NUTRIENT AND ANTI-NUTRIENT CONTENTS OF SELECTED VARIETIES OF GRAIN AND LEAFY AMARANTHS IN TANZANIA

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ABSTRACT

Assessment of nutrient and anti-nutrient composition of amaranths grown in Tanzania was conducted to come up with their nutritional information. Six selected amaranth varieties namely *Amaranthus dubius* (Bwasi jekundu), *Amaranthus cruentus* (Bwasi kijani), *Amaranthus hypochondriacus* (Lishe njano), *Amaranthus hybridus* (Lishe nyeupe), *Amaranthus hypochondriacus* (Nafaka) and *Amaranthus dubius* (White local) were analyzed for trace minerals (iron, zinc, copper and manganese), proximate composition of crude fat, crude fibre, crude protein, ash, moisture and total carbohydrate and the anti-nutrients (nitrate and oxalate). Analyses were conducted with respect to fresh leaves, dried leaves, grains and amaranth flowers from each variety. Trace mineral's results showed that dried leaves of Bwasi jekundu had significantly high ($p<0.05$) iron contents (284.384mg/100g) compared to the other varieties of dried leaves, fresh leaves and amaranth flowers. Zinc, copper and manganese were significantly high ($p<0.05$) in Nafaka (75.89mg/100g), Lishe njano (3.2837mg/100g) and White local (34.869mg/100g) respective varieties of fresh leaves. Proximate levels of crude protein, crude fibre and crude fat were significantly high ($p<0.05$) in amaranth grains. Crude protein (15.787%) was high in Bwasi kijani grains, crude fibre (13.040% and 13.163%) was high in White local and Bwasi jekundu grains respectively that had no significantly difference ($p>0.05$) in crude fibre content. Crude fat (9.273%) was high in Bwasi jekundu grains. Total carbohydrate (78.743%) was significantly high in dried leaves of Bwasi kijani. Anti-nutrients (nitrate and oxalate) were significantly high in dried leaves. Oxalate content ranged from 360.3 to 378.5mg/100g in varieties of dried leaves that were not significantly different. The nitrate content was significantly high in Lishe nyeupe (137.06µg/g) of dried leaves. All dried leaves of amaranth were also significantly high in nitrate content. The common practice of discarding immature amaranth flowers did not appear to be supported since they were found to consist of significant amount of nutrients.

DECLARATION

I, Diana Nicodemas, do hereby declare to the Senate of Sokoine University of Agriculture, that this dissertation is my own work done within the period of registration and that it has neither been submitted nor being concurrently submitted for degree award to any other institution.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF APPENDICES

LIST OF ABBREVIATIONS AND SYMBOLS

AIDS	Acquired Immune Deficiency Syndrome
ANOVA	Analysis of Variance
AOAC	Association of Official Analytical Chemists
AusAID	Australian Agency for International Development
BMI	Body Mass Index
CF	Crude Fibre
CP	Crude Protein
HIV	Human Immunodeficiency Virus
NBS	National Bureau of Statistics
PLWHAs	People Living With HIV/AIDS
TCHO	Total Carbohydrates
USAID	United States Agency for International Development
WHO	World Health Organization

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background Information

More recently, it has been noted that the production and commercialization of indigenous African vegetables are on rise (Allemann *et al.*, 1996). The most commonly consumed and fully domesticated traditional vegetables are the *Amaranthus spp*. (Pig weed), *Vigna spp*. (Cowpea leaves), *Solanum spp*. (Black nightshade), *Cleome gynandra* (Cat's whiskers), *Cucurbita spp*. (Pumpkin leaves) and *Corchorus spp*. (Jute/Bush okra) (Onyango *et al.,* 2009). In the East African region over the last 10 years, amaranth has increasingly gained popularity over the other traditional vegetables due to its high acceptability and multipurpose use (leaves and edible grain). Its tolerance to drought together with its short maturity period has led to intense production interest in East African countries (Muyonga *et al.,* 2008). According to Madakadze *et al*. (2007), amaranth is popular due to its high nutritive value and wide adaptability to diverse environments thus making it a promising crop in marginal lands and semi arid regions. Amaranth can be prepared in several ways like frying and boiling of the leaves as a vegetable and eaten as a relish and the grain ground into flour to make products such as cookies, cakes, pancakes, bread muffins, crackers, pasta and other bakery products (Teutonico and Knorr, 1985).

According to Myers (2010), amaranth is grown for its grains e.g. *A. hypochondriacus* and *A. cruentus* and for its leaves e.g. *A. tricolor.* Amaranth has the potential to address the nutritional needs of non-vulnerable and vulnerable individuals because of its high content in essential fatty acids in grains and micronutrients such as iron, zinc, selenium, copper and manganese in leaves (Escudero *et al*., 2006) which are required for normal

physiological functioning of the body. Amaranth also has medicinal value as its seeds contain oil which is good to use by individuals with hypertension and cardiovascular diseases as regular consumption reduces blood pressure and cholesterol levels, while improving antioxidant status and some immune parameters (Czerwinski *et al.,* 2004; Gonor *et al.,* 2006; Martirosyan *et al.,* 2007). Amaranth oil has shown in animal studies to lower total serum triglycerides and levels of low density lipoproteins (LDL) (Escudero *et al.*, 2006) this is due to amaranth being high in the unsaturated fat, squalene as well as heart-healthy chemicals contained in amaranth, such as soluble fiber and phytosterols. Other health benefits derived from consuming grain amaranth include helping in recovery of severely malnourished children and increase in body mass index of people formerly wasted by HIV/AIDS (Tagwira *et al*., 2006).

Kelly *et al.* (2008) reported that vegetable amaranth contains vitamin C, iron, beta carotene, calcium, and folic acid. The authors reported that amaranth leaves also contain rather high amounts of oxalic acid and nitrates and that excessive amounts of oxalic acid and nitrates are known to reduce the availability of certain minerals in the body.

1.2 Problem Statement

Lack of nutrition security is reflected in malnutrition which is affecting many Tanzanians in different forms. Despite government and development partners' efforts to address malnutrition, there has been poor progress in improving the nutritional status of children and women in Tanzania. For example, issues like stunting, which can be a result of macronutrients and micronutrients deficiency, impair immune development (Branca and Ferrari, 2002). Stunting currently affects 42% of children under five years of age, and is

only a 2% lower than it was in 2005 (NBS, 2010)[1]. The World Health Organization regards stunting (indicator of under nutrition) as 'very high' if the percent is greater than 40% in the population (WHO, 1997). The current situation of iron deficiency rates (35% of under five children (U5) and 30% women) (NBS, 2010) which is also indicating the presence of micronutrients deficiency.

More than 80% of the African population live in rural areas and depend on agriculture for their livelihoods where they produce a wide range of horticultural crops including fruits and vegetables. Of all the indigenous tropical leafy vegetables, amaranth has the largest number of species and varieties (Palada and Chang, 2003).

Studies conducted on nutrients content of amaranth have shown that amounts of protein, fats, and ash are high in amaranth grain, while the content of trace minerals (iron, zinc, selenium, copper and manganese) are especially high in amaranth leaves (Kelly *et al.*, 2008). However, amaranth leaves are also known to contain rather high amounts of oxalic acid and nitrates, high levels of oxalic acid and nitrates have been reported to reduce the availability of certain minerals in the body, most notably calcium (Kelly *et al.*, 2008). There was need to investigate the presence of these anti-nutritional factors, so as to understand the extent to which they affect bioavailability of nutrients in the body. This will help in formulating dietary guidelines and educating the public how they can maximize the benefit of amaranths by planning meals containing amaranths that can attain nutrient requirements.

[1] Although the 42% stunting of 2005 was done in a methodology that is different from the one used in 2010 to determine the prevalence of under nutrition.

Analyses of nutrients composition of amaranth varieties grown in other countries have been attempted such as in Uganda (Muyonga *et al.*, 2008), however nutrients variations in amaranth varieties grown in Tanzania has not yet been established.

In other words, while it is only said that amaranth is rich in certain nutrients, it is not clear if all varieties in Tanzania have similar nutritional composition. It is known for other plants, such as tomato grown in Nigeria, that different varieties have different nutritional composition (Olaniyi *et al.*, 2010).

Despite the numerous nutritional and health benefits associated with amaranth, research efforts to increase the commercialization of this plant, including the seeds, is still at its infancy, particularly in Tanzania. Consequently, its contributions to nutrition in local diets remain poorly understood and/or appreciated. Furthermore, there are some indications that delayed harvesting of the amaranth (one week after its maturity) when the vegetative stage ends and flowering stage begins can affect both quality and quantity of harvests (Kelly *et al.*, 2008). It is also known that consumers tend to remove the immature inflorescence part of the harvested portion. There was a need to investigate whether the young flowering part of amaranth is less nutritious than the rest of the edible part in terms of nutrient and anti-nutrients contents.

In summing up, we need to be able to recommend to consumers what variety of amaranth to consume in leaves and grains and at what stage of harvesting leaves, in order to obtain maximum amount of nutrients and minimum levels of anti-nutrients. The study therefore attempted to carry out a nutritional composition analysis of the various amaranth varieties that are commonly grown and consumed in Tanzania (and other parts of East Africa).

1.3 Justification of Study

High rates of micronutrient and other nutrients deficiencies in Tanzania leading to malnutrition have been reported (NBS, 2010). Accordingly, the prevalence of anemia is 59% among children and 41% in women of child bearing age; while 33% of children and 37% of women are estimated to have vitamin-A deficiency. A study by Allemann *et al.* (1996) showed that amaranth has the potential to be a valuable source of nutrition in areas of Africa with hot and dry conditions. The crop can grow on marginal lands and when it gets well established it can withstand acute drought conditions. Therefore, this study was conducted to come up with nutritional information on amaranth leaves and grain varieties. It is expected that the findings will establish the potential of amaranth as an important nutrient source for food and nutritional security. This will help to promote nutrient intake in households and hopefully solve various nutritional deficiencies by allowing better provision of dietary advisory service and guideline for planning meals to attain nutrient requirements. This will be in more realistic way rather than generalizing the situation by assumptions that food stuffs such as amaranth are rich in nutrients.

1.4 Study Objectives

1.4.1 Main objective

The main objective of the study was to analyze selected nutrient and anti-nutrient contents of different varieties of leafy amaranth and grain amaranth as an important step towards enhancing nutrient intake from amaranth-based dishes.

1.4.2 Specific objectives

The following specific objectives were used to achieve the mentioned general objective of the study:

i. To determine the mineral contents (focusing on iron, zinc, copper, and manganese) of different varieties of grain and leafy amaranth grown in Tanzania.

ii. To establish proximate levels of protein, fat, fibre, moisture content and ash in various genotypes of amaranth (both grain and leafy amaranth) grown in Tanzania.

iii. To determine the anti-nutrient (oxalate and nitrate) contents in different varieties of leafy amaranth and grain amaranth.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Overview of Vegetables

A vegetable as a product may be defined as a succulent plant or portion of a plant, which is consumed as a side-dish with the starchy staple (Keller, 2004). They are easy-to-grow nutrient rich foods that can help to improve nutrition and food security among communities. Vegetable production can be adopted as a strategy for improving livelihood and alleviating the nutritional status of the people. Since vegetables are affordable by many in African countries, they provide a sustainable source of nutrients to rural and urban families (Sato *et al.,* 2002).

In the Tanzanian context, there are two types of vegetables, namely indigenous vegetables and exotic vegetables. Indigenous vegetables are defined as domesticated or semi-wild vegetable crops that are grown in a particular region as an integral part of a local food system (Sukprakam *et al.,* 2005). Indigenous vegetables provide high levels of nutrients and are sometimes better nutritional sources than the exotic vegetables (Sato et *al.,* 2002; Rensburg *et al.,* 2004). Also indigenous vegetables are highly preferred to exotic vegetables. For example, a study in Tanzania showed that most of the indigenous vegetables are preferred by 50 to 90% of the people surveyed (Lyimo *et al.,* 2003), with amaranth being the most commonly consumed and fully domesticated indigenous vegetable (Onyango *et al.,* 2009).

