012). Noonan and Savage, (1999) indicated that the fibre content of the vegetables contribute to the feeling of satisfaction and prevents constipation as it assist the process of digestion. African leafy vegetables contain proteins superior to those found in fruits although inferior to those found in grains and legumes (George, 2003).

Crude protein content and crude fibre in cowpea leaves were found at 31.8% and 18.2% respectively (Muchoki, 2007) whereas Kasangi *et al.,* (2010) found crude protein and crude fibre at 35.97% and 12.76% respectively. Njoroge *et al.,* (2015) on the other hand found crude protein and crude fibre of cowpea leaves at 6.8g/100g and 4.6g/100g respectively. Ano and Ubochi (2008) found that the protein content of the cowpea leaves was comparable with the protein content of the popular grain. However, it is noted that the total dietary fibre content of cowpea leaves increases with leaf age (Nielsen *et al.*, 1997).

2.8.4 Anti-nutrient content in ALVs

Consumption of leafy vegetables provides health benefits but sometimes may turn out to be the cause of certain health problems because of presence of anti-nutrients such as tannins oxalates, phitates, nitrates, saponins, cyanide and protease inhibitors (Teutonico and Knorr, 1984; Ndidi *et al.,* 2014). In has been noted that the nitrates accumulate on the leaves rather than on the seeds (Alegbejo, 2013). Presence of such anti-nutrients may compromise digestion and absorption of vital nutrients (Uusiku *et al.,* 2010). Such inherent toxic factors or anti nutritional components in ALVs has been a major obstacle in exploiting the full benefits of their nutritional value (Akindahunsi and Salawu, 2005). African leafy vegetables that have been found to have anti-nutrient include shear butter leaves, amaranth leaves, cowpea leaves and Ethiopian kale (Muchoki *et al.,* 2010; Abimedi *et al.,* 2009; Getachew *et al.,* 2013; Yekeen *et al.,* 2013). However, it has been noted that the quantity and fraction of anti-nutrients are dependent on the crop in question (Ilelaboye *et al.,* 2013).

Phytate inhibits the functions of some digestive enzymes (Siegenberg *et al.,* 1991). Many anti-nutrient substances can reduce the amount of non-heme iron that is absorbed by the body. Thompson, (1993) also indicated that higher intake of ILVs with considerable amounts of anti-nutrients such as tannins, phytates and oxalates may impair bioavailability of nutrients and minerals or directly cause illnesses or fatality such as haemolysis, with ultimate occurrence of death when there is excessive ingestion of lectins.

To counter the effects of the anti-nutrients, several methods have been proposed. Getachew *et al.,* (2013) proposed that bulk consumption of monotype edible plant part in one meal should be discouraged. Several methods of cooking and blanching have been shown to reduce the anti-nutrient contents to levels that can be tolerated by monogastric digestive system (Fasuyi, 2006; Ilelaboye, *et al.,* 2013; Getachew et *al.,* 2013). On the other hand, adequate toxicological screening has been proposed to ensure safety of their consumption (Yekeen *et al.,* 2013).

2.9 Post-harvest losses of cowpeas and other ALVs

African leafy vegetables contain more 85% water (Muchoki *et al.,* 2007). The high moisture content deprive ALVs of long shelf life. Under room conditions, these vegetables lose water very fast and may not last for 24 hours (Abukutsa, 2010). This adversely affects the visual quality which is the major factor in attracting consumer appeal. According to Masarirambi *et al.*, (2010) post-harvest losses are estimated to contribute about 50% total losses in cowpea value chain. However, figures between 10–40%, and as high as 50–70% have been reported (FAO-World Bank, 2010). The high losses can be as a result of insufficient proper post-harvest knowledge, inefficient or expensive technologies, poor handling and lack of prioritisation in research work. Some of the measures which have been proposed include value addition and optimisation of postharvest technologies (Chavasit *et al.*, 2002).

2.10 Post-harvest management

To achieve nutrition security especially in developing countries, measures to deal with the high postharvest losses and extend the shelf life of the perishable ALVs should be initiated. Very few small holder farmer friendly measures of curbing the postharvest losses and extending shelf life have been evaluated. However, the evaluated ones could be ineffective or expensive to the resource constrained farmer.

Among the measures to curb post-harvest losses is value addition (Affognona *et al.*, 2014). According to Mibei *et al.*, (2011), value addition techniques have a great effect on the mineral composition and quality of ALVs. Traditional sun drying of the ALVs results to a significant loss of the nutrients such as vitamin C, B and beta-carotene. According to Zoro *et al.*, (2015), sun drying leads to a loss of vitamin C and beta carotene of between 58.41-100% and 62.64-100% respectively. Coupled with losses that occur during cooking (Mibei *et al.*, 2011; Kawashima and Soares, 2003), the situation could result to consumption of nutrient poor, fibre rich vegetables. On the other hand, fermentation has been evaluated as another means of value addition. Unlike sun drying, the method is effective in reducing the losses of vitamins but technicality makes it difficult for farmers (Wafula *et al.*, 2015). According to Muchoki *et al.*, (2007), fermentation coupled with solar drying is effective in managing post-harvest losses and extending the shelf life of cowpea leaves. It is indicated that beta carotene and ascorbic acid were retained at 91% and 15% respectively and by the end of three months retention had reduced to between 23-52% for beta carotene and 4-7% for ascorbic acid.On a positive note, a study evaluating the effect of blanching on the nutritive value of vegetable found that the loss of nutritive components including sugars, vitamins B_1, B_2, and C were minimal when blanching is done at 100 °C for 10 min (Jae-Yeun *et al.*, 2003). Solar drying has been modified to improve the retention capacity of vitamins in ALVs. Solar drying has been found effective in preservation of nutrients in ALVs but in most cases, it has not been evaluated alone but in combination with other measures such as blanching and fermentation (Muchoki *et al.,* 2007; Njoroge *et al.*, 2015).

Other measures to enhance the fresh shelf life of ALVs have been proposed. According to Kariuki, (2014), evaporative coolers are effective in improving the shelf life of fresh ALVs up to one week. Other evaporative coolers include pot coolers and brick coolers. Modified atmosphere packaging has been used in the past to improve the shelf life of fresh products. It has been used to lengthen the shelf life of fruits such as Mangoes, passion fruits among others (Kirakou *et al.*, 2014; Yumbya *et al.,* 2014). However, this technology has not been evaluated on ALVs.

2.11Sensory properties of AIVs

Sensory analysis has been defined as a scientific discipline used to evoke, measure, analyse and interpret reactions to those characteristics of food and materials as they are perceived by the senses of sight, smell, taste and touch (Stone *et al.,* 2012). Abimedi *et al.,*(2009) noted that the palatability of most ALVs depends on handling when prepared as vegetable or soup. In a study to determine the effects of value addition on sensory analysis by Nyambakaa *et al.,*(2004) it was found that aroma, texture and appearance influenced the choice and characteristics of fresh, sun-dried and solar-dried cowpea samples. The research established that the solar dried products do not differ in colour and tenderness from the fresh leaves, unlike sun-dried leaves which differed principally in appearance from the rest of the products.

Limited research has so far been conducted to ascertain the level of palatability of most ALVs. However local cowpea accessions have been claimed by farmers in the coastal region of Kenya to be more palatable (KARI, 2010). It has been noted that, farmers sometimes complain that improved varieties have excessively bitter or less bitter taste than the local accessions or even incidences of tough leaves which reduces its acceptability (Rowland, 1992)

CHAPTER THREE

3.0 EVALUATION OF NUTRITIONAL QUALITY ATTRIBUTES OF SELECTED SUPERIOR LOCAL LEAFY COWPEA ACCESSIONS AND AN IMPROVED VARIETY

3.1. ABSTRACT

Cowpea is one of the major ALVs produced and consumed widely at the Coast and Western regions as a dual purpose crop (grain and leaf). Although local cowpea accessions are preferred by farmers and consumers, there are still many challenges encountered. There is lack of sufficient information on nutrients and micro-nutrient densities. The development of nutrient knowledge among the local accessions can unlock the potential of the crop to combat nutrient deficiencies.The objective of this study was to evaluate and compare nutritional attributes of local cowpea accessions with an improved variety.

Four local cowpea accessions and one improved variety were planted in The University of Nairobi, field station during the short rains from October to December 2014 and long rains from March to May 2015. The cowpea leaves were randomly sampled from the experimental plots. One accession was chosen for post-harvest treatments. The treatments were,solar drying without blanching, blanching in pure water and solar drying, blanching in salty water and solar drying and fresh leaves as control. The samples were then analysed for selected proximate constituents, vitamins, minerals, anti-nutrients and sensory characteristics in The University of Nairobi and Jomo Kenyatta University of Agriculture and Technology laboratories.

Betacarotene content of M66 which is the improved variety was relatively lower at 29.71mg/100g compared to the local accessions; Katsetse had beta carotene content of 33.65mg/100g, Mnyenze 35.31mg/100g, Sura Mbaya 36.4mg/100g, Usimpe Mtu Mdogo 32.74mg/100g and Mumias-Tsimg'oli at 35.92mg/100g in season 1. On the other hand, M66 had a lower ascorbic acid content of 192.8mg/100g compared to Mnyenze at 214.7mg/100g, Sura Mbaya at 201.6mg/100g, Usimpe Mtu Mdogo at 213.1mg/100g and Mumias-Tsimg'oli at 204.4mg/100g but higher than Katsetse with 191mg/100g in season 1. M66 had the highest total phenolic content at 4744 GAE mg/100g compared to Katsetse at 4058GAE mg/100g, Mnyenze at 4588GAE mg/100g, Sura Mbaya at 4664GAE mg/100g, Usimpe Mtu Mdogo at 4000GAE mg/100g and Mumias-Tsimg'oli with 4102GAE mg/100g. The iron content of M66 was lower at 499.7PPM compared to Katsetse at 730.2PPM, Mnyenze at 1034.3PPM, Sura Mbaya at 598.3PPM and Mumias-Tsimg'oli at 564.6PPM but higher than Usimpe Mtu Mdogo at

395.9PPM.The results of the present study indicated that evaluated local cowpea accessions were comparable with each other but slightly superior to the improved variety developed by KALRO.

KEYWORDS: Cowpea accessions; nutritional quality

3.2. INTRODUCTION

Nutrition deficiency is a phenomenon that has affected children and lactating mothers in Kenya and other sub-Saharan African countries for a long time (Mibei *et al.,* 2011). In Kenya alone, it is estimated that 35% of children under the age of five years are stunted, 4% are severely underweight, 6% are wasted and 10% of infants are born with a low birth weight (UNICEF, 2009). A part from redressing these challenges, adoption, consumption and intensification of research on African leafy vegetables can reduce the intensity of malnutrition because of its inexpensive rich nutritional contents (Hallensleben *et al.,* 2009; Mwajumwa *et al.,* 1991; Irungu *et al.,* 2007; Smith and Eyzaguirre, 2007). Therefore, ALVs can become an indispensable tool when it comes to reducing the prevalence of malnutrition among resource-constrained households.

Cowpea (*Vigna unguiculata*) forms a major diet to household in Eastern and Southern Africa both as grain and leaf (Ahenkora *et al.,* 1998). In Kenya, it is ranked the third most important indigenous vegetable (HCD, 2014). The popularity in Kenya is as a result of the accompanying nutritional benefits (Rachel, 2015). Cowpea leaves have been incorporated into a variety of dishes in the country. Young leaves and shoot tips are harvested either wholly by uprooting or by removing harvestable leaves at a time (Ohler *et al.,* 1996; Saidi *et al.,* 2010).

There is a great diversity of cowpeas in Kenya. In Mombasa and Kilifi Counties alone, 32 local accessions of were identified (Hutchinson *et al.,* 2016). Most of these local accessions are popular with farmers who cite superior palatability and taste (KARI, 2010). Although popular, these local accessions have received little attention in research, more so on evaluation of their nutritional quality attributes. The diversity could be holding important nutritional superiority that could unlock improvement in consumption, management of nutrition and food security in Kenya. Therefore to realize that, more local accessions of cowpeas should be evaluated for their nutritional quality and differences. It has been noted that research work has concentrated on improved varieties and some few local accessions (Mamiro *et al.,* 2011). However, a lot of variation on the results has been noted. For instance Mamiro *et al.,* (2011) indicated that cowpeas crude protein ranges from 18 to 25%, while Okonya and Maass (2014) reported protein content of between 29.4 to 34.3%; Ono *et al.*, (1996) indicated that the crude protein content of cowpea leaves was 43%. On the other hand, results of ascorbic acid content of cowpea leaves have seen a variation of between 33.5mg/100g to 308 mg/100g (Muchoki et al., 2007; Ahenkora *et al.,* 1998; Njoroge *et al.,* 2015).

