

**EFFECTS OF BLANCHING TIME-TEMPERATURE COMBINATIONS AND SOLAR-
DRYING ON THE NUTRITIONAL AND MICROBIAL QUALITY OF INDIGENOUS
LEAFY VEGETABLES IN KENYA**

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**Thesis submitted to the Graduate school in partial fulfillment for the requirements of the
Masters of Science Degree in Food Science of Egerton University.**

EGERTON UNIVERSITY

SEPTEMBER, 2016

DECLARATION AND RECOMMENDATION

Declaration

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

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Recommendation

This thesis is the candidate's original work and has been prepared with our guidance and assistance; it has been submitted with our approval as the official university supervisors.

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DEDICATION

To my parents, Samuel Njoroge and Helen Waithera, and my siblings, Virginia, Damaris, Eutychus and Sharon Ann.

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ABSTRACT

The abundance of indigenous leafy vegetables (ILVs) during rainy seasons and accompanying poor storage systems leads to high post-harvest losses (PHL). These gluts in the rainy seasons are usually followed by scarcity in the dry seasons. Currently some limited preservation is employed involving boiling of ILVs and open sun drying, a practice that is inappropriate and done under unhygienic conditions. To reduce the huge losses, a cheaper, hygienic and locally adaptable preservation method for ILVs is required. Solar drying could be a useful dehydration option after blanching as applied in exotic vegetables, but there have been no studies on suitable combinations of blanching and solar drying protocols for preservation of ILVs. This study was conducted to determine the effects of controlled blanching time/temperature combinations followed by solar-drying on the nutritional and microbial quality of selected ILVs with a view to finding a suitable preservation technique. Three common ILVs in Kenya, spiderplant (*Cleome gynandra*), slenderleaf (*Crotalaria ochroleuca*) and cowpeas (*Vigna unguiculata*) were used in this study. The ILVs were grown at Egerton University's horticulture teaching and research station and harvested after 4 weeks. Two blanching conditions (80°C/10 min and 90°C/5 min) followed by solar-drying were tested. Blanching of ILVs at 100°C/30 min and open sun-drying was used as a control, while conventional oven drying of the ILVs was used as a standard for comparison. Analyses on fresh ILVs, blanched and dried ILVs and ILVs stored for three months, were done to determine nutrient content and microbial loads. Generally, the greatest nutrient losses were observed in ILVs blanched at 100°C for 30 min and sun-dried, while most nutrients were retained in ILVs blanched at 80°C/10 min. The total viable counts (TVC) were lowest ($\log_{10} 5.3$ - $\log_{10} 5.6$ cfu/g) in solar dried ILVs blanched at 90°C/5 min. Storage of the solar-dried ILVs for three months at 18°C led to 13-16% moisture loss, 42-44% ascorbic acid loss, 7-10% β -carotene content loss and, minor losses in iron (1.2-1.6%) and calcium (1.2-1.5%). The storage period had no significant effect on the crude protein and fibre composition of the blanched and dried ILVs. The TVC in solar-dried ILVs increased from $\log_{10} 5.9$ to $\log_{10} 6.3$ cfu/g during storage period. There were minor increases in yeasts and mould counts ($\log_{10} 1.2$ to $\log_{10} 1.4$ cfu/g) and in coliform counts ($\log_{10} 1.3$ to $\log_{10} 1.5$ cfu/g) for the solar-dried ILVs. This research indicates that blanching at 80°C/10 min followed by solar drying is a potential preservation technique for ILVs in Kenya and is therefore recommended for use by farmers to preserve ILVs.

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LIST OF ABBREVIATIONS AND ACRONYMS

AOAC	Association of Official Analytical Chemists
Conc.	Concentration
DWB	Dry weight basis
FAO	Food and Agriculture Organization
GLM	General Linear Model
ILVs	Indigenous leafy vegetables
IPGRI	International Plant Genetic Resources Institute
KALRO	Kenya Agricultural and Livestock Research Organization
KEMRI	Kenya Medical Research Institute
MoH	Ministry of Health
Mol. wt	Molecular weight
PHL	Post-harvest losses
PHH	Post-harvest handling
RDA	Recommended daily allowances
SAS	Statistical analysis for scientist
SD	Standard deviation
WHO	World Health Organization

CHAPTER ONE

INTRODUCTION

1.1 Background information

Vegetables are rich sources of vital ingredients in healthy and balanced human diets without quantitative restriction (Osuagwu, 2008). They are important low cost foods containing low levels of fat and high levels of vitamins, minerals, fibre and protein (Bolaji *et al.*, 2008), which according to Mosha *et al.* (1995), are usually in short supply in daily diet. The indigenous leafy vegetables (ILVs) are a vital constituent of African diet (Shei, 2008). As reported by Obel-Lawson (2005), ILVs contribute immensely to nutrition, health and economic well-being of both rural and urban populations. In Kenya, ILVs are often in surplus in rainy seasons leading to excessive wastage and price fluctuations accompanied by income losses to producers (Atanda *et al.*, 2011). Many African families who depend on ILVs process some through open sun-drying to be consumed during dry spells (Hodges *et al.*, 2011). The ILVs have particularly been reported to be rich in vitamin A and iron, the two nutrients that are currently believed to be deficient in the diet of people in many countries (Smith and Eyzaguirre, 2007).

The nutritional value of ILVs is highest when fresh (Nazare *et al.*, 2007) and has been reported to be superior to exotic vegetables such as cabbages (Chweya, 1997). However, it is not always possible to consume fresh vegetables throughout the year as they are only abundant during wet season while scarce during the rest of the year. Once the vegetables are harvested, they undergo a series of physical and biochemical changes that cause loss of nutritional value, flavour and they begin to rot. According to Häuser and Ankila (1997), in many developing countries large quantities (70%) of vegetables spoil due to inadequate infrastructure, insufficient processing capacities, and increasing marketing difficulties. Improving post-harvest handling (PHH) and processing of the vegetables is one way of overcoming perishability constraints and ensuring continued high quality food supply. In Kenya ILVs are rarely processed, presumably due to the general lack of basic preservation facilities for freezing, canning or dehydration.

Most of ILVs are only available during the rainy season, calling for preservation so that they can be available in the dry season. Therefore, it is important that preservation techniques be employed in an effort to increase availability all year round. The drying techniques require careful monitoring of time, temperature and hygienic conditions. Additionally, improper handling and storage could possibly enhance spoilage of dried vegetables (Hell *et al.*, 2009).

This is possible, because according to Abosede *et al.* (2013), dehydrated foods are probably never sterile. The infection of dehydrated products could be from the inhabiting microbes and other contaminants in the product, or as a result of unhygienic conditions during the storage (Mandeel, 2005).

Controlled blanching and solar drying are routinely applied in the treatment of many exotic vegetables with good preservation of nutritional and safety quality (Rawson *et al.*, 2011). There are no comprehensive reports yet on the nutritional quality and safety of ILVs preserved by controlled blanching temperatures and solar drying in Kenya. Traditional treatment of the ILVs involves boiling in water for about 30 min then sun-drying in open air (Hodges *et al.*, 2011). This traditional treatment has a lot of challenges, both in terms of hygiene and lack of temperature control. This leads to heavy losses of nutritive value and microbial spoilage. Blanching as a pre-treatment followed by hot-air oven drying is the commonly used standard for preserving ILVs (Shitandi *et al.*, 2010). Blanching inactivates the endogenous enzymes contained in the vegetables, reduces microbial load, softens and shrinks product for ease of packaging (Jose *et al.*, 2008). Various recommendations on the blanching medium (whether hot water or steam), the length of the blanching period and temperatures to be used during blanching have been presented (Sheetal *et al.*, 2008).

Generally, water blanching temperature of 70-100°C for 1-5 min for leafy vegetables is recommended (Sheetal *et al.*, 2008). However, according to Flatman (1991), this holding time is short and may stimulate enzyme actions instead of denaturing them. On the other hand, blanching the vegetables for a longer time cooks them leading to losses in vitamins, minerals, flavour and colour. Additionally, Pradeep and Susanta (2001) reported that the above stated blanching time/temperature combinations cause an undesirable change in texture and loss of nutrients especially through leaching of minerals and loss of water-soluble vitamins. Although blanching is a prerequisite for preserving leafy vegetables, information on a specific temperature and length of holding time is not available for ILVs preservation.

Conventional hot air oven-drying of ILVs has not been widely adapted in Kenya, probably because it requires electric power to run therefore relatively expensive. Most of the farmers boil the ILVs for 30 min, then the ILVs are open sun-dried for several days. Hence there is need for an alternative cheaper dehydration technique for the ILVs. In this regard, solar-drying could be a suitable option. This study was conducted to determine blanching time/temperatures

regime in combination with solar-drying that would achieve a good retention of nutritive value and least microbial load on ILVs in Kenya.

1.2 Statement of the problem

Preservation methods for ILVs are not very well developed in Kenya. Boiling and open-air sun drying is currently used. It typically involves boiling of the ILVs for 30 min and the resultant water is discarded. The high boiling temperatures and long durations of boiling destroy susceptible nutrients, especially vitamin C; proteins are denatured while mineral contents (Fe, Mg, Zn, Cu, Ca, Na and K) are lost leading to nutrient deficiencies. Sun-drying is weather dependent, time consuming, yields poor quality dried vegetables and is prone to contamination due to exposure to dust, rain, wind, insects, birds, rodents and domestic animals. In addition, the desired level of moisture content (below 15%) is rarely attained leading to nutrient instability and increased microbial loads during storage. Development of a standardized preservation method could help convert glut produce into value-added shelf-stable products. Methods such as controlled blanching and solar drying are now routinely applied in many exotic vegetables with good preservation of nutritional and safety quality. However, there are no reports yet on the nutritional and safety quality of ILVs processed by controlled blanching time/temperatures regimes in combination with solar-drying. Therefore experimentation on these would provide useful information to help achieve a good retention of nutritive value and least microbial load on ILVs in Kenya.

1.3 Objectives

1.3.1 General objective

The overall objective of this study was to contribute to increased utilization of ILVs and improved growers' incomes through processing and preservation methods for these vegetables in Kenya.

1.3.2 Specific objectives

1. To determine the effects of blanching temperature, holding time and storage on the nutritional content of solar-dried ILVs.
2. To determine the effects of blanching temperature, holding time and storage on the microbial loads of solar-dried ILVs.

1.4 Hypotheses

1. H₀: Blanching conditions (temperature and holding time) and storage have no effect on nutritional content of solar-dried ILVs.
2. H₀: Blanching conditions (temperatures and holding time) and storage have no effect on the microbial load of solar-dried ILVs

1.5 Justification

According to Kenya Agricultural and Livestock Research Organization (KALRO) (2013), consumption of ILVs in Kenya stands at 3.7% of all the greens consumed. There is no standardized processing and preservation method that will assure consistency in the supply of ILVs at all times. The inconsistency is mainly because supply peaks and gluts are high during rainy seasons but declines significantly during dry seasons owing to lack of suitable preservation methods for the excess produce. This calls for efforts to develop preservation methods that would ensure effective reduction of microbial loads and proper inactivation of endogenous enzymes. However, the developed preservation technique must retain most of the essential nutrients in the ILVs and be relatively cheap for ease of adoption countrywide. Fresh vegetables are blanched to inactivate the endogenous enzymes as a remedy of increasing the storage period. In addition, blanching reduces microbial load, and softens and shrinks product for ease of packaging. The effectiveness of the blanching process is time and temperature dependent and hence care has to be taken to avoid destruction and loss of heat sensitive nutrients and to attain acceptable levels of microbial loads. In this research, effects of optimized blanching temperatures and holding times together with solar drying were investigated. This was aimed at establishing a regime that preserves the highest amounts of nutrients and enhances safety of ILVs.

CHAPTER TWO

LITERATURE REVIEW

2.1 Indigenous leafy vegetables

ILVs are traditional edible vegetables whose leaves, young shoots and flowers are consumed as food (Abukutsa-Onyango, 2003; Maundu *et al.*, 1999). The secondary centre of origin of these vegetables is Africa and their natural habitat is in sub-Saharan Africa, thus they are also referred to as African leafy vegetables (ALVs) (Schippers, 2002).

