LEVELS OF ORGANIC AND MINERAL NUTRIENTS IN PUMPKIN FROM UNGUJA NORTH 'A' DISTRICT, ZANZIBAR

By

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DECLARATION

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

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DEDICATION

This work dedicated to my beloved mother for her great encouragement and to be my fist teacher of everything in my life.

ACKNOWLEDGEMENT

First of all, I thank Allah (SW) who creates everything in the universe and made me to finish this work. then I have to acknowledge those people that made the completion of this work. The completion has been possible because of the great contribution of my supervisors Dr Haji Makame Khamis and Prof. Haji Mwevura Haji for their positive reaction and remarks during their supervision

I do acknowledge for Kinowe Secondary school head teacher, Mr Salim Abeid Seif including his staff, my employer Ministry of Education and Vocational Training Zanzibar, Zanzibar Higher Education Loan Board, SUZA laboratory technicians and my university mates for their assistance in various steps to ensure my work enabled,

Special thanks to my family, especially my mother, my father, my wife, and my lovely children for their patience and their encouragement during whole period of working.

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ABBREVIATION AND ACRONYMS

ATPA	Adenosine Triphosphate
HPLC	High Performance Liquid Chromatograph
UV-VIS	Ultra violet visible
Wm	Wet mass

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ABSTRACT

The levels of carotenoids (lycopene and β-carotene) and mineral nutrients (K, Mg, Mn, Cu and Fe) in pumpkin (*Cucurbita maxima*) from Kidoti were determined to assess their variation and nutritive value of different parts of the pumpkin. The determination of the carotenoids in flesh and fibrous strands was carried out by combination of UV spectrophotometer and quantitative methods while the minerals in flesh, fibrous strand, rinds and seeds were determined by using multi-parameter bench photometer. Analysis revealed the presence of both carotenoid lycopene isomers (cis-lycopene and trans-lycopene) as well as β-carotene in flesh and fibrous. The levels of lycopene in flesh were slightly dominated by cis-lycopene isomer while fibrous strands showed opposite trend of slight domination of trans-lycopene over the cis-lycopene. However, in both flesh and fibrous part levels of lycopene were higher than β-carotene. Comparison of the two pumpkin parts revealed that fibrous strands are much richer in both lycopene and β-carotene than flesh part. The levels of mineral nutrients showed that all analyzed parts of pumpkins were predominated by Fe and K. The two metals contributed more than 90% of the analyzed mineral nutrients. Distribution of the measured metals gave the following trend: Fibrous strands> seed> rinds>flesh. Similar to carotenoids, fibrous strands were found to be much richer in metallic nutrient than the flesh part of the pumpkin which is highly consumed for food. Comparison with other related studies revealed that the levels of carotenoids are within the upper range while those of the metallic nutrients are within the middle range of the levels reported in similar studies conducted elsewhere. Assessment of the nutritional values indicated that, the pumpkins farmed at Kidoti carry sufficient levels of carotenoids and essential metallic nutrients of high nutritive values. These nutrients are extremely important to consumer health by preventing various diseases. Higher levels of iron in all parts of the pumpkins indicate that the fruits can be potentially used as blood supplement to address anemia problem. Among the analyzed parts of pumpkins fibrous strands were found to be of highest nutritional values although the part is not preferably consumed as food by most of the pumpkins users.

CHAPTER ONE

INTRODUCTION

1.1 Background

Pumpkin is an angiosperm flora that found in the genus *Cucurbita* and the family *Cucurbitaceae* commonly characterized by climbing herbaceous vine with tendrils (Muchugi *et al*., 2017). The genus exist in different species but the commonly cultivated species include *Cucurbita maxima Cucurbita mixta*, *Cucurbita pepo* and *Cucurbita moschota* (Mohammed *et al*., 2014). Other species in this genus include *Cucurbita ficiofolia and Cucurbita telfaria* (Ahmad and Khan, 2019) the fruits diverge in size, color, shape and weight and have a fairly hard rind, with a thick flesh, and many seeds.

Pumpkins are commonly used as food but the fruits have recently processed into different products and some of the products are considered as medicine. Although all parts of fruits are edible but flesh has been commonly used as puts and other parts are considered as wastes (Lyimo *et al*., 2012). Pumpkins are cooked and consumed innumerous ways, and most parts of the pumpkin are edible, from the flesh, shell to the seeds. In Korea, pumpkin flesh is consumed in soups and juices, or it is integrated into various foods, such as rice cakes, candies, and breads (Kim et al., 2012). Pumpkin seeds and seed oil are also commonly consumed as a sources of vitamins and minerals in some countries (Kim et al., 2012).Due to their high nutritional advantage in terms of vitamins, minerals and dietary fiber content, the fruits have attracted much attention of both food chemists and technologists.

Pumpkins have been identified to be a valuable source of carotenoids, pectin, mineral salts, ascorbic acid and other bioactive compounds, which play major roles in human nutrition. For instance, carotenoids such β-carotene are pro-vitamin A while lycopene are very important antioxidants. Current scientific studies show that a foods containing β-carotene may decrease the risk of developing cancer, provide protection against heart problems and help to prevent skin diseases and vision disorders (Blessing et al., 2011). Pumpkin consists of rind (skin), flesh (pulp), fibrous strand and seeds parts; and all these parts are edible with significant nutritive values (Lyimo *et al*., 2012).

Pumpkins are drought-tolerant and are sensitive to water logging and are commonly farmed at low scales within coral areas in Zanzibar. The crop is entirely considered as a subsistence crop with little perceptions of their potentiality of being promoted to commercial scale. Low awareness on the potential of upscaling the crop to commercial scale has been largely attributed by low understanding of its nutritive values. The internal market value of pumpkin in Zanzibar picks up during the month of Ramadhan, a period that the fruits are intensively used for breakfast (iftari). Despite such significant nutritive values and potential of promoting pumpkins to commercial there is little efforts directed in converting the crop from subsistence to commercial level. There is an urgent need to create the awareness on nutritive values and potential of promoting the crop to internally as alternative cheap source of vitamins. This awareness will also promote the crop to commercial level and boosting farmers' income in Zanzibar. Chemical analysis of their nutrients and assessment of their nutritive values can highly contribute to increase such awareness at different levels.

1.2 Problem statement

Pumpkin is considered as food crops without any significant potential to be promoted to commercial levels. In other part of the world the pumpkins fruit has been shown to carry important nutritive value and thus promoted to commercial levels. Pumpkins have considerable variations in nutrient contents depending on the cultivation environment, species and part of the fruit(Kim *et al*., 2012). Knowledge on the nutritive values of pumpkins farmed in Zanzibar is very scantly. Most people in Zanzibar consume flesh part of the fruit while seeds, rind and fibrous stands are rarely or not consumed at all. Poor understanding of the nutritive values of the fruit has largely attributed to this situation. Information on variation of nutritive among the pumpkins parts can used to provide advises to consumer on which parts should be preferred for local consumption but also for processing other commercial products. This study was aimed to assess the nutritive values pumpkins by investigating composition of organic and mineral nutrients in different parts of pumpkin.

1.3 Objectives

1.3.1 General objective

To assess various organic nutrients (lycopene and β-carotene) and minerals nutrients (such as potassium, magnesium manganese, copper and iron in different parts of pumpkin fruit (rind, flesh, fibrous strand and seeds)

1.3.2 Specific objectives

The specific objectives of this study were:

a. To determine levels of lycopene and β-carotene in flesh and fibrous of *Cucurbita maxima* pumpkin.
b. To determine levels of potassium, iron, copper, magnesium and manganese in rind, flesh, fibrous strand and seeds of *Cucurbita maxima* in different parts of pumpkin.
c. To assess variations of organic and mineral nutrients among pumpkin parts.
d. To assess nutritive value of different parts of the pumpkin (*Cucurbita maxima*) fruits.

1.4 Hypothesis

a. What are the levels of carotenoids contained in flesh and fibrous strand of *Cucurbita maxima?*
b. What are the levels of mineral nutrients contained in the rinds, flesh, fibrous strands and seeds of Cucurbita maxima**?**
c. Is their significant variation of carotenoids and minerals nutrients among pumpkin parts?
d. What are nutritional statuses of analyzed pumpkin parts?

1.5 Significance of the study

This study documents the baseline information on the levels of organic and mineral nutrients of nutritive value in pumpkins farmed in Zanzibar. It reveals their nutritive status and possibility of using pumpkins as a cheap source of protein and ensuring food security. The information can be used by Ministry of Agriculture to highlight the prospective of upscaling this local crop production to commercial level.

CHAPTER TWO

LITERATURE REVIEW

2.1 Pumpkin

Pumpkin is an angiosperm belonging to the genus Cucurbita and family Cucurbitaceae, characterized by climbing herbaceous vine with tendrils (Muchugi *et al.,* 2017). The pumpkin family exists in different species differing in properties and nature of the plants and fruits. The most common species in this genus include *Cucurbita maxima, Cucurbita mixta, Cucurbita pepo* and *Cucurbita moschota* (Mohammed *et al*., 2014). Pumpkin is native to Latin America (Chile and Argentina) and then domesticated in North America and Europe. The farming of pumpkins spread and dominated within tropical Asia climate in China, Malaysia, Philippines and Indonesia *(Hosen et al., 2021*). Pumpkins are drought resistant crop which grow well in well-drained soil and is propagated through direct sowing of seeds.

The seeds can favorably germinate at temperature of 20^0C to 35^0C and soil with pH between 6.0 and 6.5 (Ahmad, 2019). The pumpkin seedlings grow well even under dry conditions and the plants may take between three to four months to flowering depending on the varieties (BBS, 2015; Ahmad and Khan, 2019). Pumpkins are monoecious having both male and female flowers. Pumpkins fruits are harvested when they are at full maturity. The fruits are usually orange, yellow, white, red or grey and have numerous seeds. Average weights of pumpkin fruits range around 4 to 8 kilograms however very large mass reaching a weight of 34 kilograms has been report (Peter, 2011).

The pumpkin fruits have long shelf time of about six years due to wax-covered skin enables and therefore they have very little post-harvest loss (Kamarubahrin, 2018). The long shelf time of pumpkins make them as important fruits in strengthening food security to many pumpkin farmers (Nakazibwe, et al 2019).The fruits are generally used for both food by direct cooking but in recent past, there has been different innovations of value addition by processing pumpkins into flour for food coloring and making cakes, breads, bites and pickle (Dhiman et al., 2018).The innovations products are them used as additives in foods and for medical purposes.

Literature review on production of pumpkins shows that Far East region is the main producer of pumpkins and China is leading other countries contributing to nearly 58% of the world production followed by India (20%), Russia (4%) and Ukraine (4%). Other major producers of pumpkins are Mexico, Korea, Vietnam and the United State of America (U.S.A).In these countries farming of pumpkins are cultivated as commercial crop and are exported in different countries(Hosen, 2021; Rahman, 2019). Several researches have shown that pumpkin can grow well in many African countries but there have been number factors limiting their cultivation and upscaling the crop to commercial levels (Kiramana & Isutsa, 2019). Despite the existing limitation a notable cultivation has been reported in South Africa, Zimbabwe, Nigeria, Malawi and East African countries (Nakazibwe et al., 2019).In all three East African countries, Tanzania, Kenya and Uganda cultivation of pumpkins are concentrated around lake Victoria(Ondigi et al. 2008; Nakazibwe et al., 2019). According to Food and Agriculture Organization of United Nation (FAO), pumpkins are among the major sources of global world food due to their adaptability to variety of climate of the world, and its world production picked up

in 2011. It was estimated that over 24.3 million tons of pumpkins were harvested from 1.7 million hectares worldwide(Dar & Sofi, 2017). Several studies have indicated that pumpkins are potential and cheap food crops that not only can address food insecurity situation in low coming countries but the fruits are very rich in different nutrients of both nutritive and medicinal values (Dowidar et al., 2020). The nutrients include both Organic and inorganic in nature.