In this study, local cowpea accessions which have been tested for superiority in agronomic traits (Hutchinson *et al.,* 2016) were subjected to nutritional evaluations. The objective of the study was to evaluate and compare the quality attributes of five local cowpea accessions and an improved variety.

3.3.MATERIALS AND METHODS

3.3.1. Cowpea accessions under study

Four local cowpea accessions; mnyenze, usimpe mtu mdogo, katsetse and sura mbaya were collected from Mombasa and Kilifi Counties, one local accession 'Tsimg'oli' from Butere-Mumias (cream coloured seeds) and one improved variety Machakos 66 (M66) from KARLO, Industrial Crops Research Institute, Mtwapa. The four local accessions from Mombasa and Kilifi Counties had been evaluated for yield and response to agronomic traits and were found to be superior (Hutchinson *et al.,* 2016).The improved variety was chosen because of its popularity and the fact that it is a dual purpose (KALRO, 2008).

3.3.2. Site description

The experiment was conducted at the University of Nairobi field station, Kabete Campus during the short rain being season 1 (October to December 2014) and long rains being season 2 (March to May 2015). The field is located in about 15 km to the West of Nairobi city and lies at Latitude 1^0 15′S and Longitude 36^0 44′E, and at altitude of 1820 m above sea level. Kabete has a bimodal distribution of rainfall, with long rains from early March to late May and the short rains from October to December, (Opijah *et al.,* 2007). The locality receives an annual rainfall of 1,060 mm (Onyango *et al.,* 2011). The climate is typically sub humid with minimum and maximum means temperatures of 13.7^0C and 24^0C, respectively. The soils in Kabete are predominantly red humic nitisols containing 60-80% clay and are characterized as deep, well drained, dark reddish-brown to dark brown clay (Michieka, 1977; Sombroek *et al.,* 1982).

3.3.3. Experimental set up and preparation of cowpea leaves

The experiment was conducted using a Randomized Complete Block Design with three blocks. Tenders leaves of the cowpea accessions were sampled in the morning in each experimental plot, seven weeks after planting because at this stage, production for dual purpose cowpea is at its optimal point (Inaizumi *et al.,* 1999). The leaves were mixed well and packed in airtight plastic containers. The packed leaves were taken to Jomo Kenyatta University of Agriculture and Technology, Food Science laboratory within 2 hours in a cool box and stored in a deep freezer at -18^0C before analysis.

The following parameters were analyzed:

3.3.4. Moisture content

Moisture content was determined according to Association of Official Analytical Chemists (A.O.A.C) methods (A.O.A.C, 1990). Moisture dishes were placed in an oven until dry and transferred to a desiccator and their weight recorded (W_0). Five grams of the fresh sample was weighed and transferred to the dry moisture dishes and weighed (W_1). The samples were transferred to a heated oven at 105^0C for 3 hours. This time coincides with the time when a constant weight is achieved. The fresh samples and the dish are then weighed after cooling in a desiccator (W_2).

The results were calculated and expressed as a percentage from the following formula;

$$\%MC = \frac{(W1 - W2)}{(W2 - W0)} * 100\%$$

3.3.5. Ash

Moisture content was determined according to Association of Official Analytical Chemists (A.O.A.C) methods (A.O.A.C, 1990). A clean dry crucible was weighed (W0). Five grams of the fresh sample was weighed and put in the crucible and the total weight taken as (W1). The samples in the crucibles were incinerated on a mantle (charring) until it turned black (carbonisation) and no smoke was being produced. The charred sample was transferred to a furnace at 550^0C for 3 hours. The samples were allowed to cool in a desiccator and their weight measured (W2).

The ash content was determined in percentage as follows;

$$\%Ash = \frac{(W2 - W0)}{(W1 - W0)} * 100\%$$

3.3.6. Minerals

Minerals were analysed according to A.O.A.C. methods (A.O.A.C. 1990). Five grams of the sample was charred in the oven for 30 minutes then put in a muffle furnace at 550°C for 3 hours to ash. The ash was allowed to cool and diluted with 10ml of 1N hydrochloric acid. The mixture was then filtered and diluted with 100 mL of distilled water. The absorbance of the solution was read using atomic absorption spectrophy. Macro elements; potassium and calcium and micro elements; iron, zinc and manganese were analysed.

3.3.7. Crude protein content

Crude protein content was determined using the semi micro Kjeldal method (A.O.A.C. 1990). Two grams of sample was weighed into a digestion flask together with a combined catalyst of 5 grams potassium sulphate, 0.5 g of copper sulphate and 15 mL of sulphuric acid. The mixture was heated in a fume hood till the digest colour turned blue signifying the end of the digestion process. The digest was cooled, transferred to 100 mL volumetric flask and topped up to the mark with deionized water. A blank digestion with the catalyst was also made. Ten mL of diluted digest was transferred into the distilling flask and washed with distilled water. Fifteen mL of 40% NaOH was added and washed with distilled water. Distillation was done to a volume of about 60 mL. Distillate was titrated using 0.02 N HCL to an orange colour of the mixed indicator, which signified the end point.

%Nitrogen = (V1- V2) * N * F * 100/ (V * 100/S)

Where: V1 is the titre for sample in mL, V2 is titre for blank in mL; N= normality of standard HCL (0.02); F= factor of standard HCL solution; V= volume of diluted digest taken for distillation (10 mL); S= weight of sample taken for distillation (1 g);

% protein= %nitrogen* protein factor (6.25)

3.3.8. Crude fibre

Crude fibre was determined using Weende method involving acid hydrolysis (Van-Soest and McQueen, 1973). Five grams of the sample was weighed (W0). Two hundred millilitre of 1.25% sulphuric acid was added to the sample and boiled for 1 hour. The sample was filtered through a glass wool and the residue boiled in 1.25% sodium hydroxide for 1 hour. The residue was then filtered and washed with hot water, 1.25% hydrochloric acid, alcohol, petroleum ether and diethyl ether. The residue was dried in a desiccator for 30 minutes, weighed (W1) and transferred to a furnace at 550^0C for 1 hour and weighed (W2).

Crude fibre was calculated and expressed as a percentage as follows;

$$Crude\ fibre = \frac{W1 - W2}{W0} * 100\%$$

3.3.9. Beta carotenes

Beta carotene content was analysed using column chromatography and UV Spectrophotometry; using acetone and petroleum ether extraction method as described by Rodriguez-Amaya and Kimura, (2004). Two grams of fresh sample was weighed, chopped finely and placed in a mortar with 10 mL of acetone. The sample was thoroughly ground and the acetone extract transferred into 100 mL volumetric flask. The residue was extracted again with 10 mL acetone and the extract added to the contents of the volumetric flask. The extraction with acetone continued until the residue gave no colour. The combined extract was made to a volume of 100 mL with acetone. Twenty five millilitres of the extract was evaporated to dryness using a rotary evaporator. The residue was then dissolved in 10 mL petroleum ether and the solution introduced into a chromatographic column which was eluted with petroleum ether and beta carotene collected in a flask. The beta carotene elute was made to a volume of 25 mL with petroleum ether and the absorbance read at 440 nm in a UV-Vis spectrophotometer (Shimadzu model UV – 1601 PC, Kyoto, Japan). Beta carotene standards were prepared to make a calibration curve which was used to calculate the beta carotene content.

3.3.10. Ascorbic acid

The ascorbic acid content in the samples was determined by HPLC method according to Vikram *et al.,* (2005). Two grams of sample was weighed and extracted with 0.8% metaphosphoric acid. The solution was made to 20 mL of juice and centrifuged at 10000 rpm for 10 minutes at 4^0C. The supernatant was filtered and diluted with 10 mL of 0.8% metaphosphoric acid. The mixture was then passed through 0.45 µm syringe filter and 20 µL injected into the HPLC machine. Different concentrations of ascorbic acid standards were made to make a calibration curve. HPLC analysis was done using Shimdzu UV-VIS detector at a wavelength of 266 nm. The

HPLC conditions were as follows; Mobile phase: 0.8% metaphosphoric acid, Column: C18, Oven temperature: 25^0C, Flow rate: 1.0 mL/min, injection volume: 20µL.

3.3.11. Nitrates

The nitrate content in the test samples was determined by the calorimetric method using salicylic acid according to Cataldo *et al.,* (1975). Five hundred milligrams of fresh sample was weighed and put in a test tube and 10 mL of hot (90-95^0C) distilled water added. The closed tubes were placed in a water bath at 80^0C for 30 minutes and shaken. The samples were then cooled and centrifuged at 4500 rpm for 10 minutes. The supernatant was decanted and the extract weighed. Chlorophyll in leaf extract was removed by adding 0.5g magnesium carbonate ($MgCO_3$) and centrifuged again at 4500 rpm for 10 minutes. An aliquot of 0.2 ml of the filtrate was pippeted into a 50 ml beaker and 0.8 ml of 5% (w/v) salicylic acid in sulphuric acid was added and mixed thoroughly. The mixture was then allowed to stand for 20 minutes at ambient temperatures. Nineteen millilitres of 2N sodium hydroxide was added and the mixture allowed to cool for 30 minutes. The absorbance was read at 410 nm against a common blank using UV-Vis spectrophotometer (Shimadzu model UV – 1601 PC, Kyoto, Japan). The nitrate content was determined from a standard curve and expressed in mg/100 g.

3.3.12. Oxalates

Analysis was done using HPLC method as suggested by Yu *et al.*, (2002). Five hundred milligrams of the fresh sample was homogenized in 5 mL of 0.5N HCL. The homogenate was heated at 80^0C for 10 minutes with intermittent shaking. To the homogenate, distilled water was added up to a volume of 25 mL. The solution was centrifuged at 10000 rpm for 10 minutes. About 1 mL of supernatant was passed through a micro filter (0.45µm) before HPLC analysis. Standards were prepared at varying concentrations for quantification. HPLC analysis was done using Shimadzu UV-VIS detector.Condition of HPLC; Mobile phase: 0.01 N H_2SO_4, Column: C18, Oven temperature: 25^0C, Flow rate: was 0.6 mL min^{-1}.

3.3.13. Total phenolic content

Five grams of the fresh sample was crushed and weighed into a 250 mL conical flask and about 50 mL methanol added. The flask was closed securely using parafilm and covered with aluminum foil. The samples were put in a shaker and shaken for about 3 hours. They were then kept in the dark and left to extract for 72 hours. Thereafter, the samples were filtered through

Whatman No. 4 filter paper and the filtrate concentrated to dryness using a rotary evaporator, then re-dissolved in 12.5 mL of methanol and kept frozen until analysis.

Total Phenolics content was estimated by a calorimetric assay based on the procedure by Escarpa and Gonzalez (2001). A 100 µL aliquot of the extracted sample was added to 500 µL of 0.2N Folin-Ciocalteu reagent and 6 mL of distilled water. After mixing the content for 1 minute, 4 mL of saturated sodium carbonate (Na_2CO_3)was added. Samples were left to stand at room temperature for 90 minutes and absorbance measurements taken at 725 nm using a UV-VIS 1800 Shimadzu spectrophotometer (Shimadzu, Kyoto, Japan). Gallic acid was used as a reference standard and the results expressed as milligram Gallic acid equivalents (mg GAE) per 100 g extract in dry weight basis.

3.3.14. Sensory evaluation

Sensory analyses were performed on the fresh vegetables. The vegetable samples were evaluated for general appearance (colour), aroma, texture, tenderness, taste/flavour, mouthfeel and overall acceptabilityby untrained panelists, familiar with the taste of cooked cowpea leaves. The evaluation was based on a standard seven point hedonic scale (where 1 = dislike extremely and 7 = like extremely).The sampled vegetables were boiled in a stainless steel pot for 25 minutes till the leaves were tender. 500g of the fresh samples were boiled in 1.5 litre water with 12g salt (Kensalt, Salt Manufacturers Kenya Ltd). The samples were then removed and excess water drained. The vegetables were not fried to avoid change in the sensory parameters by additional ingredients. The vegetables were then served to the panelists. The samples were served hot with an accompaniment for *ugali* (a maize meal paste) as it is consumed locally. The samples served had been coded with 4-digit random number to maintain panellist neutrality. Mineral water was given to the panellists to rinse their mouths after every sample. The sensory panellists were familiar with cowpea recipe and consumption. In seasons 1 the panellists were 31 and in season 2 they were 30. The panellists were chosen among university students aged between 21 and 26 years. Women consisted of about 70% of the total panellists.

3.3.15. Statistical analysis

Data was subjected to Analysis of Variance (ANOVA) and means separated by Fisher's Least Significant Difference (LSD) at $P \leq 0.05$ using Genstat statistical package, 15^{th} edition.

