LEVELS OF SELECTED MACRONUTRIENTS IN VARIETIES OF PUMPKIN (*cucurbita spp*) GROWN IN FOUR SELECTED DISTRICTS IN LAKE VICTORIA BASIN

BY

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A thesis submitted in partial fulfillment of the requirements for the award of the degree of Master of Science in the School of Pure and Applied Sciences of Kenyatta University

OCTOBER, 2015
DECLARATION

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

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DEDICATION
To my beloved wife Jackline and sons, Enosh and Elias, from whom I derived a lot of inspiration and need for hard work.
ACKNOWLEDGEMENT

This work has been possible through the contribution of many hands without which it could have been difficult to achieve the results. Special thanks go to my supervisors Dr. Alice Ondigi for initiating the idea of the work and Prof. Hudson Nyambaka for guiding me while preparing the work.

I am grateful to VICRES for giving me financial support to supplement my research expenses without which it couldn’t have been possible.

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My special thanks go to my wife Jackline for constant encouragement and patience throughout the period of study and my sons for their concern on the progress of the program; my friends Ombui Akama and Onkoba Benjamim for moral support and encouragement to always look forward and never lose hope before achieving the target. Finally, glory to God almighty for His kindness and for granting me good health throughout the period of my studies.

Amen.
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ABBREVIATIONS AND ACRONYMS

AAS  Atomic Absorption Spectroscopy  
ADB  African Development Bank  
AES  Atomic Emission Spectroscopy  
ANOVA Analysis of Variance  
ASK  Agricultural Society of Kenya  
ATP  Adenosine Tri-Phosphate  
CV  Coefficient of Variance  
CVD  Cardiovascular Disease  
DHEA  Di Hydro Epi-Androstenedione  
DNA  Dioxyribo-Nucleic Acid  
DRI  Dietary Reference Intake  
FAO  Food Agriculture Organization  
LOD  Limit of Detection  
LVB  Lake Victoria Basin  
LVBC  Lake Victoria Basin Commission  
RDA  Recommended Dietary Allowances  
RDI  Recommended Daily Intake  
RNA  Ribose Nucleic Acid  
RNI  Reference Nutrient Intake  
RSD  Relative Standard Deviation  
SFA  Saturated Fatty Acids  
SPSS  Statistical Program for Social Scientists  
UFA  Unsaturated Fatty Acids  
UNEP  United Nations Environmental Program  
USDA  United States Department of Agriculture  
UV  Ultra-Violet  
WHO  World Health Organization
ABSTRACT

Lake Victoria Basin is one of the regions in sub-Saharan Africa which is densely populated and majority of the inhabitants are poor economically. Cases of diseases related to nutrient deficiency, child mortality, high HIV and AIDS have increased in the recent years due to malnutrition and undernourishment as the cost of basic foods have been on the rise. One way of reducing malnutrition is increased consumption of indigenous fruits and vegetables which offer variety of nutrients. The region has various indigenous foods not commonly consumed among them being pumpkins. Studies on pumpkin production, utilization and the nutritional content involving micronutrients have shown that different varieties of pumpkins contain different levels of nutrients. The need to promote varieties with higher levels of nutrients requires that levels are known. This study sought to determine varieties of pumpkins grown and the levels of selected macronutrients—proteins, carbohydrates, lipids, potassium, sodium, calcium, magnesium and phosphorus from Gucha & Busia in Kenya, Jinja in Uganda and Tarime in Tanzania. Identification of farmers was done through snowball (chain/referral) sampling method to obtain the pumpkin fruits. The levels of proteins were determined using kjeldahl method, carbohydrates using athrone method, lipids using soxhlet extraction, sodium and potassium using flame atomic emission spectroscopy, calcium and magnesium using flame atomic absorption spectroscopy and phosphorus using UV-visible spectrophotometry. Calibration curves of standards gave $R^2 > 0.995$ which were used to determine the concentrations. Samples of six pumpkin varieties identified namely banana, carnival squash, crown prince, green kabacha, butternut and bottle guard were collected for macronutrient analysis. The level of proteins in the fruit flesh was $2.525 \pm 0.620$ g per 100g sample while in seeds was $34.882 \pm 1.805$ g, carbohydrates $7.604 \pm 1.128$ g in the fruit flesh and $3.118 \pm 1.054$ g in seeds, lipids in fruit flesh was $1.737 \pm 1.031$ g and $40.581 \pm 6.543$ g in seeds, phosphorus was $29.439 \pm 2.371$ ppm in the fruit flesh and $702.468 \pm 6.910$ ppm in seeds, potassium was $326.698 \pm 20.73$ ppm in the fruit flesh and $83.981 \pm 621$ ppm in seeds, sodium was $90.034 \pm 14.23$ ppm in the fruit flesh and $36.061 \pm 4.24$ ppm in seeds, magnesium was $21.144 \pm 3.721$ ppm in the fruit flesh and $57.261 \pm 2.537$ ppm in seeds, and finally, calcium was $64.654 \pm 4.613$ ppm in the fruit flesh and $99.659 \pm 8.731$ ppm in seeds. The levels of these nutrients varied significantly with variety and crown prince was superior over the others which support the view that pumpkins can be used to supplement nutrient availability to the inhabitants if grown.
CHAPTER ONE
INTRODUCTION

1.1 Background of the study

Food security has become a major issue in the world with prices of basic foods increasing rapidly. The middle and lower class income earners are unable to provide their households with enough food and a well-balanced diet which has an impact on the health status of an individual (Mathenge, 1995). Developed world has made significant progress towards poverty reduction through application of modern methods of food production while developing countries, majority in Africa continue to experience rise in poverty. While the food security in developing countries has worsened since 1970, the population keeps on increasing; resulting in a large proportion of malnourished people ranging between 33-55 percent with upper limit being from sub-Saharan Africa (Rosegrant, 2005)

In sub-Saharan Africa, it is estimated that one out of six people in the region remain undernourished, which in turn affects children’s mental development and educational achievements (FAO, 2002). Lake Victoria Basin (LVB) which covers regions around Lake Victoria in East Africa supports an approximate population of 35 million people of which more than 50 % of the inhabitants are poor (Swallow et al., 2002). Due to increasing population, land availability has hindered large scale farming which has resulted to food shortage thus causing
underfeeding and malnutrition among the people (Africa Development Bank, 2003).

Trends in agriculture have changed from the production of indigenous food crops like millet, sorghum, potatoes and pumpkins which offer a variety of nutrients required for good human health to exotic crops like maize, rice, wheat, which have limited nutrients (Republic of Kenya, 2004). Indigenous foods contain high levels of macronutrients unlike exotic crops. The cost of production for exotic crops is high resulting to increased prices thus majority of households cannot afford them (Mathenge, 1995). The result is malnutrition and increased cases of non-communicable diseases. Promotion of some indigenous fruits and vegetables that are increasingly being ignored is likely to reverse the trend.

Little concern has been shown towards the growing of pumpkins in East Africa, perhaps due to lack of information about the nutritional content of the various varieties (Jaetzold and Schmidt, 1983). While Tanzania has paid some attention on pumpkins, Kenya has little documentation on the plant suggesting that little attention is given towards its production (Hamisy et al., 2002). Little or no step has been taken to promote its production as compared to other crops like maize, rice, where most research is concentrated. This is supported by the fact that even the farm management handbook of Kenya which gives guidelines on production
and marketing of a wide range of crops in Kenya does not give information on pumpkins.

Lake Victoria Basin has got varied climatic conditions, different soil fertilities as well as different farming practices which affects the nutritional composition of crops grown in the region. There is need therefore for more research on the pumpkin to get more information. On the nutritional composition of different varieties found in the four regions, this can attract attention towards increased production of the crop. Usually the fruit flesh, seeds and leaves are the parts which are consumed.

1.2 Problem statement and justification

Developing countries are faced with economic constraints which limit their ability towards food security (Abila, 2003). Increasing population has led to reduced land per household which has resulted to subsistence farming. High poverty levels have hindered the application of modern agricultural skills so as to produce enough food to sustain the growing population (Keith, 2008). This has resulted in underfeeding and/or poor feeding that does not supply all the essential nutrients; a situation that affects the health of the population mainly children, the aged and people living with HIV and AIDS (Awange and Obiero, 2006).
Lake Victoria basin supports more than 35 million people who face challenges of food insecurity and high incidents of malnutrition due to poverty (Abila, 2003). The malnutrition facts in the region are more aggrieving given that it has the highest HIV prevalence in East African Community (Drime et al., 2009). The food security in the region depends on maize, beans, finger millet, cassava, sorghum and bananas (Maitima et al., 2010) most of which are limited in nutrient. It is appropriate that food insecurity and malnutrition that are common in the region be addressed through intervention of indigenous crops such as pumpkins which have been neglected and underutilized.

A study by Ondigi et al. (2008) that assessed the production practices and utilization of pumpkins by small farm holders in the LVB looked at the place of pumpkin as a food crop, purpose of planting pumpkins (domestic or commercial), importance of pumpkins (nutritional or medicinal), approaches to planting, problems hampering cultivation of pumpkins and processing, storage & preservation of pumpkins among others.

The study found that farming was the main source of income in the region as the climatic conditions were favorable. Among the crops produced, pumpkins were not considered as a priority food crop as well not used to generate income by majority of the house-holds living in the region. The study recommended for
investment in value addition of pumpkins as well evaluation of nutritional composition of the crop so as to encourage people to venture into their cultivation.

In another study by Echessa (2012) which aimed at evaluating the varieties and levels of micronutrients of pumpkins grown by small holder farmers in the region, a total of 10 varieties from the three cucurbit species were identified. These include crown prince, banana, carnival squash, valenciano, bottle guard, green kabacha, connecticutfield, butternut squash, super delight and red kabacha. The nutrients included vitamins A, B (1, 2, 3 & 6), C & E and trace elements iron, zinc and selenium. From the study, there was need to determine the levels of macronutrients of these varieties so as to get the full information on the nutritional content.

This study, therefore, sought to determine the levels of selected essential macronutrients in the pumpkin varieties commonly grown in four selected districts in the LVB. These nutrients include proteins, carbohydrates, lipids (oils), phosphorus, potassium, sodium, calcium and magnesium. The results will complement those from the two studies Ondigi et al (2010) and Echessa (2012) thus form the basis for promoting the production of pumpkins especially those varieties rich in nutrients. This will help curb malnutrition and undernourishment since the pumpkin plant has a variety of nutrients, is easy to cultivate, does not need large track of land and is not labor intensive.
1.3 **Hypothesis**

Farmers in LVB know the pumpkin varieties they grow and these varieties contain significant levels of essential macronutrients.

1.4 **Objectives**

1.4.1 **General objective**

To identify varieties and determine the level of selected macronutrients in the varieties of pumpkins commonly grown in four regions of the LVB.

1.4.2 **Specific objectives**

(i) To identify the varieties of pumpkins commonly grown in Jinja, Busia, Gucha and Tarime districts in the LVB.

(ii) To determine the level of proteins, carbohydrates, lipids, in fruit flesh and seeds of pumpkins commonly grown in Jinja, Busia, Gucha and Tarime districts in the LVB.

(iii) To determine the level of sodium, potassium, magnesium, calcium and phosphorus in fruit flesh and seeds of pumpkins commonly grown in Jinja, Busia, Gucha and Tarime districts in the LVB.

1.5 **Significance of the study**

The results from the study indicates varieties of pumpkins commonly grown and their macronutrient levels, which forms the basis for sensitization on the need to
cultivate the varieties as a source of supply of nutrients leading to reduced malnutrition. Also it is with these findings that efforts can be made to promote use of pumpkins as a cheap source of nutrients to curb malnutrition and undernourishment.