2.2 Description of Amaranth in Grain and Leafy varieties

Amaranth consists of 60-70 species, 40 of which are considered native to the Americas. Amaranth fall into four categories: grain, vegetable, ornamental or weed. Many fall into

more than one category. Grain amaranth was cultivated and revered by the Aztecs in Mexico, the Mayas in Central America and the Inca in South America. Of the various species of amaranth, 3 have been selected over the years as the choice for human and animal consumption. *A. hypochondriacus* and *A. cruentus* are commonly grown for grain and *A. tricolor* is grown primarily for the leaves. *A. caudatus* that best adapted to the tropical highlands is a third type of grain species, although is often grown more as an ornamental. When used as a grain type, *A. caudatus* varieties are other vegetable amaranths represented by *A. dubius*, *A. blitum* and *A. cruentus*, weedy species are represented by *A. retroflexus* (redroot pigweed), *A. albus* and *A. spinosus* (Kelly *et al.*, 2008).

2.3 Current Knowledge About Amaranth

Amaranth which comprises the genus *Amaranthus* are widely distributed, short-lived herbs, occurring in temperate and tropical regions. There are about 60 amaranth species, several of which are cultivated as leafy vegetables, grains or ornamental plants, while others are weeds (Kauffman and Weber, 1990). The main species grown as leafy vegetable are *A. tricolor*, *A. dubius*, *A. lividus*, *A. creuntus*, *A. palmeri* and *A. hybridus* while *A. hypochondriacus*, *A. cruentus* and *A. caudatus* are the main grain species (Teutonico and Knorr, 1985). Amaranth produces a large amount of biomass in a short period of time (Kauffman and Weber, 1990) and therefore has the potential to contribute to a substantial increase in world food production.

2.3.1 How amaranth is grown

The way amaranth is grown has been described by Kelly *et al.* (2008) as following:

Sowing: To initiate amaranth production, a well-worked, firm and moist seed bed is required. It is important to firm the soil over the seed to make good contact between the

seed and the soil. Seed sown at a depth of 1.3 cm will establish a good plant stand. Seed will germinate within 3–4 days with a soil temperature of 20°C (68°F).

Transplanting: Seeds may be planted in a nursery for subsequent transplanting or sown directly where plants are to be grown. Transplanting is a very efficient use of seeds, and allows the growing area to be weeded just before the seedlings are transplanted.

Soil Fertility: Amaranth does not have a high nitrogen requirement like maize, but responds well to fertilization.

Harvesting: Leafy vegetable is harvested by repeated clippings; and grain is by three stages of harvest which include: 1) the drying of the heads, 2) threshing the grain from the head, and 3) winnowing the grain to remove the unwanted portions.

2.3.2 Use of amaranth

The leaves, shoots and tender stems are eaten as a potherb in sauces or soups, cooked with other vegetables, with a main dish or by itself. Vegetable amaranth species are utilized for food in different parts of the world. Grain amaranth can be used to make a range of products such as cookies, cakes, pancakes, bread muffins, crackers, pasta and other bakery products (Teutonico and Knorr, 1985). Kauffman and Weber (1990) provide a description of the variety of products made from amaranth in different parts of the world. These include soups and stews from whole grain.

2.3.3 Nutrient composition of grain amaranth

Amaranth seeds contain lysine, an essential amino acid, limited in other grains or plant sources. Most plant-based foods do not contain a complete set of amino acids, and thus different sources of protein must be used. Amaranth grain too is limited in some essential amino acids, such as leucine and threonine. Amaranth seeds are therefore promising complement to common grains such as wheat germ, oats and corn because these common

grains are abundant sources of essential amino acids found to be limited in amaranth. Cooked amaranth grains are a complementing source of thiamine, niacin, riboflavin, and folate, and dietary minerals including calcium, iron, magnesium, phosphorus, zinc, copper, and manganese, comparable to common grains such as wheat germ and oats (Kelly *et al.,* 2008).

2.3.4 Nutrient composition of leafy amaranth

Cooked amaranth leaves are a good source of vitamin A, vitamin C, and folate. They are also a complementing source of other vitamins such as thiamine, niacin, and riboflavin, plus some dietary minerals including calcium, iron, potassium, zinc, copper, and manganese (Kelly *et al.,* 2008). This composition of amaranth leaves is based on species grown in USA. The composition of different species grown in Tanzania is not known.

2.3.5 Nutrition and health benefits

2.3.5.1 Amaranth grains

Consumption of grain amaranth is reported to have nutritional and health benefits ranging from a general improvement in well being to prevention and improvement of specific ailments and symptoms including recovery of severely malnourished children and an increase in the body mass index (BMI) of people wasted by HIV/AIDS (Tagwira *et al*., 2006). The authors also reported that communities in Zimbabwe claimed that, eating grain amaranth made them feel healthier and that they noticed improvements in the health of their children. Specific health improvements included improvement in appetite, fast healing of mouth sores and herpes zoster and weight gain for PLWHAs (People Living With HIV/AIDS). Amaranth grains consumption was also associated with higher milk production among breast feeding mothers. Supplementation of patients with coronary heart disease with amaranth oil has been shown to contribute to a decrease or

disappearance of headaches, weakness, increased fatigability, shortness of breath during a physical activity, edema of the legs towards the evening hours and feeling of intermission of heart function in most patients (Martirosyan *et al*., 2007). In addition, decrease in body weight has also been reported. Consumption of grain amaranth has also been shown to have potential benefits to diabetics. Studies suggest that supplementation of diets with amaranth grain and amaranth oil improves glucose and lipid metabolism in diabetic rats (Kim *et al*., 2006).

2.3.5.2 Amaranth leaves

Amaranth leaves are known to be a effective astringent, and make a great wash for skin problems like eczema and acne (Kelly *et al*., 2008), due to beta-carotene content it has. Amaranth also makes an effective mouthwash for treating mouth sores, swollen gums, and sore throat. Amaranth leaves have been found to be a good home remedy for hair loss and premature graying (Kelly *et al*., 2008). Applying the fresh juice of amaranth leaves helps hair to retain its color, and keeps it soft, and is a great hair-loss treatment.

2.4 Importance of Some Selected Nutrients in the Body

2.4.1 Iron

The human body contains about 2 to 4 grams of iron. Over 65% of body iron is found in erythrocytes as hemoglobin. Up to about 10% is found as myoglobin, about 1% to 5% is found as part of enzymes and the remaining body iron is found in the blood or in storage. Hemoglobin is a molecule composed of four units, each containing one heme group and one protein chain. The structure of hemoglobin allows it to be fully loaded with oxygen in the lungs and partially unloaded in the tissues (Yip, 2001). Iron enables oxygen transportation in the body. Iron is a trace mineral that is extremely important, because a deficiency in this nutrient leads to a shortage of red blood cells, a condition known as

anemia. Anemic individuals do not have an adequate supply of oxygen in their body, which leaves them tired, pale, and short of breath (Maina and Mwangi, 2008).

2.4.2 Zinc

Zinc is an essential component of a large number (>300) of enzymes participating in all major biochemical pathways. Zinc plays a key role in protein synthesis, carbohydrate, fats, nucleic acids and other micronutrients as well as hormones metabolism also useful in the maintenance of cell membrane structure and functions (Dibley, 2001). Zinc plays a central role in the immune system, affecting a number of aspects of cellular and humoral immunity (Shankar and Prasad, 1998). Zinc deficiency can result to growth retardation, delayed sexual and bone maturation, and increased susceptibility to infections mediated via defects in the immune system (Hambridge *et al*., 1987).

2.4.3 Copper

Copper has been described as the 'workhorse mineral'. As a cofactor, copper drives a crucial array of chemical reactions that underpin human health and development. Copper is essential for the synthesis of collagen which is the main supporting and binding tissue of the body, also needed for healthy muscle tone and function and so plays a vital role in the heart, thus copper deficiency may result to heart failure (Failla *et al.,* 2001). The authors also reported that copper is necessary for the maintenance of a healthy white blood cell count, hence copper deficiency depresses the immune system. Also as a cofactor for the enzyme tyrosinase, copper is involved in the synthesis of the skin pigment melanin, and hence copper deficiency may lead to skin degeneration (albinism). They also reported that copper is crucial for the normal development of the brain and nervous system and also involved in the synthesis of neurotransmitters.

2.4.4 Manganese

Manganese is located largely in the mitochondria. Manganese activates numerous enzymes such as hydrolases, transferases, kinases, and decarboxylases and is a constituent of some enzymes associated with fatty acid metabolism and protein synthesis, and is involved in neurological function (Nielsen, 2001). The author also reported that manganese is required for normal thyroid function and is involved in the formation of thyroxin. Deficiency may lead to skeletal abnormalities, postural defects, impaired growth, impaired reproductive function, and disturbances in lipid and carbohydrate metabolism (Nielsen, 2001).

2.5 Anti-nutritional Factors in Amaranth

Kelly *et al.* (2008) reported that amaranth leaves, like some other vegetables, contain rather high amounts of oxalic acid and nitrates. Nitrates in amaranth leaves are a concern since nitrates can be changed in the digestive tract into poisonous/carcinogenic nitrosamines. Nitrites bind to the hemoglobin in blood, robbing it of the ability to carry oxygen (Kelly *et al*., 2008).

2.5.1 Role of nitrate as an anti-nutritional factor

Nitrate is an inorganic chemical that is highly soluble in water, where-by vegetables are responsible for most of the dietary intake (Caroll, 2006). Human infants and some animals have bacteria in their digestive systems which convert nitrates to nitrite. In humans, by six months old, the acid levels in the digestive system kill these bacteria (Kelly *et al*., 2008).

Nitrates may be chemically changed in the digestive tract into poisonous/carcinogenic nitrosamines (Kelly *et al.*, 2008). Once ingested, conversion of nitrate to nitrite takes

place in the saliva of human beings of all age groups, and in the gastrointestinal tract of infants. Infants convert approximately double, or 10 percent of ingested nitrate to nitrite compared to 5 % conversion in older children and adults (Grubben and Denton, 2004).

Nitrate changes the normal form of hemoglobin, which carries oxygen in the blood to the rest of the body, into a form called methemoglobin that cannot carry oxygen. High enough concentrations of nitrate in drinking water can result in a temporary blood disorder in infants called methemoglobinemia, commonly called "blue baby syndrome" (Caroll, 2006). The author also reported that in severe, untreated cases, brain damage and eventually death can result from suffocation due to lack of oxygen.

It is believed that after nitrate is converted to nitrite in the body, it can react with certain amine containing substances found in food to form nitrosamines, which are known to be potent cancer causing chemicals (Caroll, 2006). In some laboratory studies in which rodents were given high levels of nitrites along with amine-containing chemicals, cancers of the lung, liver, and esophagus were observed. However, cancer was not observed in animals given nitrate plus amines or nitrite alone without amines (Caroll, 2006).

2.5.2 Effect of oxalates as an anti-nutritional factor

Oxalate is a salt (a neutralized form of oxalic acid) and oxalic acid is a carboxylic acid. Oxalic acid and its salts occur as the end products of metabolism in a number of plant tissues. When these plants are eaten, they may have adverse effect because oxalates bind calcium and other minerals (Noonan and Savage, 1999). The authors reported that, while oxalic acid is a normal end product of mammalian metabolism, the consumption of additional oxalic acid may cause stone formation in the urinary tract when the acid is excreted in the urine. Vegetarians who consume great amounts of vegetables will have a

higher intake of oxalates, which may reduce calcium availability. This may be a risk to women who require greater amount of calcium in their diets. Human diets low in calcium and high in oxalate are not recommended, but the occasional consumption of high oxalate foods as part of nutritious diet does not pose any particular problem (Noonan and Savage, 1999).

2.6 Variation of Nutrient Content in Amaranth

2.6.1 Different genotypes

Cheruiyot (2012) reported that the mean vitamin contents of amaranth leaves are different between the species except for thiamin and riboflavin. The author reported the levels of vitamins in amaranth grain to be generally not different between the species apart from amino acids in some areas. This variation of nutrient content was seen in different species from Kenya, but the variation on nutrient content among amaranth species grown in Tanzania is not yet known. The study conducted in other countries like Uganda by Muyonga *et al*. (2008) has shown variation of nutrients composition in amaranth varieties grown in the country.

