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IMPACT OF DIFFERENT PROCESSING TECHNIQUES ON NUTRIENTS AND ANTINUTRIENTS CONTENT OF GRAIN AMARANTH (*Amaranthus albus*)

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Impact of different processing techniques on nutrients and antinutrients content of grain amaranth (*Amaranthus albus*)

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A thesis submitted in partial fulfillment for the Degree of Master of Science in Food Science and Nutrition in the Jomo Kenyatta University of Agriculture and Technology

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DECLARATION

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

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This thesis has been submitted for examination with our approval as University Supervisor.

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DEDICATION

This thesis has been dedicated to my family with lots of love

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ABBREVIATION AND ACRONYMS

AAS	Atomic absorption spectroscopy
ANOVA	Analysis of Variance.
AOAC	Association of Official Analytical Chemists
FAO	Food and agricultural organization
GC	Gas chromatography
HDL	High density lipoprotein
HIV-AIDS	Human immunodeficiency virus- acquired immunodeficiency syndrome
HPLC	High Performance Liquid Chromatography.
JKUAT	Jomo Kenyatta University of Agriculture and Technology.
SD	Standard Deviation
PPM	Parts per million
UV-Vis	Ultra violet visible spectrophotometer
VLDL	Very low density lipoprotein
WWB	Wet weight basis

ABSTRACT

Grain amaranth (*Amaranthus albus*) is a pseudo cereal consumed in various parts of the world especially in Africa, India and Nepal. It has attracted increasing interest over recent decades because of its nutritional and functional properties. It can potentially play a great role in mitigating food insecurity and reducing the prevalence of malnutrition in Kenya. Maximum utilization of the nutrient potential of the grain amaranth is limited by the presence of phytates, phenols, tannins, and oxalates which are mineral chelating agents and enzyme inhibiting anti-nutrients. The effects of these anti-nutritional factors may be reduced by some processing techniques. Traditionally, amaranth grain is boiled, popped, roasted, or milled to make gruel for consumption. This study investigated the impact of dry and wet heat processing techniques on the nutrient and anti-nutrient contents of grain amaranth *Amaranthus albus*. The dry heating processes used included roasting(160ºC 10minutes) and popping(190ºC 15seconds) while the wet heating techniques included boiling whole grains(water: seeds, 4:1) and slurries(water: flour, 6:1. Raw grains were used as the control to determine the impact on nutrient and anti-nutrient content after processing. Different processing techniques significantly ($p<0.05$) reduced the level of unsaturated fatty acids. Mineral contents did not change significantly ($p>0.05$). A pronounced reduction in the anti-nutrient content (tannins, oxalates, and phytates) was observed in the case of boiling as compared to roasting and popping. The protein digestibility of raw grain was 74.8%. The protein digestibility increased to 86.2% and 92.6% after Boiling and slurring whereas it reduced to 66.3% after roasting. Heat treated and untreated grains, had high content of lysine and arginine. Wet processing resulted to higher starch gelatinization as compared to Dry heating. Different processing techniques had different impact on nutrient and antinutrients components of grain amaranth.

Key words: grain amaranth, proximate composition, antinutrients, protein digestibility, starch gelatinization

CHAPTER ONE

1.0 INTRODUCTION

1.1Background information

Amaranth is among the oldest cultivated plant species. It originated in the American and European countries; amaranth has been cultivated for more than 8,000 years, dating back at least to the Mayan civilization of South and Central America. It was a staple of the Aztecs and incorporated into their religious ceremonies. In the 1500's the Spanish conquistadors prohibited amaranth production (Belton & Taylor, 2002).

The cultivation and production of grain amaranth were given less consideration and thus nowadays it can be classified as a forgotten, neglected and alternative crop of great nutritional value (Earnest, 2009).The use of alternative crops would result in product competitiveness and diet diversity that boosts productivity (Bavec and Bavec 2006).

Amaranth (family *Amaranthaceae*) is an underexploited plant with an exceptional nutritive value. There are more than 60 species and between 4000-6000 varieties. These species are divided into four classes as follows: grains, vegetables, ornamental and weeds (Kauffman and Weber, 1990).

Amaranths are broad-leafed non-grass plants that are easy to grow, nutrient rich and an underutilized pseudo cereal that can play an important role in actions against hunger and malnutrition. In Kenya, they are known in local languages as Terere (Kikuyu), Muchicha (Kiswahili, Giriama), Lidoodo (Luhya) and Alika (Luo)(Mburu, 2011). They are either consumed as a leafy vegetable or as grain. The grain amaranth is very versatile as a food crop and can diversify farming enterprise; as it can be used in alleviating food shortage as it is tolerant to environmental stress (Saunders and Becker 1984).

In botanical terms, amaranths, are not true cereals, they are dicotyledonous plants as opposed to most cereals (e.g. wheat, rice, barley) which are monocotyledonous. They are

referred to as pseudocereals, as their seeds resemble in function and composition of the true cereals (Alverez-Jubete, 2009). Amaranth has been touted as a miracle grain, a super grain, and the grain of the future (Matz, 1991; Evgeny, 2001). In Kenya, improved seeds have led to increased production of grain amaranth in Western and Nyanza region. The seeds contain large amounts of dietary fiber, iron, and calcium (Kariuki *et al*, 2013).

The crude protein content of grain amaranth ranges from 11 to 17.6 % dry matter (Bressani *et al.*, 1987 a; Imeri *et al.*, 1987 b; Bressani *et al.*, 1987 b). This is higher than in most common grains except soybeans. The protein quality of grain amaranth is complete containing around 5% lysine and 4% sulphur amino acids, which are the limiting amino acids in other grains. The lysine content is given as the main reason for the high protein quality of amaranth (Saunders *et al.*, 1983; Teutonico and Knorr, 1985).

Amaranth contains antinutrients like phytic acid, oxalates, phenolic compounds, protease inhibitors and saponins. These help the plant in protection against invasion. However, they bind minerals such as iron, calcium and zinc, and there is some evidence showing decreased absorption of these minerals in their presence (Hamaker, 2007). They also bind proteins and enzymes in the gastrointestinal tract. However, they also have positive effects, for instance blood cholesterol lowering effect. They are bound mainly in the hulls of grains. Like phytic acid, oxalates and tannins influence the bioavailability of some nutrients (proteins, minerals) (Matz, 1991, Hamaker, 2007). The dark seeds of amaranth contain more tannins than the light ones (Alvarez-Jubete *et al.,* 2009).

Thermal processing improves the bioavailability of macronutrient and micronutrients by destroying certain antinutritional factors mentioned (Erdman and Pneros-Schneier, 1994). For instance degradation of phytates depends on the plant species, temperature and Ph. There is some evidence reported by Perlas and Gibson (2002), Hotz and Gibson (2001) that boiling of tubers and blanching of green leaves induced moderate losses (5-15%) of phytic acids. It enhances bioavailability of nutrients by releasing them from entrapment in the plant matrix and improves starch digestion (Yadav and Sehgal, 2002).

There are different methods of dry heat techniques that are applied in processing grains. For instance popping where the seeds are subjected to intense heat at short time, toasting is done when the seeds are exposed for 60-90s at about 150 °C, usual on a hot surface and no popping occurs. Roasting g occurs when the seeds are subjected to very hot temperatures above 150 °C for longer period of time where partial popping can occur prior to roasting. Variables like moisture content, species, variety and genotype Aare typical in order to obtain a satisfactory yield (Belton and Taylor, 2002)

Heat processing has been reported to cause damage of essential amino acids resulting in decreased contents or transfer into a racemic mixture (Bressani et al., 1987b; Tovar et al., 1989). Temperatures above 100°C induce reactions in both bound and free amino acids, and especially in essential amino acids valine, leucine, isoleucine, lysine, threonine, methionine, phenylalanine and tryptophan, which then become non-utilizable for humans, thus decreasing the biological value of foodstuffs (Velíšek, 1999)

1.2 Problem statement

Malnutrition is a key issue among the poor in Kenya and therefore using the available food and land resources to build up food reserves may play a great role in reducing hunger and improving the nutritional status of vulnerable populations. Nationally, 35 % of children under five are stunted, while the proportion severely stunted is 14 %. 16 % of children under five are underweight (low weight-forage) and 4 % are severely underweight while 7 % of children are wasted and 2 % are severely wasted (KDHS, 2010)

Diets consumed by urban poor and rural people are frequently deficient in macronutrients (protein, carbohydrates and fat, leading to protein–energy malnutrition) and micronutrients (electrolytes, minerals and vitamins leading to specific micronutrient deficiencies) or both.Also, consumption of rich foods especially cereals with high levels of plant secondary metabolites (anti- nutrient factors) like phytates, tannins, saponins, oxalates, trypsin inhibitors, nitrates and proteases may not effectively alleviate malnutrition due to their presence. Therefore, for efficient utilization and assimilation of

the grain amaranth; there was need to study how each processing (popping, roasting, boiling and slurring)techniques affected the nutrients and anti nutrients components.