1.6 Scope and limitations of the study

Four districts Jinja (Uganda), Tarime (Tanzania) and Busia & Gucha (Kenya) in the LVB were selected for the study as a representative of the region. Only pumpkin varieties sampled from the four districts were analyzed.

The storage duration for the samples awaiting analysis, different weather conditions and soils from the four districts as well as the crossbreeding among the different varieties were not considered in the study. The farming practices like application of fertilizers, herbicides and insecticides that were likely to be different in the regions were not considered though this could affect the results.
CHAPTER TWO

LITERATURE REVIEW

2.1 Introduction

This chapter presents a review of the state of food security and pumpkin growing in Lake Victoria Basin, importance of pumpkins and the analytical techniques used.

2.2 Food security situation in LVB

The increase in population has an impact on the food security and general living standard of the people. These changes have affected mainly the developing countries majority of which are in sub-Saharan Africa. Availability of arable land and application of modern technology to boost food production is a challenge to these countries due to high poverty levels. Over ninety percent of food production in sub-Saharan Africa is grown under rain fed agriculture (Inter-academy Council, 2004) and are vulnerable to adverse conditions. Increased price of fertilizers and poor infrastructure has led to reduction in food production as the soils continue to degrade (Kherallah, 2002).

The LVB supports over 35 million inhabitants of which over 50 % are poor and entirely depend on agriculture for their livelihood (Awange and Obiero, 2006;
Kinuthia, 2008). The region is endowed with fertile soils and falls within a good agro-climatic zone and enjoys surplus of labor (LVBC, 2010). For instance, in Tanzania, 90% of caloric intake per person comes from vegetable products while 6% is from animal products (FAO, 2006). This shows that majority rely on crop production as a solution for food security as 1.7% comes from banana while 15 – 54% comes from cassava.

Kenya’s population in 2009 was 38.6 million which increased to 41.6 million in 2011 and it is estimated to increase to 52.6 and 65.9 million in the year 2020 and 2030 respectively (Republic of Kenya, 2011). The Western Kenya counties have the highest population densities of between 431.3 to 521.6 persons per Km². The Republic of Kenya (2011) report also gave the HIV/AIDS preference among the adult ages (19 - 49 years) as 13.9% in Nyanza with women leading by 16.0% and men by 11.4%, Western province second with 6.6% out of which 9.2% was women and 3.4% men. The two regions form part of the LVB.

The socio-economic studies of LVB have shown that there is a high reliance on indigenous vegetables as a source of nutrients (UNEP, 1995; Chadha et al., 2006). In another study carried out in the region, an estimate of 40% children and 6% mothers are malnourished (Geheb et al., 2007); thus there is need to look for cheap and reliable source of food which can supply essential nutrients as well as being easy to cultivate.
A study done by Abele et al. (2006) on the northern districts of Tanzania around the LVB show the food preference among the households in the region ranging with cassava (84%), maize (87%), sweet potato (61%), beans (30%) and banana (26%) in contrast to Uganda which depends mainly on cassava and banana for domestic food production. The study further found that although the region may have sufficient access to food in good harvest years, they are quite vulnerable in years of drought or high disease pressure. Both maize and cassava which are major food crops are prone to severe weather conditions and therefore the need for interventions in terms of considering other crops like pumpkins to boost in food security in the region.

To improve the living standards of the inhabitants, the Western countries through the World Bank and East African Community have embarked on promoting sustainable development through evaluation of indigenous crops like cassava, yams, potatoes and pumpkins with a view to curb food shortage (Partnerships for sustainable development, 2004). Other issues addressed include environmental degradation, deepening poverty and poor health standards.

### 2.3 Pumpkins cultivation in LVB

The pumpkins (*Curcubita ssp.*) belong to the family of cucurbitaceae, with approximately 30 species and nine genera that includes squash, watermelon and
cucumbers (Integrated Taxonomic Information System, 2009; Michael, 2014). It has been grown for centuries mainly in the USA and Austria and the fruit, seeds and leaves are consumed (Gerrior and Zizza, 1994). The seeds are rich in oils (Unsaturated Fatty Acids), proteins and zinc which are essential nutrients while the leaves, fruits flesh and flowers are rich in vitamins and minerals (Esquinas and Gulik, 1983). Table 1.1 gives a list of some curcubitaceae family varieties commonly grown and consumed.
Table 2.1:- Some of the varieties of *curcubitaceae* family (pumpkins)

<table>
<thead>
<tr>
<th>Species (Botanical name)</th>
<th>Variety (Common/ English name)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Curcubita maxima</em></td>
<td>Banana</td>
</tr>
<tr>
<td></td>
<td>Carnival squash</td>
</tr>
<tr>
<td></td>
<td>Crown prince</td>
</tr>
<tr>
<td></td>
<td>Super delight</td>
</tr>
<tr>
<td></td>
<td>Valenciano</td>
</tr>
<tr>
<td><em>Curcubita pepo</em></td>
<td>Connecticutfield</td>
</tr>
<tr>
<td></td>
<td>Green kabacha</td>
</tr>
<tr>
<td></td>
<td>Red kabacha</td>
</tr>
<tr>
<td></td>
<td>Autumn Gold</td>
</tr>
<tr>
<td><em>Curcubita moschata</em></td>
<td>Butternut squash</td>
</tr>
<tr>
<td><em>Lagenaria siceraria</em></td>
<td>Bottle gourd/white gourd/zucchini</td>
</tr>
<tr>
<td><em>Lumina</em></td>
<td>White pumpkin</td>
</tr>
<tr>
<td><em>Citrullus lanatus</em></td>
<td>Water melon</td>
</tr>
<tr>
<td><em>Cucumis sativus</em></td>
<td>Cucumber</td>
</tr>
<tr>
<td><em>Cucumis melo</em></td>
<td>Melon</td>
</tr>
</tbody>
</table>


Production of pumpkins in the LVB has not been given much attention since no sound literature is available to show the extent of production in spite of the fact
that the region can support agricultural activities. In Kenya for instance, pumpkins are not widely grown since even the handbook for small scale holders give shallow information relating to pumpkins compared to other food crops like maize, rice, millet, beans (Jeatzold and Schmidt, 1983).

In Tanzania, some documentation on pumpkin varieties is available (Hamisy et al., 2002). It is however observed that in much of East Africa, pumpkins are mainly cultivated as a marginal crop often on the edges of fields of crops such as maize, sorghum or millet (Hamisy et al., 2002). Less than 10% of the households in the Lake Victoria region of East Africa cultivate pumpkins as a source of food and livelihood (Republic of Kenya, 2003).

With increasing population and hiking of food prices, the cases of malnutrition and under nourishment are rising. This in turn has a devastating effect on any population because it increases both mortality and morbidity rates, diminishes the cognitive abilities of children and lowers their education attainment, reduces labor productivity and reduces the quality of life of the affected people (FAO, 2002). As a remedy, urgent measures such as dietary diversification, food sufficiency and bio-fortification are needed.

Indigenous crops like cassava, potatoes, yams and pumpkins have a variety of nutrients and can help reduce malnutrition and undernourishment as well reduces
poverty (Nansubuga, 2007). The utilization and improvement of productivity through cultivation of such under-utilized crops including pumpkins would help reduce genetic erosion of the crops (Chweya, 1997; Isutsa et al., 2013). This has not been easy to achieve especially pumpkins. A study carried out by Ondigi et al. (2008) on the production and utilization of pumpkins in the LVB indicates that farmers give pumpkin production a low attention compared to other crops like maize, cassava, beans and bananas. In terms of ranking, pumpkins were ranked sixth after maize, bananas, beans, cassava and sorghum.

2.4 Importance of pumpkins

2.4.1 Nutritional content

Analysis of nutritional composition of pumpkins has shown that pumpkins can supply a variety of nutrients most of which are essential for good human health (Roberts, 2006; Breyer, 2014). Pamplona and Roger (2004) have shown that 100 g of pumpkin fruit serving contains 0.500 % fiber, 0.800 % minerals, 6.00 % carbohydrates, 0.100 % proteins and 91.6 % water. However, these values may vary due to origin of pumpkin samples, soil and climatic conditions of the area where they are grown, and the variety of the pumpkin analyzed (Pumpkins and more, 2007; Pamplona and Roger, 2010). Table 2.2 gives percentage values of some nutrients produced by 100 g of the pumpkin fruit.
Table 2.2: Percentage value (based on 2000 caloric diet) produced by 100 g of pumpkin fruit

<table>
<thead>
<tr>
<th>Composition</th>
<th>Nutritional value (STD Units)</th>
<th>Composition</th>
<th>Nutritional value (STD Units)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy</td>
<td>109 kJ</td>
<td>Niacin</td>
<td>0.800 NE</td>
</tr>
<tr>
<td>Proteins</td>
<td>1.00 g</td>
<td>Foliate</td>
<td>16.2 mg</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>6.00 g</td>
<td>Calcium</td>
<td>21.0 mg</td>
</tr>
<tr>
<td>Fiber</td>
<td>0.500 g</td>
<td>Phosphorus</td>
<td>44.0 mg</td>
</tr>
<tr>
<td>Vitamin A</td>
<td>160 µg RE</td>
<td>Magnesium</td>
<td>12.0 mg</td>
</tr>
<tr>
<td>Vitamin B1</td>
<td>0.050 mg</td>
<td>Iron</td>
<td>0.800 mg</td>
</tr>
<tr>
<td>Vitamin B2</td>
<td>0.110 mg</td>
<td>Potassium</td>
<td>340.0 mg</td>
</tr>
<tr>
<td>Vitamin B6</td>
<td>0.061 mg</td>
<td>Total fat</td>
<td>0.100 mg</td>
</tr>
<tr>
<td>Vitamin C</td>
<td>9.00 mg</td>
<td>Sodium</td>
<td>1.00 mg</td>
</tr>
<tr>
<td>Vitamin E</td>
<td>1.06 mg</td>
<td>Zinc</td>
<td>0.320 mg</td>
</tr>
</tbody>
</table>


Studies on pumpkin seeds have also shown that the seeds have higher amounts of nutrients compared to the fruit flesh, possibly because they act as storage parts of the plant (Mansour et al., 1993; Mateljian George Foundation, 2008). The values of the nutrients have been found to differ from one study to the other depending on the origin and environment where the pumpkins are grown (Heo et al., 1998; Jang et al., 2001). Some available literatures include; Power your diet home page (2012), USDA Food & Nutrition Centre home page (USDA, 1990) and ‘The
world’s healthiest foods’ home page (Mateljian George Foundation, 2008). The values of some nutrients in pumpkin seeds are given in table 2.3.