2.2 Organic Nutrients

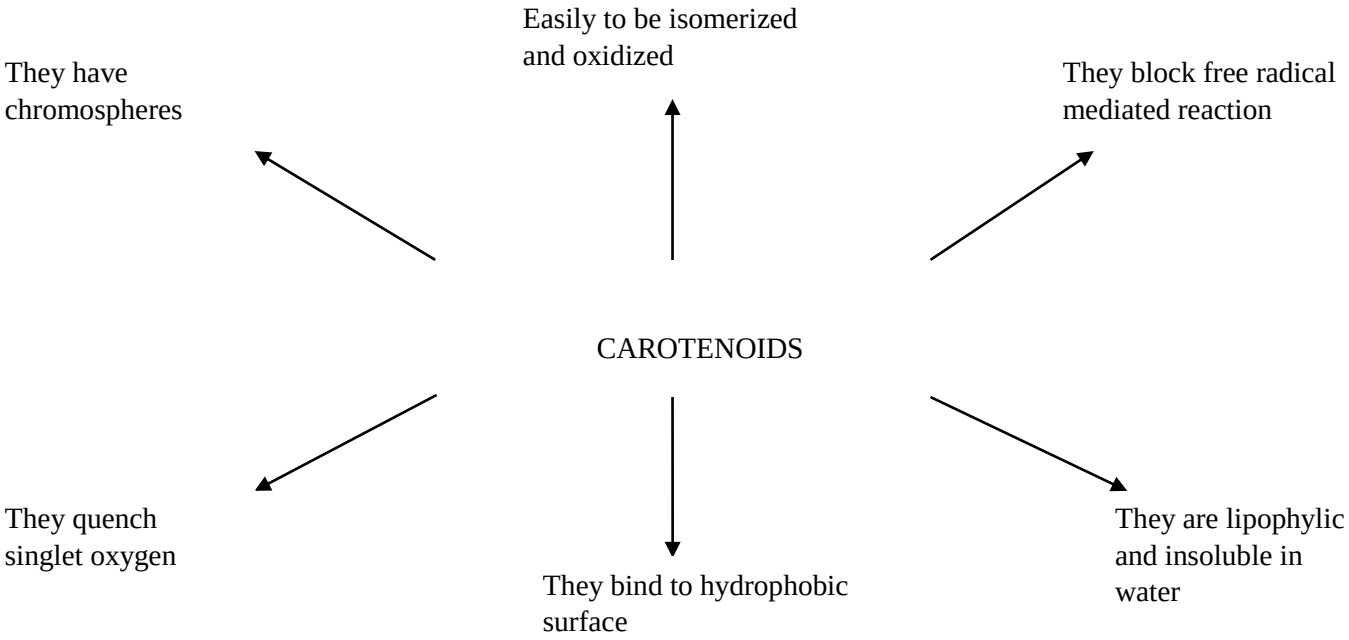
2.3 Carotenoids

The common inorganic nutrients of both nutritional and medicinal in pumpkins are referred as carotenoids. Carotenoids are fat-soluble tetraterpenoids which have coloring power for vegetables and fruits that include yellow, orange and red color. They can be classified into two groups and both groups play significant role when released into human body. The first group carotenoids are those vitamin A precursors; that can be converted to retinol (vitamin A) while the second groups are not convertible to vitamin A. The carotenoids that can be converted to retinol include alpha-carotene, beta-carotene, gamma-carotene and beta-cryptoxathin;.and those that are not converted into Vitamin A include lycopene, lutein, and Zeaxanthin which are basically function as antioxidants. Carotenoids act as antioxidants by deactivating the singlet oxygen species produced when exposed to sunlight (Papaioannou et al., 2015). Carotenoids occur extensively in nature and all colored fruits and vegetables are generally considered to be good sources of these compounds. The carotenoids can be the synthesized chemically in large amount to be used for different purpose and small amount is acquired through extraction from plants

or algae (Mezzomo & Ferreira, 2016).

2.3.1 Physical and chemical properties of properties of carotenoids

The distinguishing structural feature of carotenoids is polyene chain that means to have an extensive conjugated double bond system which consists of alternating double and single carbon-carbon bonds with chromophore that are able to absorb light, (Rodriguez-amaya, 1997). The description and physical properties of carotenoids are summarized in figure 2.1



(Rodriguez-amaya, 1997)

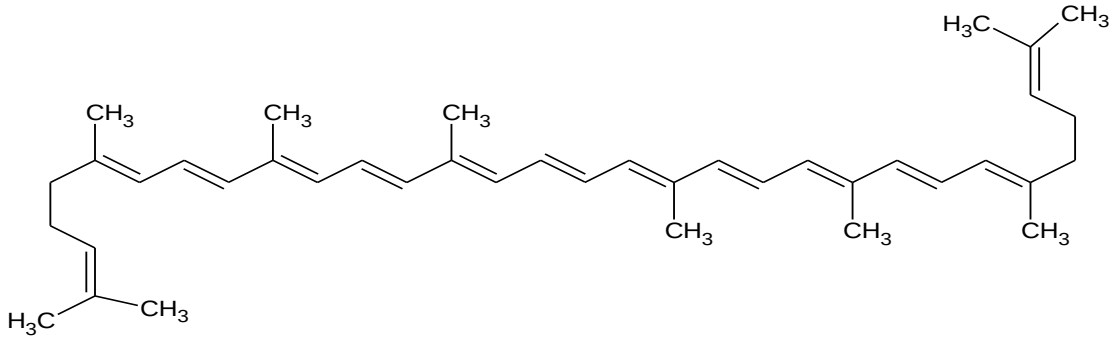
Figure 2.1: Properties of carotenoids

Carotenoids are known as bioactive compounds that are very important medicinal value to human health. The compounds have been shown to reduce risk of getting degenerative health problems such as cardiovascular diseases and cancer (Shahidan *et al.*, 2014). Many studies have revealed their effectiveness to inhibit cardiovascular, gastrointestinal,

infectious, and neurodegenerative diseases, as well as their therapeutic effect in carbohydrate metabolism disorders. Apart from human health, carotenoids are also playing major roles of being photosensitizers in conjunction with chlorophyll and in protecting the plant cells (Papaioannou *et al.,* 2015).

.3.2 Lycopene

Lycopene ($C_{40}H_{56}$) is a member of the carotenoid family of phytochemicals (i.e., chemical compounds that occur naturally in plants) and consists of a linear chain of hydrocarbons with carbon-carbon double bonds. There are two central methyl groups at the 1, 5-position and additional methyl groups at the 1,6-position. The extended system of alternating double bonds is critical to the biological activity of lycopene, which includes its susceptibility to oxidative degradation. It is an antioxidant that is synthesized by many plants and microorganisms but is not produced in humans or animals



(Substance *et al.,* 2012)

Figure 2.2 Structure of lycopene

Lycopene are abundantly found in red colored fruits and vegetables such as tomato, papaya, pink grapefruit, pink guava, pumpkin and watermelon. They occur naturally in all-trans form with chains containing seven double bond that can be isomerized to mono-cis or poly-cis when exposed with high temperatures, light, oxygen, acids, catalyst and

metal ions (Nasir *et al.*, 2015). Lycopene is a bioactive red-colored pigment that is sometimes used as a natural coloring agent in food. The color characteristic of tomatoes and other foods high in lycopene is directly linked to the presence of a high concentration of carotenoid molecules. The coloring ability of lycopene depends on its concentration, method of dispersion and formulation (Substance *et al.*, 2012).

Lycopene plays different roles in both animals and plants. Among the reported roles in human body are to treat and prevent prostate cancer, treatment of asthma, treatment of obesity, treatment of hypertension ,maintaining vision health , treatment of coronary heart diseases , prevent atherosclerosis, cure breast cancer , skin roughness, liver protection and treatment of hepatitis C, (College & Road, 2011)

2.3.3 β-carotene

β-carotene is an isoprenoid compound and one of the approximately 600 fat-soluble carotenes found in plants and micro-organisms. The compound occurs in the form of red to brownish-red to violet crystals or crystalline powder when extracted from their sources. It has chemical formula $C_{40}H_{56}$which is predominated by all-trans (Z) isomer of β-carotene with varying amounts of the cis-isomer depending on different formulations, (Gul *et al.*, 2015). The structural formula of the β-carotene is presented in figure 2.3.

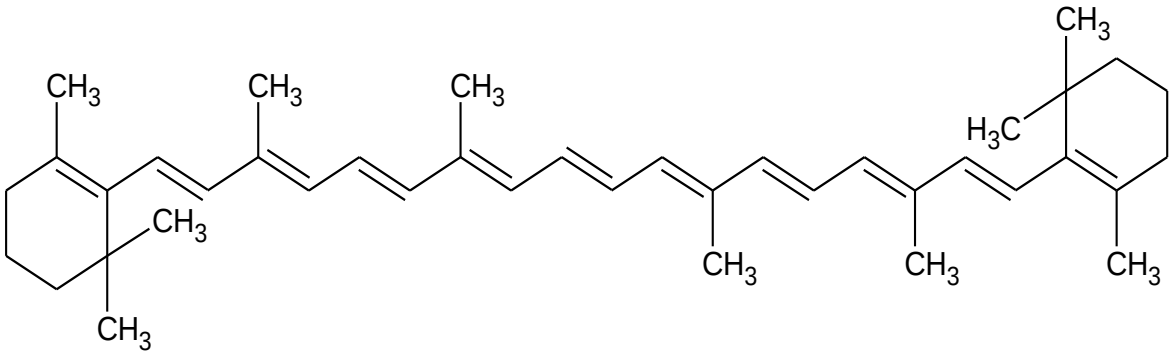


Figure 2.3: Structure of β-carotene.

According to (Jaswir et al., 2011) , the main dietary sources of β-carotene include carrot, spinach, lettuce, broccoli, tomatoes, orange, red paper, orange paper, mango, papaya, guava, pumpkin and grapefruits β-carotene is among the carotenoids that can be converted to retinol. It is widely used in food industry as a precursor of vitamin A or as a natural colorant(Gul *et al*., 2015).As the compound is very labile and easily degraded by heat, light, and oxygen, several attempts are being made to increase the shelf stability of β-carotene towards various processing conditions. Encapsulation is one of the prominent means among the several strategies of protecting β-carotene from degradation. The method has been found to be very effective in improving its stability for use in food and allied industries.

2.4 Inorganic Nutrients

The inorganic nutrients that have been widely screened and analyzed in pumpkins are the following essential metals potassium, iron, copper, magnesium and manganese. These play significant role in human and other mammalian bodies.

2.4.1 Potassium (K)

Potassium is an essential element which is widely found in cationic form in intracellular fluid. It plays a key role in maintaining cell function, particularly in excitable cells such as muscles and nerves. It is essential for cardiac-functions as well skeletal and smooth muscle contraction (Dawood, 2019). Potassium plays key role in glycogenesis process and transferring of phosphate from ATP to pyruvic acid. The metal is also important in balancing acid-base, in regulation of osmotic pressure and conduction of nerve impulse in human body (Soetan *et al*., 2010).

K is important in human in regulating functions of different biological process. Deficiency of K in human bodies has been associated with stroke, coronary heart diseases, renal diseases, loss of bone masses problems. However, high dietary intake of K decrease of in blood pressure (Weaver, 2013) Studies have also shown that presence of K is directly associated with the presence of other metals such as zinc.

2.4.2 Iron (Fe)

Iron is a biologically essential element for every living organism. It plays major roles in the formation of oxygen-transporting protein particularly hemoglobin and myoglobin, and in many iron-containing enzyme that play role in the oxidation and reduction processes in the body (Dawood, 2019). The element is required for proper myelination of spinal cord and white matter of cerebellar folds in brain and is a cofactor for a number of enzymes involved in neurotransmitter synthesis (Soetan *et al*., 2010).

Fe plays significant role in the formation of blood and ferrous containing supplements are commonly served to address the problem anemia. Deficiency of iron in the human body leads low hemoglobin content and therefore affects transportation of oxygen within human blood. On the other hand excess intake of Fe may damage various organs such as spleen, liver, heart and bone marrow (Radysh et al., 2018). Some studies have shown that deficiency of Fe may trigger absorption of toxic metals such as lead and cadmium (Goyer et al., 1997).

2.4.3 Copper (Cu)

Copper is an element which is associated with bone health, immune function and increased frequency of infections, cardiovascular risk and alterations in cholesterol

metabolism. Copper is an essential mineral for human health and but can become toxic if it is taken in excess amount such that the ingested load exceeds body tolerance limit (Araya *et al*., 2007). The most frequent clinical manifestations copper deficiency are anemia, neutropenia, and bone abnormalities. It should considered that copper is an essential nutrient with potential toxicity it is therefore need to be taken within the acceptable load range (Uauy *et al.*, 1998).

Although Cu is required in very small amount within human body but its deficiency results to number of health problems including imbalance of lipids, lipoprotein and glycolipid in blood and liver by increasing cholesterol hypertension. In some cases, deficiency of Cu may also affect immune system of the body by decreasing number of white blood cells that fight against diseases and infections. However excessive amount of Cu in human body becomes toxic (Radysh et al., 2018).

2.4.4 Magnesium (Mg)

Magnesium is an essential element required as a cofactor for over 300 enzymatic reactions and is thus necessary for the biochemical functioning of numerous metabolic pathways. It perform crucial role of preventing and treating many common health conditions including migraine headache, metabolic syndrome, diabetes, hyperlipidemia, asthma, premenstrual syndrome, preeclampsia, and various cardiac arrhythmia (Schwalfenberg & Genuis, 2017). Magnesium is a fundamental element for ATP metabolism and is required for DNA and RNA synthesis, reproduction, and protein synthesis. It is also an essential element for the regulation of muscular contraction, blood pressure, insulin metabolism, cardiac excitability vasomotor tone, nerve transmission and neuromuscular conduction (Schmidt & Kisters, 2015)

Inadequate amount of magnesium in the body has been associated with various health problems such as cardiovascular and metabolic diseases and respiratory problems; and skeletal and respiratory illness. Other health problems include stress, anxiety and depression. Mg also plays crucial role of assisting human body to absorption and vitamin D (Razzaque, 2018). Literature has reported that high magnesium intake from natural food has no effect, but effects are observed in magnesium from non-food substances that causes diarrhea and gastrointestinal effects (VKM , 2016).

3.4.5 Manganese (Mn)

Mn is one among the constituents of some enzymes and is an important element in activating numerous enzymes such as hydrolases, transferases, kinases, and decarboxylases and one of the most well-known Mn metaloen-zyme is pyruvate carboxylase, which catalyzes the conversion of pyruvate to oxalo-acetate (Watts & Ph,). Mn is an important essential element in regulating enzymatic biological processes such as regulation of insulin and glucagon. Deficiency of manganese in the human body is also associated with risk of breast cancer, tumor growth, respiratory infections including asthma in children and allergic diseases. Despite such health benefits, excess manganese in human body may affect brain functioning may overloaded result mutation of genes of the liver and brain that affects their functions (Radysh et al., 2018)

CHAPTER THREE

MATERIALS AND METHODOLOGY

Pumpkins fruits belong to *Cucurbita maxima* species were used in this study. Different parts of the fruits including flesh and fibrous strands were analyzed for both organic and inorganic nutrients. However, seeds and rinds which is the outermost part of the fruit were also analyzed for inorganic nutrients only but not organic.