1.3 Justification

In Kenya, many people are faced with severe food and nutrition insecurity. Amaranth, a traditional vegetable, is a vital source of macro/micro-nutrients and bioactive compounds of health benefits and may offer low cost complementary grain for consumption. It has high quality protein due to its essential amino acid and high levels of lysine content which is limiting in conventional cereals.

The environmental adaptability of amaranth creates an excellent potential crop to positively impact on thousands of farmers in Kenya. It is resilient to environmental stress for instance drought which is a major problem in most parts of Kenya.

Clearly clarified on how different processing techniques affected the nutritional components and antinutrients contents of grain amaranth especially at the time of consumption. Reduction of antinutrients content in the processed grain amaranth promotes better utilization and assimilation of amaranth especially proteins, starch and minerals on consumption.

1.4 Objectives

1.4.1 Main objective

The main objective of this study was to determine the impact of different processing techniques on the nutritional composition and antinutrients content of grain amaranth (*Amaranthus albus*)

1.4.2 Specific objective

The specific objectives were:

i. To determine the concentration of nutrients (proximate composition, mineral composition, fatty acid profile, amino acid composition, protein digestibility and

degree of starch gelatinized) and anti- nutrients (phytates, tannins and oxalates)in raw grain amaranth.

ii. To determine the effect of processing techniques {moist heat (boiling and slurring) and dry heat (popping and roasting)} on nutrient(proximate composition, mineral, fatty acid profile, amino acid composition, protein digestibility and degree of starch gelatinized)and anti-nutrients (phytates, tannins and oxalates)of the products.

iii. To determine protein digestibility and degree of starch gelatinization of processed grain amaranth.

1.5Hypothesis

i. Raw amaranth grain has got high levels of nutrients and antinutrients content

ii. Processing techniques have effects on nutrient and antinutrients composition of amaranth grain

iii. Degree of starch gelatinization and level of protein digestibility is affected by different processing techniques.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Introduction

Grain amaranth, an annual food and feed crop, is a dicotyledonous C4 plant belonging to Amaranthaceae, genus Amaranthus and consisting of several species. They are referred to as pseudocereals; as their seeds resemble in function and composition those of the true cereals which are monocotyledonous. It is a fast growing plant, which has high nutritional value and is resistant to stress conditions. It was a staple food in ancient Aztec culture and is still cultivated as a minor food crop in central and South America and some areas of Asia and Africa (Campbell *et al*., 1997)

This crop is now attracting worldwide attention because of its superior agronomic traits and its high potential for food and feed uses. It has been recognized as a high potential new food crop for the 21st century and in china particularly is thought that it has equal potential as a feed crop (Campbell *et al.,* 1997).

2.2 Taxonomic classification and morphology

Grain amaranth belongs to the order *Caryophyllales*, amaranth family *Amaranthaceae*, sub-family *Amaranthoideae*, genus *Amaranthus*, and according to Sauer (1967), into the section *Amaranthus*. The genus *Amaranthus* includes approximately 60 species, most of which are cosmopolitan weeds (*A. retroflexus L.,A. hybridus L., A. powellii S. Watt., A. spinosus L.*) and cultivated amaranth species which can be used as food grain, leafy vegetables, forage and ornamentals. According to the utilization of cultivated amaranths for human consumption, species can be divided into grain and vegetable amaranths.

Grain amaranth is an annual herbaceous plant which is a pseudocereal, one of the few C4 dicots, with an erect stem and enormous inflorescence of various colors. Some anatomical characteristics of amaranth and its C4-photosynthesis pathway result in increased efficiency of using CO_2 under a wide range of temperature (from 25 to 40 ºC),

under higher light intensity, and moisture stress environments. All this contributes to its wide geographic adaptability to diverse environmental conditions (Kigel, 1994).

2.2 Characteristics of grain amaranth

Top of the stem is an indefinite inflorescence (panicle). The grain is very small (about half size of millet), and may be light yellow, brown-yellow or brown black in color. The 1000seed weight is 0.6-0.9g, and 60000-100000 seeds can be produced by single plants.

Grain amaranth has four notable characteristics which includes: High protein content around 16% and quality (high lysine content); leaf protein comparable to alfalfa (Lucerne), High yield potential, it typically gives a grain yield of 2250-4500kg/ha and a fresh weight of leaf and stem of 30000-60000kg/ha, High stress tolerance, to drought, salinity, alkalinity or acidic conditions and Very low seeding rate and high germination rate, making it suitable for reclamation of barren land using aerial sowing.

2.3 Composition, nutritional and functional properties of grain amaranth

The proximate composition varies among and within the species. This depends on geographical location, season during harvesting, application of fertilizer. The range of reported data derived from different source is shown in Table 1.

Table 1: Proximate composition of grain amaranth (golden and white variety)

Nutrient	White variety (%)	Golden variety (%)
Protein	13.8-21.5	15-16.6
Carbohydrate	63-70	63.7-76.5
Lipids	5.6-8.1	5.8-10.9
Fiber	3.1-4.2	2.7-4.9
Ash	3.0-3.8	2.5-4.4

Source: Leon-Camacho *et al*, 2001, Berganza *et al*, 2003, Lupien, 2008

2.3.1 Protein

Grain amaranth has higher levels of protein than most grains and its protein is of higher quality than that of most cereals and pulses except in soybean (Bressani *et al.*, 1987 a; Imeri *et al*., 1987 b; Bressani *et al.*, 1987 b). The varieties grown in Uganda have been found to contain protein content of 12-13%, while Silva (2009) reported a range of 13.1-21.0% of crude protein, which is higher than that of most cereal grains and other common staples.

Grain amaranth contains substantial amount of essential amino acids that tend to be marginal in common cereals and pulses. Grain amaranth does not contain gluten and no allergenicity of amaranth proteins that have been recorded until now. Amaranth is unrelated to any other food crops that are commonly consumed, which makes it less likely to cause problems to people who have built up allergies due to repeated consumption of the same foods.

2.3. 2Nutritional value of Protein

Various researchers have studied the nutritional value of the proteins of amaranth. To evaluate protein quality, several indexes are used, like protein efficiency ratio (PER), net protein ratio (NPR), net protein utilization (NPU), true protein digestibility and protein biological value. Afolabi *et al.* (1991) found that the nutritional value of the protein of *A. hybridus* was low. They got a negative protein efficiency ratio, (PER-value) (-0.4), which was due to high tannin content. However, Osuntogun and Oke (1993) found a very high PER-value for the same species of amaranth. It was comparable to the value of PER of casein, 2.3 and 2.5, respectively.

This variety had very low tannin content, so it can be concluded that the nutritional value of the proteins of amaranth depends on the presence or absence of anti-nutritional factors in the seed. When amaranth seeds are processed, the nutritional value of the protein increases (Guzman-Maldonado and Paredes-Lopez (1998)). Morales *et al.* (1998) evaluated the effect of toasted, popped and flaked products of amaranth (kiwicha)

in convalescent and malnourished infants and young children. Their results indicated that the apparent nitrogen retention from amaranth was superior to that from most cereals studied and was similar to that from rice or high-lysine maize.

The protein quality of amaranth has been studied *in vitro, in vivo* and in human experiments. *In vitro* protein digestibility for raw flour of different amaranth varieties was between 73 and 76% (Muyonga *et al*,. 2008).Heat processing significantly improved the digestibility of the flour. Amino acid profile of amaranth was also studied (Bejosano and Corke (1998)) and leucine was detected as the first limiting amino acid in whole meal flour. The overall amino acid profile of *Amaranthus* protein was extremely favorable.

This attribute and its fairly good digestibility showed that *Amaranthus* is indeed a source of high quality proteins. In *in vivo* studies with growing rats, addition of amaranth to cereal flours improved the protein quality without affecting energy utilization (Ferreira and Arêas, 2004). Amaranth substitution also resulted in remarkable increases in weight gain of the experimental animals. Amaranth seems to be an effective source of protein to combine with other cereals (Pedersen*, et al*., (1987)).

The nutritional value of amaranth processed by popping and extrusion was compared with cheese in a human experiment (Bressani et al., (1993)). True digestibility results of the protein were 101.4, 89.8 and 85.5%for cheese, extruded amaranth and popped amaranth, respectively. The statistical analysis showed that the true digestibility of the protein was the same for the two products of amaranth and different from the digestibility of cheese. They also reported that the calculation of nitrogen intake for nitrogen equilibrium indicated that amaranth protein is among the highest in nutritive quality of vegetable origin and close to those of animal origin products.