2.6.2 Nutrients contents at different harvesting stage

Amanabo *et al*. (2011) reported that harvesting of the *Amaranthus cruentus* at vegetative phase (market maturity) generally reduce the levels of most of the plant toxins and still conserve most of the micronutrients in amounts to meet the dietary requirements. On harvesting amaranth when the vegetative stage ends and flowering stage begins, subsequent harvests are lower in both quality and quantity (Kelly *et al*., 2008). Short days, water or other environmental stresses may promote flowering. The stress that comes with delayed transplanting can also cause the plants to flower early. Since there is a tendency of discarding the flowering part of amaranth on preparation for consumption of

amaranth obtained from this harvesting stage (flowering stage), there is a need to study if this is supported from the varieties grown in Tanzania. Also there was a need to find whether the flowering part is not nutritious or has nutrient contents that will contribute to the nutritive value of amaranth obtained at this harvesting stage.

2.6.3 Effect of drying

Studies on other vegetables such as drumstick leaves have been conducted (Joshi and Mehta, 2010) on the effect of different methods of drying (sun, shade and oven drying) on the nutritive value of selected leaf with its fresh counterparts. The results showed significant increase ($p < 0.01$) in all the nutrients and also anti-nutrients in the dried samples of the leaves making them a concentrated source of nutrients. There was a need to study nutrient and anti-nutrient contents on the dried leaves of amaranth for comparison to fresh amaranth leaves from amaranth varieties grown in Tanzania.

CHAPTER THREE

3.0 MATERIALS AND METHODS

This chapter presents the materials and methods employed in the analysis of nutrient and anti-nutrient contents of the six selected amaranth varieties that are grown in Tanzania for grains, fresh leaves, dried leaves and amaranth flowers. The chapter is divided into two sections. The first section describes materials used while the second section describes the method used.

3.1 Materials

3.1.1 Amaranth samples

The Department of Crop Science and Production of Sokoine University of Agriculture (SUA) had previously identified amaranth genotypes grown in Tanzania by farmers in different regions. Six amaranth varieties were selected for analysis in this study. The six varieties with their local names and species names are namely: - White Local (*A. dubius*), Bwasi Jekundu (*A. dubius*), Bwasi kijani (*A. cruentus*), Lishe nyeupe (*A. hybridus*), Lishe njano (*A. hypochondriacus*), and Nafaka (*A. hypochondriacus*).

3.1.2 Land preparation for amaranth planting

Land preparation was done by land tillage and then mixing with manure. The seed beds for growing amaranths were then prepared. For the six varieties to be grown, each had three beds (three replicates) making a total of eighteen (18) beds. Amaranth seeds of each variety were sown in three replicates on different seed beds. This was done in order to avoid random error and biasness as soil characteristics may vary from one bed to another.

3.1.3 Sample collection

3.1.3.1 Amaranth leaves

Amaranth leaves were harvested after 21-24 days of maturity from each replicate of six varieties and then collected in separate plastic packages for transition to the laboratory for analysis.

3.1.3.2 Amaranth flowers

Amaranth plants were left to bolt and begin flowering stage. The flowering part of amaranth was obtained after four to seven days of market maturity in each variety then collected in separate plastic bags for laboratory analysis.

3.1.3.3 Amaranth grains

Grain amaranth were collected from the dry mature inflorescence of amaranth, there were three stages of grain harvest used which include 1) the drying of the heads under the shade 2) threshing the grain from the head which involved gently rubbing of the flower heads between hands to remove the seeds, and 3) winnowing by placing an empty bucket in a light breeze and pouring some grains in it in order to remove the chaff from the grains.

3.1.4 Samples preparation for laboratory analyses

Analyses were done on fresh amaranth leaves, dried amaranth leaves, amaranth grains and the flowering part of amaranth.

3.1.4.1 Fresh leaves

After harvesting from each replication, fresh amaranth leaves were prepared into edible portions and cut into small pieces then combining replications to obtain one homogenized sample. The homogenized sample was then packed into labeled polyethylene bags.

3.1.4.2 Dried leaves

Amaranth leaves in three replicates for each variety were dried using natural sun drying for two days then combined and blended to obtain the mixed flour of homogenized sample (Eslami *et al.,* 2007). Each sample was then packed into polyethylene bag and labeled accordingly.

3.1.4.3 Amaranth grains

Grains of six amaranth varieties were blended into flour from each replicate. The flour of the three replicates in every variety was combined to obtain homogenized samples of every variety. Each sample was then packed into polyethylene bag and labeled accordingly.

3.1.4.4 Amaranth flower inflorescence

Amaranth flowers of each of the six amaranth varieties were separated from Amaranth plant at the beginning of inflorescence and cut into small pieces then combining replications to obtain one sample (homogenized sample). The homogenized samples were then packed into labeled polyethylene bags.

3.2 Methods

3.2.1 Mineral analysis

Leafy amaranth (both fresh and dry amaranth), flowering part and grain amaranth were analyzed for the selected trace minerals (iron, zinc, copper, and manganese) using the atomic absorption spectrophotometer (Eslami *et al.,* 2007).

Amaranth leaves and amaranth grains were oven dried and blended into fine powder. Then 20ml HNO_3 was added to 10.0g of the sample portion, and allowed to stand for

15minutes. The mixture was heated until the liquid was reduced to 5ml. After cooling, 20ml of HNO_3, 10ml of H_2SO_4 and 8ml of H_2O_2 were added and the contents were evaporated to 5ml. After cooling, to eliminate residual acid, 10ml deionized H_2O was added and the mixture was boiled for 10min. After cooling the digest was filtered into 25ml volumetric flask and made up to mark with deionized H_2O. Then digestion solutions were subsequently analyzed for iron, zinc, copper, selenium and manganese using Atomic Absorption Spectrometer – Solar in the flame mode.

3.2.2 Proximate analysis

3.2.2.1 Determination of moisture content

Moisture analysis was carried out using the drying oven method (AOAC, 1990). This involved measurement of weight loss due to evaporation of water on drying and loss in weight during drying equal to the moisture content of the sample. Petri dishes were weighed and their masses recorded. In this study, 0.5 g (initial mass of the sample) of each sample was weighed into pre-weighed Petri dishes and dried in the drying oven at 105°C overnight. These were cooled in desiccators for 1 hour and re-weighed to give the mass after three hours in grams.

The percentage moisture content was determined according to the formula:

$$\text{Percentage moisture} = \frac{\text{initial mass (g) - mass after 3 h (g)}}{\text{Mass of sample}} \ \frac{100}{1} \quad \text{.....(1)}$$

3.2.2.2 Determination of ash content

Ash content was analyzed as per method outlined in the AOAC (1990).

Ash (the inorganic residue remaining after the organic matter has been burnt away) was determined by incineration of 2g of each sample in a muffle furnace (Lenton Furnaces, England) at 600ºc for 4 hours. The empty crucibles were weighed with lid on an analytical balance, then 2g of each sample were accurately weighed into the crucibles, the crucibles were heated on a hot plate until smoke ceased then the crucibles and the lid were transferred into a cool muffle furnace which was set at 600^0C for four hours. When the ash became white, the crucibles were allowed to cool until about 150^0c which were then stored in the desiccator until completely cooled. The crucibles and lids were weighed and weight of ash was determined and the percentage calculated as following:

$$\text{Percentage ash} = \frac{\text{Mass of ash (g)}}{\text{Mass of sample (g)}} \times 100 \quad \text{.....(2)}$$

3.2.2.3 Determination of protein content

The Kjeldahl method (AOAC, 1990) was used for protein analysis, which is a standard method for determining total nitrogen in foods. This method is based on the assumption that a mixture of pure proteins will contain 16% nitrogen. The nitrogen concentration was determined by converting the nitrogen present in the sample to ammonium sulphate by digesting it in concentrated sulphuric acid (H_2SO_4). The digested sample was then made alkaline with 40% sodium hydroxide (NaOH) (m/v). The ammonia was distilled into excess 4% boric acid solution and was determined by titration with standardized 0.1N H_2SO_4. Protein content was obtained by multiplying the percentage determined nitrogen by the appropriate factor, which was 6.25.

One gram of each homogenized sample was weighed and transferred into a clean dry digestion tube. The samples were digested with concentrated sulphuric acid for about 3 hours with selenium as a catalyst so as to convert organic nitrogen into ammonium ions.

Alkali (NAOH) was added and the liberated ammonia was distilled into a boric acid solution with methyl red and bromocresol green as an indicator which was then titrated against 0.1N hydrochloric acid, in which the end point was detected by grey colour.

The percentage nitrogen content was calculated as follows:

$$\% N = \frac{(\text{Sample titre in ml} - \text{blank in ml}) \times 1.4 \times \text{normality of acid} \times 100}{\text{Mass of sample} \times 10} \quad \ldots\ldots\ldots\ldots\ldots\ldots (3)$$

The percentage protein content was calculated as follows:

% Protein = 6.25 x %N

3.2.2.4 Determination of fat content

The Soxhlet method was used to determine total fat (AOAC, 1990). Crude lipid was extracted with n-hexane in a soxhlet extractor which was fitted with a reflux condenser and a 250ml round bottom flask containing 150ml of petroleum ether, 0.5g of each sample was weighed into a thimble which was previously dried in the oven then a thimble was plugged lightly with a cotton wool and then placed in the extractor. The source of heat was boiling the solvent which was left to siphon over for 8 hours. After then the condenser was detached to remove the thimble. The round bottom flask containing solvent and the extracted fat was also detached. The flask was placed in the oven at 100^0C and dried to constant weight, which was then cooled in the desiccator and weighed.

The fat percentage was determined from the following formula:-

$$\text{Percentage fat} = \frac{\text{Mass of flask after drying} - \text{initial mass of flask}}{\text{Mass of sample}} \times 100 \quad \ldots\ldots\ldots\ldots\ldots\ldots (4)$$

3.2.2.5 Determination of fibre content

Crude fiber was determined by Kirk and Sawyer (1991) method. Accordingly, 2 gm of the sample was defatted in boiled 200 cm^3 of 0.1275M sulphuric acid solution for 30 minutes with constant agitation. The boiling mixture was poured into a Buckner funnel and washed with Acetone. Then, the residue was boiled in a 0.313 M sodium hydroxide solution for 30 minutes with constant stirring. The residue was mixed with boiling water followed by 1% hydrochloric acid, then washed with boiling water until free from acid. It was dried in an oven to a constant weight.

$$\%\ \text{Fiber} = \frac{\text{Weight of residue - weight of ash}}{\text{Sample weight}} \text{ x } 100 \quad \text{.....(5)}$$

3.2.2.6 Determination of total carbohydrates

The percentage of total carbohydrate was calculated by the difference method of summing the percentage values of moisture, crude protein, ash and crude fat and subtracting the sum from 100 (McDonald *et al*., 1973).

3.2.3 Anti-nutritional factors analysis

3.2.3.1 Determination of oxalate content

The oxalate content was determined by heating 2.0 g of powdered sample in distilled water and 0.3 M HCl. The cold filtrate was treated with 2 to 3 drops of methyl red indicator and NH_4OH solution before heating the mixture to 100^0C. After cooling, the filtrate was heated further before the addition $10cm^3$ of 10% $CaCl_2$ solution and allowed to stand over night. After filtration, the precipitate formed was washed to remove traces of Ca^{2+} before dissolving in H_2SO4 solution. The solution formed was brought to near boiling by heating before titrating with 0.05M (potassium permanganate) $KMnO_4$ solution (AOAC, 1995; Daniel, 2003).