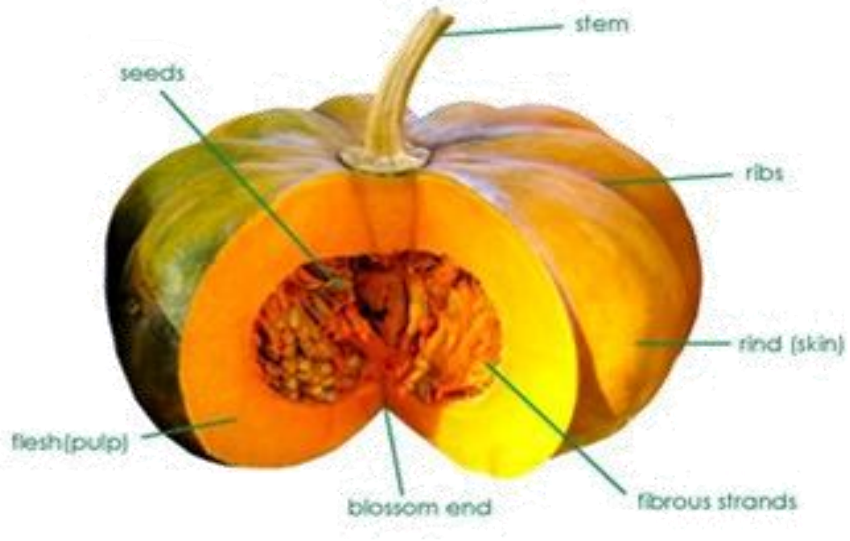


Figure 3.1 Parts of Pumpkin.

3.1 Area of study

The pumpkin samples analysed in study were collected from plots of subsistent farmers at Kidoti. Kidoti is located in North A district, North Region of Unguja Island. Kidoti is characterised by coral rag along both western and eastern coastal line but its central part is non-coral rag areas. The village is leading in production of pumpkins compared to other village within North A District. The map of Zanzibar showing the study area is presented in Figure 3.2. Pumpkins are normally farmed seasonal within coral rag areas in

the eastern part of Kidoti. The farming season start during rain period, however with the support of irrigation farming can be done in any season. The farming of pumpkins in Zanzibar in general and within the study area in particular are mainly considered to be of subsistence scale with some elements of commercial farming during the month

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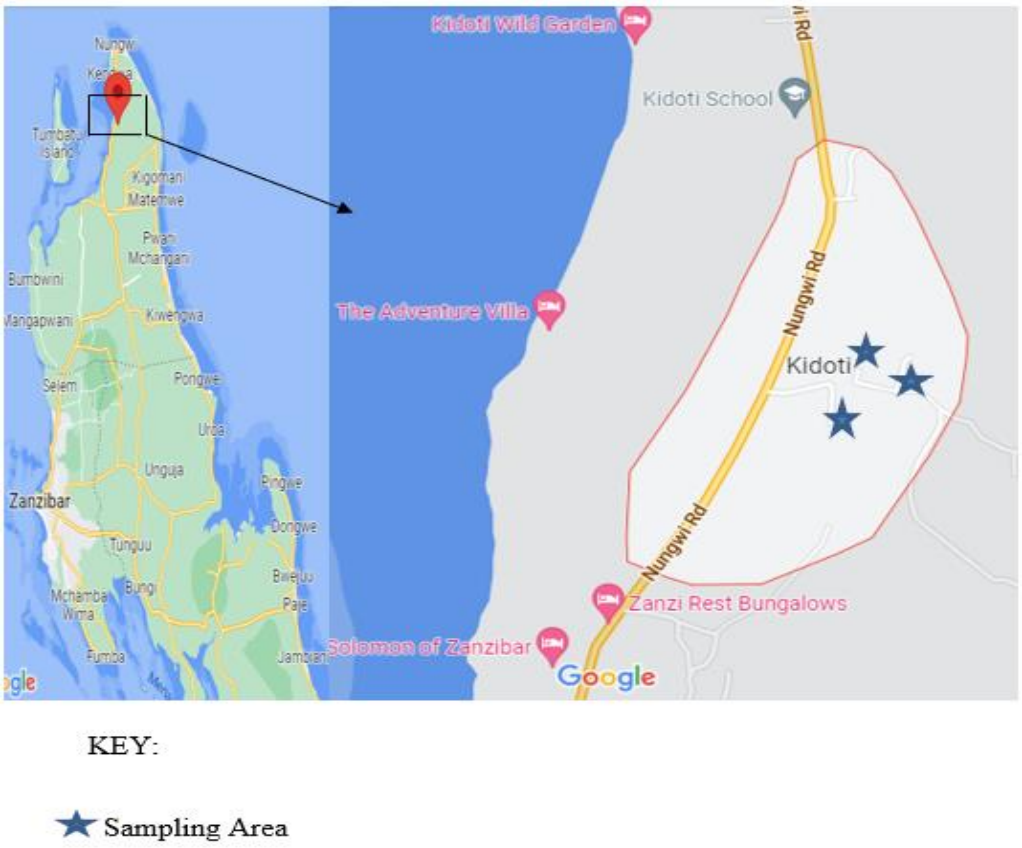


KEY:

★ Sampling Area

(Google map 2021)

Figure 3.2: The map showing study area.

3.2 Sampling

Ten *Cucurbita maxima* fruits were collected from three farms to obtain gross sample. The whole fruits were then transported to laboratory for further sample processing.

3.3 Sample Processing

Each pumpkin fruit was cut vertically ‘into ten equal portions using a pre-cleaned knife. One portion from each fruit was randomly selected for sample processing.

.3 Sample Processing

Each pumpkin fruit was cut vertically ‘into ten equal portions using a pre-cleaned knife.

Figure 3.3: Portions of pumpkin fruits.

Using the cleaned knife each selected portion was separated into rind, flesh, fibrous strand and seeds, and was then thoroughly blended to give bulk homogenized samples. For the case of flesh and fibrous strand parts two samples were measured from bulk sample; one sample of mass of 50gm for carotenoids (lycopene and β-carotene) analysis and the second sample (2g) for minerals analysis. On the other hand, one sample of 2g from each of the homogenized seeds and rinds parts was taken for mineral analysis.

3.3.1Sample Extraction of lycopene and β-carotene

Sample of mass 50g was soaked in a mixture of 100mls of ethyl acetate, acetone and hexane (1:2:4respectively) and shaken for about 15 min using overhead shaker. The

Figure 3.4: Sample with solvent mixture.

The extracts were decanted and collected into a beaker through the anhydrous sodium sulphate. The samples residues were then successively extracted twice using the same procedures. The obtained extracts from the three extraction cycles were combined. The combined extracts were then concentrated to a 3ml volume and stored into vials in a refrigerator**.**

3.3.2 Separation of lycopene and β-carotene

Lycopene and β-carotene components of organic nutrients were separated by column chromatographic method. The concentrated extract was quantitatively transferred to a 1-

cm diameter glass column packed with pre-cleaned sand (I cm) followed with silica gel (10 cm) and then capped with 2 g sodium sulfate (I cm) to dry the sample.

The column was then eluted with hexane and different portions of the eluent based on their colors were collected. Yellow orange band (*trans*-lycopene)**,** yellow band (*cis*-lycopene) and orange band (better carotene) were separately collected in different beakers. The colored eluent bands were concentrated using rotary evaporator to nearly 2 ml and then further dried by a gentle stream of nitrogen gas. The mass of the obtained dried solid was measured using analytical balance3.3.3 Sample Digestion for mineral analysis

The sample digestion was performed as described in Asdeo and Loonker (2011) with some modification on mass of sample and type of acid used for digestion. An exact mass of 2.0 g of the homogenized sample was dried sample and completely burnt in furnace at 500^{o}C. The dried samples were then digested by using 10 ml of 69 % concentrated nitric acid. The digest was then cooled and 2 ml of perchloric acid was added before being further heating at temp of 500^{o}C for 30 minutes. The sample was then filtered qualitatively transferred into volumetric flask before being topped to 50 ml with double dis

Figure 3.5: Drying of sample

3.4 Sample Analysis

3.4.1 Lycopene and β-carotene

Identification of lycopene and β-carotene was accomplished through Uv-vis scanning method using computerized Uv-visible spectrophotometer-Thermo-scientific (Evolution Bio 260) equipped with Dual Silicon Photodiode detector. The chromatographic cleaned eluent portion for Uv-vis scanning was transferred and filled into a glass cuvette to the mark. The sample in the cuvette was then scanned at wavelength ranging from 300nm to 800nm.

The characteristic spectra with wavelength were targeted and retrieved from the computer. The identification of type of carotenoids was accomplished by monitoring selected characteristic wavelength peaks.

3.4.2 Uv-vis scanning

The cleaned eluent portion for Uv-vis scanning was put into the glass cuvette to the mark. The cuvette with sample was scanned at wavelength ranging from 300nm to 800nmusing computerized Uv-visible spectrophotometer-Thermo-scientific (Evolution Bio 260) equipped with Dual Silicon Photodiode detector. The characteristic spectra with wavelength data were then recorded and retrieved from the computer. The identity of each carotenoid was confirmation by the presence of three monitored characteristic wavelength peaks. For trans- lycopene the monitored wave length peaks were 421.88, 448.44 and 475.00 nm while the monitored peaks for cis- lycopene were at wavelength of

419.53, 446.88 and 472.66. The characteristic peaks for β-Carotene were 401.56, 425.78 and 450.78

On the other hand, quantification of amount of lycopene and β-carotene was done by using quantitative method. Cleaned portion of the eluents in the pre-weighed vials were gently blown with stream of nitrogen gas to dryness. The mass of vial with dried sample was weighed by using electrical analytical balance. The mass of extracted carotenoid was then determined by subtracting mass of vial from mass of vial with dried sample. The total mass and concentration of carotenoids were then computed by multiplying with volume ratio between total extracted volume and dried volume.

3.4.3Analysis of metallic nutrients

Determination of concentrations was performed using Hanna instrument - HI 83099 COD and Multi-parameter Bench Photometer. The photometer has in-built program of methods for analysis of different metals. The program automatically adjust to required operating conditions of a metal such as wavelength once the method of analysis for the metal has been selected (Hanna instrument, 2000). The procedure for analysis was as follow: 10 ml of the blank sample containing proportions of reagents that was used in sample preparation was filled into cuvette and capped. Similarly, 10 ml of the samples to be analyzed was filled in another cuvette and then capped. The blank cuvette was then loaded into holder of the photometer and the zero key was pressed to calibrate the instrument to zero reading. Without changing the setting of the instrument, the blank cuvette was removed and the sample cuvette inserted into the holder. The timer key which provides optimum time for analyses was pressed and the instrument displayed the concentration value of the analyzed element.

3.5 Data analysis

Analysis of variations of the determined concentrations carotenoids and metallic nutrients were determined using Statistic Package for Social science (SPSS) version 20 MS excel.

CHAPTERT FOUR

RESULTS AND DISCUSSION

This chapter presents the results of the laboratory analysis of organic and minerals nutrients from four parts of *Cucurbita maxima* (rind, flesh, fibrous strands and seeds) and discussed them. The results are presented in the form of description tables and figure. The chapter also discussed the results by comparing levels of organic and minerals nutrients measured among the analyzed parts of pumpkin fruit and were compared with related studies.

4.1 Identification of lycopene and β-carotene

The identification of two isomers of lycopene and beta-carotene was accomplished by scanning their characteristic UV wavelength. These characteristic wave length for trans and cis isomers of lycopene as well as that of beta-carotene are presented in Table 4.1

Table 4.1: Lycopene and β-carotene characteristic wave length

S/No	Carotenoid	Color	Characteristic wavelength peaks (nm)
1	Trans- lycopene	Red/orange	421.88; 448.44; 475.00
2	Cis- lycopene	Yellow orange	419.53; 446.88; 472.66
3	β-Carotene	Yellow	401.56; 425.78; 450.78

The UV scan of the pumpkin flesh and strand samples between 200nm and 500 nm revealed presence of both trans and cis isomers of lycopene as well as beta carotene. The sample UV scans for cis-lycopene, trans-lycopene and beta carotene are given in figure 4.1.

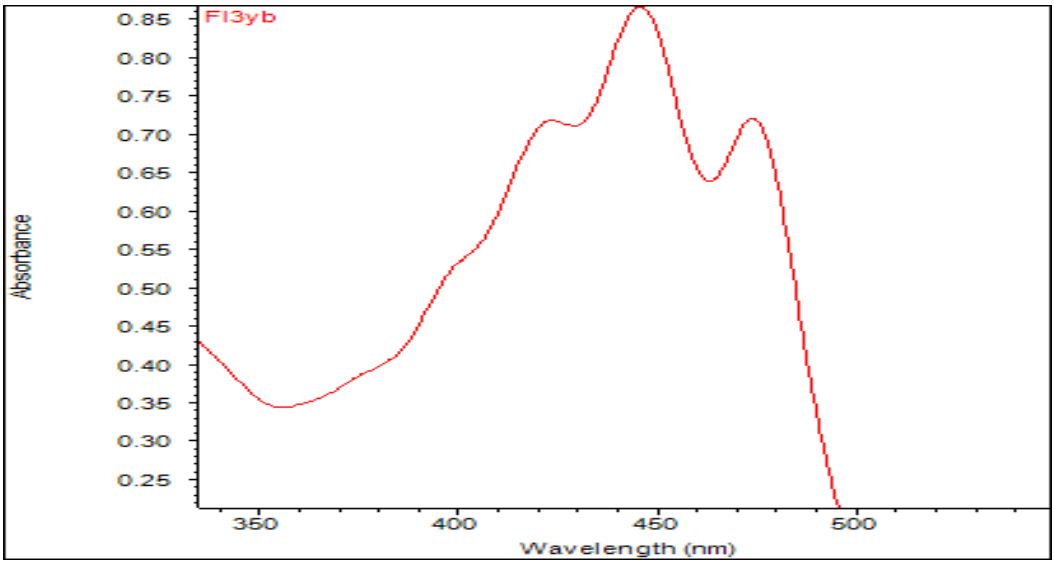


Figure 4.1: Sample UV scan of yellow colored extract for beta carotene in flesh

4.1.1 Levels of lycopene and β-carotene in pumpkin flesh

Lycopene isomers and β-carotene were measured at varying concentrations in flesh and strands of the pumpkin samples. The extracted masses of lycopene and their corresponding concentrations are presented in Table 4.2a; and descriptive statistics are given in Table 4.2b. The two isomers of lycopene, trans-lycopene and cis- lycopene were measured at concentration ranging from (200 to 5040µg/gwm,) and (540 to 2840µg/g) respectively. Their mean concentrations were 1388.89µg/g wm for cis lycopene and 1245 µg/g wm for trans-lycopene.