Table 2.3:- Nutritional content of pumpkin seeds per 100 g

<table>
<thead>
<tr>
<th>Composition</th>
<th>Nutritional value (STD units)</th>
<th>Composition</th>
<th>Nutritional value (STD Units)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proteins</td>
<td>29.0 g</td>
<td>Copper</td>
<td>1.27 mg</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>10.69 g</td>
<td>Selenium</td>
<td>9.5 mcg</td>
</tr>
<tr>
<td>Fats</td>
<td>49.02 g</td>
<td>Vitamin A</td>
<td>7.05 IU</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>1174.6 mg</td>
<td>Vitamin B1</td>
<td>0.07 mg</td>
</tr>
<tr>
<td>Potassium</td>
<td>786.6 mg</td>
<td>Vitamin B2</td>
<td>0.14 mg</td>
</tr>
<tr>
<td>Sodium</td>
<td>17.6 mg</td>
<td>Vitamin B6</td>
<td>0.10 mg</td>
</tr>
<tr>
<td>Magnesium</td>
<td>550.3 mg</td>
<td>Vitamin C</td>
<td>1.76 mg</td>
</tr>
<tr>
<td>Calcium</td>
<td>52.9 mg</td>
<td>Vitamin E</td>
<td>0.56 mg</td>
</tr>
<tr>
<td>Manganese</td>
<td>4.5 mg</td>
<td>Vitamin K</td>
<td>4.58 mg</td>
</tr>
<tr>
<td>Zinc</td>
<td>7.65 mg</td>
<td>Niacin</td>
<td>0.4 mg</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Folate</td>
<td>56.0 mcg</td>
</tr>
</tbody>
</table>

Source:- Decuypere, (2000)

Good healthy results from eating a balanced diet that supplies the body with essential nutrients both qualitatively and quantitatively. For instance, vitamins boost the immune system of the body against diseases, carbohydrates supply energy to the body tissues, and proteins help in tissue regeneration/replacement. Mineral elements help in important metabolic processes while water is essential for maintenance of shape/posture (Gibney, 2002).
Proteins are vital for the structural and metabolic operations of the human body. They form major components of the body tissues like enzymes, muscles, hormones, skin, antibodies, buffering effect and transportation of lipids. They may act as source of energy in case of insufficiency however this may cause muscle wasting and retarded growth in children; a condition known as protein – energy malnutrition (P.E.M). This condition is common with people living with HIV and AIDS, TB, Anorexia nervosa and cancer coxheixa. Other complications associated with protein deficiency include poor healing of wounds, anemia, oedema and increased susceptibility to infections. Sources of proteins include animal products (60 %) and plants (40 %) (Briony, 2001).

Carbohydrates are responsible for instant source of energy to the body depending on the state and age of an individual. Too much carbohydrate in the body can be stored as glycogen for later use (Mckinley Health Centre, 2007). Deficiency of carbohydrates will lead to conversion of either proteins or fats to meet the energy requirement since body cells cannot function without energy. Common sources of carbohydrates include cereals, potatoes, milk and fruits.

Fats (lipids) form major components of body cells both structurally, metabolically and storage. Lipids acts as a source of essential fatty acids, carriers of fatty soluble vitamins and anti-oxidants, insulation against heat loss, provide protection layer around essential organs like kidney, heart, brain, nerves and other tissues.
Usually unsaturated fatty acids (UFA) are recommended since saturated fatty acids (SFA) can lead to obesity and cardio-vascular disease (CVD) (Briony, 2001). Sources of fats include mainly meat products, milk and milk products and cereals.

Calcium is essential for the formation of skeletal structures in form of hydroxy appertite, \((\text{Ca(PO}_4\text{)}_6\text{)(OH)}_2\)). About 1% of \(\text{Ca}^{2+}\) ions are involved in membrane transportation, muscle contraction, nerve transmission and blood clotting. Absorption of the element depends on the body requirement which depend on the age of an individual. For children in ages 1-3 years, they require 350 mg/day; 4-6 years, 450 mg/day; 7-10 years 550 mg/day and 11-18 years males require 1000 mg/day while females require 800 mg/day and adults 700 mg/day. The main source of calcium includes milk and dairy products, fish, green leafy vegetables and hard water. Lack of calcium cause stunted growth and failure to achieve peak bone density which may increase the risk of osteoporosis. However, increased intake impairs absorption of zinc (Janet, 2000).

Magnesium is involved in many enzyme systems like those dealing with decarboxylation or phosphate group transfer and energy release. It contributes to skeletal development together with calcium, protein synthesis, muscle contraction and neuro- transmission (Briony, 2001). The RNI is 300 mg/day for male while females require 270 mg/day. Sources of magnesium include green vegetables as
components of chlorophyll, meat, cereals and hard water. It is usually stored in the body skeleton and deficiency may lead to muscle weakness, cramps, hypertension and cardiac arrhythmias.

Sodium forms part of extra cellular fluid and regulates fluid balance, blood pressure and trans-membrane gradients. RNI is 69-460 mg /day and deficiency may lead to anorexia and mental confusion. Potassium forms part of intracellular fluids and is involved in acid-base regulation, fluid balance, muscle contraction and nerve conduction. The RNI is 590-3500 mg/day and deficiency may lead to mental impairment, skeletal muscular weakness and heart muscle failure (cardiac arrest) (Gibney, 2002).

Phosphorous is associated with calcium in bones as hydroxy-appetite. It forms part of nucleic acids (DNA and RNA) and releases energy to cells as ATP. It acts as a medium of intracellular effects of hormones. The RNI for phosphorus is 775 mg/day for males of age 11-18 years and 625 mg/day for females while those above 19 years the daily requirement is lower (550 mg/day). Sources of phosphorus include milk and milk products, meat and meat products, vegetables and potatoes. Deficiency leads to symptoms related to myophathy, respiratory and cardiac failure, and neuropathy and tissue hypoxia. Too much phosphorus may lead to renal failure (Briony, 2001).
The United States Department of Agriculture which publishes tables of Dietary Reference Intake (DRI) of nutrients gives values similar to the Reference Nutrient Intake (RNI). However, the USDA’s DRI are slightly higher than those of RNI. Table 2.4 gives the RNI values compared to USDA’s DRI (USDA, 1990).

Table 2.4: - RNI and DRI for some elements

<table>
<thead>
<tr>
<th>Element</th>
<th>RNI</th>
<th>DRI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mg</td>
<td>270 mg/day for female and 300 mg/day for male adults.</td>
<td>350 mg/day (adult women) and 450 mg/day (adult men)</td>
</tr>
<tr>
<td>Ca</td>
<td>350 - 550 mg/day for children, 800 - 1000 mg/day for adolescents and 700 - 800 mg/day for adults.</td>
<td>2500 mg/day for children, adolescents and pregnant/lactating mothers</td>
</tr>
<tr>
<td>Na</td>
<td>69 - 460 mg/day</td>
<td>500-750 mg/day and not more than 14 g daily</td>
</tr>
<tr>
<td>K</td>
<td>590 – 3500 mg/day</td>
<td>2000 mg/daily and not more than 5900 mg/day</td>
</tr>
<tr>
<td>P</td>
<td>Children and adolescents 625 – 775 mg/day and adults 550 mg/day.</td>
<td>Infants under 6 months (100 mg/day), 7-12 months (275 mg/day), 1-3 yrs (460 mg/day) and 4-8 yrs (1250 mg/day)</td>
</tr>
</tbody>
</table>

Source: Gibney, (2002)
2.4.2 Medicinal value

Pumpkins are said to have medicinal ability whereby seeds treat kidney stones (Kirigo, 2011) perhaps due to the high level of phosphorus. It is also used to treat prostate cancer (Mateljian George Foundation, 2008) and the sap is used to treat snake bite, stomach and renal disorders (Pumplona and Roger, 2003; Pamplona and Roger, 2004). Other studies have reported that pumpkin seeds have DHEA blocking action which helps in preventing prostate cancer (Ray, 2006; Gossell-Williams et al., 2006.), and prevent learning disorders (Herbs, 2002; Earls, 2014).

The oils in seeds also contain certain phytochemical compounds which help in preventing diabetic nephropathy (Power your diet, 2012) and helps improve the health of the skin (Sitaranikhil, 2014). The unsaturated fatty acids in pumpkin oils help in maintaining healthy blood vessels, nerves and tissues (Levin and Rachel, 2008). In general, pumpkins are important both nutritionally and medically (Mukesh et al., 2010; Sarah, 2012).

2.5 Analytical techniques for measuring macronutrients

Analysis of foods requires different analytical techniques depending on the nutrients to be determined. Some of the techniques for different nutrients are discussed below.
2.5.1 Analytical technique for measuring proteins

The kjeldahl and Dumas methods are the commonly used in determining protein concentration in a food sample (Kumar, 2010). Kjeldahl method uses titrimetric technique in which a food sample is digested with a strong acid to release nitrogen to form ammonium salt. The principle behind this technique is that proteins are converted into ammonium salt which can be determined by a suitable titration to give the amount of protein using a conversion factor (Ronald and Sawyer, 1991).

The Dumas method is an automated instrumental technique used for rapid determination of protein concentration of a food sample (Iqbal, 2005). This works on the principle that proteins contain nitrogen and that the amount of nitrogen produced from a known mass of food can be used to determine the amount of protein in that food sample. The instrument is calibrated using a material of known nitrogen concentration such as EDTA (9.59 % N), (NH₄)₂SO₄ (21.9 % N) or Fe (NH₄)₃(SO₄)₂ (7.145 % N), thus the signal from the thermal conductivity detector is converted into nitrogen content.

UV- Visible spectroscopy which involves measurement of absorbance at specific wavelengths can also be used (Siegenthaler, 1999). Direct measurement at 280 nm due to the presence of tryptophan and tyrosine or the Biuret method which uses Cu³⁺ and measured at 540 nm under alkaline conditions may be used to give the concentration of proteins in a food sample. The principle utilized here is that
of Beer’s law (Price, 1997). In this study, the Kjeldah method was used for the analysis of proteins because it is conventionally accepted (Ronald and Sawyer, 1991).

2.5.2 Analytical technique for measuring carbohydrates

Carbohydrates can be determined using various methods such as chromatographic and electrophoresis, chemical methods, enzymatic method and the methods which employ physical properties like density, refractive index, IR and polarity, and the anthrone method (Sadasivam and Manickam, 1996). The anthrone method involves use of a reagent which gives a colored complex that can be measured spectro-photometrically.

The method works on the principle that glucose undergoes dehydration in the presence of concentrated sulphuric acid to form 5-hydroxymethyl furfural (Eqn. 2.1). The resulting furfural reacts with the anthrone reagent to form a blue-green complex with a maximum absorption at 620 nm (Bio-Resource, 2011; Samar, 2012). The intensity of the color of complex formed is proportional to the concentration of the carbohydrates. The technique was used in this study because the method is reliable and is not selective on the nature of the sugars, whether reducing or none reducing (Birch, 1985; James et al., 1986).


\[
\text{C}_6\text{H}_{12}\text{O}_6 \xrightarrow{\Delta \text{ Conc.}\text{H}_2\text{SO}_4} \text{C}_6\text{H}_6\text{O}_3 + 3\text{H}_2\text{O} \quad \text{Eqn. 2.1}
\]

*Principle*

Figure 2.1:-Reaction of anthrone reagent with carbohydrates (Samar, 2012)

2.5.3 Analytical technique for measuring lipids

This is a group of food compounds which are soluble in organic solvents but insoluble in water. The analytical techniques used for their determination are classified into three main categories which include solvent extraction, non-solvent extraction and instrumental methods. In this study, solvent extraction based on Soxhlet extraction technique was used (Ronald and Sawyer, 1991).