3.2.3.2 Determination of nitrates content

One gram of homogenized sample was weighed into a conical flask, and then 7 ml of distilled water and 0.25 ml of 4% NaOH was added. The content in the flask was warmed at 80^0C for 25 minutes with occasional shaking. The resulting solution was filtered into 100 ml volumetric flask and then diluted to the mark. 2 ml of aliquot was taken into the test tube cooled in ice then 0.5 ml of 5% Ag_2SO_4 solution was added followed by 3.5 ml of 98% H_2SO_4 and 0.5 ml of 5% Phenol solution. The solution was allowed to stand for 20 minutes with occasional shaking. 5 ml of Toluene was added then shaken for 5 minutes. The aqueous layer was discarded and the supernatant washed twice with 10 ml portions of distilled water by shaking for 2 minutes each time that aqueous layer was discarded. The organic phase was shaken with 10ml of 10% Na_2CO_3. The washed organic phase was read for absorbance at 407 nm (Gaya and Alimi, 2000). Nitrate content was obtained from the following formula:

$$\text{Nitrate content} = \frac{C \times 100}{Ws \times 4} \quad \text{.....(6)}$$

Where; C = Concentration of nitrate in the samples as from calibration graph (µg/ml)

W_S = Sample weight taken (g)

4 = Extract volume analyzed (ml)

100 = Volume of the total extract (ml)

3.3 Statistical Analysis

The data generated from each sample was subjected to statistical analysis using two way analysis of variance (two way ANOVA) tests and the difference in mean was compared using the Duncan's new Multiple Range test ($p \leq 0.05$) (Duncan, 1955). This was done to test for differences of nutrient contents among the test varieties and the test treatments.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

This chapter presents the results and discussion of the study. It is divided into three sections to answer the three study objectives. Section one involves results of the mineral analysis, section two presents results of the proximate analysis while section three deals with results of anti-nutrients contents and their interpretations with respect to the six different amaranth varieties, that were investigated.

4.1 Mineral Composition of Amaranth Varieties

Table 1 shows the results of minerals contents levels (mg/100g) within six amaranth varieties, and for each variety with its grains, dried leaves, fresh leaves and flowering part of amaranth. The results showed that, iron was generally high in all varieties of dried leaves of amaranth ranging from 111.858 to 284.384mg/100g. Zinc appeared high in fresh leaves of amaranth ranging from 5.750 to 75.89mg/100g. Manganese was high in fresh leaves of amaranth (9.264 - 34.869mg/100g), and copper was high in fresh leaves (1.027 - 3.284mg/100g). Zinc, copper and manganese appeared to be high in Nafaka (*A. hypochondriacus*) with 75.89mg/100g, Lishe njano (*A. hypochondriacus*) with 3.284mg/100g and White local (*A. dubius*) with 34.869mg/100g varieties of the fresh leaves of amaranth. Appendix 1 shows the best of fit curves for mineral standards indicating minerals concentration against their absorbance.

Table 1: The mineral composition of six amaranth varieties with respect to grains, fresh leaves, dried leaves and flowering part (mg/100g)

Varieties	Iron	Zinc	Copper	Manganese
Grains:				
Lishe Nyeupe	21.628 ^{t}	7.066 ^{l}	0.662 ^{j}	9.698 ^{q}
Nafaka	33.750 ^{r}	6.836 ^{mn}	0.663 ^{j}	7.997 ^{s}
Lishe Njano	45.987 ^{q}	6.857 ^{mn}	0.664 ^{j}	9.764 ^{q}
White Local	33.578 ^{r}	7.324 ^{k}	0.660 ^{j}	5.963 ^{t}
Bwasi Kijani	27.844 ^{s}	6.668 ^{n}	0.667 ^{j}	21.013 ^{ef}
Bwasi Jekundu	21.349 ^{t}	6.751 ^{n}	0.981 ^{i}	18.798 ^{i}
Fresh leaves:				
Lishe Nyeupe	60.021 ^{k}	5.750 ^{o}	2.066 ^{c}	16.506 ^{k}
Nafaka	75.884 ^{i}	75.89 ^{a}	1.318 ^{h}	11.794 ^{o}
Lishe Njano	93.258 ^{g}	9.243 ^{i}	3.284 ^{a}	25.577 ^{d}
White Local	78.250 ^{h}	9.805 ^{g}	2.251 ^{b}	34.869 ^{a}
Bwasi Kijani	55.598 ^{l}	9.171 ^{i}	1.698 ^{e}	28.730 ^{b}
Bwasi Jekundu	33.530 ^{r}	19.187 ^{b}	1.027 ^{i}	9.264 ^{r}
Dried leaves				
Lishe Nyeupe	225.838 ^{d}	9.453 ^{h}	0.976 ^{i}	20.862 ^{fg}
Nafaka	258.353 ^{b}	15.355 ^{c}	0.667 ^{j}	21.091 ^{e}
Lishe Njano	219.512 ^{e}	10.741 ^{f}	0.658 ^{j}	20.818 ^{g}
White Local	111.858 ^{f}	8.391 ^{j}	0.662 ^{j}	11.546 ^{p}
Bwasi Kijani	229.388 ^{c}	13.024 ^{e}	0.985 ^{i}	20.719 ^{g}
Bwasi Jekundu	284.384 ^{a}	13.532 ^{d}	0.642 ^{j}	20.287 ^{h}
Flowering part**:**				
Lishe Nyeupe	46.908 ^{p}	5.559 ^{p}	1.500 ^{g}	12.972 ^{n}
Nafaka	51.171 ^{m}	7.318 ^{k}	1.573 ^{f}	13.886 ^{l}
Lishe Njano	66.862 ^{j}	10.593 ^{f}	2.074 ^{c}	18.483 ^{j}
White Local	48.547 ^{o}	5.024 ^{q}	1.485 ^{g}	13.450 ^{m}
Bwasi Kijani	50.358 ^{n}	7.026 ^{lm}	1.540 ^{fg}	13.923 ^{l}
Bwasi Jekundu	59.549 ^{k}	9.324 ^{hi}	1.831 ^{d}	26.440 ^{c}

Values are expressed as mean (n=3); Values with different superscripts down the column are significantly different from each other at $p<0.05$

The mineral compositions of six varieties of amaranth in their respective products (grains, fresh leaves, dried leaves and amaranth flower) are presented in Table 1. The results showed that some varieties have significance difference ($p<0.05$) in minerals composition with respect to grains, fresh leaves, dried leaves and the flowering part, where as other varieties did not show significant difference in minerals composition with respect to grains, fresh leaves, dried leaves and flowering part of amaranth.

4.1.1 Minerals composition among varieties

Iron contents are significantly different among the six amaranth varieties ($p<0.05$). The variety that showed high iron content among the six grain varieties of amaranth is Lishe njano (*A. hypochondriacus*) of 45.987mg/100g; the same variety appears to significantly have high iron content of 93.258mg/100g in fresh leafy amaranth as well as in flowering part which is 66.862mg/100g. For the dried varieties iron was high in Bwasi jekundu (*A. dubius*) 284.384mg/100g which was also significantly high ($p<0.05$) compared to the four treatments of amaranth (fresh leaves, dried leaves, grains and flowering part) within all six varieties.

Zinc content in grains is significantly high in White local (*A. dubius*) 7.324mg/100g. In fresh leaves and dried leaves of amaranth, Nafaka (*A. hypochrondiacus*) variety was high in zinc content performing 75.89mg/100g and 15.355mg/100g respectively. Zinc among varieties of flowering part, was high in Lishe njano (*A. hypochondriacus*) variety.

Copper differed significantly among varieties as copper appeared to be significantly high in Bwasi jekundu (*A. dubius*) 0.981mg/100g among grain varieties, high in Lishe njano (*A. hypochondriacus*) among varieties of fresh leaves and flowering part which is 3.284mg/100g and 2.074mg/100g respectively. Lishe nyeupe (*A. hybridus*) was also high in zinc content 0.976mg/100g among varieties of dried leaves.

Manganese content differed among varieties of amaranth as it was found significantly high in Bwasi kijani (*A. cruentus*) 21.013mg/100g of grains varieties, high in White local (*A. dubius*) 34.869mg/100g of fresh leaves varieties, high in Nafaka (*A. hypochondriacus*) 21.091mg/100g of dried leaves varieties and high in Bwasi jekundu (*A. dubius*) 26.440mg/100g of the flowering part varieties of amaranth.

These findings are supported by the study conducted by Mnkeni *et al.* (2007) on nutritional quality of vegetable and seeds from different accessions of *Amaranthus* in South Africa which showed varietal differences in minerals composition among vegetables of amaranth. Also the study conducted by Kelly *et al.* (2008) on amaranth grain and vegetable types showed nutritional composition variation among grains and vegetable types of amaranth support the findings that minerals composition differ with different amaranth treatments as seen from the outcomes conducted in the study.

Furthermore, the mineral contents seem to vary among same species grown in different countries, like a study conducted by Akubugwo *et al.* (2007) in Nigeria, which showed that minerals such as iron and zinc do not correlate the findings of this study of the species grown in Tanzania since they appear to have a greater difference for some of the species as the varieties were grown in different countries. Some of the factors like chemical and biological processes of maintaining soil fertility in different parts of the world can lead into these differences of attaining crop nutrients at different levels (Gruhn *et al.*, 2000).

4.1.2 Minerals composition among treatments

Iron contents are significantly high in all dried amaranth varieties ($p<0.05$). Iron was high in Bwasi Jekundu dried leaves - *A. dubius* (284.38mg/100g) among the six varieties. Dried amaranth leaves were high in iron content followed by fresh amaranth leaves, then amaranth grains and lastly amaranth flowers. The findings concur with the study conducted by Joshi and Mehta (2010) on effect of dehydration on the nutritive value of drumstick leaves where by fresh leaves had iron content of 0.085 mg/100 g while iron content of the leaf powder prepared by different methods of dehydration was in the range of 19 - 24 mg/100 g which was 95 to 96% more than the fresh leaves. This makes

dehydration to be one of the most possible strategies for preservation of green leafy vegetables that are highly seasonal and perishable since dehydration technique result in concentration of iron. In that respect Bwasi Jekundu variety of dried amaranth leaves can be recommended for anemic (low red blood cells due to iron deficiency) individuals (Maina and Mwangi, 2008).

According to Emebu and Anyika (2011), iron has been reported as an essential trace metal and plays numerous biochemical roles in the body, including oxygen binding in hemoglobin and acting as an important catalytic center in many enzymes, for example, the cytochrome (Geissler and Powers, 2005).

Zinc, copper and manganese contents are highest in fresh amaranth varieties especially in Nafaka (*A. hypochondriacus*), Lishe Njano (*A. hypochondriacus*) and White Local (*A. dubius*) amaranth varieties respectively. In comparison to fresh amaranth leaves, the dried amaranth leaves are significantly low in zinc, copper and manganese. The results are also supported by a study conducted by Makobo *et al*. (2010) where by the levels of manganese, copper and zinc were high in fresh amaranth and decreased in dried amaranth. Therefore this gives good information that fresh amaranth leaves of Nafaka variety can be recommended to people with zinc deficiency (Shankar and Prasad, 1998). Fresh leaves of Lishe njano amaranth variety can be recommended to people with heart problems for supplementing them with copper due to its high copper content to avoid the risk of heart failure (Failla *et al.,* 2001). Fresh leaves of White local amaranth variety can be recommended to individuals with Goitre since manganese is high in it and is good for normal thyroid function (Nielsen, 2001).

Fresh leaves of amaranth is a good source of trace minerals zinc, copper and manganese to dried leaves of amaranth as obtained from the findings which are supported by Makobo *et al.* (2010) thus making green leafy amaranth being of nutritional potential for recommendation to cover trace minerals deficits to vulnerable people and enhancing nutrition security.

4.2 Proximate Composition

Table 2 shows the proximate levels in six amaranth varieties, and for each variety with its grains, dried leaves, fresh leaves and flowering part of amaranth. The values obtained are expressed in percentage (%). Crude protein, crude fibre and crude fat (lipid) were significantly high ($p<0.05$) in grain amaranth performing in a range of (14.530 - 15.787%), (2.520 - 13.040%), and (7.923 - 9.273%) respectively, ash was also high in amaranth grains ranging from 2.373 to 2.920%, moisture content being significantly high in fresh leaves of amaranth ranging from 85.350 to 86.523% and total carbohydrates appeared high in dried leaves of amaranth with a range from 74.123 to 78.743%. Therefore the results showed that, grains were significantly high in crude protein (especially Bwasi kijani variety), lipids (especially Bwasi jekundu variety) and crude fibre (especially White local variety), where as carbohydrates was significantly high in dried leaves of amaranth (especially Bwasi kijani variety) as shown in Table 2.