3.4.RESULTS

3.4.1. Proximate analysis

There was significant difference ($p<0.05$) in the moisture content of the accessions with usimpe mtu mdogo recording the highest value with 86.85% and katsetse the least with 85.9% in the 1st season but in the second season M66 had the least moisture content at 86.1%. The moisture content was used to convert the other parameters to dry weight basis. Although there was significant difference ($p<0.05$) among the treatments in the ash content in both the seasons, there was no trend observed. In season 1 and season 2, Machakos 66 recorded the highest percentage of crude fibre at 16.85% and 17.12%, respectively, as compared to the local accessions. Mnyenze recorded the least crude fibre content at 15% and 15.32% for seasons 1 and 2, respectively. In both seasons, usimpe mtu mdogo and M66 recorded the least percentage crude protein content compared to the other accessions at 31.44 for season 1 and 31.12 for season 2 and at 31.7% and 31.37%, respectively. On the other hand, Mumias-tsimg'oli recorded the highest percentage crude protein content at 36.16% for season 1 and 36.06% for season 2 as shown in tables 2 and 3 below.

Table 2: Selected proximate content of fresh cowpea leaves expressed in dry matter basis, Season 1.

Accession/Variety	Proximate contents (%)			
	Moisture content	Ash	Crude fibre	Crude Protein Content
Katsetse	85.9±0.01^{e}	11.61±0.23^{bc}	16.33±0.10^{ab}	33.34±0.17^{d}
Mnyenze	86.67^{b}	12.84±0.31^{a}	15±0.36^{c}	34.43±0.23^{c}
Sura Mbaya	86.57±0.04c	12.41±0.16a	15.81±0.43^{bc}	35.51±0.23b
Usimpe Mtu Mdogo	86.85±0.01a	11.6±0.24c	16.23±0.30^{ab}	31.44±0.12e
Mumias-Tsimg'oli	86.53±0.05^{c}	12.33±0.21^{ab}	16.55±0.06^{ab}	36.16±0.09^{a}
Machakos 66	86.01^{d}	12.13±0.23^{abc}	16.85±0.32a	31.7±0.13e
Means	86.422	12.152	16.13	33.762
LSD (5%)	0.0854	0.7245	0.909	0.52

*All values are mean ± standard error of the mean (n=3). Means with different letters within a column are significantly different ($P < 0.05$).

Table 3: Selected proximate content of fresh cowpea leaves expressed in dry matter basis, season 2.

Accession/Variety	Proximate contents (%)			
	Moisture content	Ash	Crude fibre	Crude Protein Content
Katsetse	86.67±0.10[bc]	11.29±0.14[b]	16.4±0.12[bc]	33.31±0.13[d]
Mnyenze	86.53±0.02[c]	12.38±0.14[a]	15.32±0.27[d]	34.49±0.14[c]
Sura Mbaya	86.79±0.04b	12.33±0.35[a]	15.99±0.10[c]	35.53±0.13[b]
Usimpe Mtu Mdogo	86.94a	11.86±0.21[ab]	15.95±0.11[c]	31.12±0.12[e]
Mumias-Tsimg'oli	86.3±0.02[d]	12.27±0.15[a]	16.75±0.14[ab]	36.06±0.20[a]
Machakos 66	86.1±0.01[e]	11.25±0.16[b]	17.12±0.25[a]	31.37±0.11[e]
Means	86.553	11.898	16.255	33.646
LSD (5%)	0.1429	0.6302	0.5521	0.4358

*All values are mean ± standard error of the mean (n=3). Means with different letters within a column are significantly different ($P < 0.05$).

3.4.2. Beta carotene, ascorbic acid and total phenols

There was significance difference among the accessions in both the seasons but a clear trend was not observed. In season 1, Machakos 66 recorded the least β-carotene content at 29.71mg/100g compared to the local accessions whereas Mumias-tsimg'oli, mnyenze and sura mbaya had the highest β-carotene content at 35.92, 35.31, 36.4 mg/100g. In season 2, Mumias-tsimg'oli had the least β-carotene content at 30.8 mg/100g whereas mnyenze and sura mbaya had the highest values at 36.55 and 36.32mg/100g respectively. Ascorbic acid varied considerably in both the seasons. However katsetse and Machakos 66 were consistently the least in ascorbic acid content at 191 and 192.8mg/100g for season 1 and 200 and 192.9mg/100g for season 2 respectively. In season 1, mnyenze recorded the highest ascorbic acid content at 214.7mg/100g while in season 2 it was usimpe mtu mdogo at 213.8mg/100g. In season 1, usimpe mtu mdogo had the least total phenolic content with 4000 GAE mg/100g whereas Machakos 66 had the highest at 4744 GAE mg/100g. In season 2, Mumias-tsimg'oli recorded the lowest total phenolic content at 3206 GAE mg/100g whereas mnyenze recorded the highest value at 4550 GAE mg/100g as indicated in tables 4 and 5 below.

Table 4: Beta carotene, ascorbic acid and total phenolic contents of fresh cowpea leaves expressed in dry matter basis, Season 1.

Accession/Variety	Antioxidants		
	Beta carotene (mg/100g)	Ascorbic acid (mg/100g)	Total Phenolic Content (GAE mg/100g)
Katsetse	33.65±0.45[b]	191±0.57[d]	4058±246.6[c]
Mnyenze	35.31±0.12[a]	214.7±1.07[a]	4588±317.7[ab]
Sura Mbaya	36.4±0.39[a]	201.6±0.42[c]	4664±58.1[a]
Usimpe Mtu Mdogo	32.74±0.71[b]	213.1±0.39[a]	4000±90.4[c]
Mumias-Tsimg'oli	35.92±0.02[a]	204.4±1.11[b]	4102±9.8[bc]
Machakos 66	29.71±0.56[c]	192.8±0.61[d]	4744±7.7[a]
Means	33.96	202.93	4359
LSD (5%)	1.366	2.322	523.9

*All values are mean ± standard error of the mean (n=3). Means with different letters within a column are significantly different ($P < 0.05$).

Table 5: Beta carotene, ascorbic acid and total phenolic contents of fresh cowpea leaves expressed in dry matter basis, Season 2.

Accession/Variety	Parameters		
	Beta carotene (mg/100g)	Ascorbic acid (mg/100g)	Total Phenolic Content (GAE mg/100g)
Katsetse	32.76±0.78[bc]	200±1.11[e]	3803±23.06[d]
Mnyenze	36.55±0.01[a]	211.7±0.55[b]	4550±69.47[b]
Sura Mbaya	36.32±0.67[a]	206.9±0.52[c]	4715±6.05[a]
Usimpe Mtu Mdogo	31.45±0.37[cd]	213.8±0.60[a]	3895±79.98[cd]
Mumias-Tsimg'oli	30.8±0.29[d]	204.4±0.43[d]	3206±7.33[e]
Machakos 66	33.43±0.26[b]	192.9±0.53[f]	3964±61.73[c]
Means	33.55	204.96	4022
LSD (5%)	1.454	2.039	157.4

*All values are mean ± standard error of the mean (n=3). Means with different letters within a column are significantly different (P < 0.05).

3.4.3. Mineral elements

All the minerals in both seasons changed considerably. All the minerals analyzed were not significantly different (P<0.05). Mnyenze recorded the highest calcium and iron content at 34764ppm and 1034.3ppm respectively. Usimpe mtu mdogo recorded the lowest calcium content at 17066ppm. The highest potassium level was recorded in sura mbaya at 34204ppm, calcium at 34764 in mnyenze, iron at 730.2ppm in katsetse, zinc at 169.8ppm in katsetse and manganese at 719.8ppm in sura mbaya for season 1. These accessions also showed the same trend in season 2 but with slightly different values as shown in tables 6 and 7.

Table 6: Selected mineral element content of fresh cowpea leaves expressed in dry matter basis, Season 1.

Accession/Variety	Mineral contents (PPM)				
	K	Ca	Fe	Zn	Mn
Katsetse	24484±2694	27496±593[b]	730.2±60[ab]	169.8±37.98	693.6±27.81
Mnyenze	28322±2600	34764±2273[a]	1034.3±148.6[a]	97.2±11.79	541.1±111.58
Sura mbaya	34204±1497	26048±1392[b]	598.3±116.5[b]	152.3±19.9	719.8±78.57
Usimpe Mtu Mdogo	26814±2264	17066±1149[c]	395.9±65.3[b]	131.9±20.85	491.5±93.83
Mumias-Tsimg'oli	24683±1565	24665±3158[b]	564.6±158.3[b]	79.9±11.51	403.7±97.74
Machakos 66	26113±2483	21407±2194[bc]	499.7±52.2[b]	130.9±17.79	569.1±80.83[a]
Means	27436	25241	637	127	570
LSD (5%)	6889.9	6106.4	335.8	67.29	264.7

*All values are mean ± standard error of the mean (n=3). Means with different letters within a column are significantly different (P < 0.05).

Table 7: Selected mineral element content of fresh cowpea leaves expressed in dry matter basis, season 2.

Accession/Variety	Mineral contents (PPM)				
	K	Ca	Fe	Zn	Mn
Katsetse	24542±2753	27639±808b[a]	740.2±36.83[ab]	165.5±33.84	689.9±27.98
Mnyenze	26235±1819	33999±1798[a]	1020.9±126.61[a]	94.5±11.9	539.2±105.95
Sura Mbaya	34149±1562	25416±1071[b]	554.7±106.67[bc]	156.4±19.07	721.5±67.36
Usimpe Mtu Mdogo	25809±1737	16836±1118[c]	402.6±53.7[c]	132.1±17.15	485.6±88.19
Mumias-Tsimg'oli	24740±1356	22026±5384[bc]	564.5±152.61[bc]	83±7.69	406.7±97.35
Machakos 66	26286±2739	21469±1717[bc]	493.5±51.16[bc]	134.4±12.53	564.3±78.78
Means	26960	24564	629	127.7	568
LSD (5%)	6375.9	7776.4	301.8	58.48	251.6

*All values are mean ± standard error of the mean (n=3). Means with different letters within a column are significantly different ($P < 0.05$).

3.4.4. Nitrates and oxalates

Nitrates and oxalates are important components in vegetables that determines their digestibility and toxicity when consumed in addition to the availability and digestibility of other nutrients in the gut. In season 1, Mumias-Tsimg'oli and M66 had the lowest nitrate content whereas Mnyenze had the highest content. In season 2, Mumias-Tsimg'olihad the lowest nitrate content whereas Usimpe Mtu MdogoUMM had the highest nitrate content as shown in table 9. Generally oxalates were high in the vegetable and the values varied considerably for the two seasons. In season1, Mumias-Tsimg'olihad the highest level of oxalates at 3816mg/100g whereas in season2, Katsetse, Sura Mbayaand Mnyenzehad no significant difference but constituted the highest peak as shown in table 8.

Table 8: Levels of selected anti-nutrients of fresh cowpea leaves expressed in dry matter basis.

Accession/ Variety	Parameters and seasons			
	Nitrates		Oxalates	
	Seasons 1	2	1	2
Katsetse	77.28±0.67[b]	78.39±0.17[d]	3392±46.84[c]	3505±17.4[a]
Mnyenze	83.76±0.25[a]	83.6±0.39[b]	3659±62.37[b]	3459±34.64[a]
Sura Mbaya	82.81±0.19[a]	81.39±0.54[c]	3395±37.97[c]	3400±48.86[a]
Usimpe Mtu Mdogo	84.6±0.72[a]	88.11±0.05[a]	3426±24.39[c]	3279±43.17[b]
Mumias-Tsimg'oli	73.31±0.18[c]	72.57±0.23[f]	3816±30.99[a]	3100±27.2[c]
Machakos 66	74.26±1.06[c]	76.58±0.21[e]	3317±50.98[c]	3163±52.2[bc]
Means	79.34	80.11	3501	3318
LSD (5%)	1.875	0.949	135.9	120.8

*All values are mean ± standard error of the mean (n=3). Means with different letters within a column are significantly different ($P < 0.05$).

3.4.5. Sensory evaluation

The sensory parameters evaluated included; appearance, aroma, texture, taste/flavor, mouth-feel and general acceptability. Generally, all the accessions were moderately accepted in both the seasons. In season 1, Mnyenze was best rated at an average of 4.71 and Machakos 66 the least with a rating of 3.52. In season 2 katstse recorded the highest rating of 5.85 and Machakos 66 the least with a rating of 3.29. M66 generally was rated inferior to the local accessions as shown in tables 9 and 10.

Table 9: Hedonic scores for sensory quality attributes of fresh cowpea leaves, season 1.