The method is an example of semi-continuous solvent extraction whereby the solvent is heated in a special flask where it evaporates through a special
evaporating thimble before being condensed. The condensed solvent trickles into the extraction chamber and surrounds the sample completely. When the solvent exceeds a certain level, it overflows back into the boiling flask where the process starts again but leaving the dissolved lipids in the flask (Sadasivam and Manickam, 1996). The contents in the flask are vacuum evaporated at 60°C and dried in an air oven at 105°C for 10 minutes and placed in a desiccator to cool before weighing. Using equation 2.2 and 2.3, the amount of lipids can be determined.

\[
\text{Lipids in ground sample} \% = \frac{\text{Wt of lipids (g)}}{\text{Wt of sample (g)}} \times 100 \quad \text{Eqn. 2.2}
\]

\[
\text{Lipids to dry weight basis} = \frac{\% \text{ lipids in ground sample}}{100 \% \text{ moisture in whole sample}} \quad \text{………Eqn. 2.3}
\]

**2.5.4 Analytical technique for measuring elemental nutrients**

Elemental analysis is usually done using various techniques (Perkel, 2012) such as flame atomic absorption spectroscopy (FAAS), flame atomic emission spectroscopy (FAES) (Taylor *et al.*, 2006), inductively coupled plasma mass spectrometry (ICPMS) (Onho, 2001), XR analyzers (Olympus corporation, 2013) and UV-Visible spectrophotometry (Lajunen, 1991). In this study, FAAS was
used for analysis of Mg and Ca, FEAS for the analysis of Na and K and UV-visible spectrophotometry for the analysis of phosphorous.

FAAS is a technique which works on the principle of absorption of radiation energy by free atoms. The concentration of an element is measured by the absorption of a radiation with characteristic frequency by free atoms of that element. Light of certain wavelength produced by cathode lamp made of element of interest emits spectral lines corresponding to energy required for excitation of atoms of the same element. Atomization of the element is achieved by introduction of fine spray of test solution through nebulizer into the air/acetylene flame (Skoog, 1998; Taylor et al., 2006).

The sample solution aspirated into the flame gets dispersed into a mist of droplets which evaporates to a dry salt. The dry salt goes into vapor which dissociates into atoms that absorb radiation from an external source. The unabsorbed radiation is led into the detector through the monochromator and the output of which is amplified, measured and recorded. The parameter measured is absorbance A, and since the relationship between absorbance and concentration is linear over a wide range of concentration (Beer’s Law, Eqn. 2.4), standards are used to obtain a calibration curve from which the concentration of analyte is determined (Price, 1997).
\[ A = \varepsilon C l \] \hspace{1cm} \text{Eqn. 2.4}

Where \( A \)-absorbance, \( C \)-concentration, \( l \)- path length and \( \varepsilon \) – constant

FAES also works on the principle that excited atoms emit energy in form of radiation as they relax to the ground state. The source of excitation energy is a flame of low-energy so that the emission spectrum is simple with few emission lines. The emitted radiation is passed through a filter which allows a characteristic wavelength depending on the element of interest (Christian, 2005). The ratio of atoms in the excited state to that in the ground state can be determined using Maxwell-Boltzman’s expression, Eqn. 2.5:

\[
\frac{N_i}{N_o} = \frac{g_e}{g_o} \times e^{\left(\frac{E_e - E_o}{2} \right) K T}
\] \hspace{1cm} \text{Eqn. 2.5}

Note:- \( N_i \) and \( N_o \) - Number of atoms in excited and ground states respectively, \( K \)- Boltman’s constant, \( T \)- absolute temperature, \( g_e \) and \( g_o \) statistical weights at the excited and ground states respectively.

UV- visible employs the use of colored complexes for quantitative determination of substances. The substance to be analyzed is reacted with a reagent which gives a colored complex that absorbs at a certain wavelength. The intensity of the color
produced is proportional to the concentration of the analyte. Its absorbance at the specific wavelength will depend on the concentration of analyte (Roberts, 2002).

In this study, inorganic phosphate was determined in the form of molybdenum-blue complex. The sample solution was reacted with sodium molybdate to form sodium phosphomolybdate which on reduction by hydrazine sulphate followed by heating gave a blue color. The concentration was determined by measuring the absorbance at 830 nm (Price, 1997).

In all these techniques (FAAS, FAES and UV-visible), standards were run alongside the sample which were used to plot a calibration curve from where the concentration of sample was determined.
CHAPTER THREE

MATERIALS AND METHODS

3.1 Study area

The LVB covers the region around Lake Victoria in Kenya, Uganda, Tanzania, Rwanda and Burundi (Fig 3.1).

Figure 3.1:- A map of Lake Victoria Basin showing study regions
The region lies between longitude 30° E and 33° E and latitudes 01° N and 03°S. It covers a total of 184,200 Km² with an approximate population of 36 million people with more than 50% of the region’s population being poor which contributes to higher incidences of HIV/AIDS and waterborne diseases (Swallow et al., 2002). About 75% of the population in the region depends on agriculture as a source of livelihood with majority of the farmers being small scale farmers. This translate to low investment in form of input, small farm size, reliance on family labor which in turn implies production of food for domestic use (Hagen and Larsen, 2002; Awange and Obiero, 2006).

The ecological conditions of the area are favorable for agricultural activities (Esquinas et al., 1983). In Tarime for instance, 78% of the economic activities consists of crop production while 22% is livestock (Ngowi et al., 2008). The common food crops cultivated includes maize, cassava, beans, bananas, sorghum and millet while cash crops includes sugarcane, pyrethrum, coffee, cotton and tea (Wikipedia, 2009). However, the pumpkin crop is rarely grown in the region with majority of farmers growing it as a hobby, mainly along the edges and margins of other staple crops like maize, tea and sugarcane (Hamisy et al., 2002).
3.2 Research design

A descriptive and diagnostic survey research design with both qualitative and quantitative approaches of data collection was applied (Bogdan and Biklen, 1982; Kombo and Tromp, 2006). Observation checklists (Appendix II) and photographs were used to collect information of the pumpkin varieties commonly grown in the area while laboratory analysis of the macronutrients was carried out to determine their levels.

3.3 Samples and sampling procedure

The farmers who grew pumpkins were purposively sampled, identified by the snow ball method and selected randomly (Borg and Gall, 1989; Mugenda and Mugenda, 1994). The farmers growing pumpkins in each of the four Districts were identified and 10% of the total number was determined. Busia District had the highest number (120) followed by Jinja (90), Tarime (70) and Gucha (60) giving a total of 340. Comparing this number with the total population of the region, this shows that pumpkins are rarely grown compared to other common food crops.

3.4 Identification of pumpkin varieties

In each household visited, four fully grown pumpkin fruits of each variety were identified using an observation checklist, collected, labeled and transferred to
Kenyatta University for laboratory analysis of nutrients. The physical features of the various pumpkin varieties identified which included color and shape of fruits and seeds were captured by the researcher for reference. Pictures of the ready fruits were also taken to assist in identification of the varieties following the procedure in www.plants.usda.gov/....classification.Servlet. (retrieved 27/9/2010)

3.5 Cleaning of apparatus

Glassware apparatus were washed with liquid detergent and hot water, rinsed with tap water followed by 5 % nitric acid solution. They were rinsed with distilled-deionized water then dried in the oven at 105ºC for two hours and allowed to cool before use. Plastic apparatus were washed with liquid detergent and tap water followed by 5 % nitric acid solution then rinsed with distilled-deionized water and allowed to dry on open racks. They were finally stored in lockable drawers.

3.6 Chemicals and reagents

Analytical grade reagents and chemicals from Sigma Aldrich Company supplied by Kobian Laboratory Suppliers Nairobi were used throughout. The chemicals included HNO₃, HCl, KH₂PO₄, HClO₃, C₆H₁₂, CH₃CH₂OH, Ca, Na, Mg, K, H₂SO₄, Anthrone reagent, D-glucose, hydrazine sulphate, petroleum ether among others.
3.7 Instrumentation

A UV-visible spectrophotometer, (Cecil CE 2041) was used for analysis of carbohydrates and phosphorous, a Buck Scientific spectrometer (model 240) for the analysis of sodium and potassium, and a Buck Scientific graphite furnace atomic absorption spectrometer (model 210 VGP) for the analysis of calcium and magnesium.

3.8 Laboratory procedures

Laboratory procedures include preparation of standards, methods validation, sample treatment and analysis procedures for the selected nutrients.

3.8.1 Preparation of standards and solutions

Commercial standard stock solutions of 1000 ppm for elements Na, K, Ca and Mg were used to prepare working solutions. Anthrone reagent (0.1 %) was prepared by taking 0.1000 g accurately in 100 mL concentrated Sulphuric acid and kept in a deep freezer for 4 - 8 hours before use. Sugar stock solution was prepared by weighing accurately 0.1000 g of D - glucose in a 100 mL volumetric flask and diluting to mark to give 1000 ppm which was used to prepare standard solution for calibration curve for the determination of carbohydrates.
Sodium Molybdate solution was prepared by dissolving 12.50 g of Na$_2$MO$_4$ in 5M H$_2$SO$_4$ in a 500 mL volumetric flask. 5M H$_2$SO$_4$ was prepared by taking 290 ml of conc.H$_2$SO$_4$ and diluting to one liter. Hydrazine sulphate solution was prepared by dissolving 1.5 g hydrazine sulphate in one liter. The phosphate stock solution was prepared by taking 0.2197 g of KH$_2$PO$_4$ and diluting to one liter. 0.1N HCl was prepared by taking 8.6 mL analytical HCl and making to one liter solution and then standardized using standard sodium carbonate. The mixed indicator was prepared by taking 0.12 g methyl red, 0.800 g methylene blue and 0.100 g bromocresol green in 95 % methanol to make 100 mL solution (Vogel, 1996).

3.8.2 Method validation

(a) Precision

Six determinations of the same sample were performed for each of the nutrients using the same reagents, apparatus and procedure to evaluate the precision of the methods in the analysis of the pumpkin fruit flesh and seeds.

(b) Recovery

The samples analyzed for precision were spiked with two levels of standards for each nutrient (twice and thrice the initial concentration) and then the
concentration determined. From the RSD (%), the recoveries of the methods were
determined.

Calibration curves were prepared and used to determine linearity of the regression
line and the limits of detection of the methods. The limit of detection, (L.O.D) for
the different macronutrients were determined using Equation 3.1,

\[ y_0 = y_B + 3 s_B \]  
\[ \text{Eqn. 3.1} \]

Where \( y_B = A \), the y-intercept in the regression line equation and \( s_B = s_{y/x} \), the
standard deviation of the slope of the regression line. The equation becomes

\[ y_0 = A + 3 s_{y/x} \]  
\[ \text{Eqn. 3.2} \]

The standard deviation of the slope \( s_{y/x} \) was calculated using the following
equation:-

\[ s_{y/x} = \sqrt{\frac{\sum (y_i - \hat{y})^2}{n - 2}} \]  
\[ \text{Eqn. 3.3} \]

The t-values were calculated using Eqn. 3.4

\[ t_c = \sqrt{\frac{r / \sqrt{(n-2)}}{\sqrt{1-r^2}}} \]  
\[ \text{Eqn. 3.4} \]
3.8.3 Sample treatment

The pumpkin fruits were washed with tap water to remove dust, rinsed with distilled water and allowed to dry. The outer cuticle was removed and the fruit divided into eight equal parts. A piece was scooped from each portion and placed in a mixer/blender and homogenized to give a paste. Part of the paste was used for the analysis of carbohydrates and proteins while the other part was dried for the analysis of lipids and elements. For seeds, the husks were removed manually and crushed in a mortar to get the paste.

3.8.4 Analysis procedures

Analysis of various nutrients was done starting with proteins, carbohydrates, lipids and elements (Ca, Mg, Na, K and P). Proteins were determined using Kjeldahl method, carbohydrates using anthrone method and lipids using soxhlet extraction. Mineral elements Ca and Mg were analysed using FAAS, Na and K using FAES and P using UV-visible spectrophotometry.