2.3.3Fats

The fat content of amaranth grain is 2-3 times higher than that of other cereals, and its shows again higher variations between species. It contains more than 75% unsaturated fatty acids. Amaranth grain obtained from farmers in Kamuli was found to contain 6.9-7.3% oil (Table 1) and the oil was made up predominantly of unsaturated fatty acids, with high levels of the essential fatty acid linoleic acid (35-55%), oleic acid (18-38%) palmitioeic acid 16% and lower in saturated fatty acids stearic acid (3-4%) and palmitic acid (20-23%)(Ayorinde *et al.,* 1989; Becker, 1994; Leon-Camacho *et al.,*2004; Berganza *et al*, 2003; Escudero *et al* ., 2004.

Based on its fatty acid profile, it can be concluded that grain amaranth is reasonably safe for consumption by individuals that are at high risk of chronic non-communicable diseases such as coronary heart disease and diabetes. Its high content of linoleic acid, an essential fatty acid, makes it suitable for consumption by children since they need essential fatty acids for proper growth and development (Muyonga, *et al*., 2008).

Amaranth grains contain high levels of squalene, a highly unsaturated open chain triterpene, which are usually only found in livers of the deep sea fish and other marine species. Squalene is mush widely used in pharmacological and cosmetic applications. The content in amaranth ranges from 2-8% (Becker, *et al,* 1981; Lyon and Becket ,1987;, Quareshi *et al.,* 1996; Leon-Camacho *et al* 2001; He *et al*., 2002, whereas in other plants oils is found in much lower amounts (e.g. olive oils 0.1-0.5% or wheat germ oil 0.1-7%). Shen *et al* (2004) found out that amaranth squalene exert a cholesterol lowering effect by increasing fecal elimination of steroids through their interference with cholesterol absorption. The effect was higher than that of shark fish squalene. In addition, amaranth oil and the grain lowered serum and hepatic cholesterol as well as triglycerides, confirming previous findings (Quareshi *et al*., 1996; Buddin *et al*., 1996; Gamel, *et al*., 2004)

2.3.4 Carbohydrates

The carbohydrates in amaranth grain consist primarily of starch made up of both glutinous and non-glutinous fractions. Amaranth starch mostly contains amylopectin (88.9 to 99.9 %), and thus classified as "waxy type" of starch with some unique characteristics: (high viscosity and gelatinization at higher temperature) in comparison to normal starches with amylose contents between 17 and 24 %. Amaranth starch granules are extremely small (0.8 to 2.5 µm) in comparison to the size of starch granules of other grains as rice (3 to 8 µm), wheat (3 to 34 µm) and maize (5 to 25µm) (Teutonico & Knorr, 1985; Bhandari & Singhal, 2001; Pal *et al*., 2001).

Smaller granules have a greater water-binding capacity, higher swelling power, lower gelatinization temperature and high resistance to amylases. Due to the facts mentioned above, amaranth starch shows good gelatinization properties and freeze/thaw stability appreciated in food industry (Breene 1991; Lopez *et al*., 1994, Williams and Brenner 1995; Pal *et al,*. 2001).

However, broad genetic diversity in physical properties of starch within and among amaranth species, and variation in all other constituents (proteins, lipids, minerals) which have an influence on starch functional properties, need to be taken into account. Amaranth starch seems to have potential for use in the preparation of custards, pastes and salad dressing (Singhal & Kulkarni, 1990a, b).

2.3.5Minerals and vitamins

Grain amaranth is known to contain substantial amounts of vitamins and minerals. It contains twice the level of calcium found in milk, five times the level of iron found in wheat and higher sodium, potassium and vitamins A, E C and folic acid than cereal grains (Becker *et al*., 1985).

Amaranth grain contains more riboflavin (vitamin B2) and ascorbic acid (vitamin C) than cereals. According to Jouci *et al*., (1997) the content of thiamin in amaranth grain is

higher than in wheat. Bressani (1994) contrary reports a lower of thiamin in amaranth than in common cereals. It is a good source of vitamin E, which among other things possesses an ant-oxidant effect and therefore prolongs the stability of the oil. Besides tocopherol (more γ than α tocopherol), tocotrienols are also present.

Amaranth grain has minerals approximately twice as high as in cereals for instance wheat (1.8%). Bran and germ have higher contents of ash than perisperm that is 66% which is found in bran and germ fractions. It is particularly higher in calcium, magnesium, iron, potassium and zinc. The ration of calcium/phosphorus is very good with a value of 1:1.9-2.7, whereby nutritionists recommend around 1:1.5 of calcium: phosphorus. Pederson *et al.,*(1987) reported that the mineral content varies considerably and is somewhat increased or decreased by different processing techniques.

2.3.6 Dietary fiber

Belton and Taylor (2002) reported that grain amaranth has got slightly lower dietary fibre than wheat. Bran fraction has higher dietary fiber than the perisperm. The fraction of insoluble dietary fiber varies 19.5-27.9% in *Amaranthus cruentus* while 33.1-49.3% in *Amaranthus hypochondriacus.* The total content of dietary fiber in *A.cruentus* was increased by different processing technique.

On the average pale-seeded amaranths contain 8 % of dietary fiber and black colored 16 % with soluble fiber rate of 30 to 40 % and 18 %, respectively (Schnetzler & Breene (1994) while Tosi *et al.* (2001) reported 14.2 % of dietary fiber in the *A. cruentus* flour (8.1 % soluble, 6.1 % insoluble).

2.4 Plant secondary metabolites and anti-nutrients components in amaranth grains

2.4.1 Phytic acid.

Phytic acid serves as a form of storage for phosphorus in the plant. In a series of investigations, the inhibitory effect of phytic acid on the digestion of starch was proven. It also has a positive blood cholesterol lowering effect, the mechanism o f which is not

fully understood. Amaranth contains 0.3-0.6% phytic acid (Brene, 1991, Bressani, 1994, Escudero *et al*., 2004; Gamel *et*., *al*., 2006).

According to Bressani (1994), phytic acid is distributed uniformly in the seeds, and is therefore hardly decreased by abrasive dehulling or extraction with water. Pederson *et al.,*(1987) described as a high phytate: zinc molar ration in amaranth, which might have a negative effect on absorption of zinc.

Phytic acids can form complexes with basic protein residues, leading to inhibition of enzymatic digestive reactions and interfere with the adsorption of minerals in particular zinc as seen earlier. Recently, it has been shown that cooking reduces the Phytates content by approximately 20%, popping 15% and germination (48hr) by 22% indicating that these approaches can be used to reduce the phytate content of amaranth grain(Gamel, et al, 2006a)

2.4.2 Tannins

Under the term tannins, several different secondary plant metabolites are included, all of which belong to the polyphenol group. They can be found mainly in the hulls of cereals and legumes in high concentrations and they can negatively influence the digestion and absorption processes by forming complexes with various nutrients and digestive enzymes. However, tannins bind proteins, carbohydrates and minerals, thereby affecting the nutritional and functional value of the bound constituents (Bressani, 1994).

Phenolics may also impart undesirable colors in grain products during food processing. Dark amaranth seeds contain more tannins than light ones 104-116mg/100g versus 80-120mg/g (Bressani, 1994). Becker, *et al* (1981) evaluated 10 different samples and found range of 80-420mg/100g. Breene 1991 gave an average of 40-120mg/100g, and whereas higher values were 410-520mg/100gwere measured by Bejosano and Corke (1998) in various amaranth species using acidified methanol instead aqueous methanol as extraction media.

Gamel *et al* (2006b) determined the phenolic compounds (expressed as tannic acid) in amaranth after extraction with acidified methanol and found values ranging from 516-524mg/100g. Moreover, thermal treatment or germination decreased the content of phenolic compounds.

2.4.3 Protease inhibitors

Many food proteins contain one or more proteases inhibitors (e.g. chymotropsin or trypsin inhibitors) that competitively inhibit the activator of protease enzymes. They have a negative effect on performance and survival of monogastric animals when it is used as the primary dietary energy source (Improta & Kellems, 2009). They form very stable complexes with proteolytic enzymes (Aguirre *et al.*, 2004). However, heat treatment can reduce their activity. Compared with other cereals amaranth grain contains very low levels of protease inhibitors.

Gamel *et al* (2006a) found trypsin inhibitors activity (TIU) ranging from 3.05-4.34TIU/mg. chymotropsin inhibitor activity (CIU) 0.21-0.26CIU/mg. and amylase inhibitor activity (AIU) ranging from 0.23 -0.27 AIU/mg. trypsin and amylase in a particular, the chymotropsin inhibitor decrease after thermal treatment or germination.

2.4.4 Saponins

They are strongly bitter tasting, surface active agents (surfactant), which can cause intensive foaming activities in aqueous solution. They can form complexes with protein and lipids (e.g. cholesterol) and posses a hemolytic effect. They are only absorbed in small amount, and their main effect is restricted to the intestinal tract. Saponins can form complexes with zinc and iron thus limiting their bioavailability (Chaunan *et al.,* 1992). With regard to health promoting effects, saponins are anti-carcinogenic, anti-microbial, cholesterol decreasing, immune modulating, as well as anti-inflammatory.