The ILVs were introduced over a century ago. Due to their long use, they have become part of the food culture in Africa. ILVs are increasingly recognized as possible contributors of both micronutrients and bioactive compounds to the diets of populations in Africa (Francisca and Eyzaguirre, 2007). Therefore, they form a significant part of the traditional diets of households and agricultural communities both in urban and the rural areas (Grubben and Denton, 2004). The ILVs species consumed and the wealth of indigenous knowledge vary with the culture, economic pursuits, species availability and level of influence by modernization.

2.2 Some common ILVs

Worldwide there are about 13,000 species of plants used as food. The Plant Resources of Tropical Africa (PROTA) reported an estimated 6,376 useful indigenous African plants of which 397 are vegetables (PROTA, 2004). It is however indicated that information is available on cultivation practices for 280 ILVs. Examples of some ILVs found in East Africa (Table 1) include African nightshade (*Solanum spp*), spiderplant (*Cleome gynandra*), amaranth (*Amaranthus spp*), slenderleaf (*Crotalaria spp*), jute mallow (*Corchorus olitorius*), cowpea (*Vigna unguiculata* L.), pumpkin (*Curcubita moschata*), African kale (*Brassica carinata* A.), *Basella alba* and *Commelina africana* among others (Abukutsa-Onyango *et al.*, 2006).

Table 1: Common ILVs in Kenya

Scientific name	Common name	Local name
<i>Gynandropsis gynandra</i> ^a	Spiderplant	<i>Chinsaga</i> ⁵ , <i>Akeyo</i> ¹ , <i>Mgangani</i> ⁷
<i>Crotalaria ochroleuca</i> ^a	Slenderleaf	<i>Mito</i> ¹ , <i>Miro</i> ² , <i>Mitoo</i> ⁵
<i>Corchorus trilocularis</i> ^c	Jute mallow	<i>Murere</i> ² , <i>Apoth</i> ¹ , <i>Mrenda</i> ³
<i>Amaranthus hybridus</i> ^a ,	African spinach	<i>Michicha</i> ³ , <i>Livokoi</i> ² , <i>Dodo</i> ⁵
<i>Vigna unguiculata</i> ^a	Cowpea	<i>Kunde</i> ³ , <i>Egesare</i> ⁵ , <i>Boo</i> ¹ , <i>Ngunyi</i> ⁶
<i>Cucurbita spp</i> ^a	Pumpkin leaves	<i>Susa</i> ¹ , <i>Lisebebe</i> ² , <i>Risosa</i> ⁵ , <i>Mareng</i> ⁴ , <i>Nenge</i> ⁶
<i>Solanum nigrum</i> ^b	Black nightshade	<i>Mnavu</i> ³ , <i>Lisutsa</i> ² , <i>Rinagu</i> ⁵ , <i>Managu</i> ⁴ , <i>Ndunda</i> ⁷

^a -Fully domesticated, ^b -Partially domesticated, ^c -Wild and/or weeds, ¹Luo, ² Luhya, ⁵ Kisii, ³ Kiswahili, ⁴Kikuyu, ⁶Kamba, ⁷Taita. **Source:** Onyango *et al.* (2000)

2.3 Indigenous leafy vegetables in the current study

2.3.1 Spiderplant

Spiderplant or cat's whiskers (*Cleome gynandra* L./ *Gynandropsis gynandra* L.) (local name *Chinsaga*) is an erect herbaceous annual herb with hairy, often purple stems and many branches. It belongs to the family *Capparaceae* (Chweya and Eyzaguirre, 1999). In Kenya, spiderplant grows from sea level to 2400 m and is a fast-growing plant that is ready for harvest in as few as three weeks (Chweya, 1997). It is believed to be a rich source of nutrients, especially proteins, vitamins (A and C) and minerals (calcium and iron).

Spiderplant has mildly bitter taste which is derived from polyphenols (Osei, 2012). In many communities, spiderplant is consumed largely by women and especially pregnant and breast-feeding mothers since it is associated with replenishment or restoration of blood supply (Schippers, 2002). An infusion of boiled leaves and/or roots is administered to facilitate childbirth, treat stomach ailments and constipation.

2.3.2 Slenderleaf

Slenderleaf (*Crotalaria spp*) (local name, *Mitoo*) has been grown and consumed in Kenya for a long time. It is a nitrogen-fixing legume and belongs to the family *Fabaceae*/

Leguminaceae (Schippers, 2002; Abukutsa-Onyango, 2004). One of the *Crotalaria* African species used as a vegetable is *Crotalaria ochroleuca*. The species is mainly found in Nyanza and Western Provinces, in Kenya. It's a short, erect perennial herb growing up to 1.5 m in height. It has bright green leaves, pale yellow flowers and bitter taste (Schippers, 2002). Its young leaves and shoots are consumed in combination with other greens because it has bitter taste. *Crotalaria ochroleuca* also has medicinal properties that help reduce the effects of stomach ailments (Schippers, 2002).

2.3.3 Cowpea

Cowpea (*Vigna unguiculata*) (local name, *Kunde*) is one of the most important legumes in Kenya. It is cultivated all over Kenya mainly for seeds but the leaves are popular as a local vegetable. In some parts of Kenya, however, some types are grown primarily for leaves. For vegetables, the leaves are harvested starting at 3-4 weeks from planting (Maundu *et al.*, 1999). The young leaves are often cooked alone or in a mixture with other leafy vegetables to prepare a side dish for *ugali* (a stiff porridge made from maize, sorghum or millet meal cooked in water). In some communities (the Kikuyu), cowpea is cooked with maize, pulse and potatoes and/or bananas and mashed to make a tasty mixture.

Cowpea is a warm-season legume grown primarily in the semi-arid tropics that includes parts of Asia, Africa, Southern Europe, Southern United States and South America (Singh, 2005). It is a dual-purpose legume in the tropics used as leafy vegetables, grain, as fresh cut-and-carry forage, and for hay and silage, especially for eastern and southern Africa (Whitbread and Lawrence, 2006). Important nutrient constituents of cowpea are minerals, vitamins especially folic acid which is a B vitamin necessary during pregnancy to prevent birth defect in the brain and spine. The folic acid content in cowpea is about 250 µg/g, which is a higher quantity compared to other ILVs (Hall *et al.*, 2003).

The cowpeas young tender leaves are cooked and eaten as vegetable while the green pods can be cooked and eaten like green beans. The seeds can be cooked when fresh and, when fully matured and dry, eaten as pulses. In African countries, cowpea is used for preparation of stew that is either used together with cereal dishes or directly mixed with the cereals as maize, wheat, sorghum and rice (Abukutsa-Onyango *et al.*, 2006).

2.4 Production and consumption of ILVs in Kenya

Production of ILVs in Kenya is mainly on a subsistence basis (Nekesa and Meso, 1995). This could partly be attributed to their neglect as vegetable research efforts have mainly been concentrated on exotic vegetables (Ndung'u *et al.*, 2004). Changing food habits, loss of indigenous knowledge and ignorance of the nutritional and health benefits associated with ILV consumption have collectively contributed to this apparent denigration. In spite of their neglect in research, a survey conducted by Abukutsa-Onyango (2003) showed that ILVs play a key role in income generation and offer a significant economic opportunity for the poor people, especially women in western Kenya. The author noted that over 70% of the traded vegetables in rural markets were ILVs while in major towns, the proportion was about 10%. Therefore, exploitation of ILVs adapted to the local environment will improve food security, nutrition, health and economy of the rural poor.

ILVs are relatively available and affordable particularly during the rainy seasons and represent one of the richest sources of biodiversity in African food systems (Chweya and Eyzaguirre, 1999). They are a potentially rich source of nutrients but are found to be among the least produced or consumed foods (Maziya-Dixon *et al.*, 2004). The decline in their production and consumption in many rural communities in Africa is believed to be due to the introduction of exotic vegetable varieties (Maundu *et al.*, 1999). Also in certain cases, there is an ill-perception that ILVs consumption is unfashionable and associated with low-class people. Moreover, some people falsely believe that ILVs are generally only a source of income for the poor and unemployed households in the urban and peri-urban slums (Mnzava, 1997). These misconceptions still linger in some places in Africa and will take time to change (Oiye *et al.*, 2009). Besides, they play an important nutritional role and food security for the disadvantaged people (Onim and Mwaniki, 2008).

2.5 Post harvest handling of ILVs

Post-harvest handling (PHH) is the final stage in the process of producing high quality fresh produce. The ability to maintain a level of freshness from the field presents many challenges. Thus a grower who can meet these challenges is able to expand his or her marketing opportunities and better the ability to compete in the market place (Janet and Richard, 2000). Most leafy vegetables are highly perishable and have a very short shelf life, lasting at best for only three days. They deteriorate very quickly in quality and flavour after harvesting and the

extent of post PHL can be serious if the crop is handled poorly. This creates problems in the marketing chain with producers, traders or consumers (Schippers, 2002). Since quality cannot be improved after harvest but only maintained, it is important to harvest vegetables at the proper stage, size and at peak quality to avoid these post-harvest constraints.

2.6 Nutritional value and potential of ILVs

2.6.1 Micronutrient and health promotion

ILVs are important sources of protein, minerals (calcium, iron, phosphorus and potassium), vitamins A (β -carotene) and C (Engle and Altoveros, 1999), as shown in Table 2. Most of ILVs have long been known and reported to have health protecting properties and uses (Ayodele, 2005; Okeno *et al.*, 2003) especially for households in poor economic settings. They are efficient sources of several important micronutrients (Table 2), both with respect to unit cost of production and per unit of land area. This includes high micronutrient content, antioxidants and medicinal properties (International Plant Genetic Resources Institute (IPGRI), 2006).

They are also rich in vitamins, minerals, trace elements, dietary fibre and protein (Mulokozi *et al.*, 2004; Mathenge, 1997). Their consumption therefore, gives diversity to daily food intake, adding flavour and appetite to the diet (Asfaw, 1997). This is important for growing children and lactating mothers because they have a great role in boosting the immune system (Abukutsa-Onyango, 2003). Effectively, they are important in food security, during times of drought or poor harvest and are also vital for income generation. However, vegetable consumption is far below the level required to meet the micronutrient requirements. Therefore, integrating micronutrient-rich foods such as vegetables into diets seem to be one of the most practical and sustainable means of alleviating micronutrient deficiency (Ali and Tsou, 1997).

Table 2: Nutritional value of some Kenyan ILVs

ILV	Crude protein (g/100 g)	β -carotene (mg/100 g)	Vitamin C (mg/100 g)	Calcium (mg/100 g)	Iron (mg/100 g)
Cowpeas	3-6	6-8	70-100	200-400	10-15
Spiderplant	5-10	6-19	130-180	434	11-15
Slenderleaf	8	4-8	170-210	270	8
African spinach	4-5	5-10	90-160	800	5-15
African eggplant	3-6	8-10	40-140	250	5-17
Pumpkin leaves	3-5	2-6	170-175	400	9-11
Ethiopian kale	5	2-6	100	250	4

Source: Abukutsa-Onyango *et al.* (2000)

2.6.2 The ILVs medicinal and health benefits

ILVs have potential nutraceutical value thus exhibit medicinal potency (Olembo *et al.*, 1995). Several of these have been used and continue to be used for prophylactic and therapeutic purposes by rural communities (Ayodele, 2005). According to a survey conducted by Kimiywe *et al.* (2007) in Nairobi, Kenya, the ILVs consumed by the respondents have medicinal values attached to them. For example, many ILVs especially the sour or bitter ones like spiderplant, slenderleaf and African nightshades have been reported to heal stomach related ailments and malaria. Some of the vegetables are also reported to cure more than one illness.