Accession/ Variety	Sensory quality attributes (7 point hedonic scale)						
	Appearance	Aroma	Texture	Tenderness	Taste	Mouth feel	Overall acceptability
Katsetse	4.95	4.86	4.81	4.52	4.52	4.48	4.52
Mnyenze	4.76	4.81	4.95	5.24	3.14	4.57	4.71
Sura Mbaya	4.19	3.76	3.76	4.43	3.52	3.95	4.24
Usimpe Mtu Mdogo	4.67	4.76	4.33	3.76	4.14	4.19	4.43
Mumias-Tsimg'oli	4.33	4.52	4.43	4.52	4.76	4.43	4.52
Machakos 66	3.86	4.05	3.76	3.71	3.14	3.38	3.52
Mean	4.46	4.46	4.34	4.37	3.87	4.17	4.33
LSD (5%)	0.748	0.726	0.78	0.863	0.839	0.832	0.873

*The values in the table represent scores on a 7-point hedonic scale (1 = dislike extremely), 2 = (dislike very much), 3 = (dislike moderately), 4 = (neither like nor dislike), 5 = (like moderately), 6= (like very much) and 7= (Like extremely).

Table 10: Hedonic scores for sensory quality attributes of fresh cowpea leaves, season 2.

Accession/ Variety	Sensory quality attributes (7 point hedonic scale)						
	Appearance	Aroma	Texture	Tenderness	Taste	Mouth feel	Overall acceptability
Katsetse	5.52	5.52	5.52	5.57	6.1	5.76	5.85
Mnyenze	4.67	4.95	4.43	4.52	5.33	4.86	4.9
Sura Mbaya	4.9	5.05	4.71	4.86	4.76	4.86	5.05
Usimpe Mtu Mdogo	5.33	5.14	5.62	5.24	5.38	5.05	5.71
Mumias-Tsimg'oli	4.76	5.1	4.86	4.95	4.76	4.62	4.86
Machakos 66	4.33	3.38	3.33	3.52	3.38	3.38	3.29
Mean	4.92	4.86	4.75	4.78	4.95	4.75	4.94
LSD (5%)	0.836	0.794	0.855	0.884	0.885	0.916	0.824

*The values in the table represent scores on a 7-point hedonic scale (1 = dislike extremely), 2 = (dislike very much), 3 = (dislike moderately), 4 = (neither like nor dislike), 5 = (like moderately), 6= (like very much) and 7= (Like extremely).

3.5.DISCUSSION

The highest amount of crude proteins, crude fibre and ash was 36.16%, 17.12% and 12.84% respectively. These results were comparable with results obtained from Muchoki *et al.*, (2007) which indicated that crude protein, crude fibre and total ash were 31.8%, 18.2% and 12.1%, respectively. Okonya and Maass (2014); and Kasangi *et al.*, (2010) also recorded results which were comparable to the present results. However, the results from the present study differed from the results obtained from Njoroge *et al*., (2015) and Chikwendu *et al.*, (2014) which indicated that the protein content was 6.8% and crude fibre at around 4.2%. The difference in the protein content can be as a result of environmental factors, age of the leaves, availability of assimilates and the ability of individual varieties to develop a symbiotic relationship with the nitrogen-fixing bacteria in root nodules (Ono *et al.*, 1996; Angessa, 2006). The accessions contain appreciable constituents of proteins and crude fibre which makes it ideal for consumption especially in supplying roughage and affordable proteins. There was no significant difference in the ash content, however crude fibre and crude protein showed significant differences. Crude protein content of M66 was inferior compared to the local accessions at 31.7% whereas its crude fibre content was superior at 16.85%.

Vitamins are important components in human diets especially in boosting immunity and scavenging for radicals (Bender, 2009). All the local cowpea accessions and the improved variety generally had high amounts of ascorbic acid and beta-carotenes ranging between 191 to 213.8 mg/100g and 29.71 to 36.55 mg/100g respectively. These results were comparable with results from Muchoki *et al*., (2007) which recorded ascorbic acid and beta-carotene at 308mg/100g and 33mg/100g, respectively. The results however, differed from the results from Chikwendu *et al.,* (2014) which recorded ascorbic acid and beta- carotene at 59.24mg/100g and 9.10mg/100g respectively and Njoroge *et al*., (2015) who found ascorbic acid at 91.0mg/100g and beta-carotene at 6.5mg/100g. The improved variety was found to be inferior in beta carotene and ascorbic acid.

Phenolics are phytochemicals which do not have direct nutritional benefits but act as strong antioxidants (Oboh and Akindahunsi, 2004; Chikwendu *et al.,* 2014; Preet and Punia, 2000; Islam *et al.,* 2002). Ishiguro *et al.,* (2004) stated that polyphenols have many physiological functions such as cancer-fighting properties. However, some researchers have categorized them as anti-nutrients (Muchoki *et al*., 2010). In this present research, sura mbaya had the highest total phenolic content at 4715 GAE mg/100g.

Minerals are important as they are used in many metabolic activities (Institute of Medicine, 2001). Generally, there was no significant difference in the content of minerals in the present study. However, calcium and iron showed some relative differences. Mnyenze had the highest calcium and iron content. The mineral contents of M66 were comparable to the local accessions except for calcium and iron.

Anti-nutrients determine the bioavailability and digestibility of some mineral elements in the gut. The leaves of the cowpeas assessed had unusually high values of oxalate (3100 to 3816ppm) compared to Muchoki *et al*., (2010) at 1889mg/100g and Chikwendu *et al.,* (2014) at 4.59%. According to (Akwaowo *et al.,* 2000) high oxalate levels may constitute human poisons. Oxalic acid also forms an insoluble salt with the essential nutrient such as calcium, iron and zinc thus inhibiting their absorption (Hodgkinson, 1997; Bothwell and Chalton, 1982). However, proper cooking significantly reduces total oxalate content (Akwaowo *et al.*, 2000). Leafy vegetables are the major source of nitrates entering the human system. All the cowpea accessions had a moderate nitrate content of between 72.57 to 83.76 mg/100g which were lower compared to Muchoki *et al*., (2010) at 771mg/100g. M66 had the lowest nitrate content but comparable with that of Mumias-Tsimg'oli. The oxalate contents of M66 was the lowest but comparable to usimpe mtu mdogo.

It has been noted that local cowpea accessions are preferred by farmers because of superior taste and palatability (KARI, 2010). It has also been noted that, farmers sometimes complain that improved varieties have excessively bitter or less bitter taste than the local accessions or even incidences of tough leaves which reduces its acceptability (Rowland, 1992). In the present study, it was found that sensory attributes of M66 were inferior compared to the local accessions. Katsetse, Mnyenze and Mumias-Tsimg'oli were superior in sensory attributes.

3.6.CONCLUSION

Local cowpea accessions were found to be superior to the improved variety in beta carotene, ascorbic acid, crude proteins and acceptance by consumers. However, the improved variety was superior in total phenolic content and crude fibre. The local accessions can be encouraged and promoted for you by consumers because of their nutritional properties.

Seasonal variation in nutritional status showed no difference because of sufficient rainfall and almost constant temperatures for the two seasons.

CHAPTER FOUR
4.0 EFFICACY OF POST-HARVEST TREATMENTS IN MAINTAINING THE QUALITY ATTRIBUTES OF COWPEA LEAVES

4.1. ABSTRACT

African indigenous vegetables are an excellent low cost source of nutrients in diets. Their availability is constrained by seasonal peaks and lows and high post-harvest losses as a result of perishability. To counter the post-harvest losses, appropriate postharvest technologies to extend the shelf life while preserving nutrient quality should be tested. The objective of this study was to evaluate the effect of blanching, solar drying and modified atmosphere packaging on the quality attributes and shelf life of cowpeas leaves. Local cowpea accessions and an improved variety were planted in The University of Nairobi, field station during the short rains from October to December 2014 and long rains from March to May 2015. One accession was chosen for post-harvest treatments. One batch of cowpea leaves was subjected to post-harvest dehydrating treatments; solar drying without blanching, blanching in pure water and solar drying, blanching in salty water and solar drying and fresh leaves as control.The samples that were blanched involved dipping the vegetables in boiling water for 2 minutes followed by immersion in cold water at 8^0C for 2 minutes before solar drying. Another batch was subjected to Modified Atmosphere Packaging (MAP) treatments; Extend® bag, ordinary polythene bag and unpackaged control. The samples were then analysed for selected proximate constituents, vitamins, minerals, anti-nutrients, sensory characteristics, colour change during processing and packaging, cumulative water loss and wilting in The University of Nairobi and Jomo Kenyatta University of Agriculture and Technology laboratories. It was found that blanching and solar drying had little effect on most proximate and mineral elements. However, vitamin and total phenolic contents were the most affected. Solar drying without blanching recorded the highest vitamin retention levels at 68.02% for beta carotene and 68.39% for ascorbic acid whereas blanching in pure water and solar drying and blanching in salty water and solar drying recorded a retention level of 55.58% and 52.78% for beta carotene, respectively and 21.08% and 20.24% for ascorbic acid, respectively. In addition, solar drying without blanching recorded the highest retention of total phenolic content at 149.91%. Blanching in pure water and solar drying and blanching in salty water and solar drying recorded retention levels of 62.58% and 65.79% of total phenolic content, respectively. On the other hand, solar drying without blanching, blanching in pure water and solar drying and blanching in salty water and solar drying recorded losses of 5.87%, 10.77% and

11.17% of oxalates and 37.22%, 69.98% and 58.7% of nitrates, respectively. The end stages of the samples which were unpackaged, packaged in ordinary polythene bag and Extend® bag under room conditions was 1 day, 4 days and 6 days, respectively. By the end stage control, ordinary polythene bag and Extend® bag had lost 28.84%, 0.93% and 3.27% cumulative weight for season 1 and 23.84%, 0.89% and 2.31% for season 2, respectively. Colour deterioration in hue angles for control, ordinary polythene bag and Extend® packaging polythene film reduced to 149.2^0, 152.8^0 and 152.12^0 respectively from 153.23^0 in season 1. Solar drying without blanching was found to be effective in maintaining the quality attributes of cowpeas. In addition, MAP was found to be effective in preserving quality of fresh cowpea leaves and improving shelf life. Therefore MAP and solar drying without blanching are simple and convenient technologies for preserving cowpea nutrients and improving shelf life.

KEYWORDS: Cowpea accessions; dehydration; MAP; nutritional quality

4.2. INTRODUCTION

African indigenous vegetables have played an immeasurable role in African nutritional requirements for decades (Smith and Eyzaguirre, 2007). The passage of culture together with preservation of sources of food from one generation to the next demonstrates the role these vegetables played in traditional food systems. That notwithstanding, these vegetables have grown to become strong economic and social pillar in recent years (Mwaura *et al*., 2013) since the growth of the venture has benefited mainly women who have become financially uplifted hence maintaining descent families. The situation has been notable in the rural areas where these vegetables are produced and consumed in large quantities (Maundu, 1997). The concept has spilled to the middle and upper class citizens in most parts of the country (Irungu *et al.*, 2007) because of the scientific evidence suggesting that the vegetables are superior nutritionally. These vegetables have also been documented to counteracting emerging lifestyle diseases and disorders (Habwe *et al*., 2008; Smith and Eyzaguirre, 2007; Abukutsa, 2003; Kimiywe *et al.*, 2007). Despite these positive aspects, notable anti-nutrient contents have been discussed (Muchoki *et al.*, 2010; Chikwendu *et al* 2014; Ajala, 2009).

In Kenya there are hundreds of ALVs grown or found in the wild (Irungu *et al.*, 2007; Otieno *et al.*, 2009). According to HCD report (2014), cowpea (*Vigna unguiculata*) is ranked as the 3rd most important vegetable among the African indigenous vegetables in production and consumption at 22% preceded by *Solanum* spp at 27% and *Cleome* spp at 23%.

The potential of cowpea leaves has not been maximized due to agronomic challenges and to a greater extent, postharvest handling limitations (Affognona *et al.,* 2014). It is estimated that postharvest losses contribute to about 50% (Masarirambi *et al.,* 2010) total losses in cowpea value chain. However, figures between 10–40%, and as high as 50–70% are regularly reported (FAO-World Bank, 2010). The losses are expressed as estimates since cowpea like the other African indigenous vegetables, has not been comprehensively documented due to its informal marketing channels and small scale production over a vast area. According to Affognona *et al.*, (2014), the real magnitude of postharvest losses is not clearly known because of the inadequacies of loss assessment methodologies available. The high postharvest losses lead to wasted resources invested in production. One factor that aggravates the post-harvest loss situation to the high postharvest losses is the seasonality characterizing production of cowpeas, such that gluts are achieved during the wet season and scarcity during the dry season (Abukutsa *et al*., 2006; Chavasit *et al.,* 2002). To alleviate food and nutrition security in Kenyan population especially among the rural and the resource constrained population, the estimated postharvest losses should be reduced to the bare minimum (FAO-World Bank, 2010). However, there are other underlying economic problems such as poor transport network and distribution systems which contribute to postharvest losses (Shiundu and Oniang'o, 2007).