In each of the determinations, duplicate sample were used with four replicates being done for each sample at different times. This was done so as to minimize both systematic and random errors on the results.
3.8.4.1 Protein analysis

Using the homogenized paste, 1.5 g of sample was weighed accurately and transferred into a clean dry Kjeldahl flask followed by addition of 2 Kjeltabs and 20 mL of concentrated sulphuric acid. The contents were mixed gently and then placed in a slanting position and heated gently until frothing stopped, then strongly for 90 minutes to digest all organic matter until the color changed to colorless. The digest was allowed to cool and then diluted with 200 mL of distilled water, 5 drops of 0.5 % phenolphthalein indicator and then mixed thoroughly.

The mixture was distilled until about 200 mL of the distillate was collected in the receiver flask which then was titrated with 0.1M HCl to a gray-green endpoint. The volume of the standard base required for complete neutralization was determined and used to calculate the amount of ammonia evolved (Vogel, 1996; Ronald and Sawyer, 1991). A blank consisting of all other reagents without sample was prepared and titrated for correction. Four replicates were performed and average determined. The nitrogen content was calculated using equations 3.5 and 3.6 (Sadasivam and Manickam, 1996).

\[
\% \text{ nitrogen} = \frac{(T - B) \times 14.007 \times 100}{\text{Weight of sample (mg)}}
\]  
Eqn. 3.5
% protein = % Nitrogen x 6.25 ………………………………………Eqn. 3.6

Where T- Sample titre          B- Blank titre

3.8.4.2 Carbohydrates analysis

About 1.0 g of sample (defatted for seeds) was mixed with 100 mL of 80% ethanol and heated to boil for about 30 minutes. The hot solution was filtered and then evaporated on a water bath for 10 minutes to remove the alcohol. The alcohol free extract was allowed to cool and reconstituted to 500 mL.

One milliliter of the extract in duplicate, duplicate blanks and a series of six diluted glucose standard solutions with 0, 1, 2, 3, 4 and 5 ppm were each transferred to different Pyrex test tubes, 5 mL of anthrone reagent added then the tubes stoppered and shaken thoroughly. The test tubes and contents were placed in a boiling water bath for 10 minutes and cooled under tap water. The absorbance of the sample, blanks and standards were measured at 630 nm and the concentration of the sample determined from a calibration curve using the following expression and eqn. 3.7. Four replicates were done and average concentration of sample determined. The actual content of carbohydrate in the sample was calculated by multiplying with appropriate dilution factor (James et al., 1986; Moonheart Infotech, 2009).
\[ C_{Un} = \frac{A_{Un} \times C_{St.}}{A_{St.}} \] \hspace{1cm} \text{Eqn. 3.7} \\
where \( C_{Un} \) = Concentration of Unknown; \( A_{Un} \) = Absorbance of Unknown
\( C_{St} \) = Concentration of Standard; \( A_{St} \) = Absorbance of Standard.

Amount of carbohydrate in 1.00 g = \( \frac{\text{mg of glucose}}{\text{volume of test sample}} \) \times 1.00 \ldots \text{Eqn. 3.8}

\subsection*{3.8.4.3 Lipid extraction}

About 5 g of the dried sample were weighed and transferred into a clean dry extraction thimble and then connected to extraction apparatus. For seeds, the husks were first removed manually. Enough petroleum ether (B.P 40-60° C) was added to the collecting flask and lipids extracted by refluxing for 5 hours. The contents in the flask were vacuum evaporated at 60° C to remove the solvent followed by addition of 20 mL of acetone and then evaporated again at 60° C. The resulting crude lipids were dried in an air oven at 105° C for 10 minutes and then transferred into a desiccator to cool to room temperature before weighing accurately. The percentage yield of crude lipids was determined using equation 3.9 (Ronald and Sawyer, 1991; Sadasivam and Manickam, 1996).

\[ \% \text{ lipids} = \left( \frac{\text{wt. of crude lipids (g)}}{\text{Wt. of sample (g)}} \right) \times 100 \] \hspace{1cm} \text{Eqn.3.9}
3.8.4.4 Elemental analysis

For elemental analysis, 1.00 g of dry, ground homogenized sample was placed in a flask followed by 10 mL of concentrated nitric (V) acid and 5 mL of concentrated sulphuric acid then digested for 30 minutes. The mixture was allowed to cool for 10 minutes then 10 mL of 70 % perchloric acid added and digested for 30 minutes until there was no fuming. The mixture was allowed to cool then reconstituted to 100 mL in a volumetric flask and kept in a deep freezer for the analysis of different elements (Ronald and Sawyer, 1991).

(a) Calcium and magnesium

Appropriate aliquots of working solution of calcium containing between 0-20 ppm were transferred into 50 mL volumetric flasks followed by 2 mL of lanthanum chloride. The contents were mixed thoroughly and then diluted to mark. 2.0 mL of sample extract previously prepared and kept in a deep freezer were treated the same way as the standards and duplicate blanks. The standards were aspirated into the graphite furnace, starting with water to Zero the instrument then one with the lowest concentration to the highest and lastly sample, successfully washing the instrument through with distilled water between each spray. The absorbance was determined at 422.7 nm using a calcium cathode lamp (Ronald and Sawyer, 1991).
The procedure was repeated with magnesium standards containing 0-10 ppm and magnesium cathode lamp. The absorbance was determined at 285.0 nm. The concentration of the Ca and Mg in the sample was obtained from the calibration curves of the standards (Appendix VI c & d).

(b) Sodium and potassium

Sodium standards with concentrations of 1, 2, 3, 4, 5, 6 and 7 ppm were prepared by transferring appropriate aliquots of 100 ppm working solution to 100 mL volumetric flask and diluting to the mark with distilled de-ionized water. Two milliliters of the digested sample were transferred to 100 mL volumetric flasks in duplicate and diluted to the mark. Using a sodium filter, the instrument was set to Zero using distilled de-ionized water and 100% using 7 ppm. The standard solutions were analyzed and results used to plot a calibration curve (Appendix VI f).

The procedure was repeated using potassium standard solutions with concentrations 1 – 12 ppm by transferring 2, 4, 6, 8, 10 and 12 mL of 1000 ppm stock solution into 100 mL volumetric flasks and diluting to the mark and potassium filter (Ronald and Sawyer, 1991). The concentration of the elements in the sample was obtained from the calibration curves prepared using the readings of the standards (Appendix VI e).
(c) **Phosphorus**

Phosphorous standard solutions were prepared by transferring 0, 5, 10, 15, 20 and 25 mL of working (stock) solution to 50 mL volumetric flasks followed by 5 mL of sodium molybdate solution and then 2 mL of hydrazine sulphate, mixed thoroughly and finally diluted to mark. 2 mL of the sample were measured from the digested sample extract and transferred to a 50 mL volumetric flask and diluted to mark. The flasks were immersed in boiling water for 10 minutes, removed and cooled rapidly in ice and the absorbance measured at 830 nm using a spectrophotometer, against a reagent blank. The procedure was repeated thrice in different days and the phosphorous content deduced from the calibration curve (Appendix VI a) obtained (Vogel, 1996; Roberts, 2002).

### 3.9 Data analysis

The data obtained from the study was analyzed using SPSS version 16.0. Analysis of variance and t-tests were done for any significant difference in the levels of macro-nutrients in the different varieties of pumpkins from the different regions and between seeds and the fruit.
CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Introduction

This chapter presents the results on available varieties of pumpkins, methods validation and levels of the macronutrients of the pumpkin varieties in the study area and discusses them. The results are presented both in tables and figures.

4.2 Varieties of pumpkins

A total of 6 varieties of pumpkins were identified across the study area as shown in table 4.1 below.

Table 4.1: Pumpkin varieties in the study area

<table>
<thead>
<tr>
<th>Variety</th>
<th>Present/absent</th>
<th>Gucha</th>
<th>Busia</th>
<th>Jinja</th>
<th>Tarime</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Common name)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Banana</td>
<td>√</td>
<td>×</td>
<td>√</td>
<td>×</td>
<td></td>
</tr>
<tr>
<td>Bottle guard</td>
<td>×</td>
<td>×</td>
<td>√</td>
<td>×</td>
<td></td>
</tr>
<tr>
<td>Butternut squash</td>
<td>√</td>
<td>√</td>
<td>√</td>
<td>×</td>
<td></td>
</tr>
<tr>
<td>Carnival squash</td>
<td>√</td>
<td>√</td>
<td>√</td>
<td>×</td>
<td></td>
</tr>
<tr>
<td>Crown prince</td>
<td>√</td>
<td>√</td>
<td>√</td>
<td>√</td>
<td></td>
</tr>
<tr>
<td>Green Kabach</td>
<td>√</td>
<td>√</td>
<td>√</td>
<td>×</td>
<td></td>
</tr>
</tbody>
</table>

√ - present  × - Absent
Figure 4.1:- Pumpkin varieties sampled in the study area

From top left: *Green kabacha, crown prince, carnival squash, banana, bottle guard and butternut.*
Crown prince was the only variety found in all the four areas possibly due to its sweet taste and ability to be stored for a long time (Sarah, 2013) which make it a favorite amongst the farmers. It also contains less water and can grow in various climatic conditions (Challock et al., 2013). Jinja district dominated the rest of the regions by having all the six sampled varieties. This could be due to the climatic conditions which are favorable throughout the year (Miti, 2010). Gucha district is among the densely populated districts in Kenya (Republic of Kenya, 2011) and this implies that the demand for food is high and therefore the farmers use the pumpkins as a means of livelihood.

Tarime district had only one variety, crown prince. Majority of the varieties found in Busia were also sampled in Jinja possibly due to proximity of the two regions. Jinja had all the varieties grown in the region. The bottle guard variety was only sampled in Jinja possibly because it is a favorite among the indigenous baganda people. It is not common with other surrounding communities due to its bitterness. Possibly the baganda know the secret of its richness in nutrients. The fruits are consumed while young before they mature. On maturity, they become hard and bitter.
4.3 Methods validation

Six determinations of the various macronutrients in a pumpkin fruit sample were performed using the same reagents and apparatus to determine the precision of the methods. Table 4.2 gives the values of coefficient of variance (CV), the regression equation, $R^2$, $t$-calculated ($t_c$) and detection limits of the calibration curve analysis.

Table 4.2: Methods validation variables of the different nutrients

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>CV (%)</th>
<th>Regression Equation</th>
<th>$R^2$</th>
<th>$t_c$</th>
<th>Detection limit (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbohydrates</td>
<td>0.921</td>
<td>$Y=0.0122+0.1909X$</td>
<td>0.9989</td>
<td>10.09</td>
<td>6.037x10$^2$</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>2.023</td>
<td>$Y=0.024+0.0784X$</td>
<td>0.9989</td>
<td>78.42</td>
<td>1.296x10$^1$</td>
</tr>
<tr>
<td>Potassium</td>
<td>1.172</td>
<td>$Y=3.2 + 8.1143X$</td>
<td>0.9974</td>
<td>39.18</td>
<td>7.292x10$^{-1}$</td>
</tr>
<tr>
<td>Sodium</td>
<td>1.960</td>
<td>$Y=39.143 +8.714X$</td>
<td>0.9955</td>
<td>32.91</td>
<td>7.057x10$^{-1}$</td>
</tr>
<tr>
<td>Magnesium</td>
<td>1.862</td>
<td>$Y=0.0086+0.1021X$</td>
<td>0.9993</td>
<td>99.98</td>
<td>1.230x10$^{-3}$</td>
</tr>
<tr>
<td>Calcium</td>
<td>1.521</td>
<td>$Y=0.0016+0.0093X$</td>
<td>0.9999</td>
<td>76.34</td>
<td>2.248x10$^{-3}$</td>
</tr>
</tbody>
</table>

The CV values range from 0.921 to 2.023 %, which fall within the recommended level an indication that the precision level of the methods were deemed reproducible and therefore valid for use in analysis (Burges, 1997). All the regression curve equations gave linear relationship with corresponding $R^2$ values.
above 0.995, indicating that over 99.5 % of instrument responses correspond to the concentrations.