Amaranth grains contain little amounts of Saponins. Dobbos (1992) found content on average 0.09 %(aescin equivalents) in various amaranth species and this has been confirmed by the investigations of Loesser *et al* (1999). It was concluded that the low

concentrations of saponins in amaranth seeds and their relatively low toxicity guarantee that amaranth derived products create no hazard to consumer.

2.4.5 Oxalic Acid

Amaranth leaves, like some other vegetables, contain rather high amounts of oxalic acid. Not only the leaves, the grains and stems contain oxalates although at relatively reasonable levels as compared with the leaves. The amount of oxalic acid in leaves is roughly the same as that found in spinach (*Spinacia oleracea*) and chard (*Beta vulgaris var. cicla*). Excessive amounts of oxalic acid may reduce the availability of certain minerals in the body, most notably calcium. This could be a concern especially if calcium intake levels are low to begin with, or if foods high in oxalic acid are consumed on a regular basis over long periods.

2.5 Nutrition and health benefits of grain amaranth consumption

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 of people formerly wasted by HIV/AIDS (SRLP, 2005; Tagwira *et al*., 2006). Tagwira *et al*., (2006) documented perceived benefits of consuming grain amaranth among communities in Zimbabwe.

The communities claimed that eating grain amaranth made them feel healthier and they noticed improvements in the health of their children. Specific health improvements noted included improvement in appetite, fast healing of mouth sores and herpes zoster, and weight gain for PLWHAs. Amaranth consumption was also associated with higher milk production among breast feeding mothers. The improvements in general well-being and health reported by people who included grain amaranth in their diets are generally explainable by its high nutritional value(Tagwira *et al*., 2006).

Some specific nutritional and health benefits of amaranth consumption are elucidated. Amaranth oil has been shown, in animal studies, to lower total serum triglycerides and levels of low-density lipoproteins (LDL) (Esculedo *et al*., 2006). Similar effects have been reported in humans (Martirosyan *et al*., 2007). High levels of serum LDL are associated with coronary heart disease. The serum LDL lowering effect of amaranth has been attributed by tocotrienols (unsaturated forms of vitamin E) and squalene in amaranth oil. These compounds affect cholesterol biosynthesis in humans (Martirosyan *et al*., 2007).

They are also believed to have anti-tumor and antioxidative activity (Kim *et al*., 2006a), pointing to potential anti-cancer effects. 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*., 2006b). The fasting serum glucose levels and the glucose tolerance of the diabetic rats were both improved

2.6 Processing amaranth grain

Grain amaranth can be used as seeds or flour to make products such as cookies, cakes, pancakes, bread muffins, crackers, pasta and other bakery products (Teutonico & Knorr, 1985). Kauffman and Weber (1990) provided a description of the variety of products made from amaranth in different parts of the world. These include soups and stews from whole grain; *alegria*, a confection made from popped amaranth in Mexico; *atolea*, a

fermented Mexican drink made from roasted amaranth flour; *chichi*, which is a form of beer made from amaranth in Peru; *sattoo*, a gruel consumed in Nepal, and *chapatti* made in different parts of Asia.

The amaranth processed under conditions that do not damage its protein and its essential amino acids availability, like moist heat cooking and extrusion, presents good protein quality, similar to casein (Mendonza & Bressani, 1987). The digestibility and the protein efficiency ratio are improved if the grain is heat processed (Kauffman & Weber, 1990). The removal of lectins by heat processing has been reported to improve the protein efficiency ratio of the amaranth flour (Singhal & Kulkani, 1988).

There are a number of viable methods for processing, including popping, toasting, heat-rolled flakes, extrusion, and wet cooking as gruel. Excessive thermal processing has been shown to reduce the quality of amaranth grain (Bressani & Elias, 1984). The potential for reducing nutritional quality is most evident when amaranth grain is processed using hot dry heat, as in toasting or popping (Bressani & Elias, 1984).

Recently, interest in amaranth has increased because of its nutritional value and functional properties (Gikonyo *et al.,* 2006).Snack foods with good acceptance and high nutritive value have been developed by extrusion cooking of the defatted flour obtained from milling the grain (Chávez-Jáuregui *et al.,* 2000).The extrusion cooking process is based on starch gelatinization and protein denaturation using high pressure and high temperature (Arêas, 1996). Such amaranth snack foods also present characteristics such as cholesterol-lowering effects in hypercholesterolemic rabbits (Plate & Arêas 2002), protein of high biologic value, and high bioavailability of calcium, zinc, and magnesium (Ferreira, 1999).

Processing is important with respect to the protein quality of amaranth grain. According to work carried out by Pederson *et al.,* (1987) on the nutritive value of amaranth grain, processing was found to increase the protein quality of amaranth grain. However if the

processing is carried out under more extreme conditions of time and temperature, it destroys the quality of the product by reducing available lysine content (Pederson *et al.,* 1987). Of interest is the extrusion process, which for *A. cruentus* and *A. caudatus* yielded cooked flour equal in protein quality to casein (Mendoza & Bressani, 1987).

2.7 Effects of heat and moisture on starch properties

The amaranth starch is unique considering the granule size. It belongs to the class of very small granule starches. Majority of granules have polygonal shape with a diameter of about 1 μm. They tend to agglomerate in round or irregular structures. Changes in starch during processing have been extensively studied for human food applications. Most starches will gelatinize upon heating to above 80◦C in excess water. Gelatinization markedly increases susceptibility for amylolytic degradation due to loss of crystalline structure (Holm *et al*., 1988; Björck *et al*., 2000; Kishida *et al*., 2001; Perez and Oliva-Teles, 2002).

In fact, Holm *et al.,* (1988) found a correlation of 0.96 between extent of gelatinization and digestion rate, indicating that the relationship is close to linear for pure starch. Donald (2001) describes gelatinization as a swelling driven process. Swelling occurs along the amorphous regions, and since the crystalline regions do not expand during swelling, stress increases at the interface between the crystalline and amorphous regions, where bonds exist between amylopectin in the crystalline regions and amylose in the amorphous regions. Thus, at a certain point in the swelling process the crystalline regions are rapidly and irreversibly broken and gelatinization is initiated.

At excess water content, this onset of the gelatinization usually occurs between 50 and 70◦C. Swelling causes nearly all amylose in the starch granule to leach out (Han & Hamaker, 2001). Viscosity increases during gelatinization, and is caused by swollen granules and gels consisting of solubilized amylose (Hermansson & Kidman, 1995). In

addition to the importance for starch digestion, the increase in viscosity during gelatinization may also affect physical quality of processed feeds positively through increased binding between feed particles.

2.8Utilization of amaranth grain in Kenya

In Kenya, amaranth is sold in some supermarkets in major towns, but in very small quantities. Its consumption is also cited in some important institutions like Kenyatta National Hospital in the private wings and in HIV/AIDS orphaned children's homes, where it is recommended for patients on special diet (Mburu *et al.,* 2011).

Farmers that grow amaranth have marketed their crop in a number of ways. Some sell small bags of the whole grain or flour mail order to consumers. Other growers sell to local or regional health food stores or restaurants. There are also a few middlemen who buy grain from the farmers and market it to the larger health food companies like INCAS.

Grain amaranth is blended with other grains (mainly maize and millet), and given to children in form of porridge (Mwangi *et al,*. 2011). Amaranth-based porridge has been adopted for the feeding normal children as a complementary food, and for feeding the sick including people living with HIV and AIDS (Ndungu *et al.,* 2014).

The main emphasis is the processing of whole grain using a state-of- the-art technology to make high quality products for health and vitality and production of high quality seed. KARI and KASAL provides improved amaranth varieties, good quality seed, research on diseases and pests, good agronomic practices through field demonstrations and technical backstopping while INCAS provides a guaranteed market and price for the farmers. Kirdi is trying to develop a miller with smaller grinder for milling these grains for better and refined products (Guptaa & Thimbaa 2009).