Water loss in fresh vegetables is a major cause of deterioration because it results in both quantitative and qualitative losses. Quantitatively, vegetables lose their saleable weight due to transpiration losses. This is the major cause of deterioration since cowpeas and other vegetables have a large surface area to volume ratio (Mampholo *et al.,* 2015). Furthermore, cowpea leaves contain about 90% water, a situation which deprives the vegetables long shelf life while fresh (Muchoki *et al.,* 2007). Under room temperatures and relative humidity, these vegetables lose water drastically and may not last for 24 hours (Abukutsa, 2010). Qualitative losses include loss in appearance due to shriveling, colour change, loss in textural and nutritional quality. A part from water loss respiration is also a major cause of deterioration in vegetables because fresh produce continue to respire after harvesting (Silva, 2010). Respiration induced losses can be minimized by using appropriate technologies to reduce the respiration rate without harming the quality of the product such as taste, texture and appearance. As a general rule, the rate of respiration can be reduced by keeping the temperature low, having lower levels of oxygen in the packaging atmosphere and increased levels of carbon dioxide. However, note should be taken to avoid anaerobic respiration as a result of too low oxygen which results to unwanted tastes and odours hence loss of quality (Karaçay and Ayhan, 2009).

To reduce the postharvest losses, other methods such as improving and lengthening shelf life and optimization of production have been proposed (Chavasit *et al.*, 2002). Some of the methods that should be employed include value addition, packaging and streamlining supply and marketing systems. Some of the technologies aim at long term lengthening shelf life through dehydration while others improve the shelf life of fresh leaves.

Drying as a means of dehydration has been the most common form of postharvest preservation in traditional systems to supplement indigenous vegetables during the dry seasons (Fellows, 2009). This was attributed to the ease in carrying out and managing the processes which didn't require specialized skills. Dehydration is achieved through several techniques including solar drying, shade drying, open air drying and oven drying. However, whichever method used, the amount of minerals and nutrients is reduced (Gupta *et al.,* 2013; Muchoki *et al.,* 2007; Frias *et al.,* 2010). The extent of reduction is dependent on the specific technique used. For instance, the sun drying technique has been documented to lead to a greater loss in vitamins than shade drying and solar drying (Muchoki *et al.*, 2007; Ndawula *et al.*, 2004). Apart from volatilization and vaporization, blanching has been cited as one of the cause of the high loss in minerals and vitamins in withered vegetables and fruits due to leaching and effects of heat on chemical degradation (Muchoki *et al.,* 2007, Tannenbaum, 1976). Optimization of blanching and dehydration methods can lead to establishment of cost effective practice in postharvest management of cowpea leaves that leads to quality products.

On the other hand, post-harvest technologies that reduce water loss, respiration, ethylene production and improve shelf life of fresh produce have been applied to reduce post-harvest losses (Kader, 1995). These technologies include cold temperature storage, controlled atmosphere storage (CAS) and modified atmosphere packaging (MAP) among others. Although limited scientific research has been published on the versatility of modified atmosphere packaging in vegetables, extensive work has been done on fruits. It has been shown that the procedure is simple, affordable and effective in preserving fresh quality of fruits (Githiga, 2012; Kirakou *et al.,* 2014; Yumbya *et al.,* 2014).

Recent advances in the design and manufacture of polymeric films with a wide range of gas permeability characteristics have stimulated the creation of MAP with flexible film packages. Additionally, incorporation of various absorbers in the polyfilm lining (for O_2, CO_2, C_2H_4 and water vapor) has increased the efficacy of the packages. An example of innovative MAP products is the Extend® which was introduced product in the Kenyan market by Amiran Kenya. According to the manufacturers, the film was designed specifically for leafy vegetables. The bag is expected to extend leafy vegetables shelf life and marketing period, ultimately reducing the postharvest losses by slowing down water loss and other deteriorative factors. Since the product is new in the market, no published work has been done its effect on vegetables, more so on ALVs.

Success of post-harvest technologies ensures near continuous availability of cowpea vegetables and other African leafy vegetables throughout the year regardless of production seasonality, improved nutrition status and income (Adebooye and Opadode, 2004). Reduction in the losses will ultimately benefit consumers as well because of stable product prices and continuous availability. The objective of the study was to evaluate the efficacy of two blanching techniques and solar drying and modified atmosphere packaging in maintaining the physical and nutritional quality of dehydrated and fresh cowpea leaves.

4.3. MATERIALS AND METHODS

One local cowpea accession (sura mbaya), superior in terms of consumer acceptability and yield, was grown at the University of Nairobi Field Station for two seasons. Short rains being season 1 (October to December 2014) and long rains being season 2 (March to May 2015). The leaves were harvested seven weeks after planting because at this stage, production for dual purpose of cowpea is at its optimal point (Inaizumi *et al.*, 1999).

The harvested vegetables were separated into four samples which were subjected to treatments;

- Solar drying without blanching,
- Blanching in pure water and solar drying,
- Blanching in salty water and solar drying and
- Control - freshly harvested leaves.

The salt content used was 10% of the weight of the vegetable and a ratio of 1:4 (vegetable to water) w/v basis. The leaves were blanched for 2 minutes in boiling water followed by immersion in cold water at 8^0C water for 2 minutes. The leaves were then dehydrated in a solar drier until they became dry to the feel. The leaves were then sealed in air tight polythene bags before analysis.

On the other hand, samples of approximately 250g of fresh cowpea leaves were sorted out, foreign matter and inconsumable leaves removed. The fresh samples were then packaged in the Extend® bag and commercial ordinary bag and the control left on a perforated tray in the laboratory under room conditions. The Extend® bag has been custom made for packaging vegetables and was introduced by Amiran as an alternative to the ordinary packaging polythene bags. Each treatment was replicated three times. Parameter measurements were recorded on a daily basis because of the highly perishable nature of the leaves. The experiment was conducted for two seasons at the university of Nairobi botany laboratory. Season 1 was conducted after the short rains while season 2 was conducted after the long rains following the cropping seasons of the planted cowpeas. The mean temperatures and relative humidity were 28^0C and 52% for season 1 respectively and 25^0C and 58% for season 2 respectively.

4.4. PARAMETERS ANALYSED

Moisture content, ash content, mineral elements, crude protein content, crude fibre, beta carotene, ascorbic acid, total phenolic content, nitrates and oxalates were analysed as described in chapter three (3.3.4 to 3.3.13). However, the weight of the samples was reduced by 80% because of the loss of moisture during solar drying.

Other parameters analysed include;

4.4.1. Sensory evaluation

Sensory analyses were performed on the dried and the fresh vegetables. The vegetable samples were evaluated for general appearance (colour), aroma, texture, tenderness, taste/flavour, mouth feel and general acceptability using untrained panelists, familiar with the taste of cooked cowpea leaves. The evaluation was based on a standard seven point hedonic scale (where 1 = dislike extremely and 7 = like extremely). Twenty eight grams for the dried samples which are equivalent to 250g of fresh sample were boiled in a stainless steel pot for 32 minutes till the leaves were tender. The samples were then removed, excess water drained and fried using the following procedure. Forty grams of finely chopped onions were weighed into an aluminum pot with 25 mL vegetable cooking oil (Rina vegetable cooking oil, Kapa Oil Refineries Ltd, Kenya). The pot was heated until the onions turned golden brown then 95g of finely chopped tomatoes were added. Six grams of salt (Kensalt, Salt Manufacturers Kenya Ltd) was also added. When a paste of the ingredients had formed, the drained sample was added and thoroughly mixed and allowed to simmer for 10 minutes. The vegetables were served hot to the panelists with an accompaniment for *ugali* (a maize meal paste) as it is consumed locally.The samples served had been coded with 4-digit random number to maintain panellist neutrality. Mineral water was given to the panellists to rinse their mouths after every sample. The number of panellists involved in the study was 29 for season1 and 31 for season 2 familiar with cowpea recipes and consumption. The panellists were chosen among university students aged between 21 and 26 years. Women consisted of about 70% of the total panellists.

4.4.2. Colour change evaluation

Leaf colour was measured when fresh, after blanching and after drying to determine the effect of processing on the colour of the product. The color of the leaf surface was measured using a potable color meter that was calibrated with a white and black standard tile. The L*, a* and b* coordinates were recorded and, a* and b* values converted to hue angle (H°). The dried samples were dipped in hot water for two minutes before the color is measured to allow rehydration.

Hue angle (H°) = arctan (b/a) (for +a and +b values)

= arctan (b/a) + 180 (for -a and +b values)

= arctan (b/a) + 180 (for -a and –b values)

Hue angle distribution is as shown below;

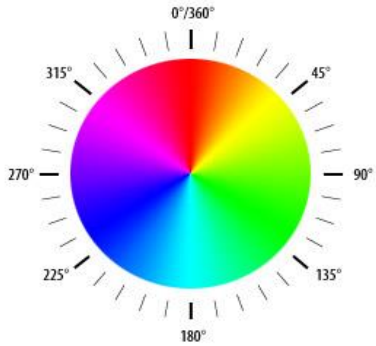

Figure 1: Hue angle distribution (in degrees).

Courtesy (www.huevaluechroma.com/012.php)

4.4.3. Cumulative weight loss (percentage)

Weight was measured using a digital weighing balance. The results were calculated and expressed as follows;

Percentage cumulative weight loss = $\frac{(Initial\ weight - final\ weight)}{Initial\ weight} \times 100\%$

4.4.4. Wilting

Wilting was measured using a wilting index on a hedonic scale ranging between 1 and 7. (1=extremely dry 2=dry 3=moderately dry 4=neither dry nor fresh 5=moderately fresh 6=fresh and 7=extremely fresh)

4.4.5. Statistical analysis

Data was subjected to Analysis of Variance (ANOVA) and means separated by Fisher's Least Significant Difference (LSD) at $P \leq 0.05$ using GenStat statistical package, 15th edition.

4.5. RESULTS

4.5.1. Proximate analysis

Moisture content, ash, crude fibre and crude proteins were analysed. There was no significant difference ($p<0.05$) in ash content for the four treatments; however, crude fibre and crude protein content showed some varied levels of significance ($p<0.05$). Fresh leaves consistently recorded the highest contents of crude fibre and protein at 15.81% and 35.51% for season 1 and 15.99% and 35.53% for season 2, respectively closely followed by solar drying without blanching, blanching in pure water and solar drying and blanching in salty water and solar drying in that order. However, there was no consistency in significance for the samples blanched in pure water and solar dried and blanched in salty water and solar dried as shown in tables 11 and 12. The moisture content was used to express the contents in dry matter basis.

Table 11: Levels of selected proximate of fresh and processed cowpea leaves expressed in dry matter basis, Season 1.

Treatments	Proximate contents (%)			
	Moisture Content	Ash	Crude fibre	Crude Protein Content
Fresh leaves	86.57±0.03[a]	12.41±0.16	15.81±0.43[a]	35.51±0.23[a]
Solar drying without blanching	12.07±0.10[b]	12.01±0.12	15.89±0.18[a]	35.02±0.05[b]
Blanching in pure water and solar drying	11.69±0.46[b]	11.52±0.26	14.70±0.18[b]	31.86±0.12[c]
Blanching in salty water and solar drying	11.92±0.25[b]	11.87±0.25	13.69±0.35[c]	30.94±0.05[d]
Means	30.56	11.95	15.02	33.334
LSD (5%)	0.875	0.674	0.992	0.4327

*All values are mean ± standard error of the mean (n=3). Means with different letters within a column are significantly different (P < 0.05).

Table 12: Levels of selected proximate of fresh and processed cowpea leaves expressed in dry matter basis, Season 2.

Treatments	Proximate contents (%)			
	Moisture Content	Ash	Crude fibre	Crude Protein Content
Fresh leaves	86.79±0.04[a]	12.33±0.35	15.99±0.10[a]	35.53±0.13[a]
Solar drying without blanching	10.98±0.12[c]	12.00±0.18	16.05±0.14[a]	34.93±0.16[b]
Blanching in pure water and solar drying	11.92±0.17[b]	11.61±0.27	14.35±0.6[ab]	31.75±0.21[a]
Blanching in salty water and solar drying	12.01±0.27[b]	11.87±0.12	13.72±0.28[b]	30.95±0.10[d]
Means	30.423	11.95	15.02	33.288
LSD (5%)	0.5601	0.801	1.135	0.5074

*All values are mean ± standard error of the mean (n=3). Means with different letters within a column are significantly different ($P < 0.05$).