The y- intercept for the regression equations were all low, passing near the origin except for sodium and potassium. The slope values were indicative of linear slope. Figure 4.2 shows the calibration curve for phosphorus standards while the others are in appendix (VI) from (a) to (f).

![Calibration curve for phosphorus](image)

Figure 4.2: Calibration curve for phosphorus

The $t_c$ values were calculated and all were greater than $t$-critical (two-tailed) of 2.78 at 95 \% confidence level. This implies that a significant correlation between

\begin{align*}
\gamma &= 0.0784x + 0.024 \\
R^2 &= 0.9989
\end{align*}
X and Y exists (Miller and Miller, 1984). The limits of detection for the different nutrients were determined where magnesium had the lowest LoD ($1.230 \times 10^{-3}$ ppm) while carbohydrates had the highest LoD ($6.037 \times 10^2$ ppm). The values were significantly low which indicates that the methods were sensitive.

### 4.4 Levels of proteins, carbohydrates and lipids

The levels of proteins, carbohydrates and lipids in different varieties of pumpkin fruits and seeds were determined and recorded as shown in table 4.3.

The results show that the levels of the nutrients varied significantly amongst the different varieties as well as between the seeds and fruit flesh for the same pumpkin variety. For instance, banana variety had the highest level of protein in the fruit flesh ($2.96 \pm 0.37$ g per 100 g) while butternut variety had the lowest ($1.55 \pm 0.13$ g per 100 g of wet sample). Likewise, green kabacha had significantly higher level of proteins in seeds ($38.06 \pm 0.06$ g per 100 g of sample while crown prince had the lowest amount ($32.87 \pm 1.49$ g per 100 g) of sample.
Table 4.3: Levels of macronutrients in different varieties of pumpkin fruit and seeds

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Concentration (g/100 g)</th>
<th>P-value</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Banana (n=40)</td>
<td>Carnival squash (n=80)</td>
<td>Crown prince (n= 50)</td>
</tr>
<tr>
<td>Proteins</td>
<td>Fruit</td>
<td>Seeds</td>
<td>Fruit</td>
</tr>
<tr>
<td></td>
<td>2.96 ± 0.37^b</td>
<td>2.88 ± 0.13^b</td>
<td>2.54 ± 0.06^b</td>
</tr>
<tr>
<td></td>
<td>33.15 ±1.13^a</td>
<td>35.95 ± 1.5^b</td>
<td>32.87 ± 1.49^b</td>
</tr>
<tr>
<td>p-value</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>Fruit</td>
<td>Seeds</td>
<td>Fruit</td>
</tr>
<tr>
<td></td>
<td>5.61 ± 0.97^abc</td>
<td>4.39 ± 0.97^ab</td>
<td>7.57 ± 2.08^cd</td>
</tr>
<tr>
<td></td>
<td>4.84 ± 0.32^b</td>
<td>3.03 ±1.09^a</td>
<td>2.88 ± 1.38^a</td>
</tr>
<tr>
<td>p-value</td>
<td><strong>0.181</strong></td>
<td><strong>0.254</strong></td>
<td>0.003</td>
</tr>
<tr>
<td>Lipids</td>
<td>Fruit</td>
<td>Seeds</td>
<td>Fruit</td>
</tr>
<tr>
<td></td>
<td>2.74 ± 0.45^b</td>
<td>1.68 ± 0.74^a</td>
<td>1.08 ± 0.40^a</td>
</tr>
<tr>
<td></td>
<td>38.16 ± 2.96</td>
<td>40.26 ± 4.28</td>
<td>46.24 ±1.79</td>
</tr>
<tr>
<td>p-value</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Mean values followed by the same small letter(s) within the same row indicate that levels do not differ significantly from one another (α = 0.05, One-way ANOVA, Duncan’s test)
The seeds in banana variety had significantly higher level of carbohydrates (4.84 ± 0.32 g per 100 g of sample) while bottle guard had the lowest (2.39 ± 0.34 g per 100 g of sample). The bottle guard variety had significantly the highest level of carbohydrates in the fruit flesh (11.96 ± 1.05 g per 100 g of sample) while green kabacha had the lowest (4.04 ± 0.68 g per 100 g sample). The green kabacha had significantly higher levels of lipids in the fruit flesh (4.26 ± 0.20 g per 100 g) and proteins in seeds being the highest (38.06 ± 0.06 g per 100 g), while crown prince variety had significantly lower levels of proteins (32.87 ± 1.49 g per 100 g) and lipids (1.08 ± 0.40 g per 100 g of sample) in the fruit flesh.

The levels of proteins and fats in seeds and carbohydrates in the fruit compare with reported values of 30.23 g, 49.02 g and 6.0 g per 100 g raw weight of flesh respectively (Indouraine, 1996). In a study done by Achu et al. (2004) on five species of curcubita family, the level of crude proteins varied from 24.3 g to 41.0 g and total lipids 42.9 g to 57.3 g per 100 g of seeds while that done by Mi et al. (2012) gave the fats level as 43.9 g to 52.4 g and that of proteins as 27.4 g to 30.8 g. The results show that the levels of nutrients in fruits vary depending on variety, (Greenfield and Southgate, 1992).

The levels of nutrients of crown prince variety which was sampled across all the selected districts were determined and analyzed for any significant difference and the results given in table 4.4. The results obtained show that the level of proteins
Table 4.4:- Levels of macronutrients in crown prince fruit and seeds from different regions.

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Concentrations (g / 100 g)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Gucha (n = 11)</td>
<td>Busia (n = 8)</td>
</tr>
<tr>
<td>Proteins</td>
<td>Fruit</td>
<td>3.09 ± 0.03&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>seeds</td>
<td>35.51 ± 1.06</td>
</tr>
<tr>
<td></td>
<td>p-value</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>Fruit</td>
<td>4.60 ± 1.64&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Seeds</td>
<td>2.94 ± 0.91</td>
</tr>
<tr>
<td></td>
<td>p-value</td>
<td>0.217</td>
</tr>
<tr>
<td>Lipids</td>
<td>Fruit</td>
<td>1.80 ± 0.91</td>
</tr>
<tr>
<td></td>
<td>Seeds</td>
<td>41.25 ± 1.46</td>
</tr>
<tr>
<td></td>
<td>p-value</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Mean values followed by the same small letter(s) within the same row indicate that levels do not differ significantly from one another (α = 0.05, One-way ANOVA, Duncan’s test)
and carbohydrates in the fruit varied significantly depending on the District where
the varieties were planted. Pumpkin fruits from Busia had protein mean level of
1.64 g ± 0.24 g while those from Jinja had 6.92 g ± 4.96 g per 100 g of fruit.
Lipids in both seeds and fruit samples from all the regions did not show any
significant difference as well as proteins and carbohydrates in seeds (P > 0.05).

Generally, the levels of proteins and lipids in pumpkin seeds and fruit differed
significantly for both variety and region (P < 0.05). In carbohydrates, there was a
significant difference in crown prince and butternut while varieties from Gucha
did not show any significant difference (Table 4.3 & 4.4).

A post hoc analysis was done to establish the regions which differed significantly
in levels of proteins and carbohydrates (Table 4.5). The differences observed in
the regions resulted from Busia and Jinja for proteins while that of carbohydrates
resulted from Gucha and Busia. The variations observed could be attributed to
difference in regions where different climatic conditions, soil type, geographical
area, cultivation practices among other factors (Garrow et al., 1998). The
nutritional content of a given plant depends on the nutritional status of the soil
where the crop grows (Woofle, 1992).
Table 4.5:- Post hoc results for proteins and carbohydrates for crown prince fruit flesh, Duncan.

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Subset for Alpha =0.05</th>
<th>Region</th>
<th>Sign.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Busia</td>
<td>Tarime</td>
</tr>
<tr>
<td>Protein</td>
<td>A 1.6350 2.0400 3.0900</td>
<td>0.494</td>
<td></td>
</tr>
<tr>
<td></td>
<td>B 3.0900 6.9192</td>
<td>0.063</td>
<td></td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>A 4.6011 4.9215</td>
<td>0.843</td>
<td></td>
</tr>
<tr>
<td></td>
<td>B 8.3700 8.1340</td>
<td>0.050</td>
<td></td>
</tr>
</tbody>
</table>

Similarly, the variety of pumpkin grown plays a greater role in determining its nutritional value. Other factors like time of harvest, duration of storage and ripeness of the fruit also affects the level of nutrients in pumpkins (Greenfield and Southgate, 1992). The post hoc results showing sources of differences in levels for the different varieties are given (Appendix IV).

The correlation coefficients of proteins, carbohydrates and lipids were done to establish whether there were any relationships between them. The results from table 4.6 indicate that carbohydrates and proteins in the fruit had a negative significant correlation at 0.01 confidence level while proteins and lipids had a significant positive correlation at 0.05 confidence level. The other nutrients did not show any relationship.
Table 4.6: Pearson's correlation coefficients for macronutrients in the pumpkin fruit and seeds.

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Protein(f)</th>
<th>Carb.(f)</th>
<th>Lipids(f)</th>
<th>Protein(s)</th>
<th>Carb.(s)</th>
<th>Lipids(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein(fruit)</td>
<td>1.000</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carb. (fruit)</td>
<td>-0.633**</td>
<td>1.000</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lipids(fruit)</td>
<td>0.004</td>
<td>-0.296</td>
<td>1.000</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Protein(seeds)</td>
<td>-0.344</td>
<td>-0.054</td>
<td>0.241</td>
<td>1.000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carb. (seeds)</td>
<td>-0.243</td>
<td>0.284</td>
<td>0.329</td>
<td>-0.074</td>
<td>1.000</td>
<td></td>
</tr>
<tr>
<td>Lipids (seeds)</td>
<td>-0.299</td>
<td>0.208</td>
<td>0.015</td>
<td>0.360*</td>
<td>-0.111</td>
<td>1.000</td>
</tr>
</tbody>
</table>

Key: ** Correlation is significant at the 0.01 level (2-tailed)
* Correlation is significant at the 0.05 level (2-tailed)
(f) - fruit flesh and (s) - seeds

4.5 Levels of mineral elements

The levels of phosphorus, potassium, sodium magnesium and calcium in pumpkin fruit and seeds were determined an recorded as shown in table 4.7.
Table 4.7: Levels of mineral elements in fruit and seeds of pumpkin varieties