A wide range of illnesses have been cited as being treated and/or managed by consumption of leafy vegetables (Olembo *et al.*, 1995). The most common illnesses cited are malaria, diarrhoea, anaemia, colds and coughs, skin infections, malnutrition, HIV/AIDS, diabetes and high blood pressure. However, as suggested by Kimiywe *et al.* (2007), there is need for further investigations to establish the scientific basis for these perceptions. This is a significant aspect since modern trends in food technological research focuses on the food-medical interface (Chweya, 1997). The key idea therefore, is to identify food sources and ingredients for the provision of both nutritional and pharmaceutical (jointly termed nutraceutical) benefits. ILVs may have an invaluable potential in this emerging area.

2.6.3 Phytochemical composition

Phytochemicals are naturally occurring, biologically active chemical compounds found in plants which have a beneficial effect on health (Rajurkar and Gaikwad, 2012). They are linked to protection against cardiovascular diseases, some forms of cancer and other degenerative diseases (Ayoola *et al.*, 2008). The most important action of these chemicals is that, they also function as antioxidants that react with the free oxygen molecules or free radicals in the body (Liu, 2003). More than 4000 of non-nutrient bioactive phytochemical compounds have been discovered from plants and it is expected that scientists will discover many more. Reports show that these compounds act as natural defense system for host plants and provide colour, aroma and flavour (Osei, 2012).

Intake of phytochemicals should be from dietary sources rather than from supplements or pills. This is because supplements and pills can only provide a few of the thousands of phytochemicals available and are thus less effective than a serving of fruits and vegetables (Liu, 2003). Therefore, since some phytochemicals are only found in plant products, it is advised that a wide variety of fruits and vegetables should be consumed in order to gain maximum benefit from the nutrients and phytochemicals they contain. Additionally, phytochemicals work in synergy, thus the effect together is stronger (Liu, 2003).

2.6.4 Antioxidant and health benefits

Antioxidants are chemical compounds or molecules present in the biotic components and high in medicinal plants (Hae-Ryong *et al.*, 2006). These compounds are necessary for human health and nutrition (Kusum and Fazlu, 2002) as they protect cells against the damaging effects of reactive oxygen species, such as singlet oxygen, superoxide, peroxy radicals, hydroxyl radicals and peroxy nitrite (David *et al.*, 2004). They play an important role in inhibiting and scavenging these free radicals and provide protection against infectious and degenerative diseases (Canadanovic-Brunet *et al.*, 2005).

High consumption of vegetables has been associated with a lowered incidence of degenerative diseases. These protective effects are attributed to the presence of antioxidants especially vitamins like ascorbic acid, tocopherol and β -carotene (Ganiyu, 2005). Antioxidants are also used to preserve food quality mainly because they arrest oxidative deterioration of lipids. Plant-based antioxidants are now preferred to the synthetic ones because of safety concerns.

2.7 Micronutrient deficiency in Kenya

Food insecurity and malnutrition is an issue of concern in Kenya and other countries in sub-Saharan Africa. Over two billion people, mostly women of childbearing age and children under five years old, suffer from malnutrition (Djuikwo *et al.*, 2011). This is due to lack of micronutrient intake, which is a major cause of weakened immunity to diseases, leading to increased mortality in vulnerable groups. Micronutrient malnutrition, particularly vitamin A, iron, and iodine deficiency disorders are major public health concerns globally (ILSI/FAO, 1997). The low level of fruit and vegetable consumption has devastating health effects (Lumpkin *et al.*, 2005) and poses a serious threat. It remains a major problem facing Kenya's poor and needy population and its impact in this population is worsened by the HIV/AIDS pandemic. If left unchecked, these deficiencies will set a vicious cycle effect that will take many generations to correct and this would translate into poor economic development.

Strategies to combat micronutrient deficiency in Kenya include food-based approach (Mibei *et al.*, 2012). The focus therefore, should be laid on programmes that intend to increase the study of micronutrient rich varieties of staple food crops (Mibei *et al.*, 2012). This is because most of the communities have traditional staple crops, which have been underutilized due to lack of information on their nutritional and medicinal value. These include the indigenous vegetables, fruits, tubers and roots, which if tapped or exploited are likely to be a more sustainable means as well as long-term solution to micronutrient deficiency elimination (MoH /KEMRI, 1999).

2.8 Changes of nutritional value of leafy vegetables during processing

Heat may improve the nutritional value of some foods by inactivating harmful substances or by liberating nutrients from otherwise stable complexes. It causes destruction of some vitamins and may change the digestibility of proteins. Thus, it is well established that heat damage and leaching are the major factors in nutrient losses from foods during processing (Lee *et al.*, 1982).

Vitamin A is mainly degraded by chemical oxidative reactions, which is accelerated by light, heat and metals such as copper (Muchoki, 2007). Its loss from foods during preparation is therefore minimal if the temperatures are kept moderate and the cooking vessel is covered. Open sun-drying of vegetables results in large losses of both vitamin A and C (Table 3 compared with Table 2).

Table 3: Nutritional composition of some open sun-dried vegetables

Vegetable	Protein (g/ 100 g)	Crude fibre (g/100 g)	Vitamin C (mg/100 g)	β -carotene (mg/100 g)	Iron (mg/100 g)	Calcium (mg/100g)
African spinach ^a	4.6	3.4	36.7	4.46	10.1	0.3
Pumpkins leaves ^a	2.2	1.4	100.6	3.86	8.1	0.5
Gnectum vegetable ^b	5.6	1.2	36.3	4.26	5.7	0.2
Slippery vine ^c	3.4	2.5	110.6	6.32	0.5	1.5
Spinach ^b	4.2	2.7	59.3	2.54	0.6	0.9
Cocoyam leaves ^c	3.3	2.4	51.7	3.02	0.4	0.6
Bush okra ^c	2.4	2.7	124.9	4.13	0.8	0.2

Source: ^a=Mepba *et al.* (2007), ^b=Masarirambi *et al.* (2010), ^c=Nyaura *et al.* (2014)

Loss of ascorbic acid during processing of vegetables depends on the method of cooking, the volume of water used and the species of vegetable (Nyaura *et al.*, 2010). Large volumes of water lead to loss of the vitamin through leaching. It has been shown that cooking vegetables in just enough water and retaining the cooking water optimizes retention of vitamin C (Krehl and Winters, 1972). Charlie *et al.* (2009), reported vitamin C to be destroyed in most dehydrated products with dehydrated vegetables retaining their vitamin values poorly.

Several minerals in the green leafy vegetables exist either free or bound to the tissue matrix of the vegetables (Osei, 2012). The main route of loss of minerals during cooking or processing is through leaching. If the cooking or processing water however, is consumed almost 100% of the minerals are ingested.

A common problem with dehydrated vegetables is the colour change (browning), which may be caused by heat damage during dehydration or poor storage conditions. If the degree of browning is not great, colour change may be the only notable effect (Ejoh *et al.*, 2007). When the changes proceeds further, the flavour, rehydration capacity and nutrient content may also be adversely affected.

2.9 The physicochemical quality of dried vegetables

The quality of dried foods is dependent on changes occurring during processing and storage (Kiremire *et al.*, 2010). Most changes involve modifications that affect texture, rehydrability and appearance. The commonly examined quality properties of dried vegetables are

textural properties (compression test, stress relaxation test and tensile test), optical properties (colour and appearance), sensory properties (aroma, taste and flavour), and nutritional characteristics (vitamins and proteins) (Babagana *et al.*, 2012). According to Natale *et al.* (2015) drying method and physical-chemical changes that occur during drying affect the quality of dehydrated products. More specifically, drying method and process conditions affect significantly the colour, texture, density and porosity characteristics of materials.

2.10 Rehydration of dried foods

The quality of dried product is reflected not only on its texture, flavour and colour, but also on its ability to rehydrate as closely as possible to the original raw material. The rehydration efficiency is determined by product preparation and the method of drying (Lewicki, 1998). During rehydration, dehydrated vegetables should be soaked in warm water for some time before cooking; otherwise, they are likely to remain tough and crinkled. The rehydration water should be used for cooking of the vegetables. Factors that affect rehydration processes of the dehydrated products are time, temperature, air displacement, pH and ionic strength (Karel, 1963).

2.11 The microbiology of dehydrated vegetables

The small-holder farmers who produce the traditional vegetables preserve them by sun-drying but generally do not adhere to the basic principles of good hygienic practices (GHPs) (Ashutosh and Verma 2009). Under these circumstances, it is suspected that dried vegetables may harbour high microbial loads and thus there is need to assess the microbiological quality of ILVs.

According to Ashutosh and Verma (2009), most microorganisms that are initially observed on vegetable surfaces are soil inhabitants belonging to a very large and diverse community of microbes. They are responsible for maintaining a dynamic ecological balance within most agricultural systems. The dominant micro-organisms of ILVs are investigated by enumerating total bacteria and moulds (Kudjawu *et al.*, 2011).

2.12 Packaging and storage of dried vegetables

Food packaging aims at protecting food against spoilage to preserve their quality and provide convenience of handling. This is through the placement of a barrier between food and the environment. The barrier should minimize causes that decrease quality, such as oxidation, permeation of gases, water vapour and aroma substances and other chemicals, or the transfer of

light and heat (Masarirambi *et al.* 2010). Foods should be packaged soon after processing to avoid recontamination or for dried foods to protect against moisture and infestation with insects. Dried vegetables have become a very popular addition to food storage programs. Dried vegetables can be found commercially in both the dehydrated form and in the freeze dried form (Schönfeldt and Pretorius, 2011). Dried foods should be sealed in airtight packages or containers to prevent them from picking up moisture from the air, thus causing them to mould or spoil. The manufacturers may use various packages to store the dried foods like plastic laminated foil pouches, metal and plastic cans or heavy plastic bags.

Home dried foods are susceptible to insect contamination and moisture absorption and must be properly packaged and stored immediately. Properly stored, dried vegetables keep well for 6-12 months (Masarirambi *et al.* 2010). However, it is recommended that the storage time for dried foods should range from 4 months to 1 year (Aulakh and Regmi, 2010). Because food quality is affected by heat, the storage temperature helps determine the length of storage; the higher the temperature, the shorter the storage time. Vegetables have about half the shelf life of fruits and can generally be stored for 6 months at 15.6°C or 3 months at 26.7°C (Schmutz and Hoyle, 1999).

CHAPTER THREE

MATERIALS AND METHODS

3.1 ILVs growing site

Certified seeds of three commonly consumed ILVs spiderplant, slenderleaf and cowpeas were purchased from Kenya Seed Company, Kenya. They were grown at the Horticulture Teaching and Research Station at Egerton University, Njoro, between the months of October 2014 and November 2014. The station lies between longitudes 35° 28' and 35° 36' east and latitudes 0°1' and 1°10' south, receives erratic rainfall with an average of 600-900 mm annually and the daily temperatures range from 22-28°C while the soil is loamy and free draining (Jaetzold *et al.*, 2007). The three ILVs used in the study were selected on the basis that they are the most commonly cultivated ILVs, they are nutritious in terms of vitamins (A and C) and minerals (iron and calcium) and most of the farmers have local knowledge on their cultivation.

3.2 Sample collection

The three ILVs were harvested 40 days after planting (Figure 1, 2 and 3). Tender leafy shoots were picked for spiderplant and slenderleaf, while trifoliolate leaves were harvested for cowpea. A cool box was used to carry the ILVs to the laboratories. Part of the raw ILVs were analyzed for total viable counts, coliform counts, moisture content, crude protein, crude fiber, vitamins (vitamin C and β -carotene), minerals (iron and calcium) before being processed. Analysis of the crude fibre contents were undertaken at the Departments of Animal Science while that of minerals and β -carotene contents were done at the Department of Chemistry laboratories, Egerton University.



Figure 1. Spiderplant, 5 weeks after planting, grown at the Horticulture Teaching and Research Station, Egerton University, Njoro.



Figure 2. Slenderleaf, 5 weeks after planting, grown at the Horticulture Teaching and Research Station, Egerton University, Njoro.