	Mass (g)					Concentration (µg/g)			
Sample	Mass	trans lycopene	Cis lycopene	Total lycopene	β-carotene	trans lycopene	Cis lycopene	Total lycopene	β-carotene
2	50	0.043	0.041	0.084	0.058	860	820	1680	1160
3	50	0.054	0.094	0.148	0.058	1080	1880	2960	1160
4	50		0.073	0.073	0.050	-	1460	1460	100
5	50	0.010	0.081	0.091	0.137	200	1620	1820	2740
6	50	0.040	0.051	0.055	0.113	800	1020	1120	2660
7	50	0.252	0.142	0.394	0.062	5040	2840	7880	1240
8	50	0.051	0.027	0.078	0.038	1020	540	1560	760
9	50	0.027	0.029	0.056	0.048	540	580	1100	960
10	50	0.021	0.087	0.108	0.041	420	1740	2160	820

Table 4.2a: levels of lycopene and β-carotene in flesh

Table 4.2b: Descriptive statistics of carotenoids in flesh

Carotenoids	**Minimum**	**Maximum**	**Mean**	**Std. Deviation**
trans lycopene	200	5040	1245.00	1562.772
Cis lycopene	540	2840	1388.89	738.520
Total lycopene	1100	7880	2495.56	2083.813
β-carotene	100	2740	1288.89	869.086

The measured isomers of lycopene in flesh part of pumpkin, gave a total lycopene concentration (Σlycopene = cis-lycopene + trans-lycopene) varying from1100µg/g to 7880µg/gwm with mean concentration of 2495.56µg/g wm. In average the total lycopene in flesh strand was slightly dominated by cis-lycopene which contributed to 55.56 % of the total lycopene. Masses of β carotene in pumpkin flesh samples and their corresponding concentrations as well as descriptive statistics are also presented in Table 4.2a and 4.2b, respectively. The β carotene was measured at relatively low concentrations compared to lycopene (Figure 4.2) and their differences were not statistically significant (p-value 0.242). It was measured at mean concentration of 1288.89µg/g wm ranging from 100 to 2740 µg/g wm.

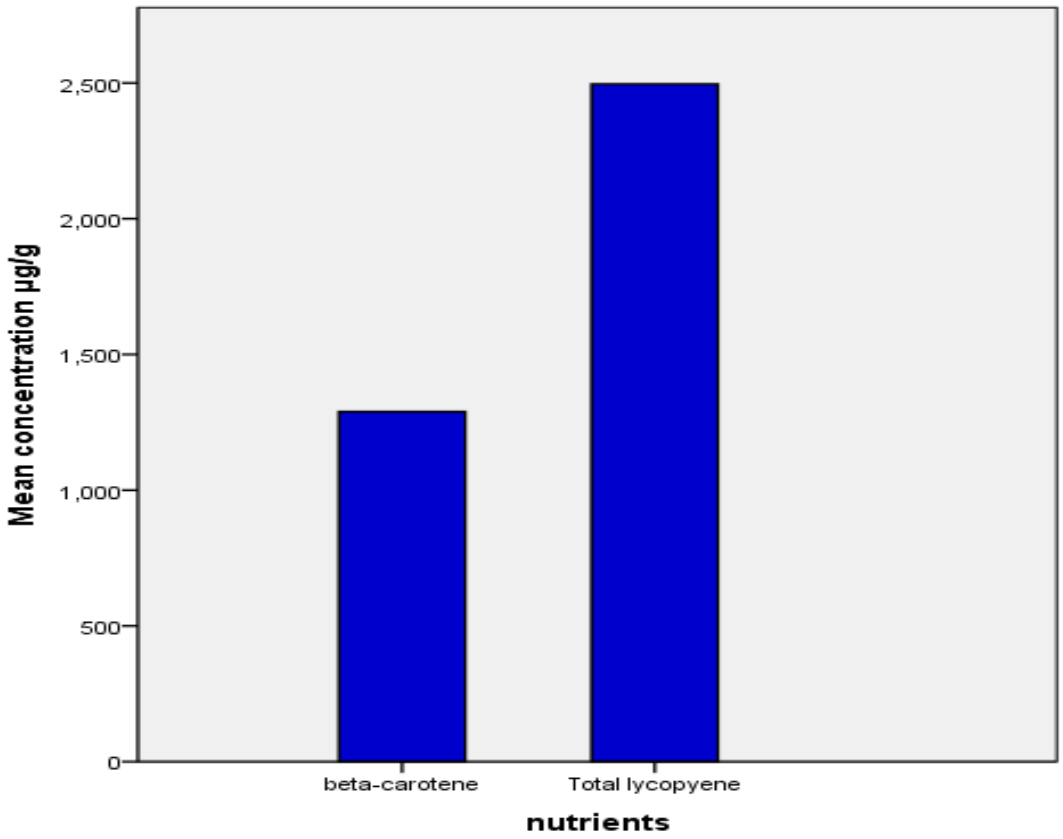


Figure 4.2: concentration of lycopene and beta-carotene in flesh.

4.1.2 Levels of lycopene and β-carotene in fibrous strand of pumpkins

Masses of lycopene isomers and β-carotene in fibrous strand part of pumpkin and their corresponding concentrations are summarized in Table 4.3a. While the descriptive statistics are presented in Table 4.3b. The levels of the two lycopene isomers were almost similar in the fibrous strand. The trans-lycopene isomer varied from 100µg/g to 6860µg/g with the mean of 1853.33µg/g while cis-lycopene varied from 220µg/g to 6680µg/g with the mean of 1788.89µg/gwm.

These levels resulted to a total lycopene concentration varying from 720 to 9060µg/g wm with the mean of 3642.22µg/g. In contrary to flesh, the total lycopene in fibrous strand was slightly dominated by trans-lycopene which contributed to 50.88% of the total lycopene. Levels of β carotene varied from 380 to 3680µg/gwith the mean of 1227.50µg/gwm. Similar to flesh, the levels of lycopene in fibrous strand were also not significantly different (p-value = 0.08)) than those of beta carotene. Figure 4.3compare the levels lycopene and beta carotene in fibrous strand

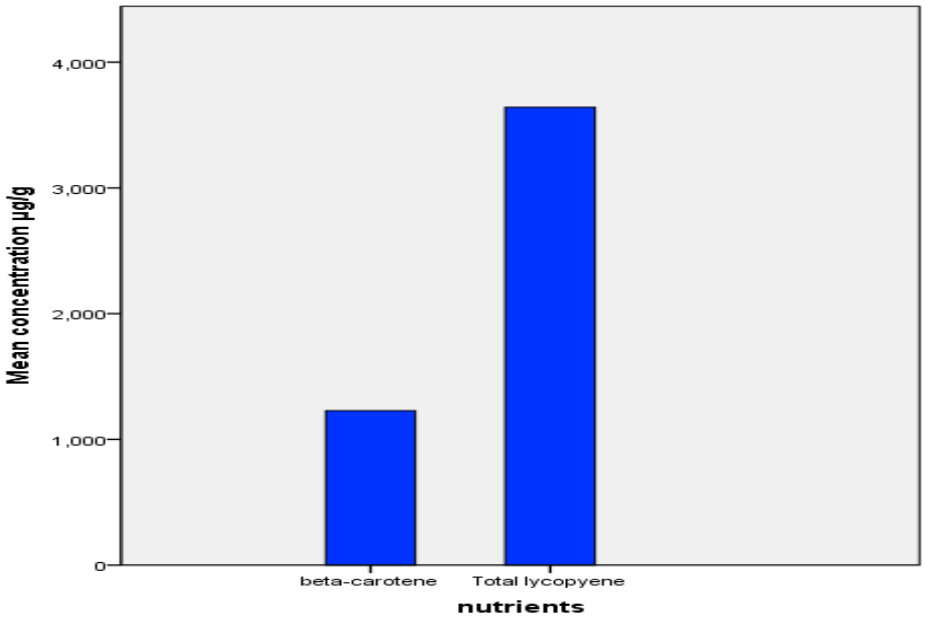


Figure 4.3: concentration of lycopene and beta-carotene in fibrous strands

Table 4.3a: levels of lycopene andβ-carotene in Fibrous strand

	Mass (g)					Concentration (µg/g)			
Sample	Mass	Trans lycopene	Cis lycopene	Total lycopene	β-carotene	Trans lycopene	Cis lycopene	Total lycopene	β-carotene
1	50	0.081	0.041	0.122	0.041	1620	820	2440	820
2	50	0.005	0.031	0.036	-	100	620	720	-
3	50	0.078	0.334	0.412	0.049	1560	6680	8240	980
4	50	0.343	0.110	0.453	0.059	6860	2200	9060	1180
5	50	0.010	0.034	0.044	0.184	200.	680	880	3680
6	50	0.038	0.011	0.049	0.040	760	220	980	800
7	50	0.050	0.022	0.072	0.024	1000	440	1440	480
8	50	0.063	0.125	0.188	0.075	1260	2500	3760	1500
9	50	0.166	0.097	0.263	0.019	3320	1940	5260	380

Table 4.3b: Descriptive Statistics of lycopene and beta-carotene in fibrous strand

Carotenoids	Minimum	Maximum	Mean	Std. Deviation
All trans lycopene	100	6860	1853.33	2104.804
Cis lycopene	220	6680	1788.89	2013.582
Total lycopene	720	9060	3642.22	3214.708
Beta-carotene	380	3680	1227.50	1054.131

4.1.3 Levels of Total analyzed carotenoids in flesh and fibrous strand

Analysis of concentrations of total quantified carotenoids (Σcarotenoid= total lycopene + β- carotene) shows that fibrous strands are much richer in carotenoids than flesh. The carotenoids levels ranged from (1100) to (12740)µg/gwm. With mean concentration of 4869.72µg/gwm, while in flesh the mean concentration of carotenoids was (3784.45)µg/gwm ranging between 1200 and 10620 µg/gwm. The mean level of carotenoids in fibrous strand was 1.3 higher than the mean value in flesh. Comparisons of levels of carotenoids between flesh and fibrous strands are presented in Figure 4.4

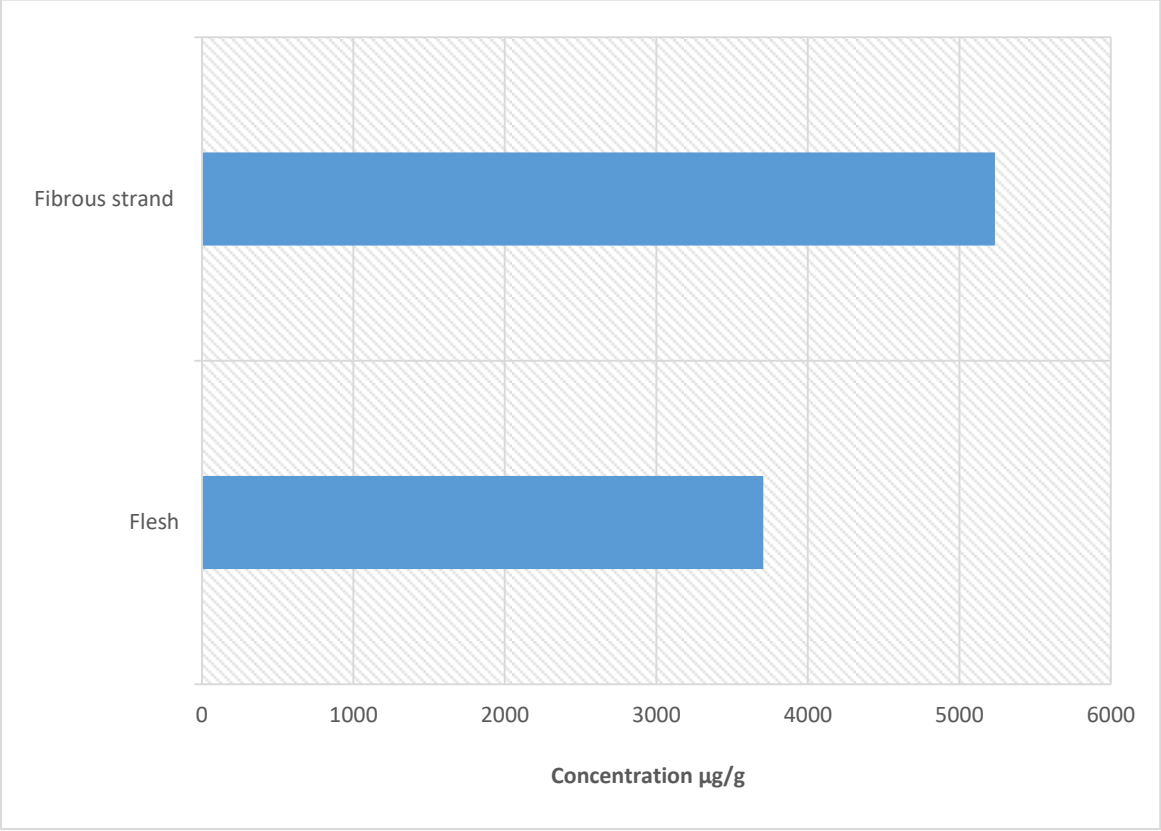


Figure 4.4: Variations of carotenoids in Flesh and fibrous strand parts of the pumpkin