4.5.2. Beta carotene, ascorbic acid and total phenols

Two water soluble vitamins (beta carotene and ascorbic acid) present in large quantities in vegetables were chosen together with total phenolic content, a phytochemical important for its antioxidant activity. Generally, there was very high significance difference ($p<0.05$) between the fresh sample and the dehydrated ones, whether blanched or not. In addition, solar dried without blanching sample recorded the highest vitamin retention levels at 68.02% for beta carotene and 68.39% for ascorbic acid unlike blanched in pure water and solar dried sample at 55.58% for beta carotene and 21.08% for ascorbic acid and blanched in salty water and solar dried sample at 52.78% β_carotene and 20.24% ascorbic acid. On the other hand, solar dried without blanching sample recorded the highest retention total phenolic content at 149.91% which surpassed the fresh sample by around 49.91%. Blanched in pure water and solar dried sample and blanched in salty water and solar dried sample recorded retention levels of 62.58% and 65.79% of total phenolic content respectively as shown in tables 12 and 14.

Table 13: Levels of beta carotene, ascorbic acid and total phenolic content of fresh and processed cowpea leaves expressed in dry matter basis, season 1.

Treatments	Antioxidants		
	Beta carotene (mg/100g)	Ascorbic acid (mg/100g)	Total Phenolic Content (GAE mg/100g)
Fresh leaves	36.4±0.39[a]	201.6±0.42[a]	4664±58.07[b]
Solar drying without blanching	24.76±0.03[b]	137.9±0.31[b]	6974±101.32[a]
Blanching in pure water and solar drying	20.23±0.07[c]	42.5±1.17[c]	2911±105.84[c]
Blanching in salty water and solar drying	19.21±0.04[d]	40.8±0.30[c]	3061±118.17[c]
Means	25.152	105.7	4402
LSD (5%)	0.6476	2.149	321.2

*All values are mean ± standard error of the mean (n=3). Means with different letters within a column are significantly different (P < 0.05).

Table 14: Levels of beta carotene, ascorbic acid and total phenolic content of fresh and processed cowpea leaves expressed in dry matter basis, season 2.

Treatments	Antioxidants		
	Beta carotene (mg/100g)	Ascorbic acid (mg/100g)	Total Phenolic Content (GAE mg/100g)
Fresh leaves	36.32±0.66[a]	206.9±0.52[a]	4715±6.05[b]
Solar drying without blanching	24.83±0.02[b]	137.2±0.32[b]	6716±130.56[a]
Blanching in pure water and solar drying	20.35±0.22[c]	41±0.13[c]	2724±119.98[c]
Blanching in salty water and solar drying	19.47±0.01[c]	40.1±0.24[c]	2972±43.91[c]
Means	25.24	106.3	4282
LSD (5%)	1.136	1.09	298

*All values are mean ± standard error of the mean (n=3). Means with different letters within a column are significantly different (P < 0.05).

4.5.3. Mineral elements

Although mineral elements were lost during processing, the losses were not significantly different ($p<0.05$) among all the treatments as shown in tables 15 and 16. However, high amounts of the minerals were generally reported in the vegetable. The highest amounts of minerals were present in the fresh sample at 34204ppm, 26048ppm, 598.3ppm, 152.3ppm and 719.8ppm for potassium, calcium, iron, zinc and manganese respectively for season 1.

Table 15: Levels of selected mineral elements of fresh and processed cowpea leaves expressed in dry matter basis, season 1.

Treatments	Mineral contents (PPM)				
	Potassium	Calcium	Iron	Zinc	Manganese
Fresh leaves	34204±1497	26048±1392	598.3±116.5	152.3±19.9	719.8±78.57
Solar drying without blanching	33717±1595	25105±908	584.2±114.7	144.5±21.33	710.7±76.2
Blanching in pure water and solar drying	32317±1790	24671±853	576.9±115.7	139.6±20.66	702.1±75.84
Blanching in salty water and solar drying	32039±1698	24345±872	563.2±111.1	137±18.16	697.8±74.68
Means	33069	25042	581	143	708
LSD (5%)	5376.9	3362.2	373.4	65.4	248.9

*All values are mean ± standard error of the mean (n=3). Means with different letters within a column are significantly different ($P < 0.05$).

Table 16: Levels of selected mineral elements of fresh and processed cowpea leaves expressed in dry matter basis, season 2.

Treatments	Mineral contents (PPM)				
	Potassium	Calcium	Iron	Zinc	Manganese
Fresh leaves	34149±1562	25416±1071	554.7±106.7	156.4±19.07	721.5±67.36
Solar drying without blanching	33577±1428	25010±1024	549.7±105.5	151.2±18.1	713.8±68.24
Blanching in pure water and solar drying	32871±1374	24636±1201	542.4±104.4	144.8±17.65	706.6±66.15
Blanching in salty water and solar drying	32018±1937	24547±1174	537±101.9	142.7±17.57	702.2±65.68
Means	34154	24903	546	148.8	711
LSD (5%)	5186.8	3650.9	341.2	59.06	218.1

*All values are mean ± standard error of the mean (n=3). Means with different letters within a column are significantly different (P < 0.05).

4.5.4. Nitrates and oxalates

Nitrates and oxalates are important components in vegetables that determine the digestibility and toxicity of vegetables when consumed. Anti-nutrients also influence the availability and digestibility of other nutrients in the gut. Oxalates were found to be higher in the vegetable and among the treatments. For instance fresh sample had an average of 3400mg/100g oxalates in dry weight basis. Solar drying without blanching, blanching in pure water and solar drying and blanching in salty water and solar drying recorded average loss oxalates of 5.87%, 10.77% and 11.17%, respectively. The highest amount of nitrate content was found in fresh leaves, season 1 at 82.81 mg/100g dry weight basis. Solar dried without blanching, blanched in pure water and solar dried, blanched in salty water and solar dried samples recorded the biggest loss of 37.22%, 69.98% and 58.7% respectively between the seasons as shown in table 17.

Table 17: Levels of selected anti-nutrients of fresh and processed cowpea leaves expressed in dry matter basis.

Treatments	Anti-nutrients (mg/100g)			
	Nitrates		Oxalates	
	Seasons 1	2	1	2
Fresh leaves	82.81±0.19[a]	81.39±0.54[a]	3395±37.97[a]	3400±48.86[a]
Solar drying without blanching	51.99±1.27[b]	52.65±0.44[b]	3196±33.24[b]	3212±14.38[b]
Blanching in pure water and solar drying	24.86±0.75[d]	26.71±0.14[d]	3030±45.98[c]	3096±76[bc]
Blanching in salty water and solar drying	36.12±0.28[c]	34.2±0.67[c]	3016±23.23[c]	2979±31.74[c]
Means	48.95	48.74	3159	3172
LSD (5%)	2.464	1.595	117.6	157.9

*All values are mean ± standard error of the mean (n=3). Means with different letters within a column are significantly different (P < 0.05).

4.5.5. Colour change during processing

Colour of fresh leaves and those take immediately after blanching did not show any significant difference ($p<0.05$). However, after dehydration, there was significant difference among the treatments with blanching in salty water and solar drying maintaining closest colour to the fresh samples at 136.3^0 and 139^0 for the two seasons, respectively followed by solar driying without blanching at around 131^0 and blanching in pure water and solar drying at around 123^0 as shown in figure 2.

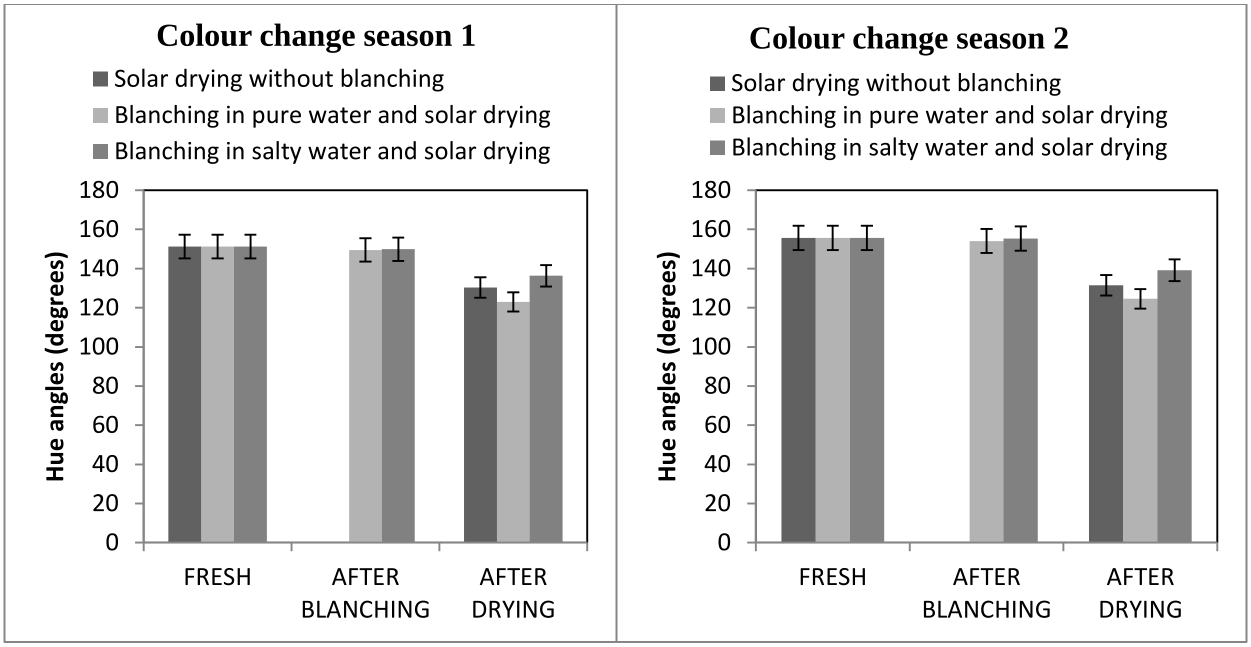

Figure 2: changes in colour (hue angle) of fresh, blanched and dehydrated cowpea leaves expressed in degrees for season 1 an 2.

*All values are mean ± standard error of the mean (n=3). Means with different letters within a column are significantly different ($P < 0.05$).

4.5.6. Sensory quality analysis

The sensory parameters evaluated included; general appearance/colour, aroma, texture, taste/flavor, mouth-feel and general acceptability. During both the seasons, fresh samples were recorded the highest average rating at 6.4 for season 1 and 6.2 for season 2 followed by solar dried without blanching at 5 for season 1 and 5.2 for season 2. Blanching in pure water and solar dryingrecorded a rating of 4.4 for season 1 and 4.6 for season 2 and blanching in salty water and solar dryingat 3.2 for season 1 and 3.4 for season 2 as shown tables 18 and 19.

Table 18: Hedonic scores for sensory quality attributes of fresh and processed cowpea leaves, season 1.

Treatments	Sensory attributes						
	Appearance	Aroma	Texture	Tenderness	Taste	Mouth feel	Overall acceptability
Fresh leaves	5.6	5.4	5.2	6.4	6.2	6.2	6.4
Solar drying without blanching	5	5.4	4.4	5	5	4.6	5
Blanching in pure water and solar drying	5.6	5.2	3.8	4	4.2	4	4.4
Blanching in salty water and solar drying	4.2	3	3.2	3.4	4	3.2	3.2
Mean	5.1	4.75	4.15	4.7	4.85	4.5	4.75
LSD (5%)	1.87	1.87	2.36	2.28	2.16	2.29	2.1

*The values in the table represent scores on a 7-point hedonic scale (1 = dislike extremely), 2 = (dislike very much), 3 = (dislike moderately), 4 = (neither like nor dislike), 5 = (like moderately), 6= (like very much) and 7= (Like extremely).

Table 19: Hedonic scores for sensory quality attributes of fresh and processed cowpea leaves, Season 2.

Treatments	Sensory attributes						
	Appearance	Aroma	Texture	Tenderness	Taste	Mouth feel	Overall acceptability
Fresh leaves	6	5.8	5	6.2	6.4	6	6.2
Solar drying without blanching	5.2	5.2	4.8	4.8	5.2	4.6	5.2
Blanching in pure water and solar drying	5.4	5.4	3.8	4.2	4	4.2	4.6
Blanching in salty water and solar drying	4.4	3.2	3	3.2	4.4	3.4	3.4
Mean	5.25	4.9	4.15	4.6	5	4.55	4.85
LSD (5%)	1.91	1.89	2.21	2.19	2.18	2.23	1.96

*The values in the table represent scores on a 7-point hedonic scale (1 = dislike extremely), 2 = (dislike very much), 3 = (dislike moderately), 4 = (neither like nor dislike), 5 = (like moderately), 6= (like very much) and 7= (Like extremely).