Figure 3. Cowpeas, 5 weeks after planting, grown at the Horticulture Teaching and Research Station, Egerton University, Njoro.

3.3 Sample preparation

The tender shoots were sorted, washed, shredded and weighed into equal amounts (2.5 kg). They were prepared for blanching at a specified time/temperature combination and drying methods to determine the effects the two processes has on nutritional quality and microbial load of solar-dried ILVs

3.3.1 Blanching of ILVs

Three blanching conditions were used. The shredded tender shoots (2.5 kg) were blanched at 80°C/10 min and at 90°C/5 min. Another batch was blanched at 100°C/30 min (in boiling water) to serve as control. Blanching was performed by bringing a large pan half-full of water to 80°C, 90°C and 100°C, respectively. The shredded vegetables were placed in wire baskets and gently lowered into the blanching water. At the end of blanching period, the baskets with vegetables were plunged into cold water at room temperature to stop the blanching process. The blanched leaves were placed in wooden boxes to drain off the water and then held at room temperature for 5 min to cool. The 80°C/10 min and 90°C/5 min blanched vegetables were then divided into two equal portions for solar-drying and for hot-air oven-drying.

3.3.2 Drying of ILVs

Three drying techniques were tested. For solar-drying, 1.25 kg of vegetables was placed in a fabricated solar dryer designed by the Department of Agricultural Engineering, Egerton University, Njoro, Kenya (Figure 4a). The dryer is designed to dry approximately 8 kg of vegetables in 2 sunny days to a moisture content of $\approx 13\%$. The solar drier was positioned in a level platform unobscured by trees and buildings so that it was fully exposed to the sun throughout the day. The drying temperature was 60°C . Moisture loss was monitored by regular weighing of the samples until a constant weight was achieved. The solar-dried ILVs are shown in Figure 4b.



Figure 4(a). ILVs placed in a solar-drier positioned on a level platform



Figure 4(b). Solar-dried ILVs

For the standard drying (oven-drying), the blanched ILVs (1.25 kg) were spread on trays and placed in a hot-air oven (Electrolux E130GF35JS Struers, Stockholm, Sweden) set at 65°C. They were dried to constant weight which took approximately 7 h with the temperature being maintained throughout. For the control, ILVs blanched at 100°C/30 min were open sun-dried (Figure 5). They were dried in the sun to constant weight, (approximately three days of drying). The blanched-dried vegetables were later ground using a mortar and pestle into fine powder then packaged in zip-lock packaging bags, labeled and stored to await chemical and microbial analyses. Figure 6 shows a summary flow chart for processing and preservation of ILVs.



Figure 5. Open sun-drying of ILVs

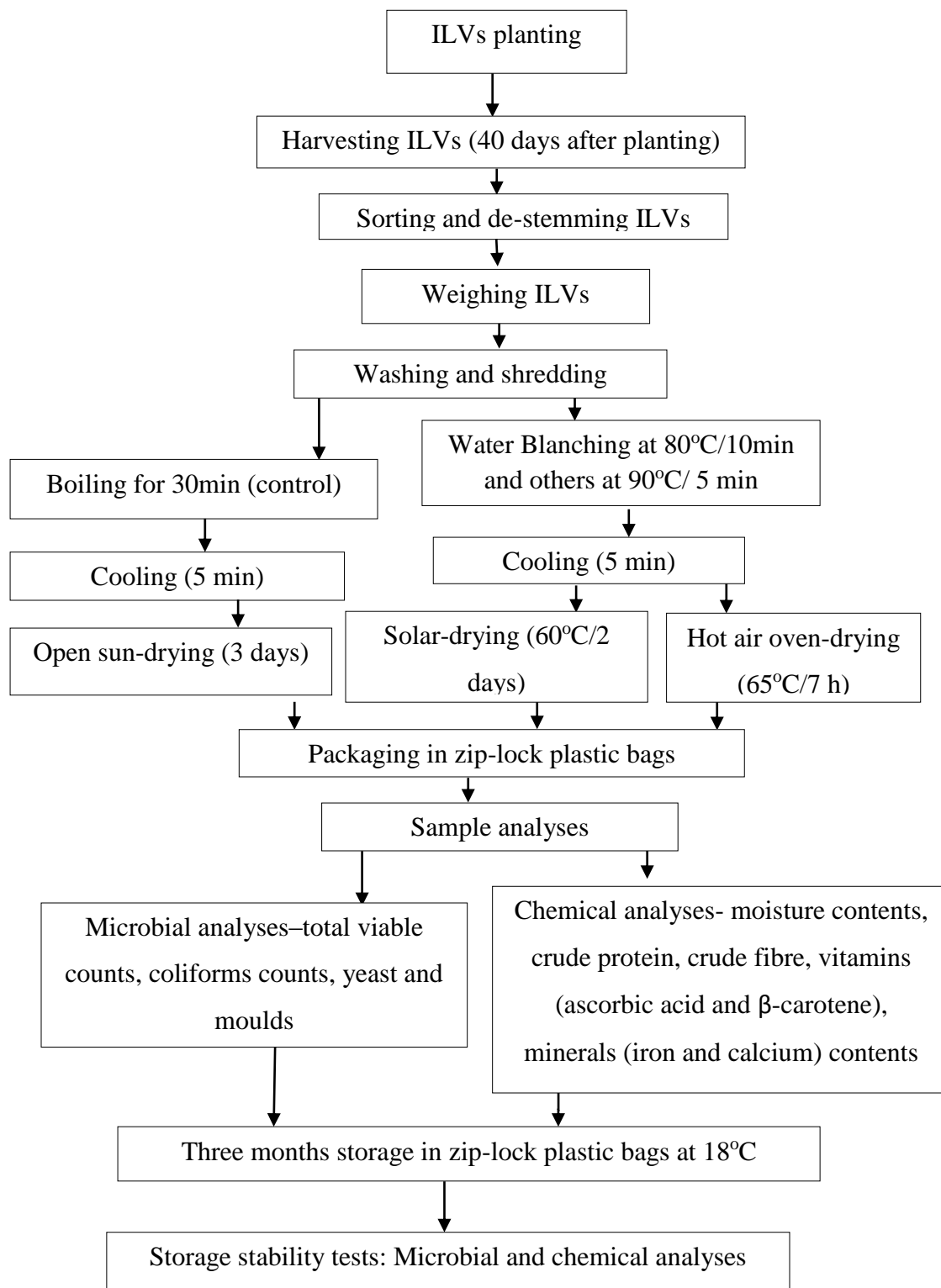


Figure 6. Flow chart for processing and preservation of ILVs

3.4 Chemical and microbial analyses

These analyses were carried out on the raw, blanched-dried and stored samples. All the analyses were performed in duplicate.

3.4.1 Chemical analyses

3.4.1.1 Determination of ILVs moisture content

Moisture content was determined by oven drying according to the Association of Official Analytical Chemists (AOAC) International (2000) Method 970.30. Samples were dried in a hot air-forced draft oven at 105°C to a constant weight then cooled in a desiccator for 10 min. Moisture content was calculated as the loss in weight expressed as a percent of the original weight of the ILVs sample. The hot-air-oven method was used to remove the loosely bound and superficial moisture.

3.4.1.2 Determination of ILVs crude protein content

Crude protein was determined by the Kjeldahl method (AOAC International, 2000) Method 991.20. Accurately, weighed sample (0.2 g) was placed in the micro-Kjeldahl digestion tubes into which 10 ml nitrogen free concentrated H₂SO₄ was added together with one selenium tablet as a catalyst per tube. The samples were digested in a DK-20S digester (Velp Scientifica, Bohemia, Italy) at 445°C for 3 h. The products of digestion were distilled using the Kjeldahl distillation unit. The distillate was collected in a 15 ml 0.1 M HCl in which a mixed indicator of methyl red and methylene blue had been added. The excess HCl was titrated against 0.1 M NaOH. The crude protein (CP) was calculated as follows;

$$\text{Crude protein (g/100 g)} = (V_1 - V_2) \times M \times 1.4 \times 6.25 / W$$

Where V₂ is volume of HCl used for test portion, V₁ is volume of HCl used for blank test

M is molarity of acid, W is weight of test portion and 6.25 is the conversion factor.

3.4.1.3 Determination ILVs crude fibre content

Crude fibre content was determined by gravimetric method according to AOAC International (2000), Method 984.04. Approximately 5 g samples were added into 25 ml 2.04 M H₂SO₄ with distilled water used to top the contents to 200 ml. The mixer was then digested in a digester (DK-20S digester, Velp Scientifica, Bohemia, Italy) at 445°C for 30 min. A glass wool rolled at the ends of the filtering stick was inserted in the suction pump to obtain the filtrate that was washed twice with hot water to make the filtrate clear. A second digestion was done using

1.78 M NaOH with similar treatment. A final washing was done in 70% ethanol and the product transferred in weighed crucibles for drying at 105°C for 3 h in an oven. Weight of the contents was recorded after the 3 h and then ashing was done in a muffle furnace at 550°C overnight and weights recorded. Crude fibre was then calculated as the difference between sample weights from furnace and that of oven.

$$\text{Fibre (g/100 g)} = (\text{Residue weight from oven} - \text{weight from ashing}) / \text{original sample weight}$$

3.4.1.4 Determination of ILVs β -carotene content

Vitamin A content was determined as β -carotene spectrophotometrically according to AOAC International (2000), Method 2000.10, as modified by Imungi and Wabule (2001). Briefly, the samples (1 g) were ground in acetone and the homogenate filtered through glass wool. The residue was ground again and rewashed several times with acetone until a colourless filtrate was observed. The volume of the combined extracts was raised to 50 ml by adding acetone. The extract (25 ml) was evaporated to dryness in an evaporator in a water bath at 65°C. Separation was then carried out in a chromatographic column packed with silica gel to 15 cm depth while the top was filled with 1 cm layer of anhydrous sodium sulphate to remove any traces of water in the samples. The evaporated samples were then dissolved in 2 ml petroleum spirit, then quantitatively spotted into the column, and eluted with petroleum spirit. The first yellow solution (eluate) was collected in a 25 ml flask and made to the mark with the petroleum spirit. The concentrations of β -carotene were measured using a CE 440 UV/Vis double beam scanning spectrophotometer (V-200-RS, London, United Kingdom), at 450 nm, calibrated with standard solutions of pure β -carotene in petroleum spirit. The β -carotene contents were calculated using the formula:

$$C_X \text{ (mg/100 g)} = [A_X \times C_S \text{ (mg/ml)} \times \text{total volume of extract (ml)}] / [A_S \times \text{sample weight (g)}],$$

Where C_X is the concentration of β -carotene, A_X is the peak area of β -carotene, C_S is the concentration of the standard and A_S is the peak area of the standard.

3.4.1.5 Determination of ILVs ascorbic acid (vitamin C) content

Ascorbic acid was determined by titration with 2, 6-dichlorophenolindophenol dye according to AOAC International (2000), Method 990.23. The samples (2 g) were homogenized in metaphoric acid solution and the extract filtered, then diluted appropriately to a concentration of 100 mg ascorbic acid/100 ml. A standard solution was prepared by dissolving 50 mg pure

ascorbic acid in 100 ml of water. Samples filtrate was titrated against the standard solution to a pink endpoint in 10 seconds. Ascorbic acid content was calculated as:

mg ascorbic acid/100 g or ml sample = $C \times V \times (DF/WT)$, where C= mg ascorbic acid/ml dye,
V= volume of dye used in titrating sample, DF= dilution factor and WT= sample weight (g).

3.4.1.6 Determination of ILVs iron and calcium content

The two minerals were determined using atomic absorption spectrophotometer (AAS) according to AOAC International (1995), Method 970.30. The spectrophotometer (2380, Perkin Elmer, Osaka, Japan) was equipped with an air acetylene flame, hollow cathode lamp and recorder. Iron readings were taken at 234 nm while for calcium were done at 248 nm. Approximately 0.5 g dried and ground samples were digested in a digester (DK-20S digester, Velp Scientifica, Bohemia, Italy) using 10 ml concentrated HCl and 20 ml concentrated HNO₃. The samples were cautiously heated at 250°C until vigorous reaction subsided. Heating continued to 450°C for 45 minutes. Filtering the distillate using Wattman filter paper No. 4 (22 µm) was done in 60 ml sampling bottles. The filtrate was filled to the mark with distilled water. The amount of each element was calculated against the standards.