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Concentration (mg/100 gm)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fruit</td>
<td>Seeds</td>
</tr>
<tr>
<td>P</td>
<td>35.17 ± 2.13</td>
<td>760.1 ± 6.0</td>
</tr>
<tr>
<td></td>
<td>35.78 ± 1.93</td>
<td>544.2 ± 6.8</td>
</tr>
<tr>
<td></td>
<td>29.88 ± 2.64</td>
<td>749.9 ± 7.2</td>
</tr>
<tr>
<td></td>
<td>28.20 ± 1.87</td>
<td>1048.4 ± 41.8</td>
</tr>
<tr>
<td></td>
<td>19.22 ± 1.69</td>
<td>377.9 ± 6.8</td>
</tr>
<tr>
<td></td>
<td>27.43 ± 1.59</td>
<td>1044.8 ± 8.1</td>
</tr>
<tr>
<td>K</td>
<td>356.66 ± 5.71</td>
<td>51.94 ± 3.53ab</td>
</tr>
<tr>
<td></td>
<td>265.72 ± 4.46</td>
<td>60.89 ± 5.72ab</td>
</tr>
<tr>
<td></td>
<td>327.25 ± 11.43</td>
<td>80.70 ± 2.47b</td>
</tr>
<tr>
<td></td>
<td>277.18 ± 7.79</td>
<td>37.71 ± 1.93a</td>
</tr>
<tr>
<td></td>
<td>355.97 ± 3.14</td>
<td>116.74 ± 5.82c</td>
</tr>
<tr>
<td></td>
<td>374.8 ± 10.94</td>
<td>121.53 ± 8.63c</td>
</tr>
<tr>
<td>Na</td>
<td>47.17 ± 2.89a</td>
<td>41.08 ± 2.04</td>
</tr>
<tr>
<td></td>
<td>74.10 ± 3.08ab</td>
<td>41.45 ± 3.28</td>
</tr>
<tr>
<td></td>
<td>119.67 ± 3.68c</td>
<td>37.17 ± 3.62</td>
</tr>
<tr>
<td></td>
<td>65.42 ± 14.23ab</td>
<td>48.02 ± 4.03</td>
</tr>
<tr>
<td></td>
<td>95.90 ± 4.53bc</td>
<td>26.16 ± 4.07</td>
</tr>
<tr>
<td></td>
<td>117.50 ± 5.13c</td>
<td>30.52 ± 1.66</td>
</tr>
<tr>
<td>Mg</td>
<td>14.52 ± 0.32a</td>
<td>34.75 ± 1.05a</td>
</tr>
<tr>
<td></td>
<td>17.42 ± 1.03a</td>
<td>65.28 ± 2.19ab</td>
</tr>
<tr>
<td></td>
<td>19.66 ± 1.07a</td>
<td>84.46 ± 7.00c</td>
</tr>
<tr>
<td></td>
<td>15.41 ± 0.70a</td>
<td>39.96 ± 1.87a</td>
</tr>
<tr>
<td></td>
<td>20.77 ± 4.04a</td>
<td>66.58 ± 4.56ab</td>
</tr>
<tr>
<td></td>
<td>34.05 ± 2.07b</td>
<td>35.36 ± 2.53a</td>
</tr>
<tr>
<td>Ca</td>
<td>35.69 ± 2.23a</td>
<td>47.47 ± 1.30a</td>
</tr>
<tr>
<td></td>
<td>49.61 ± 0.97ab</td>
<td>85.52 ± 0.88ab</td>
</tr>
<tr>
<td></td>
<td>61.64 ± 0.45bc</td>
<td>157.80 ± 2.55c</td>
</tr>
<tr>
<td></td>
<td>48.48 ± 4.53ab</td>
<td>57.24 ± 8.92a</td>
</tr>
<tr>
<td></td>
<td>73.10 ± 1.96c</td>
<td>83.76 ± 4.05ab</td>
</tr>
<tr>
<td></td>
<td>103.48 ± 5.98d</td>
<td>134.89 ± 3.00bc</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Mean values followed by the same small letter(s) within the same row indicate that levels do not differ significantly from one another (α = 0.05, One-way ANOVA, Duncan’s test)
The results indicate that the variety of the pumpkin determines the level of the mineral elements apart from P, K in fruit flesh and Na in seeds (P > 0.05). Green kabacha variety had the lowest level of K in the seeds (37.71 ± 1.69 g) while butternut had the highest (116.74 ± 5.82 g) per 100 g of sample. Banana variety had the lowest level of Na (47.17 ± 2.89 g) in the fruit flesh while crown prince had the highest level (119.67 ± 3.68 g per 100 g of sample).

The banana variety had the lowest level of Mg for both the fruit flesh and seeds while bottle guard and crown prince respectively had the highest levels. For Ca, banana variety also had the lowest levels for both fruit flesh (35.69 ± 2.23 g) and seeds (47.47 ± 1.30 g) while bottle guard (103.48 ± 5.98 g) and crown prince (157.80 ± 2.55 g) respectively had the highest levels in the fruit flesh and seeds. The values show a similar trend with respect to the varieties.

Bottle guard had the highest levels for most of the elements (K, Na, Mg and Ca) followed by crown prince. Green kabacha and carnival squash had moderate levels while butternut and banana varieties had the least. The results show that the levels of sodium and potassium varied significantly between the seeds and fruit while the seeds had significantly higher levels of phosphorus, magnesium and calcium compared to the fruit. It is therefore possible to associate the level of the elements with the variety of pumpkin analyzed (Greenfield and Southgate, 1992).
The levels of the elements in crown prince variety were also compared with respect to the regions and Table 4.8 gives the summary of the results. The results show that there was no difference in levels of P in both the fruit flesh and seeds across the regions. Potassium levels only varied significantly in seeds with samples from Gucha having the lowest level (45.06 ± 2.59 mg) while Busia had the highest level (101.44 ± 5.96 mg per 100 g). Na varied significantly in the fruit flesh only with samples from Tarime having the highest level (120.89 ± 2.91 mg) while those from Gucha had the lowest level (57.69 ± 1.50 mg per 100 g of sample).

Magnesium levels varied significantly in both the fruit flesh and seeds. Samples from Gucha had the lowest level in the fruit flesh (13.48 ± 1.67 mg) and those from Jinja had the highest level (24.43 ± 1.01 mg per 100 g of sample). Samples from Tarime had the highest level in seeds while those from Jinja had the lowest level. Finally for Ca, samples from Gucha had the lowest level in both the fruit flesh and seeds while those from Busia and Tarime had the highest levels in the fruit flesh and seeds respectively.

In general the results indicate that pumpkins from Tarime had a significant difference in levels (subsets with b and c) compared to those from other regions. Those from Gucha had the lowest levels for most nutrients (Ca, Mg and Na in the fruit) as the means belong to the lower subset (a).
Table 4.8: Levels of mineral elements in crown prince fruit and seeds from the four regions

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Concentration (mg/100 g)</th>
<th>Gucha (n = 11)</th>
<th>Busia (n = 8)</th>
<th>Jinja (n = 9)</th>
<th>Tarime (n = 22)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>P</td>
<td>Fruit</td>
<td>29.56 ± 2.28</td>
<td>25.61 ± 3.68</td>
<td>28.75 ± 3.47</td>
<td>34.52 ± 1.82</td>
<td>0.916</td>
</tr>
<tr>
<td></td>
<td>Seeds</td>
<td>616.43 ± 9.10</td>
<td>596.40 ± 8.86</td>
<td>745.76 ± 5.82</td>
<td>531.92 ± 6.40</td>
<td>0.444</td>
</tr>
<tr>
<td>K</td>
<td>Fruit</td>
<td>370.35 ± 2.38</td>
<td>337.47 ± 6.21</td>
<td>360.79 ± 7.99</td>
<td>334.16 ± 1.06</td>
<td>0.104</td>
</tr>
<tr>
<td></td>
<td>Seeds</td>
<td>45.06 ± 2.59^a</td>
<td>101.44 ± 5.96^b</td>
<td>100.18 ± 4.63^b</td>
<td>83.90 ± 2.35^b</td>
<td>0.001</td>
</tr>
<tr>
<td>Na</td>
<td>Fruit</td>
<td>57.69 ± 1.50^a</td>
<td>110.19 ± 8.20^b</td>
<td>92.65 ± 3.03^b</td>
<td>120.89 ± 2.91^b</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Seeds</td>
<td>45.45 ± 1.96</td>
<td>33.03 ± 2.10</td>
<td>33.62 ± 6.47</td>
<td>30.96 ± 1.81</td>
<td>0.182</td>
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<tr>
<td>Mg</td>
<td>Fruit</td>
<td>13.48 ± 1.67^a</td>
<td>23.83 ± 2.73^b</td>
<td>24.43 ± 1.01^b</td>
<td>23.41 ± 1.08^b</td>
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<td>43.37 ± 1.53^a</td>
<td>81.79 ± 2.02^b</td>
<td>37.54 ± 7.10^a</td>
<td>103.33 ± 2.13^b</td>
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<td>Ca</td>
<td>Fruit</td>
<td>45.47 ± 1.91^a</td>
<td>77.47 ± 2.77^b</td>
<td>71.81 ± 2.83^ab</td>
<td>64.91 ± 1.08^ab</td>
<td>0.035</td>
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<td>58.07 ± 1.23^a</td>
<td>132.56 ± 6.9^bc</td>
<td>88.23 ± 4.99^ab</td>
<td>164.13 ± 7.33^c</td>
<td>0.001</td>
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</table>

Mean values followed by the same small letter(s) within the same row indicate that levels do not differ significantly from one another (α=0.05, One-way ANOVA, Duncan’s test)
The variation of mineral elements observed may result from difference in soil content (Torelm and Damubon, 1988). Mineral elements usually are absorbed by plants directly from the soil where they grow implying that the status of the soil where the pumpkins grow will determine the availability of the elements in the fruit or seeds (Garrow et al., 1998). In a study on the mineral content of the dry matter of sweet potatoes, it was found to vary widely depending on the cultivar, geographical area, climate, soil type and cultivation practices (Woofle, 1992).

Analysis of Variance (ANOVA) to determine whether there were any significant differences in the levels of elements obtained from the various regions show no significant difference in the levels of phosphorus, potassium in fruit and sodium (seeds) while the rest of the elements differed significantly (P < 0.05).

The correlation between the elements in the pumpkin fruit and seeds was determined using Pearson’s correlation coefficients at 95 % confidence level and table 4.9 gives a summary of the results.
Phosphorus shows a weak negative correlation with other element both in the fruit and seeds. Sodium, potassium, magnesium and calcium had positive relationship with Ca and Mg having the highest coefficient (0.885). For seeds, calcium had a significant positive correlation with potassium and magnesium while phosphorus had a weak positive correlation. Phosphorus had a negative relation with potassium and magnesium, and also sodium with potassium and magnesium. Negative correlation refers to when the levels of two elements change reversibly, that is to say as one increases, the other decreases and vice versa.

The average levels of elements in the pumpkin fruit flesh and seeds in crown prince variety determined were compared with the USDA Nutrient Database

Table 4.9: Pearson’s correlation coefficients for the elements in the pumpkin fruits and seeds.

<table>
<thead>
<tr>
<th>Nutrient (fruit)</th>
<th>P</th>
<th>K</th>
<th>Na</th>
<th>Mg</th>
<th>Ca</th>
<th>Nutrient (seeds)</th>
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<tr>
<td>P</td>
<td>1.000</td>
<td>-0.115</td>
<td>0.237</td>
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<tr>
<td>K</td>
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<td>1.000</td>
<td>-0.283</td>
<td>0.191</td>
<td>0.442**</td>
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<td>0.559**</td>
<td>0.759**</td>
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<tr>
<td>Ca</td>
<td>-0.134</td>
<td>0.565**</td>
<td>0.766**</td>
<td>0.885**</td>
<td>1.000</td>
<td>Ca</td>
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</tbody>
</table>

**- Correlation is significant at the 0.01 level (2-tailed test)
(Table 4.10) indicate that pumpkin seeds can provide sufficient amount of phosphorus and magnesium while the flesh can provide substantial amount of potassium and sodium. This implies that frequent consumption of pumpkins (both the fruit and seeds) can supply the body with these elements thus assist to prevent malnutrition.