Table 2: Proximate composition of six amaranth varieties with respect to grains, fresh leaves, dried leaves and flowering part

Varieties	Moisture	*CP	*CF	Lipid	Ash	*CHO
Grains:						
Lishe Nyeupe	9.643^{j}	14.753^{cd}	6.557^{b}	8.473^{bc}	2.373^{d}	51.640^{h}
Nafaka	9.640^{j}	15.280^{b}	3.903^{d}	8.447^{c}	2.147^{e}	56.677^{f}
Lishe Njano	10.503^{i}	14.530^{d}	2.520^{f}	8.933^{ab}	2.740^{b}	58.257^{e}
White Local	9.397^{j}	14.743^{cd}	13.040^{a}	7.923^{d}	2.473^{cd}	39.387^{i}
Bwasi Kijani	10.130^{i}	15.787^{a}	4.867^{c}	8.510^{bc}	2.920^{a}	52.923^{g}
Bwasi Jekundu	9.483^{j}	14.980^{c}	13.163^{a}	9.273^{a}	2.380^{d}	37.557^{j}
Fresh leaves:						
Lishe Nyeupe	86.523^{a}	4.420^{i}	1.757^{ij}	1.470^{j}	2.737^{b}	1.337^{no}
Nafaka	86.513^{a}	3.837^{j}	1.963^{hi}	1.697^{j}	1.913^{f}	2.113^{m}
Lishe Njano	86.203^{ab}	3.613^{jk}	1.313^{l}	4.530^{g}	2.653^{bc}	0.373^{p}
White Local	85.917^{bc}	4.247^{i}	1.490^{kl}	2.433^{i}	1.323^{g}	3.100^{kl}
Bwasi Kijani	85.597^{cd}	4.153^{i}	1.623^{jk}	2.320^{i}	1.403^{g}	3.283^{k}
Bwasi Jekundu	85.350^{d}	5.240^{g}	2.297^{fg}	2.617^{i}	1.420^{g}	0.780^{op}
Dried leaves:						
Lishe Nyeupe	9.270^{jk}	4.827^{h}	1.877^{hi}	1.713^{j}	2.460^{cd}	77.977^{b}
Nafaka	10.217^{i}	3.510^{k}	1.907^{hi}	4.540^{g}	1.863^{f}	76.053^{c}
Lishe Njano	8.937^{k}	3.543^{k}	2.933^{e}	4.843^{g}	2.557^{bcd}	74.253^{d}
White Local	9.343^{j}	4.163^{i}	2.130^{gh}	6.767^{e}	1.843^{f}	75.833^{c}
Bwasi Kijani	8.507^{l}	5.770^{f}	1.563^{jk}	2.387^{i}	1.467^{g}	78.743^{a}
Bwasi Jekundu	11.367^{h}	4.713^{h}	2.487^{f}	2.357^{i}	2.473^{cd}	74.123^{d}
Flowering part:						
Lishe Nyeupe	82.793^{e}	4.800^{h}	2.870^{e}	1.637^{j}	2.630^{bc}	2.400^{lm}
Nafaka	81.993^{f}	4.263^{i}	5.057^{c}	0.470^{k}	2.127^{e}	1.027^{nop}
Lishe Njano	82.233^{f}	3.430^{k}	1.557^{jk}	5.370^{f}	2.530^{cd}	3.320^{k}
White Local	78.413^{g}	5.603^{f}	2.543^{f}	6.767^{e}	1.843^{f}	2.287^{m}
Bwasi Kijani	82.127^{f}	6.230^{e}	2.043^{h}	2.450^{i}	1.500^{g}	3.607^{k}
Bwasi Jekundu	82.133^{f}	5.657^{f}	2.097^{gh}	3.537^{h}	2.403^{d}	1.683^{mn}

Values are expressed as mean (n=3); Values with different superscripts down the column are significantly different from each other at $p<0.05$; *CHO: Carbohydrate; * CF: Crude fibre; *CP: Crude protein

The proximate composition of the six varieties of amaranth in their respective products (grain amaranth, fresh leafy amaranth, dried leafy amaranth and amaranth flower) is presented in Table 2.

4.2.1 Proximate composition among varieties

The results of the study showed that there was a significant difference ($p<0.05$) of proximate composition in different amaranth varieties. There is also a significant difference in proximate contents within grains, fresh leaves, dried leaves and the flowering part of amaranth. Other varieties did not show significant difference in proximate composition with respect to grains, fresh amaranth leaves, dried amaranth leaves and flowering part of amaranth (Appendix 2).

The study conducted by Mnkeni *et al*. (2007) on nutritional quality of vegetable and seed from different accessions of amaranthus in South Africa, had shown that there were significant differences ($p< 0.05$) in crude protein and crude fat content between the vegetables from the different accessions which supports the findings of the proximate levels of crude protein, crude fibre, crude fat (lipids) and carbohydrates differing among amaranth varieties.

Crude protein was found to be significantly high in Bwasi Kijani (*A. cruentus*) variety among grains, dried leaves and flowering part having 15.787%, 5.770% and 6.230% respectively. Crude protein was also significantly high in Bwasi jekundu (*A. dubius*) 5.240mg/1000g among varieties of fresh leaves.

Crude fibre was high in two grain varieties that did not differ significantly at $p<0.05$ which are Bwasi Jekundu (*A. dubius*) 13.163% and White local (*A. dubius*) 13.040%. Crude fibre was also high in Bwasi jekundu (*A. dubius*) 2.297% among the fresh leaves varieties, also high in Lishe njano (*A. hypochondriacus*) 2.933% among the dried leaves varieties and high in Nafaka (*A. hypochondriacus*) 5.057% and varieties of flowering part.

Crude fat (Lipid) was found to be high in Bwasi Jekundu (*A. dubius*) among grains and fresh leaves varieties which are 9.273% and 2.617% respectively. Lipid was high in White local (*A. dubius*) 6.767% of dried leaves and flowering part varieties which showed no significance difference in lipid composition as shown in Table 2. The total carbohydrates content was high in White local (*A. dubius*) 58.257% among varieties of grains and was also high in Bwasi kijani (*A. cruentus*) among varieties of fresh leaves, dried leaves and flowering part performing 3.283%, 78.743% and 3.607% respectively.

4.2.2 Proximate composition among treatments

The proximate composition appears to significantly differ among the four treatments (grains, fresh leaves, dried leaves and flowering part of amaranth). The crude protein (15.787%) and ash content (2.920%) were significantly high in amaranth grain and were especially high in Bwasi kijani (*A. cruentus*) grains variety compared to other grain varieties. As it was expected, the amaranth grain varieties are of significantly high crude protein compared to fresh leaves, dried leaves and the amaranth flower. The ash content in other grain amaranth varieties was not significantly different from amaranth fresh varieties. This supports the studies conducted by Kelly *et al*. (2008) on the nutritional content of amaranth and showing that amounts of crude protein and crude fibre are high in amaranth grain. It was also seen in White local (*A. dubius*) grains and Bwasi Jekundu (*A. dubius*) grains that there is no significantly different in fibre composition, these grain varieties are good source of fibers compared to other varieties, thus making them important to be considered for people who suffer from elevated cholesterol levels and in helping to cleanse the colon (Zhao *et al*., 2007). According to Emebu and Anyika (2011), components of plants such as dietary fiber have beneficial effects in lowering blood cholesterol levels aside from the decreased intake of saturated fat and cholesterol that occurs with high intakes of plant foods (Ekumankama, 2008). Ash composition was not

significantly different from grain amaranth and fresh amaranth, specifically Lishe Njano (*A. hypochondriacus*) grains and Lishe Nyeupe (*A. hybridus*) fresh leaves.

A study conducted by Kelly *et al.* (2008) support the finding that Crude fat is significantly high in grain amaranth varieties compared to fresh leafy amaranth, dried amaranth and flower amaranth, crude fat is specifically high in Bwasi jekundu (*A. dubius*) grains of amaranth variety (9.273%), this is good to provide the body with fatty acids as bodies cannot live fat free. We need significant amounts of essential fatty acids to function properly and enhance immunity (Meyers, 2005).

Grains of amaranth being of high protein content, crude fibre content and crude lipid content as seen from the findings of the study, makes grain amaranth gain potential of its contribution in addressing the nutritional needs of vulnerable people and/malnourished individuals. Amaranth grain has more protein (13.56g/100g) than corn (9g/100g), and the protein is of an unusually high quality. It is high in the amino acid lysine, which is the limiting amino acid in cereals like maize, wheat and rice (Kelly *et al.*, 2008).

The same study support the fact that fresh amaranth has high moisture content to grain amaranth, dried amaranth and amaranth flower. The moisture content is especially high in two varieties that are not significantly different, which are Lishe nyeupe (*A. hybridus*) and Nafaka (*A. hypochondriacus*) varieties of fresh amaranth. Since it is known that products that have high moisture contents normally have low fat values, and moisture content is a widely used parameter in the processing and testing of food, thus it is used as an index of water activity of many foods (Emebu and Anyika, 2011). The observed values in Lishe nyeupe (86.523%) and Nafaka (86.513%) fresh amaranth varieties, implies that they may

have a short shelf life compared to other fresh amaranth varieties since microorganisms that cause spoilage thrive in foods having high moisture content.

Bwasi kijani (*A. cruentus*) dried leaves had high carbohydrate content which is attributed to its lowest moisture content (Oko and Ugwu, 2010). Carbohydrate was even high to all dried amaranth varieties compared to fresh amaranth, grain amaranth and amaranth flower. These findings are supported by the study conducted by Joshi and Mehta (2010) on effect of dehydration on the nutritive value of drumstick leaves and found that the carbohydrate content in the dehydrated powder increased from 55.33 to 56.10% from the fresh sample of drumstick leaves and therefore energy content in the dehydrated powder increased from 65.74 to 66.15% from the fresh sample of drumstick leaves. According to Emebu and Anyika (2011) carbohydrates are pivotal nutrients required for adequate diet. Their prime role is to produce energy required for the smooth functioning of the body (USDA, 1984), this makes amaranth also play a role in energy contribution to the body by incoperation in meal on insuring balanced diet.

As far as vegetables are concerned, some of them are rich sources while others contain traces of the nutrient. Bwasi kijani variety is the best variety that can be recommended to individuals with PEM (Protein Energy Malnutrition) since PEM results from a diet that lacks sufficient sources of protein and/or energy therefore as the Bwasi Kijani grains will supply with protein due to its highest protein content that makes it suitable for consumption, as a necessity for body development (Emebu and Anyika, 2011). The grains can be recommended to PLWHAs as 100 grams of amaranth grain flour per day for consumption in form of porridge for six months was proved improve weight (Tagwira *et al.,* 2006). Compared to other amaranth vegetable varieties and Bwasi kijani dried amaranth due to its highest carbohydrate content compared to other varieties to supply

with energy from vegetables consumption as apart of balanced diet, since PEM is a condition that refers to different levels of malnutrition caused by not having enough of the foods that would normally supply energy and protein to the body (WHO, 2003).

Bwasi jekundu grain was the best variety compared to other amaranth grain varieties that can be recommended to diabetic individuals for consumption of grain products due to its high fibre (13.163%) and lipid (9.273%) content while low carbohydrates compared to other grain varieties. Diabetic individual require much fibre for cleansing the digestive tract, by removing potential carcinogens from the body and prevents the absorption of excess cholesterol. Fibre also adds bulk to the food and prevents the intake of excess starchy food and may therefore guard against metabolic conditions such as hypercholesterolemia and diabetes mellitus (Emebu and Anyika, 2011).

4.3 Anti-nutrient Contents

Table 3 shows the anti-nutrients contents (nitrate and oxalate) in the six amaranth varieties, with respect to grains, dried leaves, fresh leaves and flowering part of amaranth. The results showed that, oxalate was significantly high at ($p<0.05$) in all six varieties of the dried amaranth leaves ranging from 360.3 to 378.5mg/100g compared to grains, fresh leaves and flowering part of amaranth. Oxalate content within six varieties of dried amaranth leaves did not show significant difference in their levels. Nitrate content was seen to be high in dried amaranth leaves which show significant difference in nitrate levels within varieties ranging from 115.23 to 137.06µg/g. Other varieties did not show significant difference in nitrate content among different treatments such as Lishe njano grain (119.15µg/g), Bwasi kijani fresh leaves (118.41µg/g), White local dried leaves (118.81µg/g) and Bwasi kijani flower (119.05µg/g). With these findings, dried amaranth was seen to contain high levels of anti-nutrients (oxalate and nitrate) as shown in Table 3.