3.4.2 Microbial analyses

Microbiological analyses were performed on duplicate samples of the raw and blanched-dried ILVs. Samples were analyzed for total viable counts, coliforms and yeast and mould counts. Serial decimal dilutions were prepared using peptone water. The isolation of microorganisms was done by cultivating the samples on different media. The inoculated media were cultured at different temperatures. Pure cultures of the microorganisms were identified using the standard procedures (Bridson, 2006). The test employed for the identification of isolates was gram staining, cell morphology, and catalase tests.

3.4.2.1 Determination of total viable counts on the ILVs

The total viable counts were determined by following AOAC International (2010), Method 986.33 for microbiological testing of dried fruits and vegetables. Homogenized ground sample (10 g) was added to 90 ml peptone solution as diluent. A tenfold serial dilution was prepared and 1 ml of each sample was transferred to a Petri-dish that had been appropriately labelled with marker. From the appropriate 10-fold dilutions, total bacteria were enumerated by use of standard plate count agar incubated at 37°C for 48 h. Coliform bacteria were enumerated

on violet red bile agar (VRBA) incubated at 37°C for 24 h, and confirmed in brilliant green bile lactose broth incubated at 37°C for 24 h. Potato dextrose agar was used for moulds and yeast and incubated at 25°C for 46 h. All the Petri-dishes were incubated upside down, and after the stated incubation period, the microbial count was carried out. Colonies appeared as clusters and each plate was counted and the averages computed for each sample. The isolates were sub-cultured to obtain pure cultures. The plates were incubated at 30°C for 24 h and 72 h for bacteria and fungi respectively. The bacterial isolate was identified using morphological and biochemical characteristics. Moulds were identified using microscopy and cultural characteristics.

3.4.2.2 Isolation and identification of microorganisms in ILVs

This was performed according to Bridson (2006). Five isolates were taken from the highest dilution plates and continually streaked on agar to obtain pure colonies.

3.4.2.3 Identification of moulds and yeast

Moulds and yeast isolates were identified in potato dextrose agar incubated at 25°C for 48 h by colony and cell morphology (Himelbloom and Crapo, 1998).

3.4.2.4 Confirmation of coliforms

Coliforms were confirmed in brilliant green bile lactose broth incubated at 37°C for 24 h (Bridson, 2006).

3.5 Evaluation of ILVs storage stability

The blanched and dried ILVs were packaged in zip-lock plastic bags prior to storage for three months in a cool dry room at 18°C. Assessment of the effect of storage time on the product quality and safety was determined. Chemical and microbial analyses were done at the beginning of the storage and at the end of the third month to determine the effects the three months had on the stability of nutrient composition and microbial population of ILVs.

3.6 Statistical analysis

The experiments were performed in duplicates and repeated at least twice. The experiments were carried out in a Completely Randomized Design (CRD) with five treatments. The analysis of the ILVs was performed on fresh, blanched-dried and on the ILVs stored after the three month storage at 18°C. The data were analyzed by one way analysis of variance (ANOVA) as outlined by Gacula and Singh (1984) using sample as the independent variable and

the measured parameters as the dependent variables. Data was analyzed using SAS statistical package with mean separations done with Fisher's least significant difference (LSD) at $P \leq 0.05$ to identify significant differences among treatment means.

CHAPTER FOUR RESULTS AND DISCUSSION

4.1 Nutritional composition of raw ILVs

The nutritional composition of the raw ILVs is presented in Table 4. The moisture content and nutritional composition of the three species was significantly different ($P < 0.05$) for each species. Raw slenderleaf had the highest moisture content (81.8%) followed by spiderplant leaves (81.1%) and cowpeas (71.1%). The high water contents in the vegetables suggest the need for appropriate preservation measures since they are prone to deterioration. Hence, this may be the reason why most of the farmers open sun-dry their vegetables after boiling at the farm level. The lower moisture content in the present study when compared to other studies using similar ILVs may be attributed to a difference in maturity stage and harvesting time. Previously, a review by Uusiku *et al.* (2010) documented moisture content of spiderplant and cowpeas to be 83% and 78 % for leaves harvested three weeks after planting.

The protein content in the fresh ILVs was significantly different among the three ILVs. It was found to be highest in spiderplant (8.3 g/100g) and lowest in slenderleaf (6.4 g/100 g). The difference in crude protein contents among the species may be attributed to vegetable type and age of leaves at harvesting as noted by Bruinenberg *et al.* (2001). However, the three ILVs can thus play a significant role in providing cheap and affordable protein for rural communities.

The levels of crude fibre was significantly different ($P < 0.05$) in all the three ILVs. Slenderleaf recorded the lowest fibre content (3.5 g/100 g) of the three species, probably because of the leaf tenderness, early stage of harvesting and structural components of the leaves. Additionally, the difference in crude fibre contents might have also been attributed to early harvesting time from the convectional harvesting time of 12 weeks where the leaves are considered as over matured becoming too fibrous (Kader, 1998). Fibre adds bulk to the food and prevents the intake of excess starchy foods and may thus guard against metabolic conditions such as diabetes mellitus (Mensah *et al.*, 2008).

The vitamin C content in the fresh ILVs was significantly different among the three ILVs. The three species in their fresh form meets the RDA for vitamin C (75 mg/day) for an average adult (Gropper *et al.*, 2009). However the insignificant differences ($P < 0.05$) observed may be attributed to the difference in stages of maturity and time between harvest and preparation of the

vegetables before the analysis. The recorded amount of vitamin C for spiderplant (178 mg/100 g) is higher than (143 mg/100 g) value reported by Hassan and Hassan (2008).

Spiderplant recorded the highest amount of β -carotene (10.3mg/100g) with slenderleaf and cowpeas recording an almost similar amounts of β -carotene (6.6 mg/100g and 6.5 mg/100g) respectively. This β -carotene is determined as a precursor of vitamin A where, 6–12 μ g of β -carotene is equivalent to 1 μ g of vitamin A. Nutritionally, the total amount of vitamin A in foods is expressed as μ g retinol activity equivalents (RAE), calculated from the sum of μ g of preformed vitamin A + $1/12 \times \mu$ g β -carotene (Gibney *et al.*, 2009). It is recommended that an average person consume 700 μ g/ 100 g/ day amounts of vitamin A. Thus, fresh spiderplant recorded the highest amount of β -carotene 10.3 mg/100 g (858 μ g RAE/100 g) which is a value almost two times greater than of cowpeas 6.5 mg/100g (542 μ g RAE/100 g). Spiderplant's vitamin A content (858 μ g RAE/100 g) meets the vitamin A requirement for an adult. Therefore, it would be expected to improve vision, eye health and immune system of adults who regularly consume the ILVs. In Africa, about 30% of all preschool children are affected by vitamin A deficiency and in some countries this figure can reach more than 60% (Standing Committee on Nutrition, 2004).

The level of iron was significantly different ($P < 0.05$) in all the three ILVs. Slenderleaf had the highest level of iron (46.5 mg/100 g), while spiderplant and cowpeas levels of iron (21 mg/100 g and 10.8 mg/100 g) are almost similar to those reported by Weinberger and Msuya (2004), (29 mg/100 g and 14.5 mg/100 g). The high amount of iron in these vegetables makes them a potential source of iron for vulnerable groups such as children less than five years whose iron needs increases due to rapid growth (Nutrition and Gastroenterology Committee, 2012). Additionally, according to micronutrient information centre (2013), pregnant and lactating women require 27 mg/day iron to support growth and development of the foetus and placenta and to meet the increased demand for red blood cells to transport oxygen.

Spiderplant recorded the highest amount of calcium (281 mg/100 g), followed by cowpeas (175 mg/100 g) and finally by slenderleaf (44 mg/100 g). The contents in spiderplant was higher than those obtained by Muhammad *et al.* (2014) (170 mg/100 g) probably due to tender harvesting stage.

Table 4: Nutritional composition of raw ILVs

ILVs	Moisture (%)	Crude protein (g/100 g)	Crude fibre (g/100 g)	Vitamin C (mg/100g)	β-carotene (mg/100g)	Iron (mg/100g)	Calcium (mg/100g)
Spiderplant	81.1 ^b ±0.6	8.3 ^b ±0.1	4.2 ^b ±0.2	178.0 ^c ±0.2	10.3 ^b ±0.5	21.0 ^b ±0.4	281 ^c ±1.4
Slenderleaf	81.8 ^b ±0.3	6.4 ^a ±0.1	3.5 ^a ±0.2	160.0 ^b ±0.1	6.6 ^a ±0.5	46.5 ^c ±1.1	44 ^a ±1.4
Cowpeas	71.1 ^a ±0.2	6.8 ^a ±0.0	4.6 ^c ±0.2	91.0 ^a ±1.5	6.5 ^a ±0.4	10.8 ^a ±0.2	174 ^b ±0.8

Values are means ± standard deviations, n=2. Values in a column followed by different letter notations are significantly different (P≤0.05).

Generally, the fresh leaves compositional content were slightly higher in amounts than those reported by Abukutsa-Onyango (2003) and Maundu *et al.* (1999) which may be due to effect of controlled environment and the difference in soil nutrients available for the formation of nutrients in leaves. More so, nutrient content of raw plant foods vary widely and are affected by factors such as variety or cultivar, part of the plant consumed, stage of maturity, geographic site of production or climate, harvesting and post-harvest handling conditions and storage conditions (Kader, 1998). Comparing nutrient content of leaves from different data sources is therefore challenging. Common errors introduced during sampling procedures, sample preparation and analytical methods can also affect the reported nutrient values.

4.2. Effects of blanching time-temperature regimes and drying methods on nutritional composition of ILVs

Heat treatment and drying methods significantly affected the nutritional composition of the leaves (Table 5). The three drying methods resulted in different amount of moisture content among the three species. Open sun-drying was the least effective method while oven drying (standard method) was the most efficient in dehydrating the ILVs. Solar-dried ILVs had moisture contents very similar to the level obtained with oven-drying. The mean moisture content for the solar dried ILVs was 13.3% which was close the oven-dried ILVs (12.4%). The two drying methods were significantly different ($P < 0.05$) from the open sun-dried ILVs whose mean moisture content was 16.2%. The differences in the amount of moisture between the solar-dried and open sun-dried ILVs may be attributed to the ability of the solar-drier to generate heat through solar radiations that were absorbed by the ILVs, thus, the rate of moisture removal was fast unlike in the open-sun dried ILVs.

Additionally, the removal of moisture from the ILVs in the solar drier may have been contributed by the transparent polythene paper that probably created a greenhouse effect in the drier thereby enhancing air exchanges and ensuring a balanced air regulation in the drier. The agronomic reasons such as difference in maturity stage and harvesting time may also have contributed to a difference in moisture content of the dried ILVs as suggested by Uusiku *et al.* (2010). A faster moisture removal from the ILVs is expected to hinder susceptibility to microbial attack during drying and this would increase their shelf stability making them available throughout the year (Ngoddy and Ihekoronye, 1985). The solar-dried mean moisture content of

the ILVs in the present study (13.3%) is almost similar to 13% reported by Oboh *et al.* (2005) for solar dried traditional leafy vegetables in Nigeria.