Table 4.10: - Levels of some elements in 100 g serving of crown prince fruit and seeds

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Mean level SD (mg/100 g)</th>
<th>RDI Nutrient Database USDA (2004) (mg)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Fruit</td>
<td>Seeds</td>
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<tr>
<td>P</td>
<td>29.88 ± 2.64</td>
<td>749.9 ± 7.20</td>
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<tr>
<td>K</td>
<td>327.25 ± 11.43</td>
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<td>Na</td>
<td>119.67 ± 3.68</td>
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<tr>
<td>Mg</td>
<td>19.66 ± 1.07</td>
<td>84.46 ± 7.00</td>
</tr>
<tr>
<td>Ca</td>
<td>61.64 ± 0.45</td>
<td>157.80 ± 2.55</td>
</tr>
</tbody>
</table>

A comparison of the mean levels of the macronutrients in the different varieties of pumpkins analyzed (Tables 4.3 and 4.6) was done with a view to identify the variety with higher levels. This was done by considering the fruit flesh and seeds with maximum and minimum significant levels of each of the nutrients analyzed (Table 4.11). From the frequency of each variety, bottle guard lead in dominance followed by crown prince, Green kabacha, carnival squash and lastly banana and butternut.
Table 4.1: Relative dominance of macronutrients in different varieties of pumpkins

<table>
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<th>Nutrient</th>
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<tr>
<td></td>
<td>Minimum</td>
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<td>Proteins</td>
<td>Butternut</td>
<td>Banana</td>
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<tr>
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<td>Bottle guard</td>
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<td>Lipids</td>
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<td>G/ kabacha</td>
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<td>Butternut</td>
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<tr>
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<td>Carn. Squash</td>
<td>Bottle guard</td>
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<tr>
<td>Sodium</td>
<td>Carn. Squash</td>
<td>Crown prince</td>
</tr>
<tr>
<td>Magnesium</td>
<td>Banana</td>
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<tr>
<td>Calcium</td>
<td>Banana</td>
<td>Bottle guard</td>
</tr>
</tbody>
</table>

However, since crown prince was sampled from all the four regions it is therefore common perhaps because it adapts to any climatic conditions apart from being sweet (Michael et al., 2000; Challock et al., 2013). The fruits on maturity have low moisture content and can be stored for a long time without being affected (Sarah, 2013). It should be noted that due to cross pollination, new varieties may be coming up which could result to further variation of the expected characteristics.
CHAPTER FIVE

CONCLUSIONS AND RECOMMENDATIONS

5.1 Introduction

The chapter gives the conclusions and recommendations made from the study and areas for further work.

5.2 Conclusion

Six different varieties of pumpkins were grown by the farmers, with crown prince dominating in all the four regions in the LVB. The levels of proteins, carbohydrates, fats, phosphorus, potassium, sodium, magnesium and calcium determined were found to be significantly higher in seeds compared to the fruit flesh and also varied significantly with variety. Therefore, seeds will supply relatively greater amount of the nutrients if consumed compared to the fruit flesh.

5.3 Recommendations

Since seeds were found to contain higher levels of macronutrients, it is recommended that seeds be consumed together with the fruit flesh so as to improve the nutritional status of the individual.
5.4 Recommendation for further work

1. From the findings of the study, the possibility of growing all the varieties under similar conditions is important so as to assess the effect of similar growing conditions on nutritional content. This will form the basis for promoting the production of a specific pumpkin variety rich in nutrients as a solution to food security and remedy to malnutrition as the crop can be grown locally.

2. It is important to develop recipes containing pumpkin seeds/fruit and determine macronutrient bio-availability.
REFERENCES


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APPENDICES

(I) Letter of introduction

Dear Sir/Madam,

I am a student in Kenyatta University undertaking a Masters of Science degree. The title of the study is “Levels of selected macronutrients in varieties pumpkin (Cucurbita spp) grown in four selected districts in Lake Victoria Basin.” You have been selected to take part in the study by responding to the questions in the questionnaire. The questionnaires are only meant for research purposes and the responses will be treated confidentially. Kindly assist me by answering these questions as honestly and precisely as possible.

Thank you for your co-operation.

Yours sincerely,

[Signature]

Evans C. Choi
(II) Observation checklist

(For use by Researcher for identification)

PART (I) (For participating farmers)

1. Name of the place under investigation: ..............................................

2. Pumpkin species/varieties observed

(a) ........................................

(b) ........................................

(c) ........................................

(d) ........................................

(e) ........................................

PART (II) (For Researcher)

3. Pumpkin details for each variety/species:

...............Color of fruit

...............Shape of fruit

4. Botanical name............................................
(III) ANOVA Tables for proteins, carbohydrates and lipids in different varieties.

### Descriptives

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<td>10.90</td>
</tr>
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<td>Total</td>
<td>31</td>
<td>40.5813</td>
<td>6.54265</td>
<td>1.17510</td>
<td>38.1814</td>
<td>42.9812</td>
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</tbody>
</table>
(IV) Post hoc tests for proteins, carbohydrates and lipids for the different varieties

**Homogeneous Subsets**

**a) protein (fruit)**

<table>
<thead>
<tr>
<th>Variety</th>
<th>N</th>
<th>Subset for alpha = 0.05</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Butternut</td>
<td>6</td>
<td>1.5483</td>
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<tr>
<td>Crownprince</td>
<td>5</td>
<td>2.5400</td>
</tr>
<tr>
<td>Carnivalsquash</td>
<td>8</td>
<td>2.8825</td>
</tr>
<tr>
<td>Greenkabacha</td>
<td>2</td>
<td>2.9300</td>
</tr>
<tr>
<td>Banana</td>
<td>4</td>
<td>2.9575</td>
</tr>
<tr>
<td>Bottleguard</td>
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<td>2.5900</td>
</tr>
<tr>
<td>Sig.</td>
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<td>.189</td>
</tr>
</tbody>
</table>

Means for groups in homogeneous subsets are displayed.

**b) carbohydrates (fruit)**

<table>
<thead>
<tr>
<th>Variety</th>
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<th>Subset for alpha = 0.05</th>
</tr>
</thead>
<tbody>
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<tr>
<td>Bottleguard</td>
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<td></td>
</tr>
<tr>
<td>Greenkabacha</td>
<td>2</td>
<td>4.0400</td>
</tr>
<tr>
<td>Carnivalsquash</td>
<td>8</td>
<td>4.3912</td>
</tr>
<tr>
<td>Banana</td>
<td>4</td>
<td>5.6125</td>
</tr>
<tr>
<td>Crownprince</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Butternut</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Sig.</td>
<td></td>
<td>.187</td>
</tr>
</tbody>
</table>

Means for groups in homogeneous subsets are displayed.
c) **carbohydrates (seeds)**

<table>
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<th>N</th>
<th>1</th>
<th>2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bottleguard</td>
<td>6</td>
<td>2.3900</td>
<td></td>
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<tr>
<td>Crownprince</td>
<td>5</td>
<td>2.8800</td>
<td></td>
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<tr>
<td>Butternut</td>
<td>6</td>
<td>2.9633</td>
<td></td>
</tr>
<tr>
<td>Carnivalsquash</td>
<td>8</td>
<td>3.0300</td>
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<tr>
<td>Greenkabacha</td>
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<td>3.2700</td>
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<td>Banana</td>
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<tr>
<td>Sig.</td>
<td></td>
<td>.184</td>
<td>1.000</td>
</tr>
</tbody>
</table>

Means for groups in homogeneous subsets are displayed.

---

d) **lipids (seeds)**

<table>
<thead>
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<th>Variety</th>
<th>N</th>
<th></th>
<th>subset for alpha = 0.05</th>
</tr>
</thead>
<tbody>
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<td>Bottleguard</td>
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<td>36.5800</td>
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<td>Banana</td>
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<tr>
<td>Sig.</td>
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<td>.055</td>
<td></td>
</tr>
</tbody>
</table>

Means for groups in homogeneous subsets are displayed.
(V) Graphs of variation of nutrients against different pumpkin varieties

a) Protein in fruit flesh

b) Carbohydrates in fruit flesh
c) Carbohydrates in seeds

![Graph showing carbohydrate content for different varieties of fruit]

- Banana
- Carnivalsquash
- Crownprince
- Greenkabacha
- Butternut
- Bottleguard

d) Lipids in fruit flesh

![Graph showing lipid content for different varieties of fruit]

- Banana
- Carnivalsquash
- Crownprince
- Greenkabacha
- Butternut
- Bottleguard
e) Lipids in seeds
(VI) Calibration curves of standards for nutrients

a) Phosphorus calibration curve

\[ y = 0.0784x + 0.024 \]
\[ R^2 = 0.9989 \]

b) Carbohydrates calibration curve

\[ y = 0.1909x + 0.0122 \]
\[ R^2 = 0.9989 \]
(c) Calcium calibration curve

\[ y = 0.0093x + 0.0016 \]
\[ R^2 = 0.9999 \]

(d) Magnesium calibration curve

\[ y = 0.1021x + 0.0086 \]
\[ R^2 = 0.9993 \]
(e) Potassium calibration curve

\[ y = 8.1143x + 3.2 \]
\[ R^2 = 0.9946 \]

(f) Sodium calibration curve

\[ y = 8.7143x + 39.143 \]
\[ R^2 = 0.9955 \]
KENYATTA UNIVERSITY
GRADUATE SCHOOL

E-mail: dean-graduate@ku.ac.ke
Website: www.ku.ac.ke

P.O. Box 43844, 00100
NAIROBI, KENYA
Tel. 8710901 Ext. 57530

Our Ref: 156/CE/15655/05

DATE: 17th September. 2013

The Permanent Secretary,
Ministry of Higher Education, Science & Technology,
P.O. Box 30040,
NAIROBI

Dear Sir/Madam,

RE: RESEARCH AUTHORIZATION EVANS CHOI CHOI – REG. NO.
156/CE/15655/05

I write to introduce Mr. Evans Choi Choi who is a Postgraduate Student of this University. He is registered for M.Sc degree programme in the Department of Chemistry.

Mr. Choi intends to conduct research for a M.Sc proposal entitled, “Levels of Macronutrients in Pumpkin Varieties (cucurbita spp) Grown in Lake Victoria Basin.”

Any assistance given will be highly appreciated.

Yours faithfully,

MRS. LUCY N. MBAABU
FOR: DEAN, GRADUATE SCHOOL
KENYATTA UNIVERSITY
GRADUATE SCHOOL

E-mail: dean-graduate@ku.ac.ke
Website: www.ku.ac.ke

P.O. Box 43844, 00100
NAIROBI, KENYA
Tel. 810901 Ext. 57530

Internal Memo

FROM: Dean, Graduate School
TO: Evans Choi Choi
C/o Zoological Sciences Department

DATE: 17th September, 2013
REF: 156/CE/15655/05

SUBJECT: APPROVAL OF RESEARCH PROPOSAL

This is to inform you that Graduate School Board, at its meeting of 11th September, 2013, approved your Research Proposal for the M.Sc Degree Entitled, “Levels of Macronutrients in Pumpkin Varieties (cucurbita spp) Grown in Lake Victoria Basin.”

You May Now Proceed with Data Collection.

Thank you.

DAVID NJOROGE
FOR: DEAN, GRADUATE SCHOOL

cc. Chairman, Department of Chemistry

Supervisors:

1. Prof. Hudson Nyambaka
   C/o Department of Chemistry
   Kenyatta University

2. Dr. Alice Ondigi
   C/o Department of Hospitality Management
   Kenyatta University