Comparisons of levels of carotenoids revealed that in both flesh and fibrous strands the total carotenoids were dominated by total lycopene however fibrous strand samples were

much richer in lycopene compared to flesh samples. In fibrous strand, lycopene contributed 75% of the total carotenoids as oppose to contribution of 66% of the lycopene

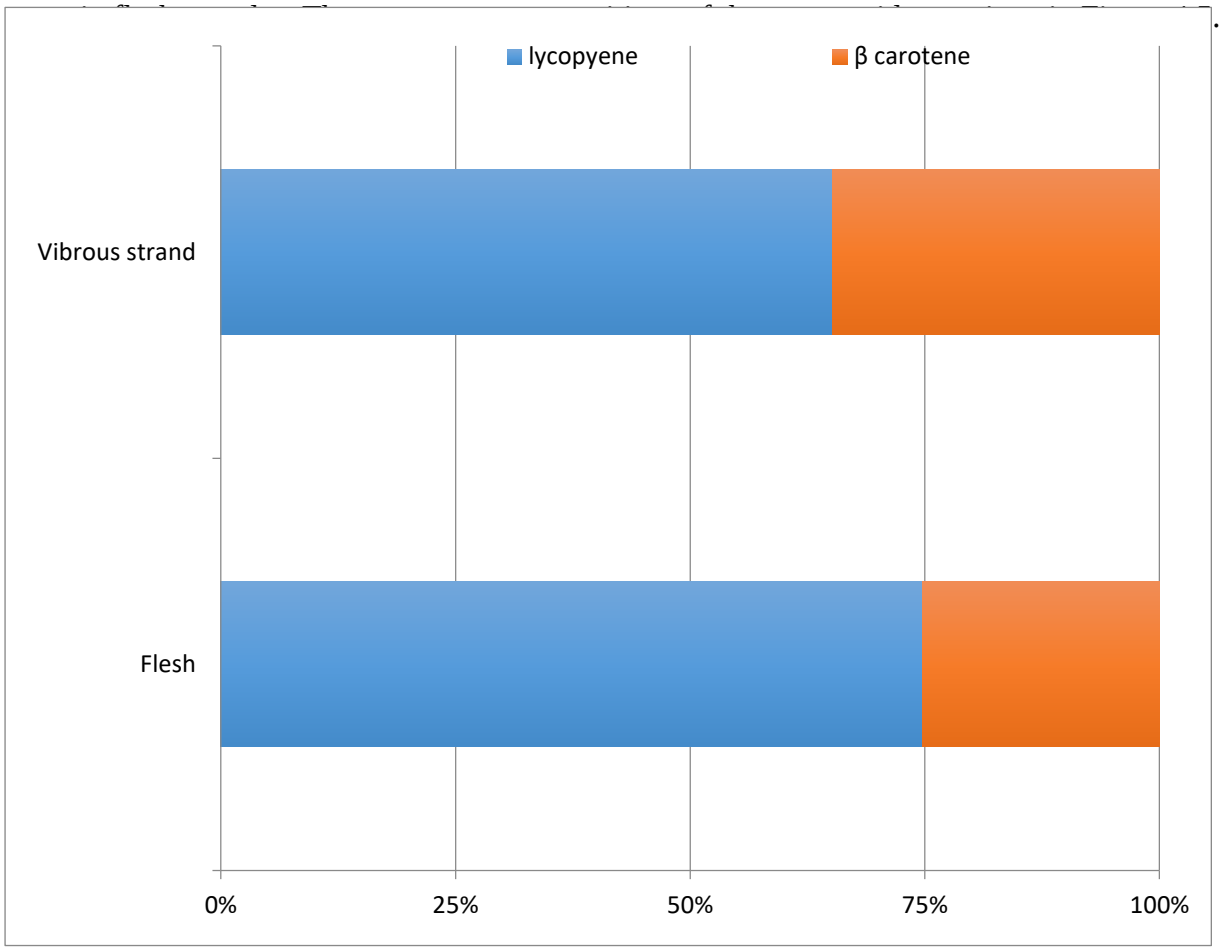


Figure 4.5: Concentrations of lycopene and beta carotene in flesh and fibrous strand

4.2 Levels of minerals nutrients

Levels of five elements including K, Mg, Mn, Cu and Fe were measured in different parts of pumpkin. The metals like K and Mg are essential macronutrients while Fe, Cu, and Mn are essential micronutrients for plant that are necessary to facilitate metabolism process in the plants Since trace metals are essential micronutrients for plant growth, there presence in low concentrations is not surprising, however pumpkins may accumulate these metals

beyond the plant requirement if they were grown in the environment rich in pollutant load (Njoku-Tony et al., 2020). In some studies, however, there were no correlations between metal concentrations in soil and those measured in pumpkin suggest that the transfer of metals from soil to plant is primarily determined by their bioavailability (Danilcenko et al. 2016).

The measurement results of the metals in rind, flesh, fibrous strand and seeds are presented in Table 4.4a, 4.5a, 4.6a, 4.7a respectively and their corresponding descriptive statistics are given in Table 4.4b, 4.5b, 4.6band 4.7b.

4.2. 1 Levels of minerals nutrients in rind of pumpkins

Analysis revealed that the metal Fe was dominant metallic nutrients in rinds followed closely by K and the other elements were measured at relatively low concentrations. Levels of K was measured at mean concentration of 817 μg/g

Table 4.4a: Levels of minerals nutrients in rinds (μg/g)

Sample number	K	Mg	Mn	Cu	Fe
1	900.00	47.50	4.75	2.80	1227.50
2	700.00	42.50	4.50	8.45	1077.5
3	750.00	35.00	3.00	3.05	975.00
4	750.00	40.00	2.50	2.90	1222.50
5	1000.00	40.00	1.75	3.00	895.00
6	725.00	42.50	4.00	2.60	1225.00
7	900.00	47.50	2.25	0.55	742.5.00
8	725.00	35.00	2.75	4.50	547.500
9	925.00	47.50	4.50	5.10	1192.50
10	800.00	35.00	3.25	2.75	1130.00

Table 4.4b: Descriptive Statistics of concentration of minerals in rinds

	Minimum	Maximum	Mean	Std. Deviation
K	700.	1000	817.50	104.782
Mg	35.0	48.0	41.30	5.355
Mn	2.00	5.00	3.20	1.033
Cu	1.00	8.00	3.60	1.838
Fe	548.00	1228	1023.50	233.032

In general, the mean concentrations of the metallic nutrient depicted the following concentration trends Fe (1023.50µg/g)> K(817.50µg/g)> Mg(41.30µg/g)> Cu(3.60µg/g)> Mn (3.20µg/g).The log-transformed values of the individual samples are given in Figure 4

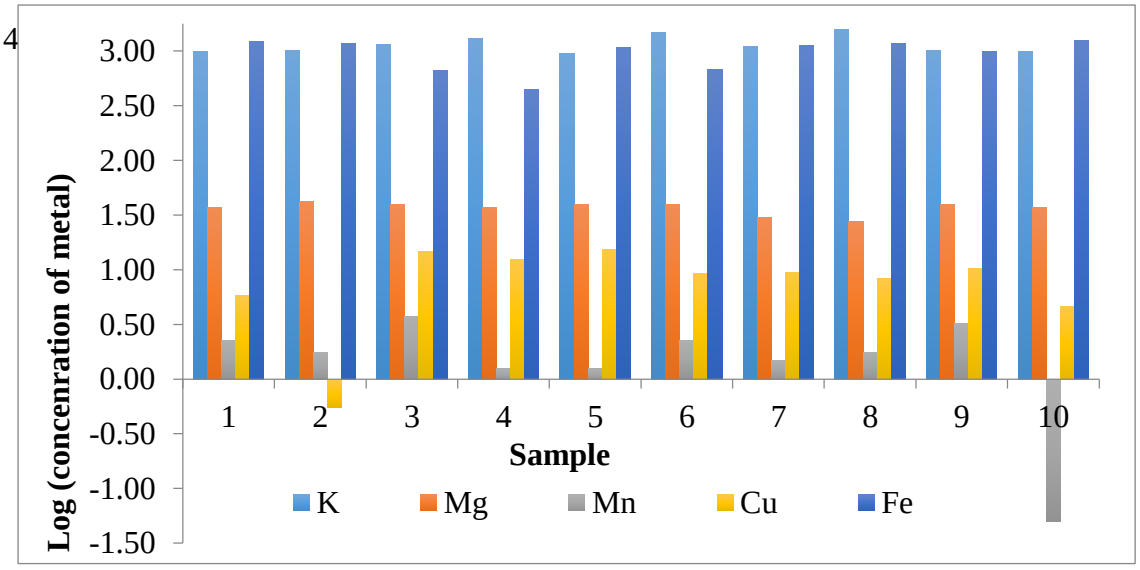


Figure 4.6: Concentrations of metallic nutrients in rinds

4.2.2 Levels of minerals nutrients in flesh of pumpkins

The levels of metallic nutrients in flesh samples are presented in Table 4.6a and their descriptive statics in Table 4.6b. Similar to rinds, metals in flesh depicted similar trends of domination of Fe and K concentration. However, the levels of metals in flesh were slightly lower than the levels measured in rinds with the exception of Cu and Mn which recorded slightly elevated mean levels in flesh than in rinds. The observed trend of metallic concentration in flesh is Fe (927.70µg/g)> K(537.50µg/g)> Mg(38.80µg/g)> Cu(10.60µg/g)> Mn(5.40µg/g). The levels of these mineral nutrients are presented in log-transformed Figure 4.7a

Table 4.5a: Levels of minerals nutrients in flesh (µg/g)

Sample number	K	Mg	Mn	Cu	Fe
1	700	42.5	1.00	10.95	1060
2	675	35.0	6.60	12.85	935
3	550	40.0	7.50	10.50	1140
4	550	45.0	2.50	12.05	667.5
5	300	32.5	9.50	15.15	762.5
6	625	40.0	4.50	9.90	710
7	325	37.5	2.50	10.55	982.5
8	450	32.5	4.75	12.05	970
9	625	42.5	8.25	1.70	962.5
10	575	42.5	7.25	9.85	1087.5

Table 4.5b: Descriptive Statistics of concentration of minerals in flesh.

	Minimum	Maximum	Mean	Std. Deviation
K	300.000	700.000	537.500	138.067
Mg	32.000	45.000	38.800	4.467
Mn	1.000	10.000	5.400	3.062
Cu	2.000	15.000	10.600	3.406
Fe	668.000	1140.000	927.700	162.115

The results are also presented in long transformed concentrations in Figure 4.7

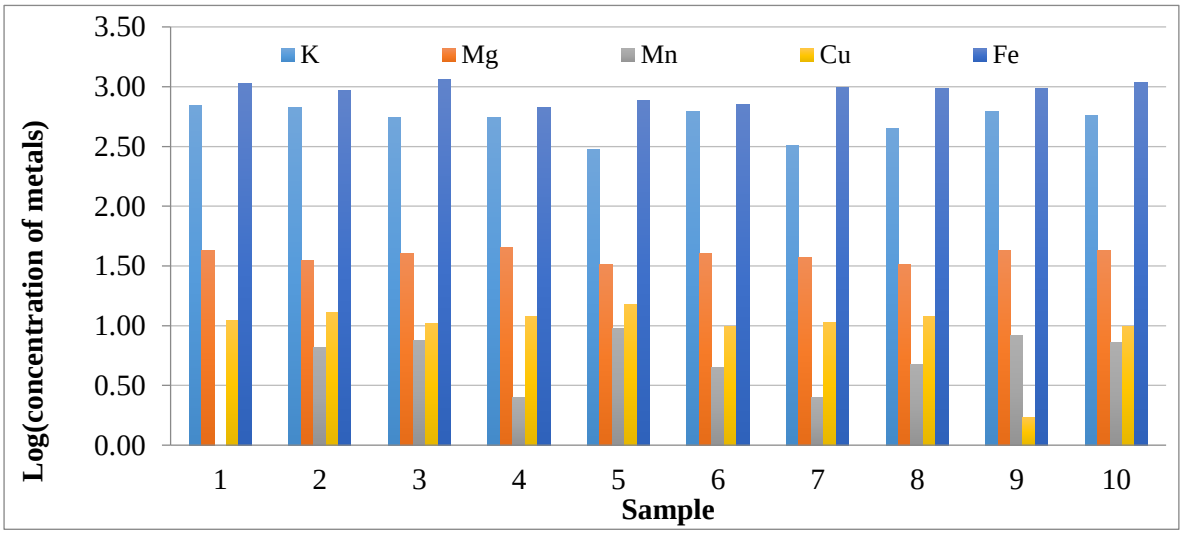


Figure 4.7: Concentrations of metallic nutrients in flesh

4.2.3 Levels of minerals nutrients in fibrous strand of pumpkins

Levels of metals measured in fibrous strand part of the pumpkin are given Table 4.7a and their descriptive statics in Table 4.6b.