Table 3: **Anti-nutrient contents of six amaranth varieties with respect to grains, fresh leaves, dried leaves and amaranth flower**

Varieties	Nitrate(µg/g)	Oxalate(mg/100g)
Grains**:**		
Lishe Nyeupe	92.44 ^{l}	108.0 ^{j}
Nafaka	96.00 ^{k}	111.6 ^{ij}
Lishe Njano	119.15 ^{e}	98.8 ^{jk}
White Local	113.14 ^{gh}	90.3 ^{jk}
Bwasi Kijani	101.56 ^{j}	77.9 ^{k}
Bwasi Jekundu	98.10 ^{k}	115.6 ^{ij}
Fresh leaves**:**		
Lishe Nyeupe	117.34 ^{ef}	230.0 ^{d}
Nafaka	122.14 ^{cd}	298.9 ^{b}
Lishe Njano	103.07 ^{ij}	189.3 ^{ef}
White Local	112.66 ^{gh}	270.6 ^{c}
Bwasi Kijani	118.41 ^{e}	175.3 ^{f}
Bwasi Jekundu	115.30 ^{fg}	177.8 ^{f}
Dried leaves**:**		
Lishe Nyeupe	137.06 ^{a}	378.5 ^{a}
Nafaka	127.26 ^{b}	360.8 ^{a}
Lishe Njano	123.47 ^{c}	368.8 ^{a}
White Local	118.81 ^{e}	371.8 ^{a}
Bwasi Kijani	119.70 ^{de}	360.3 ^{a}
Bwasi Jekundu	115.23 ^{fg}	364.5 ^{a}
Flowering part**:**		
Lishe Nyeupe	112.18 ^{h}	209.9 ^{de}
Nafaka	122.14 ^{cd}	136.6 ^{hi}
Lishe Njano	127.78 ^{b}	235.7 ^{d}
White Local	105.45 ^{i}	149.9 ^{gh}
Bwasi Kijani	119.05 ^{e}	170.4 ^{fg}
Bwasi Jekundu	114.44 ^{gh}	229.1 ^{d}

Values are expressed as mean (n=3); Values with different superscripts down the column are significantly different from each other at $p<0.0$

4.3.1 Anti-nutrient contents in different varieties of amaranth

Nitrate was significantly high in Lishe njano (*A. hypochondriacus*) variety among grains and flowering part (119.15µg/g and 127.78µg/g respectively). It was also high in Nafaka - *A. hypochondriacus* (122.14µg/g) among fresh leaves and high in Lishe nyeupe - *A. hybridus* (137.06µg/g) of the dried leaves.

Nitrate content was significantly low in Lishe nyeupe - *A. hybridus* (92.44µg/g) of amaranth grain, in Lishe njano - *A. hypochondriacus* (103.07 µg/g) of fresh leaves, in Bwasi jekundu - *A. dubius* (115.23µg/g) of dried leaves and also in White local - *A. dubius* (105.45µg/g) of flowering part (Appendix 4). The findings of nitrate content varying with varieties are supported by the study conducted by Mnkeni *et al.* (2007).

Oxalate appeared to be significantly high ($p<0.05$) in grains of Bwasi jekundu - *A. dubius* (115.6mg/100g), fresh leaves of Nafaka - *A. hypochondriacus* (298.9mg/100g), and flowering part of Lishe njano - *A. hypochondriacus* (235.7mg/100g). Oxalate was significantly high in dried amaranth leaves of all six varieties of amaranth (Table 3).

Oxalate content was significantly low in grains and fresh leaves of Bwasi kijani - *A. cruentus* (77.9mg/100g and 175.3mg/100g respectively). It was also significantly low in flowering part of Nafaka (*A.hypochondriacus*) 136.6mg/100g (refer to Appendix 3).

4.3.2 Anti-nutrients contents among different treatments

The anti-nutrients nitrate and oxalate were high in dried leaves of amaranth compared to grain amaranth, fresh amaranth and amaranth flower. Nitrate was high in Lishe nyeupe dried leaves (137.06 µg/g). Oxalate contents were high in dried amaranth leaves of all the six varieties ranging from 360.3 to 378.5mg/100g. This is supported by the study conducted by Joshi and Mehta (2010) on effect of dehydration on the nutritive value of drumstick leaves, where by oxalate content increased from 101mg/100g in fresh leaves to 430mg/100g in sundried leaves.

For this reason, due to high oxalate content dried amaranth varieties are not recommended to people with bones problem as higher intake of oxalates, which may reduce calcium

availability (Noonan and Savage, 1999). Since the higher the level of anti-nutrient, the lower the bioavailability of the nutrient and minerals contained and even affecting bones and this has to be carefully observed to people with bones problem as the anti-nutrients reduce calcium availability making bones development worse.

In human diet low in calcium and high in oxalate are not recommended, but the occasional consumption of high oxalate foods as part of nutritious diet does not pose any particular problem (Noonan and Savage, 1999). That is, too much consumption of high oxalate foods posses health effect.

Also dried amaranth being of high nitrate content, it is not recommended to anemic people and pregnant women since it changes the normal form of hemoglobin, which carries oxygen in the blood to the rest of the body, into a form called methemoglobin that cannot carry oxygen.

In severe, untreated cases, brain damage and eventually death can result from suffocation due to lack of oxygen (Caroll, 2006). Otherwise the way of preparing dried amaranth matter a lot before consumption, this is when the method cooking by discarding water is applied after boiling of the dried leaves, which will remove potential nitrate and oxalate toxins, it is where it can be consumed with minimal nitrate effects (Kelly *et al.*, 2008) and thus can be recommended to for pregnant women to eliminate anaemia. Therefore cooking and discarding water will remove potentially harmful oxalates and nitrates leaving dried leaves of amaranth a good source of iron that is less harmful.

For the flowering part of amaranth was seen to have less anti-nutrient in most of the varieties (four varieties) out of six varieties from the study compared to fresh amaranth

vegetable at market maturity, this keeps discouraging the common practice of discarding amaranth flower from amaranth plant obtained at that stage (stage of heading/flowering stage) as it is also seen to have nutrients that are not so far less from fresh amaranth. These findings are supported by Amanabo *et al*. (2011) who conducted a study on effect of heading on some micronutrients, anti-nutrients and toxic substances in *Amaranthus cruentus* grown in Minna, Niger State, Nigeria.

CHAPTER FIVE

5.0 CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

It is believed that the results of this study will help to stimulate consumption or utilization of amaranth as good sources of nutrients needed for healthy growth. Different amaranth varieties have different nutritional values and different maximum nutrients that make them of potential in combating nutritional deficiencies according to nutritional needs of individuals towards reducing malnutrition cases and enhancing nutrition security.

Generally, the findings of the study have shown that iron content is high in dried amaranth leaves making it best to supplement with iron and meeting iron needs/requirements of the body. Zinc, copper and manganese contents are high in fresh amaranth leaves making them of nutritional importance to cover the required levels in the body on consumption. Proximate levels of crude fat, crude fibre and crude protein are high in grains of amaranth. Anti-nutrients (oxalate and nitrates) are high in dried amaranth leaves which are disadvantageous as it lowers the bioavailability of the micronutrients like minerals in the body. Anti-nutrients lower bioavailability of micronutrients in the human body by binding to micronutrients and preventing its absorption, this is due to saturation of the transport system and reduced absorption of the other nutrients caused by the anti-nutrients

5.2 Recommendations

The study has observed that drying of the amaranth leaves resulted in concentration of anti-nutrients and iron. Since dehydration is one of the most possible strategies for preservation of green leafy vegetables, on requirement of certain nutrients such as iron

due to its quality of iron content that will increase blood levels, then the dried amaranth can be used for supplementation but with a cooking method of discarding water after boiling the dried leaves. This can lower anti-nutrients content so as to minimize its effect of lowering nutrients bioavailability.

The common practice of discarding amaranth flowers when preparing amaranth leafy vegetable don't appear to be supported since the study has shown that the nutrients composition are not so far different from the fresh leaves. When vegetative stage ends and flowering begins, subsequent harvests are lower in nutritional quality, so at this harvesting stage incase of obtaining this kind of amaranth it is recommended not to discard the flower heads of amaranth as it will contribute into nutritive value of the harvest instead of removing and making them less.

The best identified Amaranths varieties from this study can serve as good starting materials for plant breeders to come up with more desired plant line composed of more different nutrients widening its potential in nutrition security. Also amaranth grain can serve as a good option in fortification of staples like maize flower, sorghum flower and wheat, which are nutritionally low in protein and other essential nutrients.

Since this research was done to only six varieties, where as there are so many varieties of amaranth in Tanzania, there is opportunity for further identification of nutritional values in other varieties, as there can be other varieties best than the ones on study in combating malnutrition and attaining food and nutrition security of which is not known.

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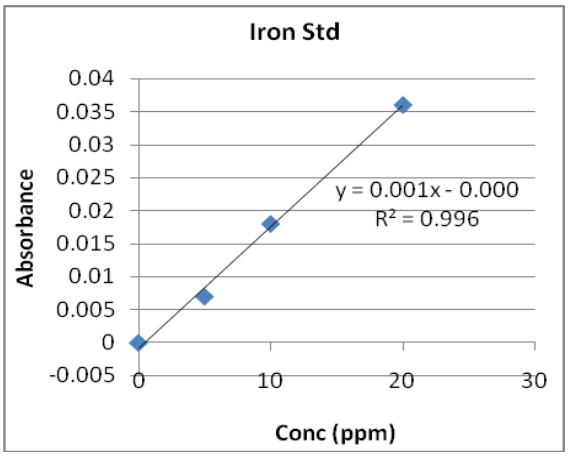
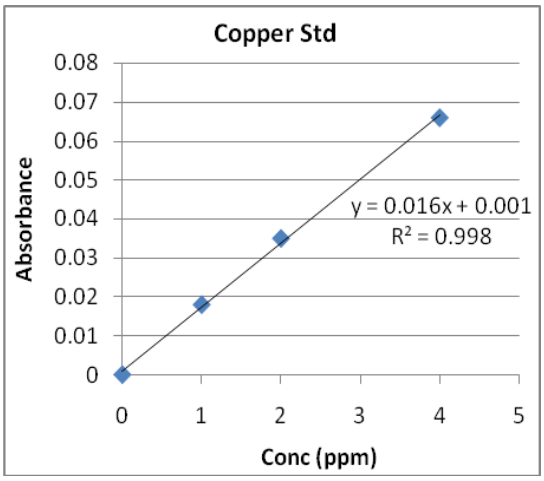
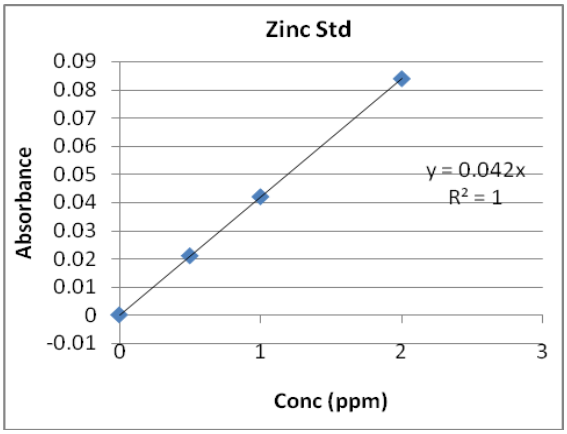
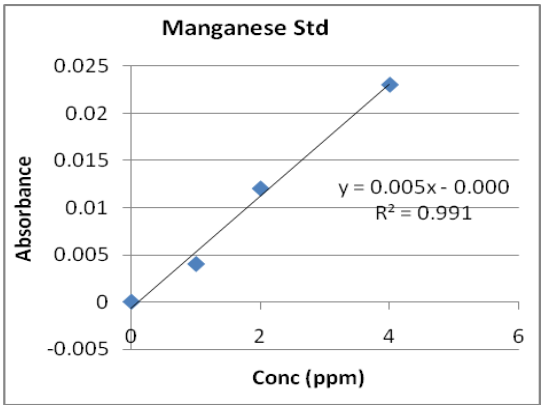
APPENDICES

Appendix 1: Best of fit curve of minerals standards (Iron, Zinc, Copper and Manganese).