After blanching and drying the ILVs, there was a slight loss in protein amounts in all processed ILVs (Table 5) which was not expected. However, the solar-dried spiderplant leaves blanched at 80°C/10 min had the highest amount (5.5 g/ 100 g) of crude protein while the cowpea leaves boiled with open-sun drying had the lowest crude protein amounts (4.1 g/ 100 g). This slight loss in crude protein was probably due to loss of water soluble nitrogen-containing compounds in the ILVs such as free amino nitrogen, nucleic acids, nucleotides and certain water soluble vitamins during blanching. Less loss in crude protein was observed for solar and oven dried ILVs, while greatest loss was recorded for open sun-dried ILVs. This was probably because of relatively mild blanching conditions used for solar and oven-dried ILVs. The FAO/WHO RDA for protein is 0.8 g/kg/day (Gropper *et al.*, 2009). An average adult weighing 64 kg requires 51.2 g/kg/day which may not be obtained from ILVs under study. Relatively, more benefit could be achieved by practicing solar-drying in order to have better protein retention, unlike open sun-drying of boiled ILVs. Additionally, the amounts obtained after the preservation can play a significant role in providing cheap and affordable protein supplement for rural communities.

After processing, crude fiber content across all ILVs was almost similar. Slenderleaf blanched at 90°C/5 min with solar-drying had the highest amount of crude fiber (4.3 g/100 g). Slenderleaf and cowpeas boiled then open sun-dried had lower amounts (3.2 g/100 g) of crude fiber than solar-dried samples (4.5 g/100 g). The difference in crude fiber contents may be due to difference in leaves structure and age of leaves at harvesting as noted by Bruinenberg *et al.* (2001). Crude fiber content for both fresh and processed ILVs was lower than the FAO/WHO RDA for an average adult (38 g/day) (Gropper *et al.*, 2009). Combining the three species could supply the body with the FAO/WHO RDA/day. Fiber adds bulk to the food and prevents the intake of excess starchy foods and may thus guard against metabolic conditions such as diabetes mellitus (Mensah *et al.*, 2008).

Table 5. Effects of blanching time-temperature combinations and drying methods on nutritional composition of ILVs

ILVs	Treatment	Moisture (%)	Protein (g/100 g)	Crude fibre (g/100 g)	Vitamin C (mg/100 g)	β-carotene (mg/100 g)	Iron (mg/100 g)	Calcium (mg/100g)
Spiderplant	H810	11.8 ^a ±0.0	5.5 ^g ±0.6	3.4 ^a ±0.0	82.9 ^k ±1.9	3.7 ^d ±0.0	1.2 ^c ±0.0	7.6 ^g ±0.0
	H905	12.2 ^b ±0.7	4.9 ^e ±0.6	3.9 ^c ±0.2	79.0 ^j ±0.2	3.3 ^b ±0.1	0.9 ^b ±0.0	7.2 ^f ±0.2
	S810	13.3 ^d ±1.6	5.5 ^g ±0.5	4.1 ^c ±0.2	103.7 ⁿ ±0.0	3.5 ^c ±0.0	1.4 ^c ±0.7	7.1 ^f ±0.2
	S905	12.5 ^c ±0.3	4.7 ^d ±1.1	4.3 ^d ±0.0	84 ^l ±0.3	3.7 ^d ±0.0	0.9 ^b ±0.0	6.7 ^e ±0.1
	O3B	16.7 ^g ±0.0	1.3 ^a ±0.3	3.6 ^b ±0.2	79.1 ^j ±0.6	3.1 ^b ±0.1	1.1 ^b ±0.0	7.0 ^f ±0.1
Slenderleaf	H810	12.5 ^c ±0.0	5.0 ^e ±0.7	4.0 ^c ±0.2	53.6 ^e ±0.9	3.2 ^b ±0.0	2.2 ^e ±0.2	1.6 ^b ±0.0
	H905	11.9 ^a ±0.0	5.4 ^f ±0.7	4.3 ^d ±1.2	51.8 ^d ±0.8	3.3 ^b ±0.0	1.8 ^d ±0.7	1.4 ^b ±0.1
	S810	12.5 ^c ±1.4	4.5 ^d ±1.1	3.4 ^a ±0.7	26.8 ^a ±1.9	3.1 ^b ±0.0	1.7 ^d ±0.2	1.1 ^a ±0.0
	S905	12.3 ^b ±0.3	4.6 ^d ±0.7	3.9 ^c ±0.7	61.9 ^g ±1.6	2.7 ^a ±0.2	1.3 ^c ±0.0	1.1 ^a ±0.0
	O3B	16.0 ^f ±0.8	2.3 ^b ±1.1	3.2 ^a ±0.0	69.8 ⁱ ± 1.2	3.6 ^c ±0.0	1.8 ^d ±0.0	0.9 ^a ±0.0
Cowpeas	H810	12.0 ^a ±0.7	5.2 ^f ±0.3	4.2 ^d ±0.4	48.9 ^c ±0.8	3.4 ^c ±0.0	0.6 ^a ±0.0	6.7 ^e ±0.2
	H905	12.0 ^a ±0.0	4.7 ^d ±1.0	4.7 ^e ±0.1	45.1 ^b ±0.5	3.4 ^c ±0.0	0.6 ^a ±0.0	6.5 ^e ±0.0
	S810	12.1 ^b ±0.0	3.9 ^c ±0.7	4.9 ^e ±0.4	91.6 ^m ±1.0	3.2 ^b ±0.0	0.4 ^a ±0.0	6.2 ^d ±0.0
	S905	12.3 ^b ±0.3	4.7 ^d ±0.3	4.3 ^d ±0.2	64.8 ^h ±0.7	3.1 ^b ±0.2	0.6 ^a ±0.0	5.8 ^c ±0.1
	O3B	14.8 ^e ±0.3	4.1 ^c ±0.7	3.2 ^a ±0.0	57.9 ^f ±0.7	3.1 ^b ±0.2	0.5 ^a ± 0.0	6.2 ^d ±0.0
RDA*	-	-	0.8**	38**	75***	5.8***	20***	1000***

Values are means ± standard deviations, n =2. Values in a column followed by different letter notations are significantly different P≤0.05.

* =WHO/FAO RDA (average adult) (2008). ** = RDA g/day, ***=RDA mg/day. H810- Blanched at 80°C /10 min hot air-dried, H905-Blanched at 90°C /5 min hot air dried, S810- Blanched at 80°C /10 min solar-dried, S905-Blanched at 90°C/5min solar-dried, O3B- Blanched at 100°C/30 min Open sun-dried.

There were significant losses on the content of ascorbic acid upon blanching and drying. A loss in ascorbic acid of 44.4%, 39.2% and 32.3% for spiderplant, slenderleaf and cowpeas, respectively, blanched at 100°C/30 min with open sun-drying, was observed. The above losses were higher when compared to solar-dried spiderplant and cowpeas (37% and 41%) blanched at both 80°C/10 min and 90°C/5min. Thus these results indicate that boiling of ILVs with open sun-drying can result in significant reduction in levels of ascorbic acid compared to blanching at 80°C/10 min with solar drying. Losses in ascorbic acid during drying have been reported for other vegetables (Mziray *et al.*, 2000). Ascorbic acid is the most commonly assayed nutrient in blanching probably because its high solubility and heat susceptibility make it a conservative indicator of nutrient retention (Jose *et al.*, 2010). Subhash and Neeha (2014) reported ascorbic acid to be destroyed during blanching and drying of vegetables.

It is thus generally recognized that dehydration of leafy vegetables results in losses of vitamins, the extent of loss depending on the type of vegetable (Gareth *et al.*, 1998). The difference in loss of ascorbic acid in the ILVs treated under similar conditions could be attributed to difference in ILVs internal tissues structures (size and shape), mechanical damage, fresh ILVs ascorbic acid content and enzymatic activities. It can be observed that spiderplant and cowpeas blanched at both 80°C/10 min and 90°C/5min with solar-drying meets the RDA (75 mg/day) for ascorbic acid.

Amount of β -carotene for the leaves blanched at 80°C/10 min and 90°C/5 min blanched/solar-dried samples was found to be higher than the control and this might be due to the effect of direct sun radiations on the vitamin. The total β -carotene significantly decreased ($P < 0.05$) which correlates with a study done by Gayathri *et al.* (2004), who found that boiling resulted in the greatest loss of β -carotene in *Amaranthus* species. The vitamin is not a heat-sensitive nutrient, although heat facilitates the breakdown of cell walls to release β -carotene making it more readily available for absorption into the bloodstream (Gayathri *et al.*, 2004). Intake of the three species in combination could meet the β -carotene RDA/day. Alternatively, since the solar-dried leaves attains almost similar β -carotene as the standard hot air-oven drying, it could be advantageous to solar-dry the leaves so as to obtain higher amounts of β -carotene.

Comparing the iron content among the three blanching time/temperature combinations, slenderleaf blanched at 80°C/10 min had the highest iron content (2.2 mg/100g) while cowpeas blanched at 80°C/10 min had the lowest iron content (0.4 mg/100g). As expected, blanching of

the leaves decreased the content of iron. This may be because more iron could have leached out in the blanching water during the heat treatment (Vorster *et al.*, 2002).

Blanching and drying caused significant ($P < 0.05$) reductions in calcium content of the three species. This suggests consumption of large quantities of the ILVs to meet the RDA for calcium. For instance, adult minimum calcium requirement for health set by the FAO/WHO RDA is 1000 mg daily (Gropper *et al.*, 2009). In meeting the RDA amount of calcium from blanched spiderplant, 71 mg/100g would be required from leaves blanched at 80°C/10 min. Generally, solar dried ILVs had higher mineral content than the open sun-dried vegetables for all the minerals. This may be because solar dried foods are dried at high air, temperature and low humidity. This hastens the drying rate and helps to retain more minerals as well as enable the products to be stored longer. This is further corroborated by reports of Fuller (1991) and Ruel (2001), that solar drying helped retain more minerals in fruits and vegetables. Blanching and drying resulted into drastic mineral losses among the three ILVs which contributed into the processed ILVs not meeting the nutritional requirement.

4.3. Microbial quality of raw ILVs

The microbial load of the three species is given in Table 6. Slenderleaf moisture content (81.8%), spiderplant (81.1%) and cowpeas (71.1%) show that the three species are high in moisture content.

Table 6: Microbial population of freshly harvested ILVs

ILVs	Total viable counts (log ₁₀ Cfu/g)	Yeasts and moulds counts (log ₁₀ Cfu/g)	Coliform counts (log ₁₀ Cfu/g)
Spiderplant	8.4 ^a ±0.8	3.7 ^b ±1.4	1.5 ^a ±1.2
Slenderleaf	8.3 ^a ±0.9	2.4 ^a ±0.6	1.4 ^a ±0.4
Cowpeas	8.0 ^a ±0.9	2.8 ^a ±1.0	1.9 ^a ±1.7
Limit	5.0	3.0	3.0

Values are means (log₁₀ cfu/g) ± standard deviation of ILVs. Cfu/ g = colony forming unity per gram. Values in a column followed by different letter notations are significantly different ($P \leq 0.05$).

The high total viable bacterial counts, coliform counts and fungal counts may be connected to the high moisture contents and a rich chemical composition of the ILVs since

microorganisms utilize the nutrients to multiply in number which probably leads to spoilage of stored ILVs (Table 6). The presence of the fungal isolates on the three samples suggests possible contaminations by spores in the air, since their spores are numerous available in the air (Arotupin *et al.*, 2003).

The high total viable counts 8.4 CfU/ g indicate contamination during handling, since microbes are known to be a normal flora of man (Onuorah *et al.*, 1987) and to the fact that most microbes are widely distributed in air, dusts and soils. Hot air oven-drying of the leaves at 60°C (Table 6) attained a mean moisture content of 12% at the seventh hour while solar-drying attained 13% at ninth hour and open sun-drying attained 16% after 2 days. This probably explains why the open sun-dried samples were susceptible to microbial attack as a result of longer drying period.

The faster moisture removal in oven dried leaves hinders susceptibility to microbial attack during drying and this invariably increases the shelf life of the dried vegetable and makes it available throughout the year (Ngoddy and Ihekoronye, 1995). The trend of the total bacterial count obtained was significantly high thus indicating a large extent of contamination of the open sun-dried samples which is liable to predispose consumers to some physiological effects.