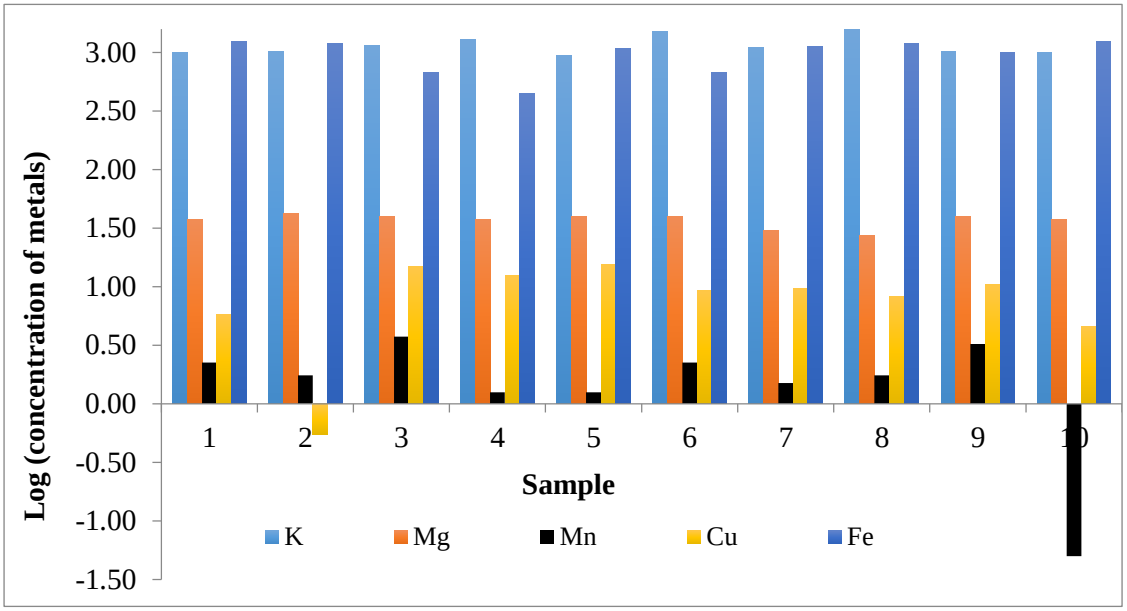
Table 4.6a: Levels of mineral nutrients in fibrous strand (µg/g)

Sample number	K	Mg	Mn	Cu	Fe
1	1000.00	37.50	2.25	5.80	1240.00
2	1025.00	42.50	1.75	0.55	1185.00
3	1150.00	40.00	3.75	14.80	670.00
4	1300.00	37.50	1.25	12.40	450.00
5	950.00	40.00	1.25	15.35	1082.50
6	1500.00	40.00	2.25	9.20	677.50
7	1100.00	30.00	1.5	9.55	1125.00
8	1575.00	27.50	1.75	8.30	1187.50
9	1025.00	40.00	3.25	10.35	1000.00
10	1000.00	37.50	0.05	4.60	1245.00

Table 4.6b: Descriptive statistics of concentration of minerals in fibrous strand

	Minimum	Maximum	Mean	Std. Deviation
K	950.000	1575.000	1162.500	221.814
Mg	28.000	42.000	37.400	4.624
Mn	0.000	4.000	1.900	1.101
Cu	1.000	15.000	9.100	4.383
Fe	450.000	1245.000	986.300	283.382

In average the levels of metals in fibrous strand were highly dominated by K followed by Fe resulting to different trend from those observed in flesh and rinds. Rinds and flesh part of the pumpkin were highly dominated by Fe followed by K. The metal concentration trends in fibrous strand is K(1162.5µg/g)> Fe(986.3µg/g)> Mg(37.4µg/g)> Cu(9.1µg/g)> Mn(1.9µg/g). Figure 4.8 Present the log-transformed concentrations of the measured levels of metals.



4.3.3 Levels of minerals nutrients in seeds of pumpkins

Table 4.7a and 4.7b present concentrations of metals measured in seed individual samples and their corresponding descriptive statistics, respectively

Table 4.7a: Levels of minerals nutrients in seeds (µg/g)

Sample number	K	Mg	Mn	Cu	Fe
1	600.00	70.00	7.50	2.80	877.50
2	750.00	60.00	6.75	4.00	1217.50
3	700.00	85.00	12.50	0.40	1050.00
4	1050.00	212.50	8.75	3.00	77.50
5	675.00	270.00	7.00	3.25	1185.00
6	875.00	110.00	8.25	2.90	967.50
7	1050.00	117.50	12.50	0.60	1017.50
8	950.00	90.00	8.75	1.75	1137.50
9	525.00	117.50	6.75	2.30	950.00
10	500.00	150.00	6.25	3.50	987.50

Table 4.7b: Descriptive Statistics of concentration of minerals in seeds

	Minimum	Maximum	Mean	Std. Deviation
K	500	1050	767.50	204.481
Mg	60	270	128.30	66.255
Cu	0	4	2.50	1.269
Mn	6	12	8.50	2.068
Fe	78	1218	947.10	323.912

Like in rinds and flesh, the metal Fe was measured in highest concentration followed by K in seeds; however, in seeds the mean concentration of Mn was higher than the recorded mean of Cu. In average the levels in seeds depicted the following trend Fe (947.10µg/g)> K(767.50µg/g)> Mg(128.30µg/g)> Mn(8.50µg/g)> Cu(2.50µg/g). The levels of metals are also presented in log-transformed form in Figure 4.9

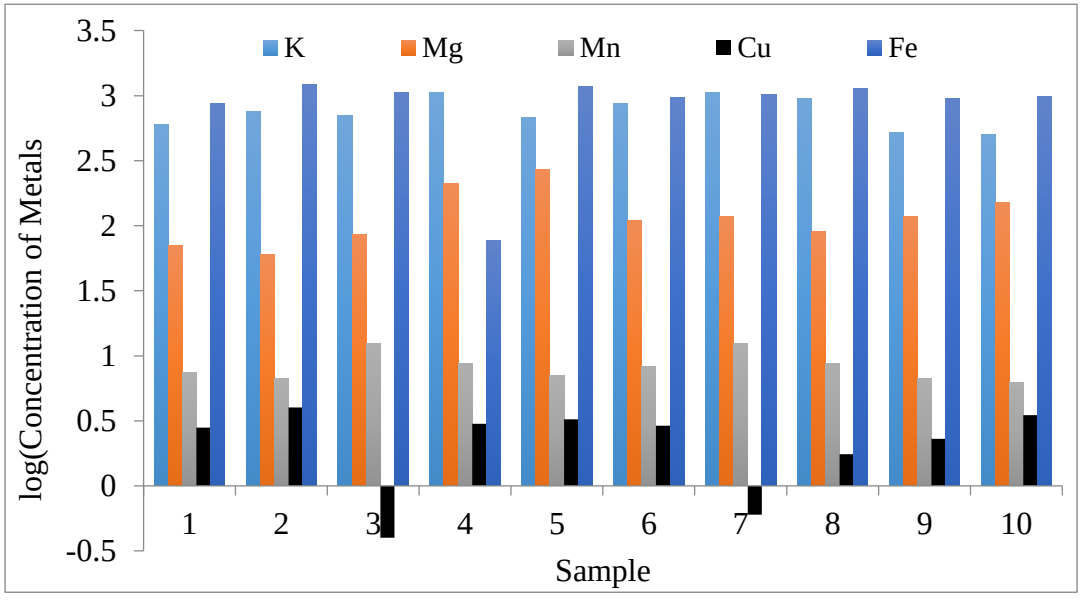


Figure 4.9: Concentrations of metallic nutrients in seeds

4.3.4 Comparison of metallic nutrients in cucurbita maxima parts

The measured total concentrations of the metals in different analyzed parts of pumpkins are summarized in table 4.8

Table 4.8 Total concentrations of metallic nutrients in pumpkin

	Total concentrations of metals (µg/g)		
Pumpkin part	Minimum	Maximum	Mean
Rinds	950.3	2182.6	1814.9
Flesh	1120	1814.5	1520.24
Fibrous strands	1801	2800	2197
Seeds	1351.75	2198.1	1852

The total metallic in nutrients in rinds ranged from 950.3 to 2182.6µg/g while in flesh the range of total concentrations of the nutrients was 1120 – 1814.5µg/g. Fibrous strands on the other hand had a total metallic nutrient level ranging between 1801 and 2800µg/g whereas the range of total concentration of the metallic nutrients was 1351.75 – 2198.1µg/g. In average, the fibrous strands depicted higher concentrations than the other analyzed parts of pumpkins while flesh part which is the main edible part had lowest average concentrations of metallic nutrients (Figure 4.10). The overall trend of the average concentrations was Fibrous strands> seeds> rinds> flesh.

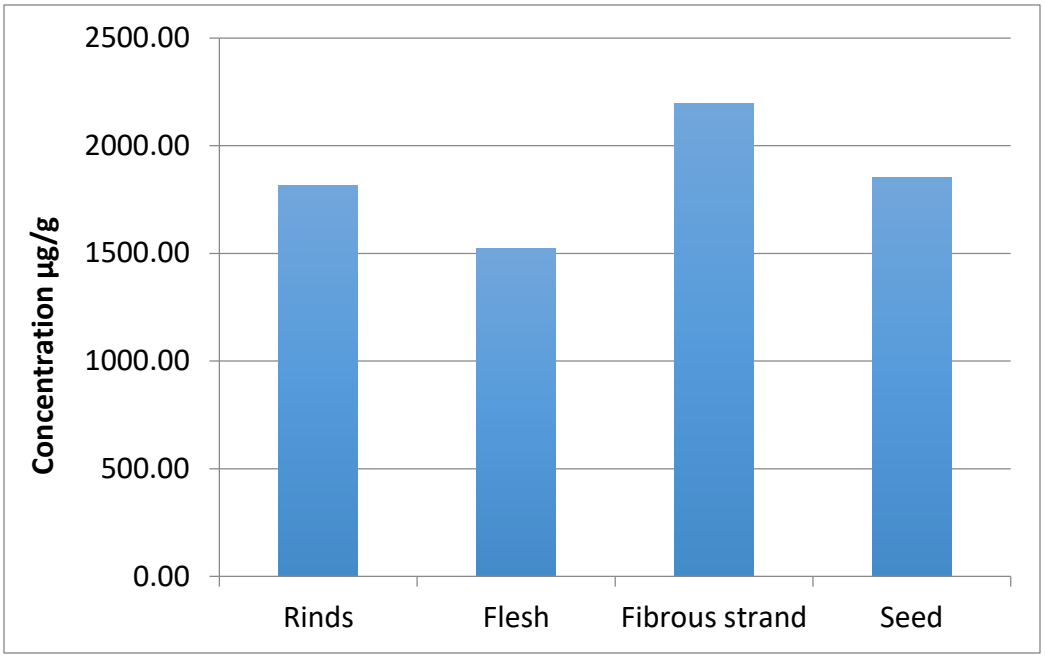


Figure 4.10: Variation of concentrations of total metallic nutrients among pumpkin parts

The average compositions of metals in different parts are presented in Figure 4.11. Analysis revealed that the measured metallic concentrations in all parts of pumpkins are predominated by Fe except in fibrous strands in which K was higher than Fe. In general, Fe contributed between 45% to 60% of the total measured metals. The metal Fe was followed up by K in all parts of the analyzed pumpkins. The contribution of K in total metals ranged between 35% to 53 of the total measured metals in the pumpkin parts. The ANOVA statistical analysis revealed that both Fe and K in all analyzed parts were significantly higher ($p < 0.05$, Appendix 11 -16) than the other metals but there was no significant difference between them.

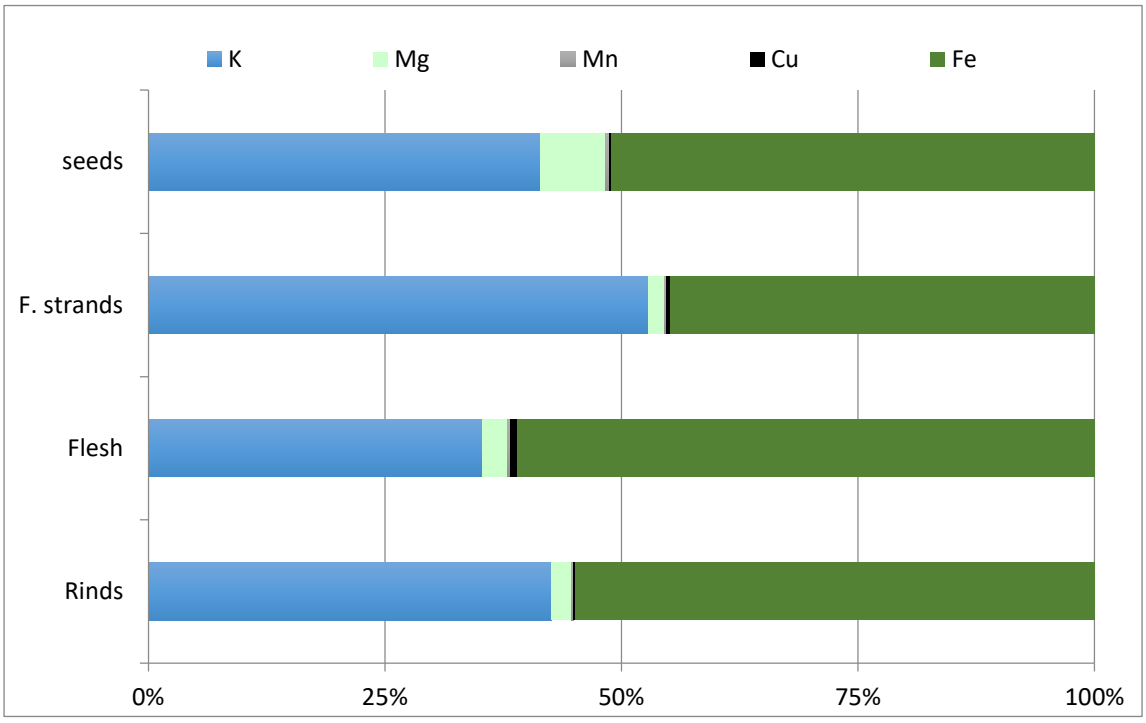


Figure 4.11: Average contribution of analyzed metals in different parts of the Pumpkin.