4.5.7. Cumulative weight loss

All the treatments led to weight loss with time but the extent at which they were losing weight differed. The control reached the end stage at day 1, ordinary polythene bag at day 4 and Extend® bag at day 6 after packaging for both seasons. At each end stage, control, ordinary polythene bag and Extend® bag had led to cumulative weight losses of 28.84%, 0.93% and 3.27% for season 1 and 23.84%, 0.89% and 2.31% for season 2, respectively as shown in figure 3.

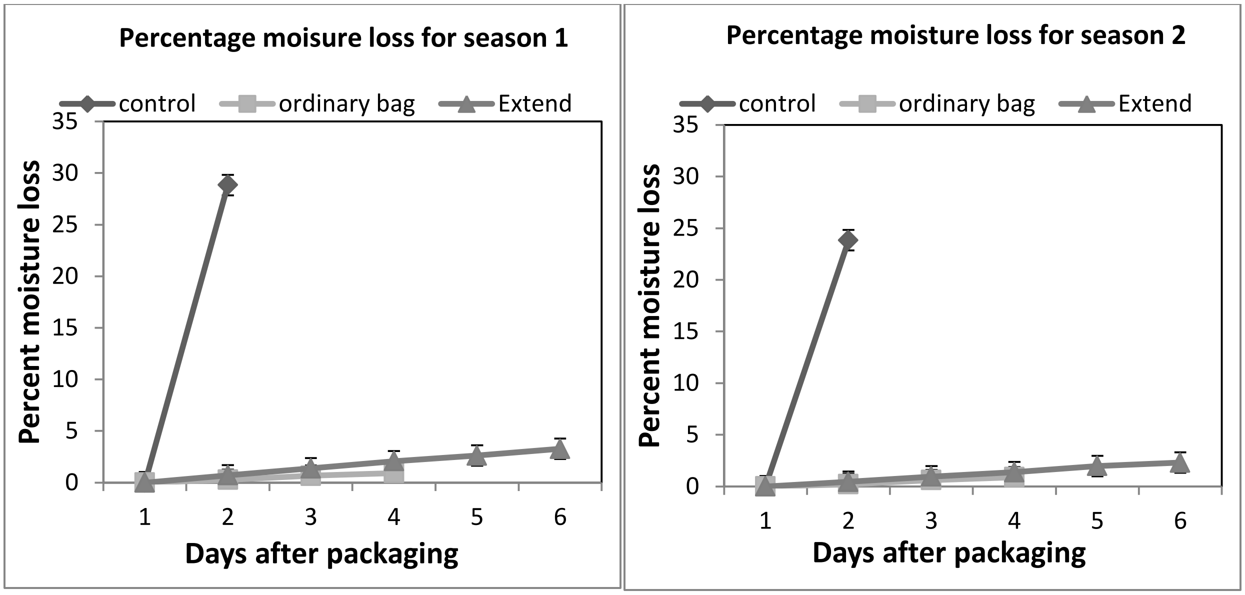

Figure 3: Percentage Cumulative weight loss of cowpea leaves packaged in Extend® bag and ordinary polythene bag and control season 1 and 2.

4.5.8. Wilting

All the treatments led to deterioration in the hedonic scale over packaging and/or storage time. The control reached the end stage at day 1, ordinary polythene bag at day 4 and Extend® bag at day 6 after packaging for both seasons. Since this was a subjective measurement, the behaviour of the treatments for the two seasons was the same. At each end stage, leaves in control, ordinary polythene bag and Extend® bag had deteriorated to 1, 7 and 6 in the hedonic scale as shownin figure 4.

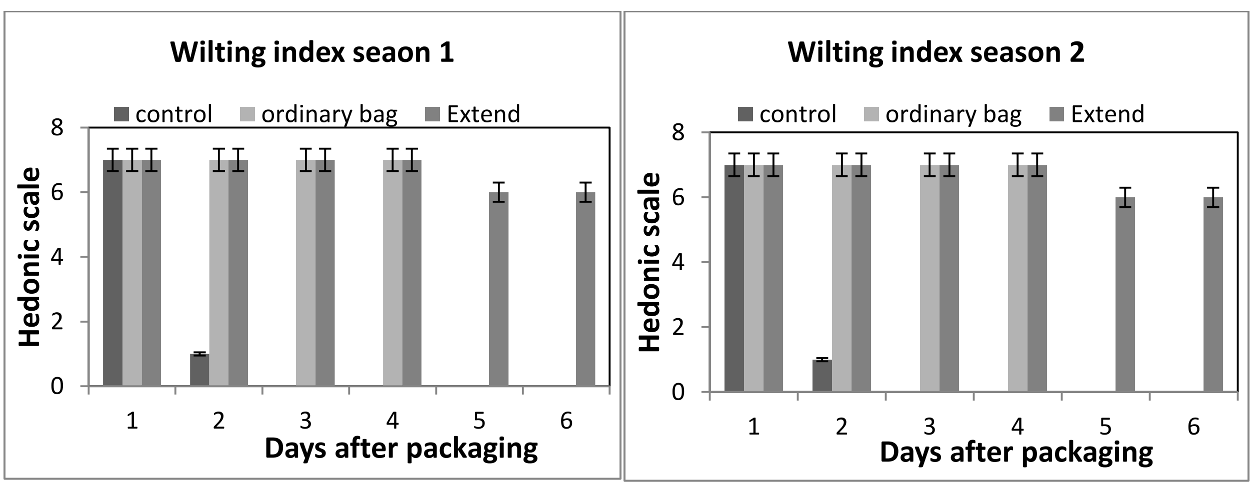

Figure 4: Wilting indices of cowpea leaves packaged in Extend® bag and ordinary polythene bag and control season 1 and 2.

4.5.9. Colour change of fresh cowpea leaves in MAP

The modified atmosphere packaging treatments showed small change in colour in both seasons. At end stages, sample in the ordinary polythene bag, Extend® bag and control reduced colour to 152.89^0, 152.12^0 and 149.2^0, respectively from 153.23^0in season 1. In the season 2, ordinary polythene bag, Extend®and control samples had reduced colour to 150.79^0,$150.32^0$and $149.82^0$from 151.11^0, respectively as shown in figure 5. The end stages for control, ordinary polythene bag and Extend® was 1, 4 and 6, respectively.

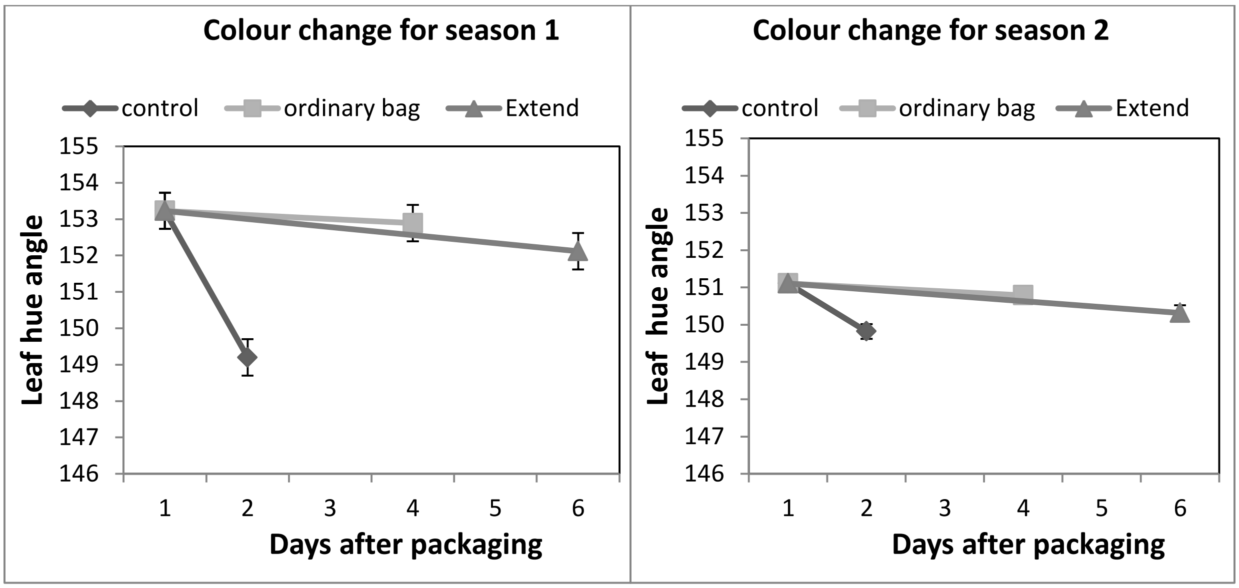

Figure 5: Colour change of cowpea leaves packaged in Extend® bag and ordinary polythene bag and control season 1 and 2.

4.6. DISCUSSION

The richness of foodstuff is measured by the quantities of nutrients it has. The higher the nutrient content the more it's considered high quality. The results obtained for crude proteins, crude fibre and total ash in this study were in the same range with results from Muchoki, *et al.*, (2007), Chikwendu, *et al.*, 2014 and Kasangi, *et al.*, (2010). The significant losses ($p<0.05$) in crude proteins for the solar dried and blanched samples can be as a result of leaching of water soluble protein contents and denaturation of heat sensitive proteins. Njoroge, *et al.*, (2015) suggested that the slight loss in crude protein was probably due to loss of water soluble nitrogen-containing compounds in the African indigenous vegetables such as free amino acids, nucleic acids, nucleotides during blanching. This is evident because the blanched samples had the highest loss in crude protein content as compared to the non-blanched samples. Crude protein content was least for blanching in salty water and solar dryingat 30.95% and highest for the fresh samples at 35.53%. Although total ash slightly varied among the treatments, there was no significant differences ($p<0.05$). This could be as a result of minimal loss in mineral elements which constitute the ash content. On the other hand, crude fibre showed significant differences ($p<0.05$) among the treatments with fresh samples having the highest percentage of crude fibre content and blanched in salty water and solar dried samples the least. The blanched samples recorded some losses in crude fibre which could be as a result of losses of the water soluble components of the crude fibre.

Water soluble vitamins are very important in human metabolic functioning. They are important in improving immunity and supporting physiological processes and scavenging for free radicals (Gareth *et al*., 1998 and FAO, 1995). ß-carotene is the principal precursor of vitamin A, which is involved in vision, cell differentiation, synthesis of glycoproteins, mucus secretion from the epithelial cells, and overall growth and development of bones (Guerra-Vargas *et al*., 2001). Relatively high levels of beta carotene and ascorbic acid were recorded in the vegetable, however losses as a result of processing were significant ($p<0.05$) from the fresh leaves. Solar drying without blanching, blanching in pure water and solar drying, blanching in salty water and solar dryingretained an average of 68.02%, 55.58% and 52.78% for β_carotene and 68.39%, 21.08% and 20.24% for ascorbic acid, respectively relative to the fresh leaves. Aruna *et al*., (1999) explained the loss of beta carotene to be due to non-oxidative changes involving cis-trans isomerization, epoxide formation or heat degradation of tissues or oxidative changes on exposure to light and oxygen. The losses in beta carotene and ascorbic acid compared well with results from Singh *et al.,* (2003), Oboh and Akindahunsi, (2004) and Gupta *et al.,* (2013). Gupta *et al.,* (2008) explained that ascorbic acid is liable to heat, sensitive to light, oxygen and oxidizing agents. The extent of loss of beta carotene was low compared to that of ascorbic acid because of the insolubility of beta carotene in water. The loss in ascorbic acid could have resulted from leaching during blanching, effects of the processing temperatures or due to enzymatic and chemical degradation. Negi and Roy (2000) came to a conclusion that during blanching, vitamin losses are as a result of thermal degradation, diffusion and leaching.

African indigenous vegetables have been documented to contain substantial amounts of phytochemicals (Habwe *et al*., 2008; Smith and Eyzaguirre, 2007; FAO, 1995; Abukutsa, 2003).