4.4. Effects of blanching time-temperature regimes and drying methods on microbial quality of ILVs

The ILVs blanching time-temperature treatment had a significant reduction on the number of counted micro-organisms when compared with freshly harvested ILVs (Table 7). The effects of blanching and drying of ILVs led to 26-34% loss in the number of total viable counts. This implies that the two treatments contribute to safety of the processed ILVs by reducing the number of viable counts. The drying methods had a significant effect on the moisture content of the dried ILVs which may have also reduced the number of counted micro-organisms. However, despite the low moisture content of the blanched-dried vegetables, microbial counts were recorded in all the three samples. The results showed that microbial load in solar dried vegetable samples were significantly lower ($P < 0.05$) than that of sun dried samples at the end of the drying period (Table 7).

Table 7: The microbial quality of processed ILVs

ILVs	Treatment	Total viable counts (log ₁₀ Cfu/g)	Yeasts and moulds counts (log ₁₀ Cfu/g)	Coliform counts (log ₁₀ Cfu/g)
Spiderplant	H810	6.1 ^a ±1.1	2.2 ^a ±1.2	0.9 ^a ±0.7
	H905	5.8 ^a ±0.9	2.0 ^a ±1.7	0.8 ^a ±0.6
	S810	6.3 ^b ±0.5	2.1 ^a ±1.3	1.3 ^a ±0.3
	S905	5.4 ^a ±0.8	1.8 ^a ±0.9	1.1 ^a ±0.9
	O3B	6.7 ^b ±0.4	2.6 ^b ±1.2	1.4 ^a ±0.2
Slenderleaf	H810	6.0 ^a ±0.9	2.1 ^a ±1.1	0.7 ^a ±1.1
	H905	5.2 ^a ±0.7	2.0 ^a ±0.3	0.7 ^a ±0.8
	S810	6.2 ^a ±1.2	2.3 ^a ±0.4	1.6 ^b ±1.1
	S905	5.3 ^a ±0.5	2.1 ^a ±1.1	1.5 ^a ±0.8
	O3B	6.4 ^b ±0.7	2.6 ^b ±0.7	1.8 ^b ±0.7
Cowpeas	H810	6.2 ^a ±0.3	2.4 ^a ±0.9	1.0 ^a ±0.2
	H905	5.8 ^a ±0.8	2.0 ^a ±0.5	0.8 ^a ±0.2
	S810	6.0 ^a ±1.2	2.2 ^a ±1.2	1.6 ^b ±1.1
	S905	5.6 ^a ±0.6	1.9 ^a ±0.8	0.9 ^a ±0.2
	O3B	6.3 ^b ±1.1	2.8 ^b ±0.4	1.9 ^b ±0.3
Recommended criteria	-	5.0	3.0	3.0

Values are means (log₁₀ cfu/g) ± standard deviation of blanched-dried ILVs. Cfu/ g =colony forming unity per gram. Values in a column followed by different letter notations are significantly different P≤0.05, H905-Blanched at 90°C /5 min hot air dried, S810- Blanched at 80°C /10 min solar-dried, S905-Blanched at 90°C/5min solar-dried, O3B- Blanched at 100°C/30 min Open sun-dried.

The higher counts in open sun-dried ILVs may be attributed to inappropriate conditions such as open air that is composed of dust and insects which contaminates the ILVs. The number of total viable counts for solar-dried ILVs (5.3-6.3 cfu/g) was almost similar to that of hot air-dried ILVs (5.2-6.2 cfu/g), whose values are higher than of the recommended value (5.0 cfu/g). The higher value than what is recommended may have been attributed by the packaging of dried ILVs in zip-lock bags that were used in storing the samples as they awaited analysis. Probably the packaging materials should have been sanitized before the ILVs were stored. Mean

concentrations (10^1 - 10^3 cfu/g) for the total bacteria in all the samples was done with the solar-dried samples blanched at 90°C/5 min recording slightly lower populations. This could be attributed to the higher blanching temperatures and enclosure of the samples in a solar-drier. Counts for moulds, yeast and coliforms were done at concentration 10^1 to 10^4 cfu/g.

The yeasts and moulds results (Table 7) shows small differences in total viable counts for blanching done at 90°C/5 min with solar-drying compared to those blanched at 100°C with open sun-dried samples. This could be due to the effect of heating since most of them are heat sensitive. Blanching and drying caused a 32-38% loss in the number of yeast and molds leading to counts (1.8-2.8 cfu/g) that are less than the maximum recommended value (3.0 cfu/g). The open sun-dried ILVs recorded higher counts (2.6-2.8 cfu/g) in the number of yeast and molds probably due to higher levels of moisture content (15.4-16.6%) that accelerated their growth contributing to their multiplication. The occurrence of coliforms in high numbers in most of the dried vegetables is an indication of poor handling of the vegetables during processing (Ezeronye and Ubalua, 2005). Factors which could be responsible for the counts may include drying vegetables on exposed surfaces and packing them in containers not adequately cleaned (Kudjawa *et al.* 2011). The results further showed that microbial load was not high (3-5 cfu/g) to harm the body and that the vegetables could be preserved over a considerable period of time. FAO (2004) reports showed that solar dryers are free from microbial contamination and are better preservers and give good quality products than sun dried products. In general, microbial load in raw ILVs decreased after the blanching and drying treatments. This could be due to the water content declining which result from the loss of humidity in samples, causing the inhibition of microbial growth (Adom *et al.*, 1996).

4.5 Effects of storage time/temperature on nutritional quality and microbial safety of ILVs

4.5.1 Nutritional composition of stored ILVs

Moisture content of dried vegetables is very important for the shelf life. The lower the vegetable moisture contents, the better the storage stability (Butt *et al.*, 2004). From Table 8, it can be depicted that the moisture contents of the ILVs decreased at 18°C during the storage period. Relative humidity and temperature during storage are two major factors that affect overall quality of the product. Low humidity gives rise to low moisture content, which does not encourage enzymatic hydrolysis of the fat present in food products (Mridula *et al.*, 2009). During

storage at 18°C for the three months, all the stored samples in zip-lock packaging bags showed varying degrees of moisture loss. There was a 6-8% moisture content decrease for hot air-oven-dried ILVs, 3.5-5% decrease for solar-dried ILVs while a 2-3% decrease was experienced on open sun-dried ILVs (Table 8). Moisture loss from the ILVs suggest that the growth of micro-organisms is limited which reduces the spoilage of the vegetables. Thus the vegetables may be considered safe after 3 months of storage. The ILVs initially sealed in airtight zip-lock plastic bags were protected from picking up moisture from the air. Although there were decreases in amounts of moisture content, the hot air oven-dried ILVs had the lowest moisture content mean value (9.24%) followed by solar-dried ILVs (11.4%) and the highest moisture content was recorded for open sun-dried ILVs (14.5%). The moisture reduction may be attributed to the cool dry place of storage and rigidity of the packaging material which is a flexible film of high density polyethylene (HDPE) that is resistant to moisture.

Table 8. Effects of storage time/temperature on dried ILVs after three months of storage at 18°C

ILVs	Treatment	Moisture (%)	Protein (g/100 g)	Crude fibre (g/100 g)	Vitamin C (mg/100 g)	β-carotene (mg/100 g)	Iron (mg/100 g)	Calcium (mg/100 g)
Spiderplant	H810	8.75 ^a ±0.35	5.40 ^c ±0.28	3.15 ^a ±0.07	76.40 ^g ±1.55	3.10 ^{ab} ±0.00	1.01 ^a ±0.14	5.05 ^c ±0.35
	H905	9.45 ^a ±0.63	4.15 ^{ab} ±0.91	3.45 ^a ±0.49	74.65 ^g ±1.34	3.20 ^b ±0.14	0.45 ^a ±0.07	5.40 ^d ±0.98
	S810	11.25 ^b ±1.06	3.70 ^a ±0.28	3.15 ^a ±0.35	68.40 ^f ±1.27	3.40 ^b ±0.00	1.20 ^a ±0.14	5.80 ^d ±0.14
	S905	11.50 ^b ±0.70	4.70 ^b ±0.28	3.35 ^a ±0.01	94.12 ^h ±2.28	3.35 ^b ±0.07	0.95 ^a ±0.07	4.50 ^b ±0.14
	O3B	14.70 ^c ±0.28	2.80 ^a ±0.28	2.75 ^a ±0.21	77.55 ^g ±2.61	2.95 ^a ±0.07	1.03 ^a ±0.07	5.75 ^d ±0.21
Slenderleaf	H810	9.35 ^a ±1.20	4.15 ^{ab} ±0.91	3.00 ^a ±0.28	49.60 ^c ±0.98	3.15 ^{ab} ±0.07	1.55 ^b ±0.07	0.65 ^a ±0.07
	H905	9.15 ^a ±0.49	3.60 ^a ±0.42	2.01 ^a ±0.14	48.80 ^c ±0.98	2.85 ^a ±0.07	1.48 ^c ±0.21	1.30 ^a ±0.84
	S810	11.75 ^b ±1.06	4.35 ^b ±0.63	3.10 ^a ±0.28	44.10 ^b ±2.26	2.76 ^a ±0.07	1.60 ^b ±0.14	1.55 ^a ±0.21
	S905	11.15 ^b ±1.20	3.22 ^a ±0.14	3.45 ^a ±0.07	53.45 ^d ±1.76	2.30 ^a ±0.28	1.25 ^a ±0.21	1.68 ^b ±0.39
	O3B	14.15 ^c ±0.21	4.45 ^b ±0.21	2.70 ^a ±0.14	61.70 ^c ±1.12	3.00 ^a ±0.00	1.75 ^b ±0.00	0.35 ^a ±0.21
Cowpeas	H810	9.25 ^a ±0.35	4.30 ^b ±1.27	2.55 ^a ±0.07	38.40 ^a ±0.81	3.00 ^a ±0.00	0.52 ^a ±0.07	4.90 ^b ±0.28
	H905	9.50 ^a ±0.70	2.85 ^a ±0.21	2.90 ^a ±0.14	35.60 ^a ±0.52	3.10 ^{ab} ±0.00	0.60 ^a ±0.00	4.30 ^b ±0.28
	S810	11.50 ^b ±1.41	4.35 ^b ±0.63	2.65 ^a ±0.07	74.00 ^g ±1.01	3.20 ^b ±0.00	0.30 ^a ±0.00	4.32 ^b ±0.56
	S905	11.45 ^b ±0.76	3.20 ^a ±0.14	3.20 ^a ±0.41	44.20 ^b ±0.70	3.05 ^a ±0.21	0.06 ^a ±0.21	5.47 ^d ±0.28
	O3B	14.75 ^c ±0.07	4.45 ^a ±0.21	2.70 ^a ±0.14	46.30 ^b ±0.70	3.15 ^{ab} ±0.07	0.45 ^a ±0.07	5.05 ^c ±0.49

Values are means ± standard deviations for ILVs stored at 18 °C for three months, n =2. Values in a column followed by different letter notations are significantly different P≤0.05, H905-Blanched at 90°C /5 min hot air dried, S810- Blanched at 80°C /10 min solar-dried, S905-Blanched at 90°C/5min solar-dried, O3B- Blanched at 100°C/30 min open sun-dried.

As expected, the changes in crude proteins and fibre were negligible (Table 8) after three months of storage. The storage period had no significant effect on the crude protein and fibre composition of the differently blanched-dried ILVs. Pradyuman *et al.* (2013) reported that amounts of crude fibre content are not significantly affected by packaging material and storage time. The amount of crude fibre decrease was insignificant (1.1%) across stored ILVs. The observed slight protein value decrease (1.3%) in stored ILVs probably may be due to slight browning reactions that probably occurred during protein hydrolysis at storage.

Johnson (1998) reported that low amount of moisture is retained by the vegetables stored in polyethylene bags which cause a dilution effect, thus reducing the extent of browning. Vitamin C content decreased during storage and it ranged from 44.1-94.1 mg/100 g. Spiderplant values remained relatively higher, followed by slenderleaf and cowpeas respectively (Table 8). The solar-dried ILVs decrease was significant ($P < 0.05$) which was 17.3% while that of open sun-dried ILVs was 12%. The loss thus indicated that the quality and shelf life of ILVs is hindered by storage duration and temperatures. The losses in the amount of stored ascorbic acid could probably be due to the effect of the residual oxygen retained in the packaging material during the initial packaging and light due to transparent polyethylene bag (Mziray *et al.*, 2000).