CHAPTER THREE

3.0 MATERIALS AND METHODS

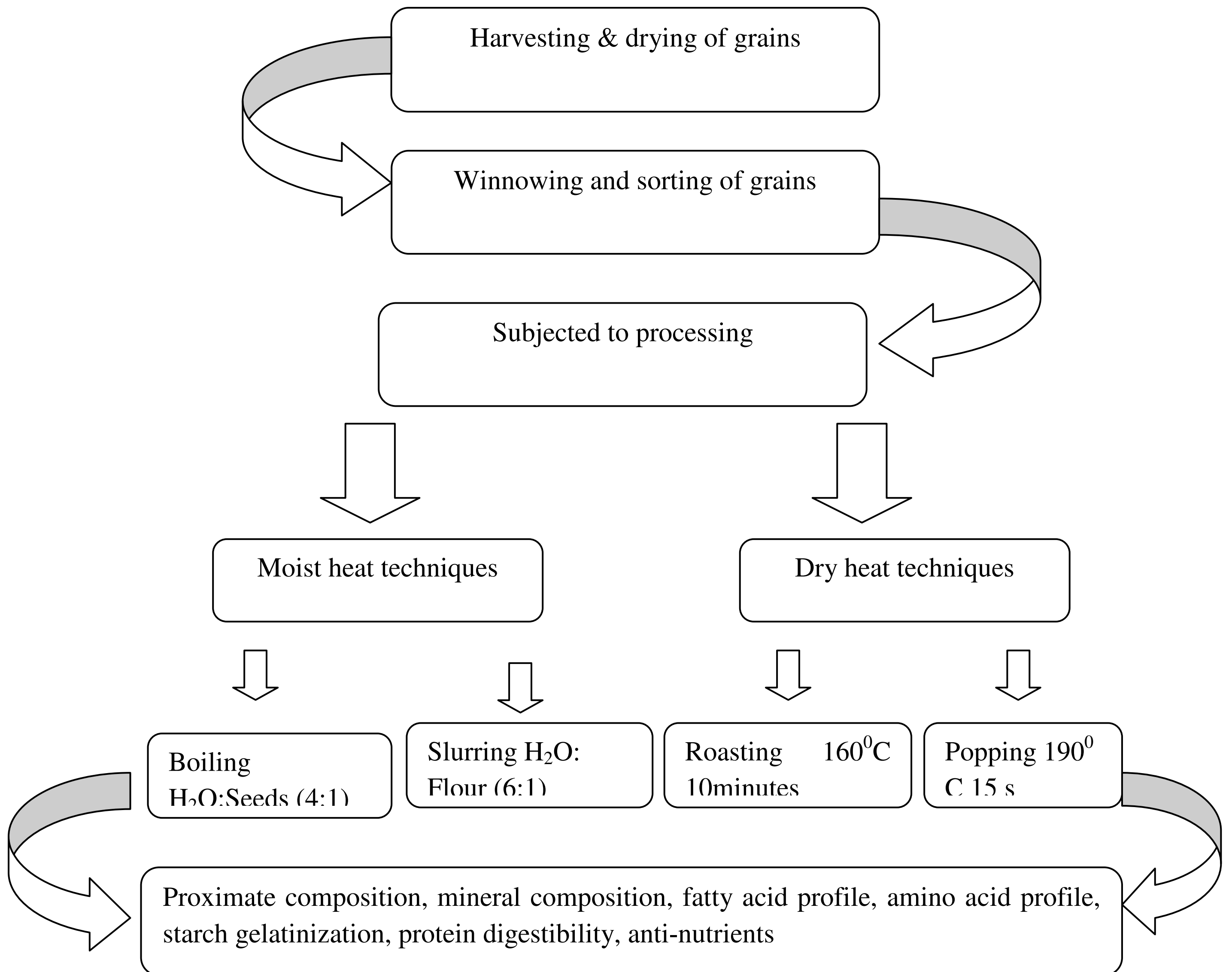

Figure 5: Flow chart of the whole task carried out

3.1 Research site

The grains were obtained from JKUAT farm; processing and analysis was carried out in JKUAT laboratories.

Figure1: ***Amaranthus albus*** **plant**

Source: Njoki, 2013

Figure2: Mature grain amaranth

Figure3: Different varietal yield

Figure4: Different varieties of grain amaranth

Source: Njoki, 2013

3.2 Sample selection

Ten kilograms of grain amaranth(*Amaranthus albus* variety) were obtained from JKUAT farm. They were sorted and cleaned from chaff and debris. The sorted sample was divided into five equal proportions which were later subjected to popping, roasting, boiling and slurring.

3.3 Research design

The complete randomized block design was used, whereby different processing techniques were the blocks while the anti-nutrients (phytates, tannins and oxalates), nutrients (proximate analysis), protein digestibility and degree of starch gelatinization were the assessments carried out.The grains were randomly selected prior to processing and during analysis.

3.4 Sample preparation and Experimental treatment*:*

The raw grains were subjected to thermal and hydrothermal processing methods viz: cooking, slurring, popping and roasting. Each of these processing methods served as

experimental treatment groups to be compared with control (raw grains). The different thermal and hydrothermal processes are described as thus:

3.4.1Popping

Clean grains wereput on the heated pan at 190ºC and spread to form a thin layer to enhance uniform popping.The grains popped and changed from dark yellow to white. Popping took 15seconds.

3.4.2Roasting

The oven was preheated at around 160^0C. The sorted grains were placed in a single layer on a baking sheet. The grains were roasted for 10 minutes, the oven was opened to stir them and progress checked after every two minutes. The roasted grains were cooled and packaged in an air tight package awaiting analysis to be carried out.

3.4.3 Boiling

Distilled water (1000 ml) was placed in a saucepan and brought to a boiling (100° C). The seeds (250 g lots) weretransferred to the boiling water, then the saucepan was covered and boiling continued for 30 min. Timing began when the cooking water regained100^oC after introduction of the seeds. The boiled seeds were drained using a sieve.

3.4.4 Slurring

The raw grains were milled using local miller commonly used to mill millet whose final particle size of the flour was250micrometers

Cold water was mixed with grain amaranth flour to form a smooth paste. Some water was put on a saucepan and placed on a hot plate until it boiled. Smooth paste was added and mixed thoroughly using a wooden spoon until no lumps were formed. The slurry was cooked for 25 minute, stirring was done occasionally. The ration of water to slurry is 6:1.

3.5 Proximate analysis

3.5.1 Determination of moisture content

Moisture was determined according to AOAC methods specification 950 46, method 925.10-32.10.03 (AOAC, 1995).

Sample of 5g was accurately weighed into a moisture dish was transferred in a hot-air oven previously heated to around 130^oC and then drying done for one hour. Final weight of sample was taken after drying and cooling in a dessicator. The residue was taken as the total solids and loses in weight as the moisture content of the sample. This formulawas adopted:

% Moisture= $\frac{W1-W2}{W} \times 100$

W1~weight of sample before drying

W2~weight of sample after drying

W~weight of sample before drying

3.5.2Determination of protein content

Protein was determined using the semi-micro kjeldahl method, specification 950.46. method 20.87-37.1.22 (AOAC, 1995). Two grams of sample was weighed into a digestion flask together with a combined catalyst of 5g K_2SO_4 and 0.5g of $CuSO_4$ and 15ml of concentrated H_2SO_4. The mixture was heated in a fume hood until the digest color turned blue. This signified end of the digestion process.

The digest was cooled, transferred to 100ml volumetric flask and topped up to the mark with deionized water. A blank digestion with the catalysts was also made. 10ml of diluted digest was transferred into the distilling flask and washed with about 2ml of distilled water. 15 ml of 40% NaOH was then added and this washed with 2ml of distilled water. Distillation was done to a volume of about 60ml distillate. The distillate was titrated using 0.02N HCl to an orange color of the mixed indicator, which signified the end point.

$$\%N = \frac{\frac{[(V_1 - V_2) \times N \times F \times 100]}{V \times 100}}{S}$$

Where: V_1 is titer for sample in ml, V_2 is titer for blank in ml

N is normality of standard HCl solution (0.02), f is the factor of the standard HCl solution, V is the volume of diluted digest taken for distillation (10ml),

S is weight of sample taken (1g),

Protein factor (PF) = 6.25

% Crude protein= $N \times PF$

3.5.3 Determination of crude fat content

Crude fat was done using the soxhlet method 920.85-32.1.13, (AOAC, 1995). This gave an intermittent extraction of oil with excess of fresh condensed organic solvent used. 5g of sample was weighed into extraction thimbles and initial weight of extraction flasks taken. Fat extraction was done using petroleum spirit in Soxhlet apparatus for 8 hours. The extraction solvent was rotary evaporated and the fat extracted dried in a hot air oven for 15 minutes before the final weight of flasks with extracted oil taken.

% Crude fat= $\left(\frac{W1}{W2}\right) \times 100$

W1~weight of fat extracted

W2~weight of the sample

Extraction of fat from slurry and boiled sample

It applies the principle of the Weibul Berntop Method. 20g of the sample portion was digested by boiling it with distilled HCL. The hot digest was then filtered through a wetted filter paper. Fatty substances present in the digest are retained on the filter paper. The filter paper is dried in the oven at 40°C. Then the fat is extracted from it using soxhlet extraction as previewed above.

3.5.4 Determination of crude ash

Ash content was determined by incinerating in a muffle furnace (AOAC, 1995) method 923.03-32.1.05. Sample weights of about 5g were weighed in pre-conditioned crucibles. First, the sample was charred by a flame to eliminate carbons before being incinerated at 550^0C in a muffle furnace, to the point of white ash. The residues were cooled in a dessicator and the weights taken.