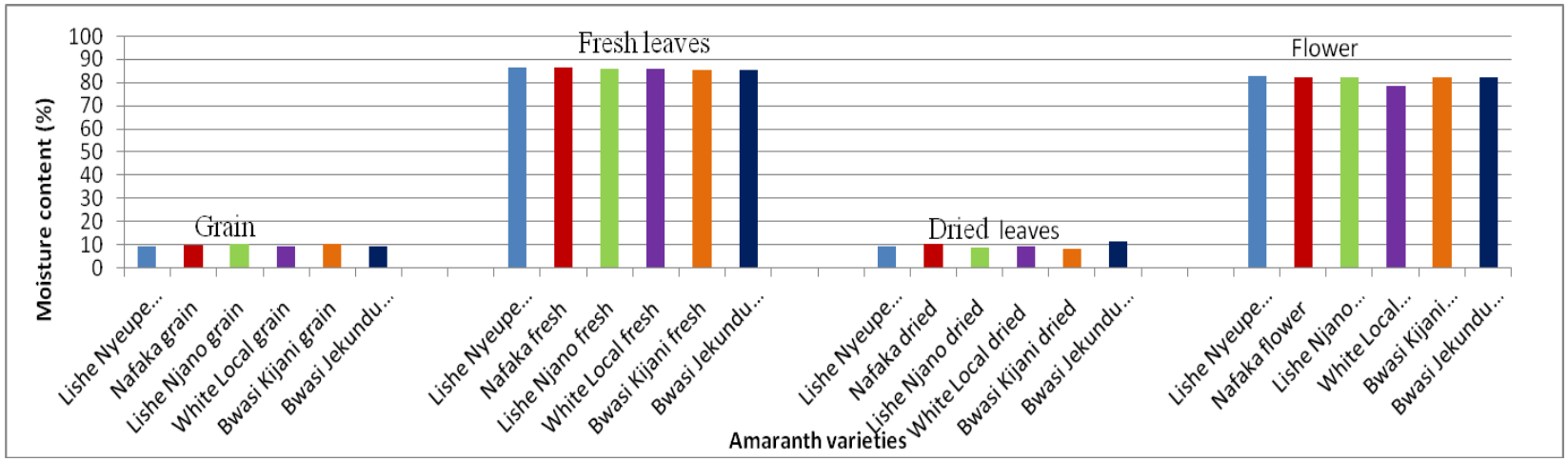


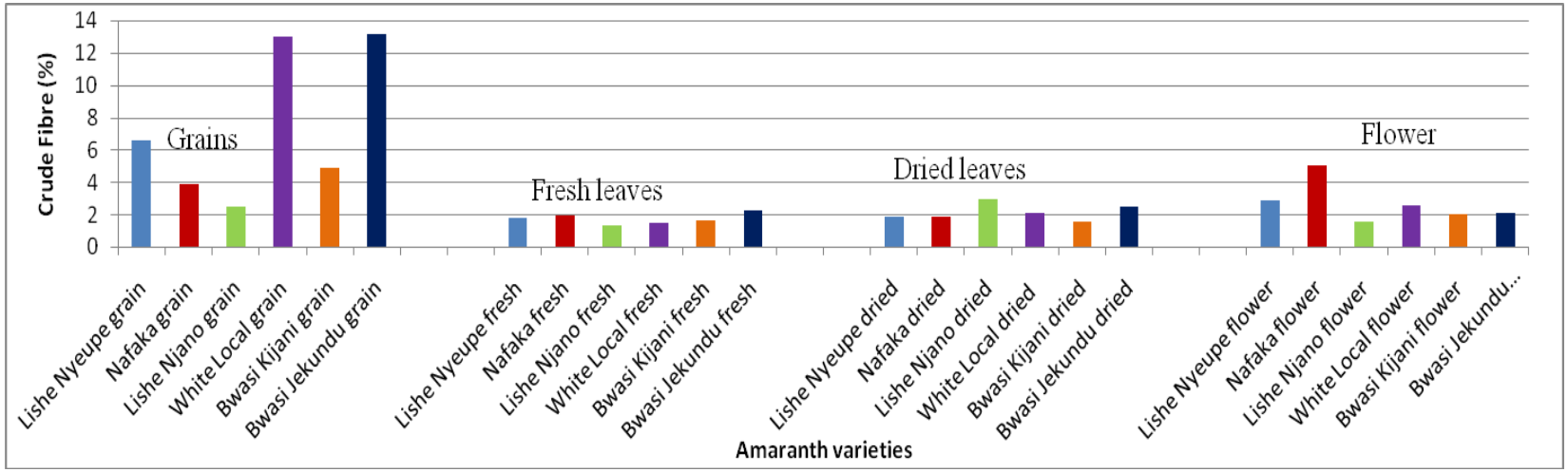


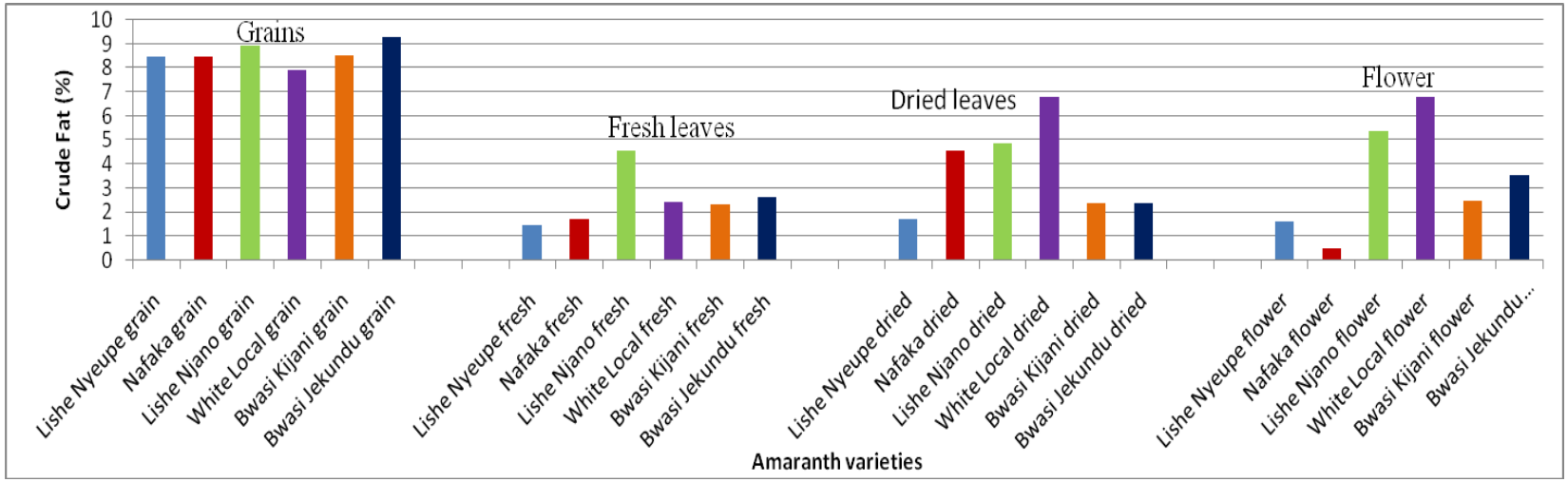


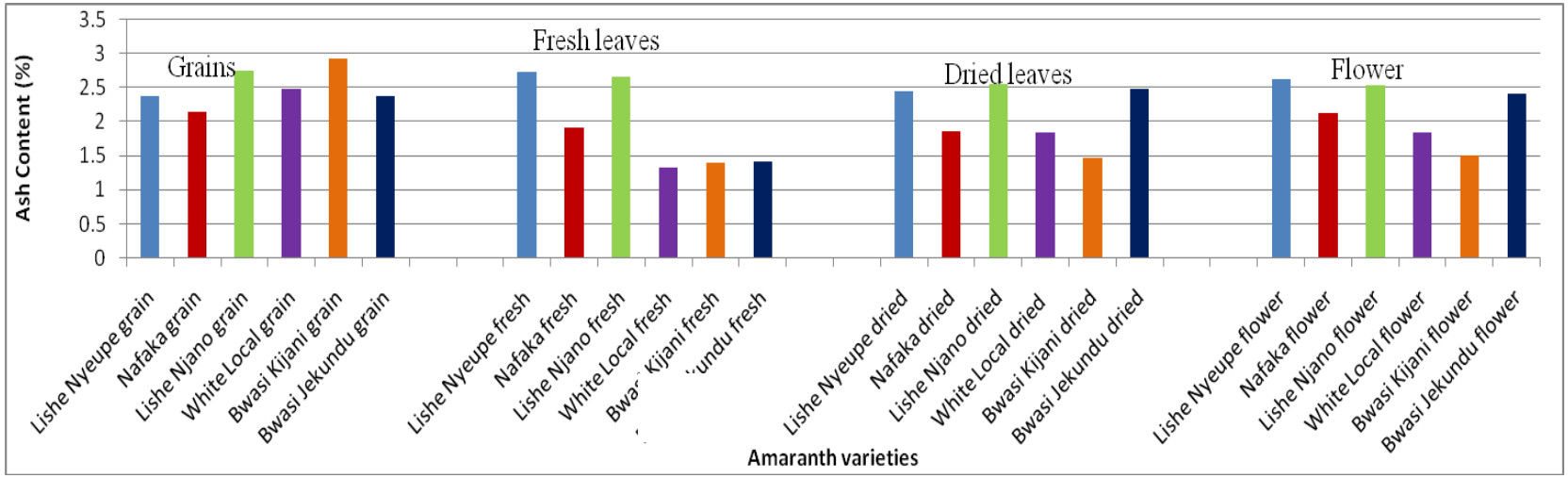


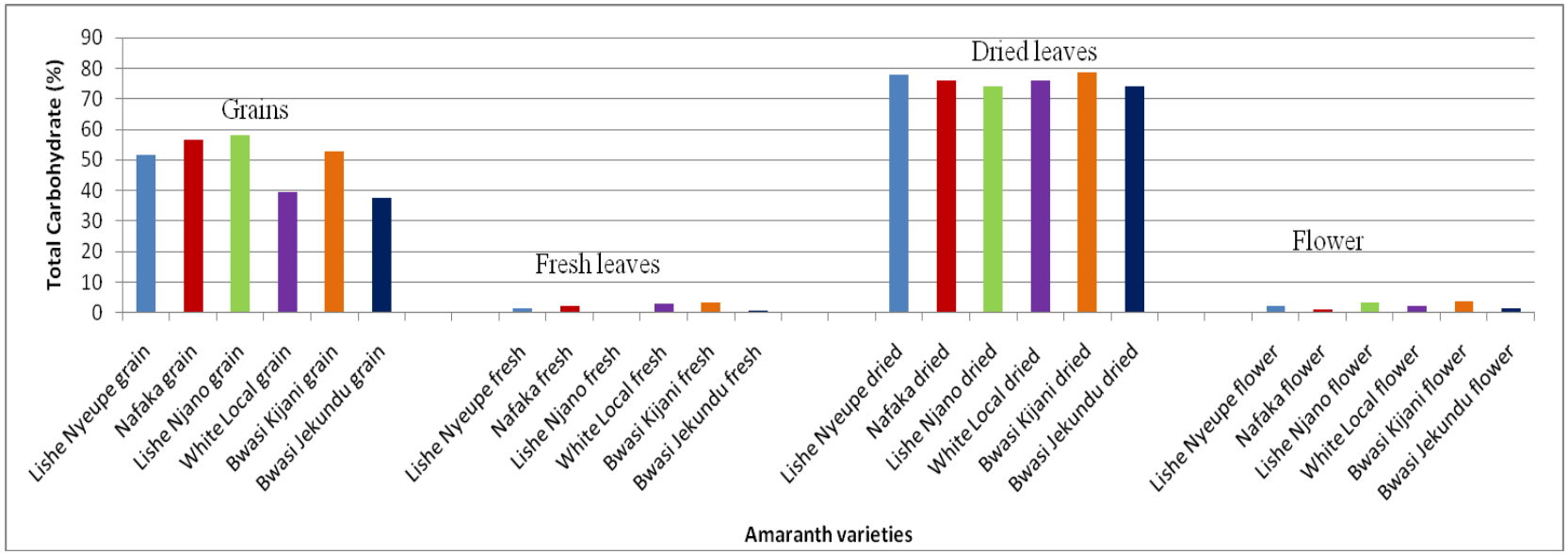
Appendix 2: Proximate composition of Amaranth varieties in fresh leaves, dried leaves grains of amaranth and amaranth flower