On the other hand, the metals Mn and Cu had very minimum contributions that ranged between 0.09 to 0.46% and 0.13 to 0.69% respectively. Similarly, Mg metal showed a very small contribution in total metal ranging between 1.7 and 6.92%. The statistical analysis on comparison of Cu, Mn and Mg showed no significant differences ($p>0.05$) among them but were significantly lower than Fe and K.

The distribution of metals in pumpkin parts in this study showed both resemblance and differences with the results reported in same species from Bangladesh. While in this study levels of copper were very low in all analyzed pumpkin tissues, a notable amount of Cu was measured in seeds from in Bangladesh pumpkins (Amin et al.,2019). However, the two studies resemble in the sense that both of them found a notable and dominance of K and Fe in the flesh part. As highlighted elsewhere that both K and F are

biologically essential element for living tissues (Dawood, 2019), their dominance in pumpkins may add the nutritive values of the fruit. Deficiency of these macronutrients is associated with number of health problems and is normally addressed by prescribing the nutrients supplements. For instance, Fe plays a major role in the formation of hemoglobin and its deficiency is always associated with anemia. The anemia cases are largely addressed by serving Ferrous salts such as ferrous fumarate, ferrous gluconate and ferrous sulfate. Although these chemical-based supplements are considered to be the best remedy for Fe deficiency but they might carry other side effects (Okam *et al*., 2017). To reduce such side effects organically and food-based sources of Fe such as pumpkins and vegetables can provide ideal sources of Fe to address the deficiency.

4.3 Comparisons with other studies

Comparison of the levels of carotenoids (lycopene andβ-carotene) measured flesh and fibrous strands in this revealed that most of the study from elsewhere report levels in flesh and the levels in fibrous trends were very rare. The studies concentrated on flesh rather than fibrous strands because the fibrous strands are not commonly utilized as food and most of time are thrown as wastes. However, it is widely acknowledged that fibrous strands can be much richer lycopene and β-carotene compared to other parts pumpkins (Sharma et al., 2018). The literature review also revealed that even in flesh part analysis, most of the studies focused on β-carotene instead of lycopene because β-carotene is of unique importance as vitamin A precursor (Sharma et al., 2020).

The comparison showed that the levels of carotenoids in pumpkins from Zanzibar are within the upper range of the levels reported in various study elsewhere. The summary of the comparison is given in Table 4.9

Table 4.9 Comparison of mean levels of carotenoids of related studies

Country	Tissue	Mean concentration (µg/g)		Reference
		Lycopene	β-carotene	
Zanzibar	Flesh	2495.56	1288.89	This study
	Fibrous strands	3642.22	1227.50	
Kenya	Flesh	NA	538.0	Monica *et al.*, 2008
Italy	Flesh	19.25	49.29	Bergantin et al., 2018
Poland	Flesh	8.1	NA	*Kulaitie*n *et al.*, 2014
Korea	Flesh	NA	17.04	(Kim et al., 2012)
Pakistan	Flesh	NA	61.8	(Hussain et al., 2021)
Mexico	Flesh	NA	2670	(Jacobo-valenzuela et al., 2011)

From Table 4.9, it is clear that the mean concentrations of β-carotene measured in flesh of pumpkins from Zanzibar were lower than that reported in Mexico which were of *Cucurbita mashkota* (Jacobo-valenzuela et al., 2011) and higher than those reported in Kenya (Monica *et al.*, 2008), Italy (Bergantin et al., 2018),Poland. (*Kulaitie*n*et al.*, 2014) , Korea (Kim et al., 2012),and Pakistan(Hussain et al., 2021). In case of lycopene their concentrations were higher than those reported in Italy (Bergantin et al., 2018) and Poland. (*Kulaitie*n*et al.*, 2014).

For the case of mineral nutrients, the mean levels found in rinds, flesh and fibrous strands and seeds in pumpkins from Zanzibar were general within the middle range of those reported in elsewhere. The summary of the comparison is presented in table 4.10

Table 4.10 Comparison of mean levels of minerals in rinds of pumpkins (µg/g)

Country	K	Mg	Mn	Cu	Fe	Reference
Zanzibar	817.50	41.30	3.20	3.60	1023.5	This study
Nigeria	1851.2	NA	219.9	100.0	559.8	Paiko et al., 2014a
Bangladesh	6874.6	33.53	3.60	0.25	40.04	Amin *et al.*, 2019
Pakistan	4577	NA	NA	NA	40.5	Hussain et al., 2021
Mexico	2255.53	3441.8	7.33	5.4	63.98	Jacobo-valenzuela et al., 2011

The mean concentration of Fe found in rinds part of Zanzibar pumpkin were higher than the levels reported in rinds part of pumpkins from Nigeria, Pakistan, Mexico and Bangladesh. In contrary the mean levels of K and Mn measured in Zanzibar were lower than those from Nigeria (Paiko *et al.*, 2014), Pakistan (Hussain et al., 2021), Mexico(Jacobo-valenzuela et al., 2011) and Bangladesh (Amin *et al.,* 2019). For the case of Cu the mean level in rinds from Zanzibar were lower than the mean concentration reported in pumpkins from Nigeria (Paiko et al., 2014a) and Mexico (Jacobo-valenzuela

et al., 2011) but higher than the mean level found in Bangladesh (Amin *et al.,* 2019).For the case of Mg found that the concentration were lower than that reported in Mexico and higher than that reported in Bangladesh. These studies indicate that, although the rind part of pumpkins is not preferred for eating but they seem to be a good source of K and Fe to mammals.

Table 4.11 Comparison of mean levels of minerals in flesh of pumpkins (µg/g)

Country	K	Mg	Mn	Cu	Fe	Reference
Zanzibar	537.50	32.00	5.400	10.6	927.70	This study
Nigeria	1843.4	NA	203.3	120	536.70	(Paiko et al., 2014a)
	160.31	189.91	0.50	1.37	3.710	Farombi & Oyekanmi, 2013
Bangladesh	16163.94	56.43	4.5	0.6	420.7	Amin *et al.,* 2019
Pakistan	15920	NA	NA	NA	451	(Hussain et al., 2021)
Mexico	42194	1590.40	3.33	8.44	31.69	(Jacobo-valenzuela et al., 2011)

Comparison of mean concentrations in flesh part of pumpkins (table 4.11) depicted same finding of higher concentration of Fe in flesh part of Zanzibar pumpkin compared to mean values reported elsewhere. In contrary the mean value of Mg from Zanzibar were smaller than the values of mean concentrations report from other part of world. On the other hand, the mean values of Cu and Mn in flesh of *cucubita maxima* of Zanzibar were higher than their corresponding mean reported in flash from Niger State in Nigeria (Farombi& Oyekanmi, 2013) and Bangladesh (Amin *et al.,* 2019) but lower than the mean value reported in another study from Osun state in Nigeria ((Paiko et al., 2014a))

As mention elsewhere there are limited studies on determination of concentrations of both carotenoids and mineral concentration in fibrous strands. Only one study on mineral

concentrations was located in Anhui China (Li *et al.,* 2021) that was conducted in different *cucubita pepo* species. The mean levels of K mineral reported in Chinese study (2370µg/g) was higher than the mean value reported in fibrous strands of the maxima pumpkin from Zanzibar. However the mean levels Fe showed opposite trend where the mean values of reported in Chinese study (1.92µg/g) was notably lower than the mean Fe levels measured in Zanzibar (986.30µg/g). Table 4.12 summarizes the comparisons of mean values of the metals measured in pumpkin seeds from Zanzibar and other related study on the same maxima specie

Table 4.12 Comparison of mean levels of minerals in seeds of pumpkins (µg/g)

Country	K	Mg	Mn	Cu	Fe	Reference
Zanzibar	767.50	128.30	6.25	2.50	947.10	This study
Nigeria	3975.3	NA	18.6	120.1	78.0	Paiko *et al.*, 2014
Bangladesh	7800	349.00	18.00	70.00	298.00	Habib *et al.,* 2015
Kenya	2169.0	598.95	61.50	13.15	24.68	Karanja *et al.,* 2013
Pakistan	3877	NA	NA	NA	61.6	Hussain et al., 2021

The concentration of K, Mg, Mn and Cu found in pumpkin seeds from Zanzibar were lower than those reported in Nigeria (Paiko*et al.*, 2014), Bangladesh (Habib *et al.,* 2015) Kenya(Karanja *et al.,* 2013) and Pakistan (Hussain et al., 2021).But in contrary the mean concentration of Fe in pumpkin seeds measured in Zanzibar were notably higher than those reported elsewhere. For instance the mean value reported in Zanzibar is more three times higher than the levels reported in Bangladesh (Habib *et al.,* 2015). Similarly the level of Fe in pumpkin seeds from Zanzibar were more than ten times than the mean

levels reported in pumpkin seeds from Nigeria (Paiko*et al.*, 2014), Kenya (Karanja *et al.*, 2013) and Pakistan (Hussain et al., 2021).

NUTRITIVE VALUE OF PUMPKIN

Analysis of different parts of pumpkin in this study has revealed presence of both organic and mineral nutrients. These nutrients are of high nutritional and health benefit. Several studies have shown that food rich in carotenoids (β-carotene and lycopene) have strong medical therapeutic (Dar & Sofi, 2017), and they contain antimicrobial agents that hinder growing diseases causing microbes (Hussain et al., 2021). The carotenoid, β-carotene is considered to be protein A precasor which is easily converted to protein A in the body. Presence of carotenoids, minerals, and other food substances have been also shown to play significant role in protecting human body against difference health complications such as hypertension, diabetes, cancer and coronary heart diseases. also pulp of pumpkin fruits used to relive dyspepsia , stomach disorder and intestinal inflammation (Amin et al., 2019)

CHAPTER FIVE

CONCLUSION AND RECOMMENDATION

5.1 Conclusion

Based on the summary of the results of this study, the following conclusions can be drawn:

a. Pumpkins (*Cucurbita maxima*) from Kidoti contain carotenoids (lycopene and β-carotene) and mineral nutrients falling within upper and middle ranges of the levels reported elsewhere.
b. Carotenoids measured in flesh and fibrous parts of the pumpkins are dominated by lycopene whereas mineral nutrients measured in all parts of the pumpkins are predominated by both Fe and K.
c. Fibrous part of the pumpkin is much richer in both carotenoids and mineral nutrients than the other analyzed parts of the pumpkins.
d. Fibrous part of the pumpkins has higher nutritive value than flesh which is the commonly eaten part of the pumpkin.
e. Pumpkins (*Cucurbita maxima*) from Kidoti carry very high nutritive and can be used as supplement to address lack of vitamins A and anemia.

5.2 Recommendation

Following the results revealed in this study the following recommendations are given:

- As the study revealed that pumpkins carry a good level of beta carotene which is a pre-casor of Vitamin A. It is recommended that the fruit should be used in a special campaign to address deficiency of vitamin A.
- As fibrous strand part which is not commonly used as a food was the richest part in terms of nutrients. It is recommended that this part should be considered to be used as food parallel to flesh. Or the fibrous part should be considered for preparation of animal feed where there are large scale productions of pumpkins.
- A further study is recommended to investigate on effects of preparations methods and cooking conditions that affect the difference in nutrients distributions in *Cucurbita maxima* parts.

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APPENDIX

Appendix 1:Independent Samples Test comparison of mean difference of total lycopene and beta carotene in flesh

		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
									Lower	Upper
concentration	Equal variances assumed	1.478	.242	1.603	16	.128	1206.667	752.595	-388.763	2802.096
	Equal variances not assumed			1.603	10.701	.138	1206.667	752.595	-455.440	2868.774

Appendix 2: Independent Samples Test comparison of mean difference of total lycopene and beta carotene in fibrous strands

	Levene's Test for Equality of Variances		t-test for Equality of Means					
	F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference

									Lower	Upper
concentration µg/g	Equal variances assumed	9.330	.008	2.024	15	.061	2414.722	1193.230	-128.588	4958.033
	Equal variances not assumed			2.128	9.887	.059	2414.722	1134.531	-117.086	4946.530

Appendix 3: ANOVA comparison of mean difference of total metallic in rinds

Concentration µg/g

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	9991189.477	4	2497797.369	219.894	.000
Within Groups	499800.156	44	11359.094		
Total	10490989.633	48			

Multiple Comparisons

Appendix 4: Dependent Variable: concentration µg/g

Bonferroni

(I) nutrients in rinds	(J) nutrients in rinds	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
K	Mg	776.200*	47.664	.000	635.33	917.07
	Mn	814.300*	47.664	.000	673.43	955.17
	Cu	813.900*	47.664	.000	673.03	954.77
	Fe	-237.278*	48.970	.000	-382.01	-92.55
Mg	K	-776.200*	47.664	.000	-917.07	-635.33

Mn	Mn	38.100	47.664	1.000	-102.77	178.97
	Cu	37.700	47.664	1.000	-103.17	178.57
	Fe	-1013.478*	48.970	.000	-1158.21	-868.75
	K	-814.300*	47.664	.000	-955.17	-673.43
	Mg	-38.100	47.664	1.000	-178.97	102.77
	Cu	-.400	47.664	1.000	-141.27	140.47
	Fe	-1051.578*	48.970	.000	-1196.31	-906.85
	K	-813.900*	47.664	.000	-954.77	-673.03
Cu	Mg	-37.700	47.664	1.000	-178.57	103.17
	Mn	.400	47.664	1.000	-140.47	141.27
	Fe	-1051.178*	48.970	.000	-1195.91	-906.45
	K	237.278*	48.970	.000	92.55	382.01
Fe	Mg	1013.478*	48.970	.000	868.75	1158.21
	Mn	1051.578*	48.970	.000	906.85	1196.31
	Cu	1051.178*	48.970	.000	906.45	1195.91

The mean difference is significant at the 0.05 level.