% Crude ash= $(\frac{W1}{W2}) \times 100$

W1~weight of ash

W2~weight of the sample

3.5.5 Determination of crudefiber

Crude fiber was determined according to (AOAC, 1995, Method 920.86-32.1.15).Approximately 2g (W) of sample was weighed into a 500ml conical flask. About 200ml of boiling 1.25% H_2SO_4 was added and boiling done for 30min under reflux condenser. Filtration was done under slight vacuum with Pyrex glass filter (crucible type) and the residue washed to completely remove the acid with boiling water. Approximately 200ml of boiling 1.25% NaOH will be added to the washed residue and boiling done under reflux for another 30min. Filtration was done using the same glass filter previously used with the acid. The residue was rinsed with boiling water followed by 1% HCl and again washed with boiling water to rinse the acid from the residue. The residue was washed twice with alcohol and thrice with ether. It wasthendried in a Hot-air oven at 105ºC in a porcelain dish to a constant weight (W_1). Incineration will be done in a muffle furnace at 550ºC for 3hrs, the dishwill then be cooled in a dessicator and the final weight (W_2) taken Calculations was done as shown below.

% Crude fiber=$\frac{W1-W2}{W} \times 100$

Where W_1= weight of acid and alkali digested sample, W_2= weight of incinerated sample after acid and alkali digestion and W= weight of sample taken.

3.5.6 Total carbohydrate

Carbohydrate was estimatedbased on Müller and Tobin (1980) method.

Total carbohydrates %=

$$100 - (Moisture\ content + crude\ protein + crude\ fiber + ash + crude\ lipid)$$

3.6 CHEMICAL ANALYSIS

3.6.1 Determination of Minerals

Mineral analysis was determined according to AOAC(1995) method.The ash that was previously determined (refer to determination of crude ash) was cooled. Then 15ml of 6N HCl was added to samples in crucibles before transferring to 100ml volumetric flasks. Distilled water was used to top up to the mark (100ml). Atomic Absorption Spectroscopy (AAS) was used for all the minerals except Potassium and Sodium where Flame Emission spectrophotometer were used (Model A-6200, Shimadzu, Corp., Kyoto, Japan).

Standards for sodium and potassium were prepared from their salts of chloride while the others were already in their standard form as purchased from the supplier.

3.6.2 Determination of Fatty acid

The fatty acid profile was determined according to Dyer and Blair (1959) method

One gram of ground sample of grain amaranth was weighed into a 50ml glass stopered centrifuge tube and denatured over boiling water for 3 min and 2ml of water and 7.5ml

of 2:1(v/v) methanol- chloroform mixture was added. The mixture was shaken thoroughly and left at room temperature for several hours with intermittent shaking.

After centrifugation, the supernatant was decanted into another centrifuge tube and the residue re-suspended in 9.5ml 0f methanol-chloroform-water (2:1:0.8v/v).The homogenate was shaken and centrifuged. This was repeated twice and the supernatants combined with the first extract. About 7.5ml of each chloroform and water will be added to the combined extract, shaken and centrifuged.

The chloroform phase was withdrawn using a Pasteur pipette and brought to dryness using a vacuum rotary evaporator at low temperature. The residue in the flask was dried under vacuum in desiccators and weighed. Finally, the residue was reconstituted using 2ml of 1:2 methanol chloroform mixtures and stored in the freezer until required. Gas liquid chromatography (GC) was used to quantify the methyl esters of the fatty acids.

About 0.1ml of oil extractwas put into the conical flask. 4ml of methanolic HCL solution was added. Then, the samples were heated under reflux for 2 hours. The refluxed samples were cooled under tap water.

The extract methyl esters in solution were transferred into a separating funnel and 4ml of hexane was added and shaken vigorously and let stand. The hexane fractions layer wascollected return the aqueous layer and extractionrepeated one more time.

Filtration was done using defatted cotton wool and anhydrous sodium sulphate to remove water.The filtrate was concentrated using rotary evaporator at 40 ^{0}C. The sample will be injected into the GLC. Column 15 Degs used wasused; mobile gas was nitrogen, flame gas hydrogen and oxygen, Flame ionization detector. Injecting temperature 220^{0}C, column initial 170^{0}C, column final 170^{0}C, detecting temperature 240^{0}C and attenuation range 2

3.6.3 Determination of Amino acid profile

Pre-column derivatization of aminoacids with *o*-phthalaldehyde (OPA) followed by reversed-phase HPLC separation with fluorometric detection was used according to method described by Maria and Federico (2006), with modifications.

Samples were pretreated whereby 5g of raw and processed amaranth was mixed with 25ml hexane and let to stand for about 20 minutes, centrifuged at 3000rpm for 10 minutes, filtered and supernatant discarded.

Protein extraction was done according toMethod described by Sumner*et al*., (1980) with modification as described by Aguilera and Garcia (1989). The defatted sample was subjected to protein extraction. The protein from the samples was extracted twice with 100 ml H2O, adjusted to pH 9 with 1N NaOH, while stirring for 20 min. Each extract was separated by centrifuging at 1000 rpm for 20 min.

The extracts were combined and the protein precipitated by adjusting to pH 4.5 with 1N HCl and separated by centrifuging. The precipitate was redispersed in 100 ml H2O, dissolved at pH 9 and re-precipitated at pH 4.5. After separation of the protein by centrifuging, it was given two 50 ml washes at pH 4.5. The flour was slurried and converted to protein extract by adjusting to pH 6.5-7 with 1N NaOH. The protein isolates were freeze-dried. Each trial was carried out in triplicate.

About 0.2g protein isolate of each sample was weighed in the clean and dry glass pyrextest tube, 6N HCl containing 5% of phenol was added. The tubes were held in dry ice-acetone bath where pressure in the tube was reduced by use of a vacuum pump, and sealed off. Hydrolysis took place at 110^{0}C for 24 hours in an oven, later cooled and centrifuged at 2000rpm for five minutes to force water drops on the inner wall of the test tube to come down to the solution level.

The test tubes were opened and the resultant solution transferred into eggplant-shaped flask. The test tubes were rinsed with fresh 6N HCl and the washing added to the flasks,

and then rotary evaporated with rotary evaporator at 50^0C. HCL remained after drying was further dried using NaOH pellets in dessicator under vacuum for 12hour.

Sample diluent: This was prepared by weighing 9.8g of sodium citrate dissolved in 400ml distilled water in a 500ml volumetric flask. 8ml of perchloric acid and 0.05 of n-caprylic acids were added, and the volume topped to the mark with distilled water. The pH of the solution was adjusted to 2.2 with perchloric acid.The hydrolyzed sample was dissolved in 2ml of sample diluent

Standard amino acid solution: Mixed amino acid solution (H-type from Sigma Chemicals) was diluted by taking 0.5ml of the standard into 10 ml sample diluent prepared earlier. The H-type mixed solution available commercially contains 17 amino acids and ammonia each by 2.5µmol/ml. Therefore the concentration in the diluted solution was 0.125µmol/ml which was subsequentlymixed with OPA solution at ratio of 1:1 for HPLC analysis.

Chromatography condition was in accordance with the Agilant method (Mengerink *et al.,* 2002). Briefly, the hydrolyzed samples and amino acid standards solutions were automatically derivatized with OPA solution at a ratio of 1:1. OPA solution was prepared by mixing 0.5mg/ml of OPA to borate diluent (pH 10.4), 0.1% (v/v) 2-mercaptoethanol and 0.1% (v/v) of Brij 35.

After derivatization, an amount equivalent to 10µl of each sample was injected on a ODS column 5µm, 250 x 4.6 mm at 40^0C, with detection at 350nm excitation wavelength and 450nm emission wavelength. Mobile phase A was 40mM KH_2PO_4, adjusted to pH 7.8 with NaOH, while mobile phase B was acetonitrile/methanol/water (45/45/10 v/v/v). The separation was obtained at a flow rate of 1ml/minute with a gradient program that allowed for 1.9min at 0% followed by a 16.3 min step that raised eluent B to 53%. The washing at 100% B and equilibration at 0% B was performed in a total of 30 minutes.

Analysis were performed using a HPLC (Model LC-10AS, Shimadzu Corp., Kyoto, Japan) PE series 400 liquid chromatography fitted with a binary pump delivery system, column thermostat and a fluorescence detector. Amino acids were detected based on the retention time established for the individual aminoacid under defined experimental conditions.

Area=$height \times width(\frac{1}{2height})$

NE~ norleucine equivalent = Area of norleucine peak/Area each Amino acid

S standard= NEstd*Molecular weight*µmAmino acid standard

Cg/100g protein=$NH \times \left(\frac{NH}{2}\right) \times S$

g/16gNprotein=$\left\{\frac{D \times 16}{SW}\right\} \times N\% \times \left(\frac{V}{NH}\right) \times W(norleucine)$

D~ dilution

SW~ sample weight

V~ volume loaded

NH~ net height

C~ concentration

S~ constant for each amino acid in standard mixture

3.7 PLANT SECONDARY METABOLITES

3.7.1Determination of Phytates

Analysis of phytic acid in grain amaranth was done by HPLC combining the column/mobile phase conditions established by Tanjendjaja *et al.,* (1980), with modification as detailed by Camire and Clydesdale (1982**).**

A known amount of the sample was weighed into a 125 ml Erlenmeyer flask, extracted with 25 ml of 3% sulphuric acid for 30 minutes on a shaker bath at medium speed and at room temperature. The slurry was filtered through fast filter paper (Whatman #41) and rinsed using a fine jet stream from a squeeze bottle, with a small volume of extracting solvent.