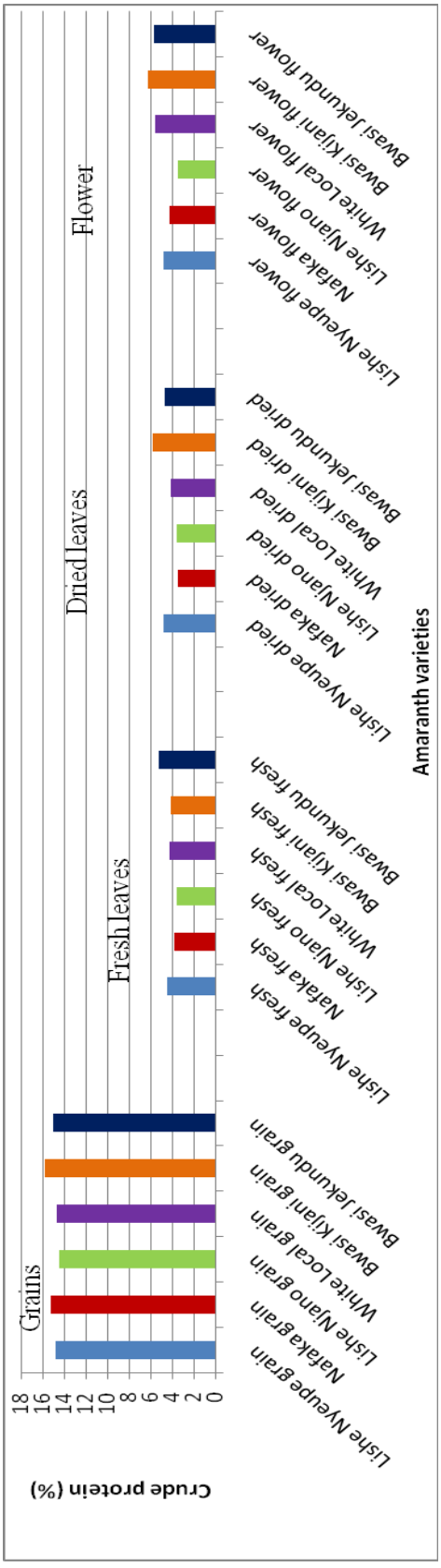



Crude Fibre (%)
14
12
10
8
6
4
2
0
Grains
Fresh leaves
Dried leaves
Flower
Lishe Nyeupe grain
Nafaka grain
Lishe Njano grain
White Local grain
Bwasi Kijani grain
Bwasi Jekundu grain
Lishe Nyeupe fresh
Nafaka fresh
Lishe Njano fresh
White Local fresh
Bwasi Kijani fresh
Bwasi Jekundu fresh
Lishe Nyeupe dried
Nafaka dried
Lishe Njano dried
White Local dried
Bwasi Kijani dried
Bwasi Jekundu dried
Lishe Nyeupe flower
Nafaka flower
Lishe Njano flower
White Local flower
Bwasi Kijani flower
Bwasi Jekundu...
Amaranth varieties

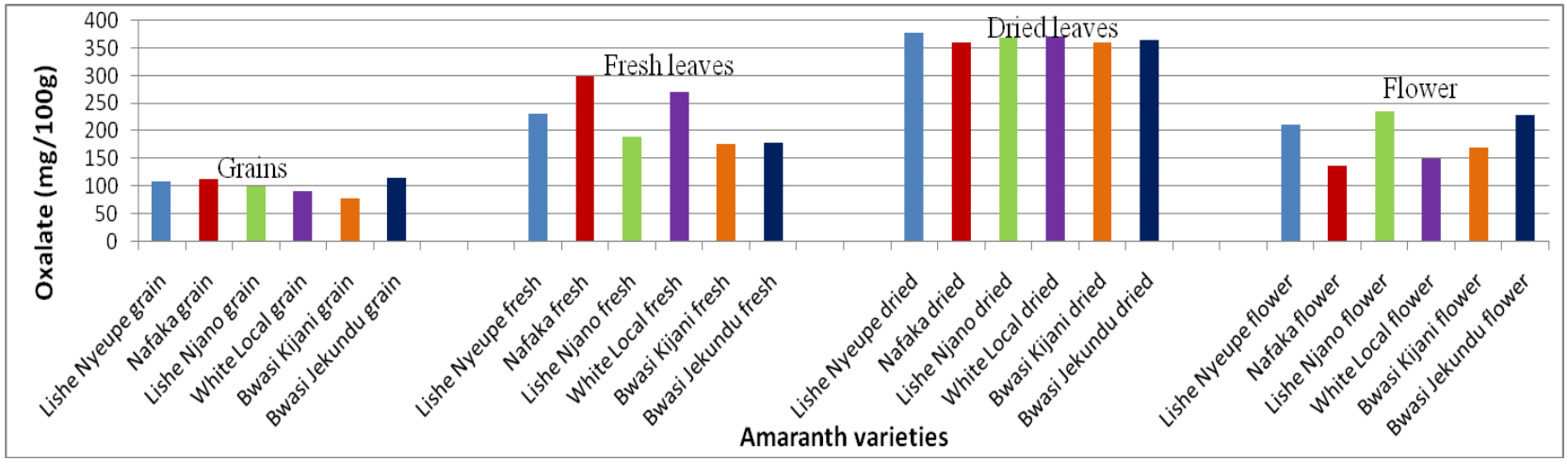

Crude Fat (%)
10
9
8
7
6
5
4
3
2
1
0
Grains
Fresh leaves
Dried leaves
Flower
Lishe Nyeupe grain
Nafaka grain
Lishe Njano grain
White Local grain
Bwasi Kijani grain
Bwasi Jekundu grain
Lishe Nyeupe fresh
Nafaka fresh
Lishe Njano fresh
White Local fresh
Bwasi Kijani fresh
Bwasi Jekundu fresh
Lishe Nyeupe dried
Nafaka dried
Lishe Njano dried
White Local dried
Bwasi Kijani dried
Bwasi Jekundu dried
Lishe Nyeupe flower
Nafaka flower
Lishe Njano flower
White Local flower
Bwasi Kijani flower
Bwasi Jekundu...
Amaranth varieties


Ash Content (%)
3.5
3
2.5
2
1.5
1
0.5
0
Grains
Fresh leaves
Dried leaves
Flower
Lishe Nyeupe grain
Nafaka grain
Lishe Njano grain
White Local grain
Bwasi Kijani grain
Bwasi Jekundu grain
Lishe Nyeupe fresh
Nafaka fresh
Lishe Njano fresh
White Local fresh
Lishe Nyeupe dried
Nafaka dried
Lishe Njano dried
White Local dried
Bwasi Kijani dried
Bwasi Jekundu dried
Lishe Nyeupe flower
Nafaka flower
Lishe Njano flower
White Local flower
Bwasi Kijani flower
Bwasi Jekundu flower
Amaranth varieties

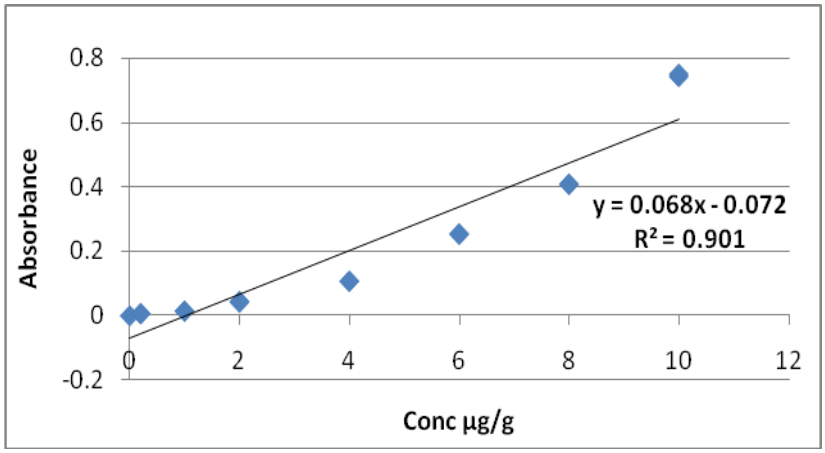

Total Carbohydrate (%)
90
80
70
60
50
40
30
20
10
0
Grains
Fresh leaves
Dried leaves
Flower
Lishe Nyeupe grain
Nafaka grain
Lishe Njano grain
White Local grain
Bwasi Kijani grain
Bwasi Jekundu grain
Lishe Nyeupe fresh
Nafaka fresh
Lishe Njano fresh
White Local fresh
Bwasi Kijani fresh
Bwasi Jekundu fresh
Lishe Nyeupe dried
Nafaka dried
Lishe Njano dried
White Local dried
Bwasi Kijani dried
Bwasi Jekundu dried
Lishe Nyeupe flower
Nafaka flower
Lishe Njano flower
White Local flower
Bwasi Kijani flower
Bwasi Jekundu flower
Amaranth varieties


Crude protein (%)
18
16
14
12
10
8
6
4
2
0
Grains
Fresh leaves
Dried leaves
Flower
Lishe Nyeupe grain
Nafaka grain
Lishe Njano grain
White Local grain
Bwasi Kijani grain
Bwasi Jekundu grain
Lishe Nyeupe fresh
Nafaka fresh
Lishe Njano fresh
White Local fresh
Bwasi Kijani fresh
Bwasi Jekundu fresh
Lishe Nyeupe dried
Nafaka dried
Lishe Njano dried
White Local dried
Bwasi Kijani dried
Bwasi Jekundu dried
Lishe Nyeupe flower
Nafaka flower
Lishe Njano flower
White Local flower
Bwasi Kijani flower
Bwasi Jekundu flower
Amaranth varieties

Appendix 3: Oxalate composition of amaranth varieties in grains, fresh leaves, dried leaves and amaranth flower



Appendix 4: Best of fit curve of nitrate standard



Appendix 5: Six varietal Analysis of Variance (ANOVA) of the proxim-0ate composition of fresh leaves, dried leaves grains and amaranth flower)

Source of Variation	Df	Moisture		Ash		CP		Fat		CF		CHO	
		F-v	F-p	F-v	F-p	F-v	F-p	F-v	F-p	F-v	F-p	F-v	F-p
Replication	2	59.48	0.001	5538.97	0.001	181.79	0.001	206.26	0.001	884.85	0.001	532.23	0.001
Variety	5	6.016E+05	0.001	4540.43	0.001	19816.49	0.001	1814.89	0.001	6350.87	0.001	1.062E+05	0.001
Treatment	3	53.39	0.001	4684.88	0.001	33.56	0.001	75.43	0.001	931.01	0.001	532.16	0.001
Variety*Treatment	15												
Residual	46												
Total	71												

F-v: F-value; F-p: F-probability; Df-Degrees of freedom

Appendix 6: Six varietal Analysis of Variance (ANOVA) of the minerals composition of grains, fresh leaves, dried leaves and amaranth flower

Source of variation	Df	Iron		Zinc		Copper		Manganese	
		F-v	F-p	F-v	F-p	F-v	F-p	F-v	F-p
Replication	2								
Variety	5	22107.49	0.001	52410.92	0.001	441.99	0.001	8652.38	0.001
Treatment	3	1.240E+06	0.001	67226.97	0.001	5580.53	0.001	26096.76	0.001
Variety*Treatment	15	29725.38	0.001	43316.16	0.001	441.47	0.001	14852.33	0.001
Residual	46								
Total	71								

F-v: F-value; F-p: F-probability; Df-Degrees of freedom

Appendix 7: Six varietal Analysis of Variance (ANOVA) of the anti-nutrient composition of grains, fresh leaves, dried leaves and amaranth flower

Source of Variation	Df	Nitrate		Oxalate	
		F-v	F-p	F-v	F-p
Replication	2				
Variety	5	40.29	0.001	8.74	0.001
Treatment	3	553.38	0.001	1044.20	0.001
Variety*Treatment	15	106.86	0.001	19.30	0.001
Residual	46				
Total	71				

F-v: F-value; F-p: F-probability; Df-Degrees of freedom