Like vitamins, Phenolics have been found to have strong antioxidant activity. Meyer *et al.*, (1998) stated that the antioxidant activities of phenolics in different vegetables markedly vary due to the differences in the phenolic compound structures primarily related to their hydroxylation and methylation patterns. Total phenolic content in the treatment which was solar dried without blanching increased by 149.9% for season 1 and 142.2% for season 2. This can be attributed to concentration of phenolics in the dehydrated vegetable (Zoro *et al.*, 2015). The increase in the phenolic content of the dehydrated leaves was comparable with results from Oboh and Akindahunsi, (2004) and Zoro *et al.*, (2015). On the other hand blanched samples recorded a significant decrease ($p<0.05$) in phenolic content from the fresh and the solar dried without blanching samples. Blanched in pure water and solar dried samples recorded a retention capacity of 37.4% whereas blanched in salty water and solar dried samples recorded a decrease of 34.2%. The loss can be attributed to diffusion and leaching (Negi and Roy 2000). Amic *et al.*, 2003 indicated that consumption of dried powdered leafy vegetables could be advantageous for lower cellular ageing process in human body because phenolics are known for their antioxidant and scavenging properties

Macro and micro nutrients are important constituents of vegetables. Although there was a decrease in all the mineral contents analyzed from processing, the losses were not significantly different ($p<0.05$) from the fresh vegetables. This can be attributed to the fact that mineral elements are embedded in the cell structures, of which are hard to be removed hence lowered solubility in water.

Most plant species contain nutritional stress factors also known as anti-nutrients that increase the loss of essential nutrients from the body. Their presence interferes with the metabolism of absorbed essential nutrients. The common anti-nutrients in vegetables include nitrates and nitrites, oxalates, cyanogenic glycosides, glucosinolates, tannins and saponins (Teutonico and Knorr 1984). In this research, there was significant differences ($p<0.05$) in nitrate and oxalate contents among the treatments. Generally, the fresh sample exhibited the highest levels of both anti-nutrients followed by the solar drying without blanching sample, blanching in salty water and solar drying and blanching in pure water and solar drying. These results were consistent with the results from (Muchoki *et al*., 2010). The oxalate content in the blanched samples did not show any significant differences ($p<0.05$). The loss in the anti-nutrients can be as a result of leaching and volatilization since both are water soluble.

Colour is the first physical impression that determines the acceptability of a product to consumers. Processing is thought to interfere with colour property either positively or negatively depending on the treatment. In this case, colour change was tracked from the fresh vegetables, after blanching and after dehydration as explained in the procedure. Chlorophyll is the major component contributing to the green colour in cowpeas and other green leaves. There was no significant difference ($p<0.05$) in colour between the fresh and blanched samples. However, there were significant differences ($p<0.05$) between the first two stages (fresh sample and blanched sample) and the last stage (dehydrated sample). The colour of the vegetables changed from dark green ($151\text{-}155^0$) for the fresh and blanched samples to pale green at 131.5^0, 124.6^0 and 139.2^0 for fresh leaves, solar drying without blanching, blanching in pure water and solar drying and blanching in salty water and solar drying, respectively. The slight loss in colour can be attributed to the loss in chlorophyll content because leaching and chlorophyll forming pheophytins being broken down (Rodoni *et al.,* 1997).

Processing of any food whether through dehydration or blanching is expected to produce some changes in the sensory qualities of the product. Bunger *et al.,* (2003) stated that use of salts during blanching modifies the sensory characteristics improving colour, increasing external crispness and internal softness. Fresh leaves scored the highest average score of all the sensory attributes in the hedonic scale followed by solar dried leaves, leaves blanched in pure water and solar dried and leaves blanched in salty water and solar dried.

Cowpea leaves subjected to modified atmosphere packaging exhibited varied post-harvest characteristics. The end stage of the cowpea leaves in control, ordinary polythene bag and Extend® bag were reached at 1, 4 and 6 days. Although the leaves in ordinary polythene bag and Extend® bag remained fresh past 10 days after packaging, the 4^{th} and 6^{th} day respectively was chosen because of the start of anaerobic respiration which resulted to bad odours. The increased post-harvest longevity as a result of modified atmosphere packaging was caused primarily by reduced rate of water loss from the leaf tissue. The polyfilm creates a barrier between the internal and external the environment therefore developing a modified internal atmosphere with elevated CO_2, reduced O_2 and increased relative humidity (Kader *et al.,* 1989; Batu and Thompson, 1996). The elevated relative humidity leads to a significant reduction in water loss and therefore reducing weight loss in addition to slowing wilting and green colour loss. The permeability in MAP films determines the rate at which water vapour and gases move between the internal and external environment to achieve equilibrium.

In other research works, high CO_2, low O_2 and high relative humidity conditions created by modified atmosphere packaging have been reported to lead to reduction in weight loss, respiration rate, ethylene production and sensitivity, as well as retard changes related to the ripening process in fruits (Díaz-Mula *et al.,* 2011, Kirakou *et al.,* 2014; Yumbya *et al.,* 2014). However limited scientific work has been documented on the effect on vegetables.

4.7. CONCLUSION

Value addition through dehydration led to reduced nutrient contents especially ascorbic acid and beta carotene. The loss of the other nutrient elements a part from the vitamins was minimal. However, the results of the study indicate that solar drying without blanching was the better option for preserving quality and increasing shelf life of cowpea vegetable. This is because, the loss of ascorbic acid and beta carotene is generally minimal compared to the other techniques which in addition had high rating by the sensory panelists, high phenolic content which has a strong anti-oxidative activity and simplicity of the process.

On the other hand modified atmosphere packaging was found effective in increasing the shelf life of fresh cowpea vegetables. Extend® bag extended shelf life and maintained cowpea leaf quality for 6 days because of better permeability unlike ordinary polythene bag which lead to anaerobic respiration by the 4th day. Weight loss and deterioration in modified atmosphere packaging is significantly reduced due to the ability of polymeric films to prevent diffusion of water vapor from the package by creating saturation in the package with water vapor pressure hence reducing transpiration induced water loss from the tissues.

CHAPTER FIVE

5.0. OVERALL CONCLUSION AND RECOMMENDATION

Cowpea leaves are rich sources of proteins, fibre, beta carotene, ascorbic acid, antioxidants and minerals like potassium, calcium, iron, zinc and manganese. Though they are underutilized, cowpea leaves nutritive value can nourish the ever increasing human population. In this study, all the local accessions were found to be superior to the improved variety.It was also found that value addition through dehydration leads to loss of nutrients. However, the loss of the nutrients is dependent on the techniques used. It was concluded that solar drying without blanching is the effective in preserving quality and improving the shelf life of cowpea vegetable. This is because, the loss of ascorbic acid and beta carotene is generally minimal compared to the other techniques which in addition had high rating by the sensory panelists. It was also found that losses of other nutrient elements a part from vitamins was minimal in dehydration. On the other hand, modified atmosphere packaging was found effective in improving the shelf life of fresh cowpea leaves because of increased humidity in the package.

More studies should be done on the effects of edaphic factors and fertilizer application on the nutritional and physical quality of cowpea leaves.

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8.0. APPENDICES

Appendix 1: Sensory evaluation form

NAME…………………………………….…………………GENDER (M/F)……………………

You are provided with coded samples of cowpea accessions. Please rate the samples (1-6) according to the scale provided below by filling in the table against each sample attribute.

1. Dislike extremely (worst)
2. Dislike very much
3. Dislike moderately
4. Neither like nor dislike
5. Like moderately
6. Like very much
7. Like extremely

ACCESSION COMPARISON

Sample ID	General appearance	Aroma	Texture	Tenderness	Taste/flavour	Mouth feel	General acceptability
XA0.260							
XA0.990							
XA0.773							
XA0.791							
XA0.198							
XA0.570							

Additional comments

………………………………………………………………………………………………
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Appendix 2: Analysis of Variance (ANOVA) table for the effect of processing cowpea leaves on crude protein content, season 1.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	3	46.35743	15.45248	292.61	<.001
Residual	8	0.42247	0.05281		
Total	11	46.77989			

Appendix 3: Analysis of Variance (ANOVA) table for the effect of processing cowpea leaves on crude protein content, season 2.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	3	46.61643	15.53881	213.96	<.001
Residual	8	0.58100	0.07262		
Total	11	47.19743			

Appendix 4: Analysis of Variance (ANOVA) table for the effect of processing cowpea leaves on beta carotene content, season 1.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sample_ID	3	558.6216	186.2072	1574.03	<.001
Residual	8	0.9464	0.1183		
Total	11	559.5680			

Appendix 5: Analysis of Variance (ANOVA) table for the effect of processing cowpea leaves on beta carotene content, season 2.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sample_ID	3	540.8534	180.2845	495.22	<.001
Residual	8	2.9124	0.3641		
Total	11	543.7658			

Appendix 6: Analysis of Variance (ANOVA) table for the effect of processing cowpea leaves on ascorbic acid content, season 1.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sample_ID	3	55322.134	18440.711	14158.21	<.001
Residual	8	10.420	1.302		
Total	11	55332.554			

Appendix 7: Analysis of Variance (ANOVA) table for the effect of processing cowpea leaves on ascorbic acid content, season 2.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sample_ID	3	59135.4795	19711.8265	58812.01	<.001
Residual	8	2.6813	0.3352		
Total	11	59138.1609			

Appendix 8: Analysis of Variance (ANOVA) table for the effect of processing cowpea leaves on total phenolic content, season 1.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sample_ID	3	32111026.	10703675.	367.77	<.001
Residual	8	232832.	29104.		
Total	11	32343858.			

Appendix 9: Analysis of Variance (ANOVA) table for the effect of processing cowpea leaves on total phenolic content, season 2.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sample_ID	3	30765080.	10255027.	409.31	<.001
Residual	8	200433.	25054.		
Total	11	30965514.			

Appendix 10: Analysis of Variance (ANOVA) table for the effect of processing cowpea leaves on oxalates, season 1.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sample_ID	3	283230.	94410.	24.20	<.001
Residual	8	31205.	3901.		
Total	11	314435.			

Appendix 11: Analysis of Variance (ANOVA) table for the effect of processing cowpea leaves on oxalates, season 2.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sample_ID	3	289742.	96581.	13.73	0.002
Residual	8	56266.	7033.		
Total	11	346008.			

Appendix 12: Analysis of Variance (ANOVA) table for the effect of processing cowpea leaves on calcium, season 1.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sample_ID	3	4919955.	1639985.	0.51	0.684
Residual	8	25509647.	3188706.		
Total	11	30429602.			

Appendix 13: Analysis of Variance (ANOVA) table for the effect of processing cowpea leaves on calcium, season 2.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sample_ID	3	1417311.	472437.	0.13	0.942
Residual	8	30078389.	3759799.		
Total	11	31495701.			

Appendix 14: Analysis of Variance (ANOVA) table for the effect of processing cowpea leaves on iron, season 1.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sample_ID	3	1932.	644.	0.02	0.997
Residual	8	314592.	39324.		
Total	11	316524.			

Appendix 15: Analysis of Variance (ANOVA) table for the effect of processing cowpea leaves on iron, season 2.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sample_ID	3	551.	184.	0.01	0.999
Residual	8	262720.	32840.		
Total	11	263271.			

Appendix 16: Analysis of Variance (ANOVA) table for crude fibre on cowpea accessions, season 1.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sample	5	6.3748	1.2750	4.89	0.011
Residual	12	3.1307	0.2609		
Total	17	9.5055			

Appendix 17: Analysis of Variance (ANOVA) table for crude fibre on cowpea accessions, season 2.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sample	5	6.14938	1.22988	12.77	<.001
Residual	12	1.15567	0.09631		
Total	17	7.30505			

Appendix 18: Analysis of Variance (ANOVA) table for ascorbic acid content on cowpea accessions, season 1.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sample	5	1467.831	293.566	172.38	<.001
Residual	12	20.436	1.703		
Total	17	1488.267			

Appendix 19: Analysis of Variance (ANOVA) table for ascorbic acid content on cowpea accessions, season 2.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sample	5	891.695	178.339	135.74	<.001
Residual	12	15.766	1.314		
Total	17	907.461			

Appendix 20: Analysis of Variance (ANOVA) table for nitrate content on cowpea accessions, season 1.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sample	5	376.986	75.397	67.91	<.001
Residual	12	13.324	1.110		
Total	17	390.310			

Appendix 21: Analysis of Variance (ANOVA) table for nitrate content on cowpea accessions, season 2.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sample	5	450.3920	90.0784	316.86	<.001
Residual	12	3.4114	0.2843		
Total	17	453.8034			

Appendix 22: Analysis of Variance (ANOVA) table for manganese content on cowpea accessions, season 1.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sample	5	217046.	43409.	1.96	0.157
Residual	12	265730.	22144.		
Total	17	482777.			

Appendix 23: Analysis of Variance (ANOVA) table for manganese content on cowpea accessions, season 2.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sample	5	216244.	43249.	2.16	0.127
Residual	12	240039.	20003.		
Total	17	456283.			