The filtrate was transferred to a 50 ml screw cap centrifuge tube and placed in a boiling water bath (BWB) for 2-5 minutes (to aid in the precipitation of ferric phytate), before addition of 3 ml of a ferrous chloride solution containing 6mg ferric iron per ml in 3% sulphuric acid. The tubes were heated in a BWB for 45 minutes to allow for complete precipitation of the ferric phytate complex. This was centrifuged at 2,500 rpm for 10 minutes and supernatant discarded, while the precipitate was washed once with 30 ml of distilled water, centrifuged again and supernatant discarded.

The remaining content in the tube was added 3 ml of 1.5 N NaOH and 1 ml of distilled water. A glass rod was used to break the precipitate and then sonicated to completely disperse the precipitate, which was then topped to 30 ml. The samples were cooled, centrifuged and the supernatant quantitatively transferred to 50ml volumetric flask. The precipitate was rinsed once with approximately 10ml of distilled water, centrifuged and added to the volumetric flask.

Stock solution containing 10 mg/ml of sodium phytate in distilled water was prepared. Serial dilutions were made to contain from 1 g/100 ml to 100 mg/100 ml. The sample and standard dilutions were injected to the HPLC using a 20-µl sample loop.

Analysis was performed using a HPLC Model LC-10AS, Shimadzu Corp., Kyoto, Japan equipped with a UV detector at 205-340 nm filter, 250 mm X 4.6 mm ID column containing spherisorb ODS C18 10 µ packing and oven temperature 35^0C. Mobile phase is 0.005M sodium acetate, flow rate 0.5 ml/minute and injection volume 20 µl. Shimadzu software was used to calculate the peak areas.

$$Mg/100g=\{\frac{\frac{Y}{m}}{SW} \times \frac{1000}{100}\}$$

Y~ height of the peak

M ~ gradient of the standard curve

SW~ sample weight

3.7.2 Determination of Tannins

Tannins were determined by the FolinsDenis colorimetric method described by Kirk and Sawyer(1998). About 0.5g of the samples were boiled in 20ml of water in attest tube and then filtered through Whatman No.42 filter paper. A few drops of 0.15mg ferric chloride were added. A brownish green or a blue-black coloration indicated the presence of tannins.

Tannin content was determined by the same method (Kirk and Sawyer, 1998). About 5g were dispersed in distilled water and shaken. The mixture was allowed to stand for about 30 minutes 28ºC before it was filtered through Whatman No.42 filter paper. 2ml of the extract and the standard tannin solution (tannic acid) will 0, 0.1, 0.2, 0.3, 0.4 and 0.5mg/ml were dispersed into a 50ml flask. Similarly, 2ml of distilled water was put in a separate volumetric flask as a blank to calibrate the instrument to zero. 2 ml of Folins Denis reagent was added to each of the flasks followed by 2.5ml of saturated Na_2CO_3 solution.

The content of each flask was made to 50ml with distilled water and allowed to incubate at 28^0 C for 90min. Their respective absorbance was measured in a UV-vis spectrophotometer (UV mini 1240 model, Shimadzu Corp., Kyoto, Japan) at 760nm.

$$Mg/100g=\{\frac{\frac{Y}{m}}{SW} \times \frac{1000}{100}\}$$

Y~ absorbance

M ~ gradient of the standard curve

SW~ sample weight

3.7.3 Determination of Oxalates

The analysis was made according to Libert (1981) with modifications (Yu et al., 2002). Aliquots of 0.5 of the sample were homogenized in 4ml of 0.5N HCL. The homogenate was heated at 80 ^{0}C for 10min with intermittent shaking. Distilled water was added to the homogenate up to a volume of 25ml. About 3ml of the solution was withdrawn and centrifuged at 12000g for 10min. 1ml of supernatant was passed through a filter (0.45µm) before HPLC analysis. Standards were prepared at varying concentrations for quantification.

$$Mg/100g=\{\frac{\frac{Y}{m}}{SW} \times \frac{1000}{100}\}$$

Y~ absorbance

M ~ gradient of the standard curve

SW~ sample weight

3.8 Determination of Protein digestibility

The pepsin digestion method was used based on that of Hamaker et al (1987). Accurately weighed samples (200 mg) were digested with P7000100G pepsin, activity 863 units/mg proteins for 3 hours at 37°C and products of digestion was pipetted off using a Pasteur pipette. The residues were washed with distilled water and clear supernatant pipetted off. The residues were then dried in an oven at 100°C overnight. The residual protein was determined by the Dumas combustion method (AACC International 2000) method.

Protein digestibility (%)= $\{(\text{Totalprotein} - \text{Residualprotein}) \div \text{Totalprotein}\} \times 100\%$

3.9 Starch staining and microscopic analysis

Light microscopy with iodine staining was used to analyze using Mastersizer 2000 produced by Malvern, with water as dispersant.

100g slurry of grain amaranth samples was added to 600ml of 0.25% NaOH solution and mixed in Waring Blendor (Dynamics Corp of America), New Hart CT for 6minutes at 25° C, then filtered through a 200mesh screen and washed with limited water. The filtrate was centrifuged 3000rpm for 6minutes. The supernatant containing the starch was decanted and centrifuged at 1000rpm for 10minutes. Then, the supernatant was discarded. The starch cake surface was washed with sufficient water to remove cloudy solution on the surface of the starch. Residue starch was then slurried in water (600ml) and centrifuged 1000rpm for 20minutes to remove any remaining salt. The final wet starch cake was suspended in a 95% ethanol solution and filtered using a separating glass funnel.

The filtrate was stained with an iodine solution(0.74g of resublimated iodine I_2 and 1.48g of potassium iodide KI dissolved in 400ml of distilled water) and observed under light microscope (400X)

3.10 Data management and analysis

Each analytical process was carried out in three independent replications; the data obtained was subjected to Analysis of Variance (ANOVA) using Genstat (14th edition - 2012). Mean comparisons for treatments were made using Duncan's Multiple Range Tests. Significant difference was accepted at $P<0.05$. (Steel and Torrie, 1980). The values reported are on wet weight basis because that is how they are generally consumed.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 Changes in nutritional characteristics of grain amaranth during processing

Table 2 shows the gross chemicals composition of raw amaranth and grain amaranth processed in different ways. The results of the samples are in the form consumed by the humans.

4.1.1 Moisture content

The dry heating techniques used, namely roasting and popping showed significant ($p<0.05$) reduction of moisture content; with roasting affording the lowest moisture content. Due to their lower moisture content, the roasted and popped amaranth products may be more resistant to spoilage by molds than the raw amaranth. During popping, hot air makes water expand all at once; thereby expanding the grain, while roasting water is lost without expansion. The moisture content of the moist heat techniques was higher than the raw grains due to absorption of water during cooking which had a dilution effect on all other nutrients (Rehman & Khalil, 1988, Mubarak, 2005).

Table 2: Chemical composition of raw and processed grain amaranth (%) wwb

Parameters	Moisture content	Crude protein	Crude fat	Crude ash	Crude fiber	Carbohydrate
Raw grain	9.74^{c}±0.17	14.44^{c}±0.15	7.09^{d}±0.13	3.18^{d}±0.13	4.27^{e}±0.13	66.28^{e}
Popped grain	6.38^{b}±0.08	14.35^{b}±0.04	6.87^{c}±0.14	3.07^{c}±0.05	3.36^{d}±0.09	65.17^{d}
Roasted grain	5.78^{a}±0.15	14.15^{bc}±0.14	7.00^{c}±0.14	2.88^{c}±0.12	4.09^{c}±0.17	64.95^{c}
Boiled grain	73.99^{d}±0.09	3.53^{a}±0.08	1.69^{b}±0.06	1.32^{b}±0.06	2.09^{b}±0.21	17.38^{b}
Slurry	86.37^{e}±0.04	2.81^{a}±0.13	1.34^{a}±0.2	0.88^{a}±0.05	1.57^{a}±0.1	7.03^{a}

NB: Means in columns with the same superscript are not significantly different (P>0.05)

4.2.2 Protein

The crude protein (14.4%) of raw grains was within the range that has been reported(13.1 – 21%)(Leon-Camacho *et al.,* 2001, Berganza*et al.,* 2003, Kariuki*et al.,* 2013).In comparison with common cereals, grain amaranth has relatively high protein content with excellent composition of essential amino acids. The crude protein decreased significantly (p<0.05) after processing as compared with raw grains. These

results have agreed with Shaker *et al*. (1995) who reported that nutrients loss might be attributed to the leaching of soluble nitrogen, mineral and other nutrients into desired solution especially in boiling. Similar reports had been given earlier for boiled soybean and mungbean flour as well as fluted pumpkin seed flour (Fagbemi, 2007). Roasting reduced crude protein content from 14.40% to 14.15%, this may be due to prolonged time of roasting. Similar report was made by Fagbemi (2007) on toasted fluted pumpkin seed flour.