Appendix 5: ANOVA comparison of mean difference of total metallic in flesh concentration µg/g

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	6124178.096	4	1531044.524	58.621	.000
Within Groups	1175289.904	45	26117.553		
Total	7299468.000	49			

Multiple Comparisons

Appendix 6: Dependent Variable: concentration µg/g

Bonferron

(I) nutrients in flesh	(J) nutrients in flesh	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
K	Mg	499.056*	74.254	.000	279.85	718.26
	Mn	528.600*	72.274	.000	315.24	741.96
	Cu	527.200*	72.274	.000	313.84	740.56
	Fe	-306.773*	70.612	.001	-515.23	-98.32
Mg	K	-499.056*	74.254	.000	-718.26	-279.85
	Mn	29.544	74.254	1.000	-189.66	248.75
	Cu	28.144	74.254	1.000	-191.06	247.35
	Fe	-805.828*	72.638	.000	-1020.26	-591.40
Mn	K	-528.600*	72.274	.000	-741.96	-315.24
	Mg	-29.544	74.254	1.000	-248.75	189.66
	Cu	-1.400	72.274	1.000	-214.76	211.96
	Fe	-835.373*	70.612	.000	-1043.83	-626.92
Cu	K	-527.200*	72.274	.000	-740.56	-313.84
	Mg	-28.144	74.254	1.000	-247.35	191.06
	Mn	1.400	72.274	1.000	-211.96	214.76
	Fe	-833.973*	70.612	.000	-1042.43	-625.52
Fe	K	306.773*	70.612	.001	98.32	515.23
	Mg	805.828*	72.638	.000	591.40	1020.26
	Mn	835.373*	70.612	.000	626.92	1043.83
	Cu	833.973*	70.612	.000	625.52	1042.43

*.

The mean difference is significant at the 0.05 level.

Appendix 7: ANOVA comparison of mean difference of total metallic in fibrous strand

Concentration µg/g

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	13601415.520	4	3400353.880	131.238	.000
Within Groups	1165938.800	45	25909.751		
Total	14767354.320	49			

Multiple Comparisons

Appendix 8: Dependent Variable: concentration µg/g

Bonferroni

(I) nutrients in strands	(J) nutrients in strands	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
K	Mg	1125.100*	71.986	.000	912.59	1337.61
	Mn	1160.600*	71.986	.000	948.09	1373.11
	Cu	1153.400*	71.986	.000	940.89	1365.91
	Fe	176.200	71.986	.183	-36.31	388.71
Mg	K	-1125.100*	71.986	.000	-1337.61	-912.59
	Mn	35.500	71.986	1.000	-177.01	248.01
	Cu	28.300	71.986	1.000	-184.21	240.81
	Fe	-948.900*	71.986	.000	-1161.41	-736.39
Mn	K	-1160.600*	71.986	.000	-1373.11	-948.09
	Mg	-35.500	71.986	1.000	-248.01	177.01
	Cu	-7.200	71.986	1.000	-219.71	205.31
	Fe	-984.400*	71.986	.000	-1196.91	-771.89
Cu	K	-1153.400*	71.986	.000	-1365.91	-940.89
	Mg	-28.300	71.986	1.000	-240.81	184.21
	Mn	7.200	71.986	1.000	-205.31	219.71
	Fe	-977.200*	71.986	.000	-1189.71	-764.69
Fe	K	-176.200	71.986	.183	-388.71	36.31

	Mg	948.900*	71.986	.000	736.39	1161.41
	Mn	984.400*	71.986	.000	771.89	1196.91
	Cu	977.200*	71.986	.000	764.69	1189.71

*. The mean difference is significant at the 0.05 level.

Appendix 9: ANOVA comparison of mean difference of total metallic in seeds

Concentration µg/g

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	7765173.525	4	1941293.381	62.886	.000
Within Groups	1358283.822	44	30870.087		
Total	9123457.347	48			

Multiple Comparisons

Appendix 10: Dependent Variable: concentration µg/g

Bonferroni

(I) nutrients in seeds	(J) nutrients in seeds	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
K	Mg	639.200*	78.575	.000	406.97	871.43
	Mn	759.000*	78.575	.000	526.77	991.23
	Cu	765.000*	78.575	.000	532.77	997.23
	Fe	-175.056	80.728	.356	-413.65	63.54
Mg	K	-639.200*	78.575	.000	-871.43	-406.97
	Mn	119.800	78.575	1.000	-112.43	352.03
	Cu	125.800	78.575	1.000	-106.43	358.03
	Fe	-814.256*	80.728	.000	-1052.85	-575.66
Mn	K	-759.000*	78.575	.000	-991.23	-526.77
	Mg	-119.800	78.575	1.000	-352.03	112.43
	Cu	6.000	78.575	1.000	-226.23	238.23
	Fe	-934.056*	80.728	.000	-1172.65	-695.46

Cu	K	-765.000*	78.575	.000	-997.23	-532.77
	Mg	-125.800	78.575	1.000	-358.03	106.43
	Mn	-6.000	78.575	1.000	-238.23	226.23
	Fe	-940.056*	80.728	.000	-1178.65	-701.46
Fe	K	175.056	80.728	.356	-63.54	413.65
	Mg	814.256*	80.728	.000	575.66	1052.85
	Mn	934.056*	80.728	.000	695.46	1172.65
	Cu	940.056*	80.728	.000	701.46	1178.65

*. The mean difference is significant at the 0.05 level.

Appendix 11: ANOVA comparison of mean difference of K in pumpkin parts

concentration of K (µg/g)

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1998687.500	3	666229.167	22.014	.000
Within Groups	1089500.000	36	30263.889		
Total	3088187.500	39			

Dependent Variable: concentration of K (µg/g) Bonferroni						
(I) pumpkin parts	(J) pumpkin parts	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
RIND	FLESH	280.000*	77.800	.006	62.79	497.21

	FIBROUS STRAND	-345.000*	77.800	.000	-562.21	-127.79
	SEEDS	50.000	77.800	1.000	-167.21	267.21
FLESH	RIND	-280.000*	77.800	.006	-497.21	-62.79
	FIBROUS STRAND	-625.000*	77.800	.000	-842.21	-407.79
	SEEDS	-230.000*	77.800	.033	-447.21	-12.79
FIBROUS STRAND	RIND	345.000*	77.800	.000	127.79	562.21
	FLESH	625.000*	77.800	.000	407.79	842.21
	SEEDS	395.000*	77.800	.000	177.79	612.21
SEEDS	RIND	-50.000	77.800	1.000	-267.21	167.21
	FLESH	230.000*	77.800	.033	12.79	447.21
	FIBROUS STRAND	-395.000*	77.800	.000	-612.21	-177.79
*. The mean difference is significant at the 0.05 level.						

Table 22: the mean and concentration range of Mg in pumpkin parts

Descriptive Statistics

	Minimum	Maximum	Mean	Std. Deviation	Variance
RIND	35	48	41.30	5.355	28.678
FLESH	32	45	38.80	4.467	19.956
FIBROUS STRAND	28	42	37.40	4.624	21.378
SEEDS	60	270	128.30	66.255	4389.789

Appendix 12: ANOVA comparison of mean difference of Mg in pumpkin parts

Concentration of Mg (µg/g)

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	59663.700	3	19887.900	17.837	.000
Within Groups	40138.200	36	1114.950		
Total	99801.900	39			

Multiple Comparisons

Dependent Variable: Concentration of Mg (µg/g)

Bonferroni

(I) pumpkin parts	(J) pumpkin parts	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
RINDS	FLESH	2.500	14.933	1.000	-39.19	44.19
	FIBROUS STRAND	3.900	14.933	1.000	-37.79	45.59
	SEEDS	-87.000*	14.933	.000	-128.69	-45.31
FLESH	RINDS	-2.500	14.933	1.000	-44.19	39.19
	FIBROUS STRAND	1.400	14.933	1.000	-40.29	43.09
	SEEDS	-89.500*	14.933	.000	-131.19	-47.81

FIBROUS STRAND	RINDS	-3.900	14.933	1.000	-45.59	37.79
	FLESH	-1.400	14.933	1.000	-43.09	40.29
	SEEDS	-90.900*	14.933	.000	-132.59	-49.21
SEEDS	RINDS	87.000*	14.933	.000	45.31	128.69
	FLESH	89.500*	14.933	.000	47.81	131.19
	FIBROUS STRAND	90.900*	14.933	.000	49.21	132.59

*. The mean difference is significant at the 0.05 level.

Appendix 13: the mean and concentration range of Mn in pumpkin parts

Descriptive Statistics

PUMPKIN PARTS	Minimum	Maximum	Mean	Std. Deviation
RIND	2	5	3.20	1.033
FLESH	1	10	5.40	3.062
FIBROUS STRANDS	0	4	1.90	1.101
SEEDS	6	12	8.50	2.068

Appendix 14: ANOVA comparison of mean difference of Mn in pumpkin parts

ANOVA

Concentration of Mn(µg/g)

	Sum of Squares	df	Mean Square	F	Sig.

Between Groups	229.299	3	76.433	16.757	.000
Within Groups	164.201	36	4.561		
Total	393.500	39			

Multiple Comparisons

Dependent Variable: Concentration of Mn(µg/g)

Bonferroni

(I) pumpkins parts	(J) pumpkins parts	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
RIND	FLESH	-2.022	.981	.280	-4.76	.72
	FIBROUS STRAND	.836	.933	1.000	-1.77	3.44
	SEEDS	-5.300*	.955	.000	-7.97	-2.63
FLESH	RIND	2.022	.981	.280	-.72	4.76
	FIBROUS STRAND	2.859*	.960	.031	.18	5.54
	SEEDS	-3.278*	.981	.012	-6.02	-.54
FIBROUS STRAND	RIND	-.836	.933	1.000	-3.44	1.77
	FLESH	-2.859*	.960	.031	-5.54	-.18
	SEEDS	-6.136*	.933	.000	-8.74	-3.53
SEEDS	RIND	5.300*	.955	.000	2.63	7.97

FLESH	3.278*	.981	.012	.54	6.02
FIBROUS STRAND	6.136*	.933	.000	3.53	8.74

*. The mean difference is significant at the 0.05 level.

Appendix 15: the mean and concentration range of Cu in pumpkin parts

ANOVA

concentration of Cu (µg/g)

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	479.700	3	159.900	17.866	.000
Within Groups	322.200	36	8.950		
Total	801.900	39			

Dependent Variable: concentration of Cu (µg/g)

Bonferroni

(I) pumpkin parts	(J) pumpkin parts	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	Upper Bound
RIND	FLESH	-	1.338	.000	-10.74	-3.26

		7.000*				
	FIBROUS STRAND	-5.500*	1.338	.001	-9.24	-1.76
	SEEDS	1.100	1.338	1.000	-2.64	4.84
	RIND	7.000*	1.338	.000	3.26	10.74
FLESH	FIBROUS STRAND	1.500	1.338	1.000	-2.24	5.24
	SEEDS	8.100*	1.338	.000	4.36	11.84
	RIND	5.500*	1.338	.001	1.76	9.24
FIBROUS STRAND	FLESH	-1.500	1.338	1.000	-5.24	2.24
	SEEDS	6.600*	1.338	.000	2.86	10.34
	RIND	-1.100	1.338	1.000	-4.84	2.64
SEEDS	FLESH	-8.100*	1.338	.000	-11.84	-4.36
	FIBROUS STRAND	-6.600*	1.338	.000	-10.34	-2.86

Appendix 16: the mean and concentration range of Fe in pumpkin parts

ANOVA

concentration of Fe (µg/g)

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	54363.500	3	18121.167	.273	.845

Within Groups	2392287.600	36	66452.433
Total	2446651.100	39	

Multiple Comparisons

Dependent Variable: concentration of Fe (µg/g)

Bonferroni

(I) pumpkin parts	(J) pumpkin parts	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
RINDS	FLESH	95.800	115.284	1.000	-226.07	417.67
	FIBROUS STRAND	37.200	115.284	1.000	-284.67	359.07
	SEEDS	76.400	115.284	1.000	-245.47	398.27
FLESH	RINDS	-95.800	115.284	1.000	-417.67	226.07
	FIBROUS STRAND	-58.600	115.284	1.000	-380.47	263.27
	SEEDS	-19.400	115.284	1.000	-341.27	302.47
FIBROUS	RINDS	-37.200	115.284	1.000	-359.07	284.67

STRAND	FLESH	58.600	115.284	1.000	-263.27	380.47
	SEEDS	39.200	115.284	1.000	-282.67	361.07
	RINDS	-76.400	115.284	1.000	-398.27	245.47
SEEDS	FLESH	19.400	115.284	1.000	-302.47	341.27
	FIBROUS STRAND	-39.200	115.284	1.000	-361.07	282.67

Multiple Comparisons

Dependent Variable: concentration of Fe (µg/g)

Bonferroni

(I) pumpkin parts	(J) pumpkin parts	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
RINDS	FLESH	95.800	115.284	1.000	-226.07	417.67
	FIBROUS STRAND	37.200	115.284	1.000	-284.67	359.07
	SEEDS	76.400	115.284	1.000	-245.47	398.27
FLESH	RINDS	-95.800	115.284	1.000	-417.67	226.07
	FIBROUS STRAND	-58.600	115.284	1.000	-380.47	263.27

	SEEDS	-19.400	115.284	1.000	-341.27	302.47
	RINDS	-37.200	115.284	1.000	-359.07	284.67
FIBROUS STRAND	FLESH	58.600	115.284	1.000	-263.27	380.47
	SEEDS	39.200	115.284	1.000	-282.67	361.07
	RINDS	-76.400	115.284	1.000	-398.27	245.47
SEEDS	FLESH	19.400	115.284	1.000	-302.47	341.27
	FIBROUS STRAND	-39.200	115.284	1.000	-361.07	282.67