As storage progressed for the three months, the residual oxygen in the package may have decreased and therefore the rate of oxidation of ascorbic acid also decreased. The loss of ascorbic acid during storage of fruits and vegetables has been reported by Mutegi (2002). However, the level of spiderplant after three months of storage meets the RDA for ascorbic acid. To meet the RDA for slenderleaf and cowpeas, it would require that the two ILVs be combined in their intake.

The β -carotene loss during storage of blanched-dried ILVs is presented in Table 8. The β -carotene content of immediately dried ILVs was 2.7-3.7 mg/100 g. During storage, 7% β -carotene loss was found on solar-dried ILVs while 12.3% was observed for open sun-dried ILVs stored in zip-lock plastic bags (Table 8). This could have resulted from oxidation due to oxygen retained in the package and light due to transparent polyethylene bags used. The higher rate of oxidation of β -carotene can be attributed to reaction kinetics, where there was more β -carotene initially (as reactant) which decreased with oxidation thus lowering the reaction rate during the storage. Gareth *et al.* (1998) reported that losses of β -carotene in stored dehydrated vegetables are usually due to oxidation mainly by the oxygen retained in the package and catalyzed by light.

At the end of three months storage, the retention ranged between 2.3-3.4 mg/100 g in sample stored at 18°C and packaged in zip-lock plastic bags.

There were negligible losses in the levels of iron and calcium during storage (Table 8). The mineral changes were not expected during storage since minerals are generally stable during storage (Severi *et al.*, 1997). The solar-dried ILVs blanched at 80°C/10 min retained higher levels of iron in all the ILVs despite a 1.2% loss during the storage. The ILVs subjected to 100°C/30 min in combination to open sun-drying recorded a 1.3% loss in the level of iron contents. There was a 1.5% decrease in the amount of calcium for solar-dried ILVs while a 1.6% was found in open sun-dried ILVs after the three months of storage. The RDA of calcium is 1000 mg /day while that of iron is 20 mg/day. The observed values for both elements immediately after drying and further reduction during storage suggest that other ingredients which provide additional quantities of the two nutrients may be included when cooking the ILVs or after cooking, fortification may be needed.

4.5.2 Microbial population of stored ILVs

Vegetables are brittle and crunchy when dry and do not need cooling before storage in zip lock plastic bags (Severi *et al.*, 1997). The dried vegetables should be stored in air-tight containers and in a dark place to prevent them from absorbing the moisture in the air. There was a slight increase in the number of total microbial counts during the storage period (Table 9). Additionally, during the storage, coliforms microbial load increased. There was a 6% increase in the number of total viable counts for the solar-dried ILVs. This could be due to stored ILVs nutritive value which might have provided nutrients to microorganisms leading to an increased metabolism among the micro-organism and multiplication. The increase was low compared to 11% reported by Agbo *et al.* (2014) on dried and three months stored okra leaves sold in markets. A 5% increase in the number of total viable counts for open sun-dried ILVs was observed. The effect of storage on yeast and moulds growth was negligible with the range being from 1.8-2.9 log₁₀ cfu/g to 1.6-2.6 log₁₀ cfu/g. The low counts could indicate a good drying although the fact that yeasts and moulds were identified during the storage shows that their growth is possible in dried ILVs. A 26% increase in the number of coliform counts was observed, suggesting that possibility of ILVs spoilage after the 3 months of storage may occur.

The growth rate of the micro-organisms is responsible for spoilage which depends on storage temperature, relative humidity and gas compositions of the surrounding atmosphere. The

protection of packaged food from attack by micro-organisms depends on the mechanical integrity of the package e.g. the absence of breaks and seal imperfections, and on the resistance of the package to penetration by micro-organisms (Schönfeldt and Pretorius, 2011).

Table 9. The microbial quality in log₁₀ cfu/ g of stored ILVs

ILVs	Treatment	Total viable counts	Yeasts and moulds counts	Coliform counts
		(log ₁₀ cfu/g)	(log ₁₀ cfu/g)	(log ₁₀ cfu/g)
Spiderplant	H810	6.8 ^b ±0.9	2.0 ^a ±1.3	1.4 ^a ±0.1
	H905	6.1 ^a ±1.1	2.3 ^a ±1.5	1.3 ^a ±1.6
	S810	6.4 ^b ±0.9	1.8 ^a ±1.8	1.5 ^a ±0.8
	S905	5.9 ^a ±0.3	1.6 ^a ±0.6	1.2 ^a ±0.5
	O3B	7.6 ^c ±0.7	2.3 ^a ±1.3	1.9 ^a ±1.2
Slenderleaf	H810	6.4 ^b ±1.1	2.5 ^b ±1.9	1.6 ^a ±1.5
	H905	5.5 ^a ±0.4	1.9 ^a ±0.5	1.8 ^a ±1.2
	S810	6.5 ^b ±1.6	2.5 ^b ±0.9	1.5 ^b ±1.4
	S905	5.7 ^a ±0.8	2.3 ^a ±1.6	1.8 ^a ±0.9
	O3B	6.9 ^b ±1.2	2.4 ^b ±0.9	2.0 ^b ±1.3
Cowpeas	H810	5.8 ^a ±0.7	2.1 ^a ±1.5	1.5 ^a ±0.4
	H905	6.2 ^a ±0.4	1.8 ^a ±0.6	1.8 ^a ±0.9
	S810	6.3 ^b ±0.2	2.6 ^b ±1.1	1.3 ^a ±1.3
	S905	5.9 ^a ±0.6	2.1 ^a ±0.3	1.7 ^a ±0.6
	O3B	6.8 ^b ±1.0	2.5 ^b ±0.5	1.8 ^a ±1.3

Values are means (log₁₀ cfu/g) ± standard deviation of three months stored ILVs. CfU/ g =colony forming unity per gram. Values in a column followed by different letter notations are significantly different P≤0.05, H905-Blanched at 90°C /5 min hot air dried, S810- Blanched at 80°C /10 min solar-dried, S905-Blanched at 90°C/5min solar-dried, O3B- Blanched at 100°C/30 min Open sun-dried.

Additionally, since the bacterial isolate was identified using morphological and biochemical characteristics (Gram staining, oxidase and catalase tests). The organisms

characterised to be present were *Staphylococcus aureus*, *Bacillus spp.* *Flavobacterium spp.*, *Micrococcus spp.* and lactic acid bacteria from the dried ILVs (Table 10).

Table 10. Gram's reaction and biochemical characteristics of bacterial isolates

Lab code	Gram reaction	Catalase	Oxidase	Probable identity
SPH8	GPC	+	-	<i>Staphylococcus aureus</i>
SPS9	GPC	+	-	Lactic acid bacteria
SPO3	GNR	+	-	<i>Flavobacterium spp</i>
SLH9	GPR	-	+	<i>Bacillus spp.</i>
SLS8	GPC	+	+	<i>Micrococcus spp</i>
SLO3	GNR	+	-	<i>Flavobacterium spp</i>
CH9	GPC	+	+	<i>Micrococcus spp</i>
CS8	GPC	+	-	<i>Staphylococcus aureus</i>
CO3	GPR	-	+	<i>Bacillus spp.</i>

GPC- Gram positive cocci; GNR-Gram negative rod; GPR- Gram positive rod, SP-Spiderplant, SL-Slenderleaf, C-Cowpeas, H-Hot air oven-dried, S-Solar-dried, O-Open sun-dried, 8- Blanched at 80°C/10 min, 9-Blanched at 90°C/5 min, 3-Blanched at 100°C/30 min.

Staphylococcus aureus bacteria were isolated from the stored spiderplant and cowpeas initially blanched at 80°C/10 min. The organism is very resistant to heat, drying and radiation. *Micrococcus spp.* was isolated from slenderleaf and cowpeas that had been blanched at both 80°C/10 min and 90°C/5 min although the organism is not pathogenic; it is primarily on mammalian skin and in soil.

The stored open sun-dried ILVs initially blanched at higher temperatures with a longer time (100°C/30 min) had a higher possibility of being contaminated with *Flavobacterium spp.* (Table 10), thus, the organisms seems to be resistant to higher temperatures of heat. *Bacillus spp.* was able to tolerate storage at 18°C for the three months while lactic acid bacteria were only found to be present in spiderplant blanched at 90°C/5 min. The presence of the microbial organisms after the three months of storage is an indication that processing and packaging methods can have an effect on microbial contamination. The relative humidity, moisture content

of the product, storage temperature and conditions influence the growth of micro-organisms on dried vegetables (Pitt and Hocking, 1997).

Additionally, prolonged storage in poorly ventilated containers increases moisture content of the commodity, rendering the stored products more susceptible to mould growth and toxin production (Chourasia, 1995). It is evident that blanched-dried ILVs can support growth of micro-organisms during storage, thus consumers in the community need to be informed about proper storage in order to improve product quality and reduce microbial contamination.

CHAPTER FIVE

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

Solar-drying especially after blanching at 80°C/10 min, is an effective dehydration method for ILVs; it retains a good proportion of vital nutrients in the final product except the minerals. Additionally, the microbial load is significantly lower in solar dried ILVs and very similar to those recorded in the standard hot air drying methods. This study shows that blanching at 80°C/10 min and solar drying is a potential option that can be used as a local preservation technique for ILVs in Kenya. Storing blanched-dried vegetables for up to three months leads to minimal losses of moisture, β -carotene, ascorbic acid, crude protein, crude fibre and minerals.

5.2 Recommendations

In view of the results of this study, the following recommendations are made:

1. In order to get a more clear understanding of the impact of blanching and solar drying on the quality of the ILVs, further studies to evaluate the quality of the cooked ILVs should be examined.
2. A study should be carried out on the storage of the dried ILVs in other types of packaging material, other than the zip lock bags.
3. Storage of the dried vegetables at higher temperatures than 18°C and for longer periods than the three months in this study should be carried out to determine the nutritional losses and microbial quality of longer stored ILVs. Similar studies should be carried out on other popular traditional vegetables.
4. A study to determine why the number of total viable counts in blanched-dried ILVs is higher than the recommended counts is also suggested.
5. The technology tested in the study should be adopted by the local communities and women groups for preservation of ILVs. Additionally, a promotion program to increase acceptability and consumption of solar-dried ILVs among the rural communities is recommended.

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APPENDIX

Appendix I: Publications

1. Njoroge, E.W., Matofari, J., Mulwa, R.M.S. and Anyango, J.O. (2015). Effects of blanching time/temperature combination coupled with solar-drying on the nutritional and microbial quality of indigenous leafy vegetables in Kenya. *African Journal of Food Science and Technology*. **6 (7)**: 209-219
2. Njoroge, E.W., Matofari, J., Mulwa, R.M.S. and Anyango, J.O. (2015). Effects of blanching time-temperature combinations and drying methods on nutritional composition of indigenous leafy vegetables in Kenya. Paper presented at the 9th Egerton University International Conference, Kenya, March, 2015.
3. Esther Wangari Njoroge, Joseph Matofari, Richard M.S. Mulwa and Joseph Anyango. 2015. Effects of Blanching Time- Temperature Combinations and Drying Methods on Nutritional Composition of Indigenous Leafy Vegetables in Kenya. In: Proceedings of the KAPAP CGS Collaborative Research Stakeholders Conference. 10th – 12th June, KALRO Headquarters, Nairobi, Kenya pp 185 - 191.
4. Esther Wangari Njoroge, Richard M.S. Mulwa and Joseph Matofari. (2016). Effects of blanching time/temperature combinations on the microbial and storage quality of solar-dried indigenous leafy vegetables in Kenya. Paper presented at the 10th Egerton University International Conference, Kenya, March, 2016.