4.2.3 Fat

Crude fat of the raw sample (7.09%) fell within the range (5.60 to 10.9%) reported by Mlakar *et al* 2009, Kariuki *et al*., 2013 and it differed significantly($p<0.05$) from processed samples. Moist heat processing methods decreased fat content. Mubarak (2007) reported reduction of fat content on mungbean seeds and the decrease was attributed to their diffusion into cooking water or volatilization of fats during boiling. Crude fat in both popped and roasted grains is significantly different ($p<0.05$) from raw sample, this may be due to oxidation of fats during dry heating.

4.2.4 Ash

Crude ash of raw grain amaranth (3.18%) is within the range (2.5 to 4.4%) reported by Mlakar *et al.,* (2009). Low levels of ash content in slurried samples are attributed due to dilution of cooking water.

4.2.5 Fiber

Crude fiber of raw grain amaranth (4.27%) is within the range (3.1 to 5%) as reported by Kariuki *et al*., 2013. There is reduction of crude fiber during processing as stipulated from Table 5.

4.2.6 Carbohydrates

The grain amaranth is rich in carbohydrates (66%) which are within the range that has been reported (55–69%) by Kariuki et al., 2013. During popping and roasting much of the free and bound water in grains is lost and thus increase the concentration of other

nutrients particularly carbohydrates, while in moist heat much water is taken in and thus tend to lower carbohydrate contents in samples.

4.3 Change of Mineral composition during processing

Results in Table 3 indicate that grain amaranth is a rich source of both major and trace elements. Comparatively, it has been observed that moist heat techniques (boiling) decreased the mineral content more than the dry heat method. This occurred probably because soluble minerals leached into the processing water which was decanted. This result agreed with the research of Fox and Cameron (1984) and Edem *et al*., (1994) that soluble minerals leach by dissolving into cooking water which is normally decanted.

In general, cereals high in phytates content tend to have higher iron content. From table 6 raw grains have high levels of iron (35.02mg/100g). This indicates that amaranth can be used to mitigate iron deficiency among women of reproductive age and anemic people through formulation of composite flour using amaranth flour as an ingredient. Processing has led to reduction of iron significantly. This is possible due to oxidation of ferrous to ferric iron during heating

The high phytate content of amaranth grains may affects the bioavailability of non-haem iron. A number of factors including phytate and fibre, tannins, lectins and phosphate may contribute to the inhibition of non-haem iron absorption. The high levels of phytate contribute most to the inhibition of non-haem iron. To eliminate its inhibitory effect on non-haem iron absorption, phytate must be almost totally removed. This has been achieved by subjecting this grains to different processing techniques which have significant ($p<0.05$) in reduction of the anti-nutrients.

Table 3: The mineral composition of raw and processed grain amaranth (mg/100g)

parameters	Raw grain	Popped grains	Roasted grains	Boiled grains	slurry
Iron	$35.02^{e}\pm0.09$	$23.90^{d}\pm0.1$	$18.12^{c}\pm0.2$	$15.50^{b}\pm0.12$	$7.26^{a}\pm0.06$
Magnesium	$653.27^{e}\pm0.12$	$578.49^{d}\pm0.10$	$546.44^{c}\pm0.03$	$473.73^{b}\pm0.17$	$326.78^{a}\pm0.14$
Calcium	$578.24^{e}\pm0.01$	$562.53^{d}\pm0.09$	$545.15^{c}\pm0.06$	$374.75^{b}\pm0.13$	$318.86^{a}\pm0.09$
Zinc	$5.33^{e}\pm0.13$	$5.15^{d}\pm0.02$	$3.61^{c}\pm0.07$	$1.79^{b}\pm0.02$	$1.35^{a}\pm0.04$
Potassium	$729.69^{e}\pm0.02$	$714.98^{d}\pm0.07$	$720.54^{c}\pm0.04$	$620.54^{b}\pm0.05$	$604.86^{a}\pm0.03$
Sodium	$94.537^{e}\pm0.11$	$90.86^{d}\pm0.02$	$87.96^{c}\pm0.03$	$69.67^{b}\pm0.08$	$65.42^{a}\pm0.07$
Phytate/calcium	0.001377	0.001347	0.001453	0.0008	0.00063

NB: means in rows with the same superscript are not significantly different (P>0.05),

Also the high phytate content of grains may reduce the availability of calcium for absorption because the phytate forms insoluble complexes with calcium. The combined effect of low calcium content, a low Ca/Mg ratio, and probably low Ca/P may lower bioavailability of calcium via high phytate content posing problems for healthy bone development.

The content of zinc (5.3mg/100g) in grain amaranth is quite higher than reported values (3.6-4mg/100g) by Kariuki *et al*., 2013. Probably there was an error especially in standards preparation. The zinc content of the processed grain was significantly ($p<0.05$), this may be due to dilution effect during wet cooking.

4.4Fatty acid composition

The oil extracted from raw and processed grains contains mainly unsaturated fatty acids (Table4). The predominant acids in the oil are oleic, linoleic and palmitic. Total unsaturated acids ranged from 76.2% to 77.6% and saturated fatty acids 22.4% to 22.8%. Linolenic acid was present at low concentration (1.2%). The percent unsaturation in amaranth fats is reported as 65% by Opute (1979), 74% by Lorenz (1985) and 76% by Saunder and Becker (1984). These are comparable with the research findings,

76%.Berger *et al.,* 2003 reported that amaranth oil significantly reduced non-HDL cholesterol and raised HDL cholesterol, as well as lowering very low density lipoprotein cholesterol (VLDL cholesterol) by 21–50%. Amaranth grains can therefore be recommended as a functional food product for the prevention and treatment of cardiovascular diseases.

Table 4: Percentage of fatty acids composition of raw and processed grain amaranth

parameter	Raw grain	Popped grain	Roasted grain	Boiled grain	Slurry
Lauric	0.80^{b}±0.1	1.23^{c}±0.34	1.37^{c}±0.56	0.64^{ab}±0.01	0.46^{a}±0.02
Myristic	0.28^{d}±0.01	0.19^{c}±0.12	0.55^{b}±0.27	0.17^{b}±0.02	0.10^{a}±0.07
palmitic	24.26^{ab}±0.34	25.67^{b}±1.2	25.032^{ab}±0.32	23.57^{a}±0.06	23.83^{ab}±0.45
Stearic	1.2^{c}±0.01	1.58^{e}±0.9	1.90^{d}±0.23	0.76^{a}±0.03	0.21^{b}±0.13
Oleic	36.71^{b}±1.6	36.36^{c}±0.21	36.16^{c}±0.12	36.82^{ab}±0.21	36.86^{a}±0.745
linoleic	36.5^{b}±1.4	34.48^{a}±0.17	34.30^{a}±0.34	36.54^{b}±0.18	36.94^{b}±0.89
Linolenic	1.22^{a}±0.3	and	and	1.46^{ab}±0.21	1.56^{a}±0.19
Saturated Fatty Acid	25.57^{a}	28.66^{b}	28.84^{b}	25.18^{b}	24.6^{a}
Unsaturated Fatty Acid	74.43^{a}	71.34^{b}	71.16^{b}	74.82^{a}	75.4^{a}

Nd= not detected

NB: Means in rows with the same superscript are not significantly different (P>0.05)

Popping, roasting significantly $p<0.05$ reduced the contents of poly unsaturated fatty acids (linoleic and α linolenic).

Oleic acid, with only one double bond in its structure, was more thermo-stable, which is indicated by the statistically significantly increased content of oleic acid in grain amaranth products.

According to the obtained results it can be concluded that high temperature did not affect the content of saturated palmitic acid. Results of the stearic acid changes under the impacts of increased temperatures cannot be observed separately from the results of the analysis of unsaturated fatty acids. Stearic acid, as a saturated fatty acid, is very stable.

4.5 Amino acid profile

Results of amino acid profile of both raw and processed samples of grain amaranth are shown in Table 5.The obtained amino acid levels (Table 5) in raw amaranth grains are consistent with the data from the literature (Tovar *et al*., 1989; Bressani *et al.,* 1992; Dodok *et al*., 1997; Jarošová *et al*., 1997; Gorinstein *et al*., 2002) although slightly higher than results reported by Kariuki *et al* (2013).

Generally, there is high content of lysine and arginine was detected in both heat treated and untreated grains, as well as satisfactory content of cystein and lower levels of methionine, valine, isoleucine and leucine.

Table 5: Amino acid profile of raw and processed grain amaranth (g/16Ng)

parameter	Raw grain	Popped grain	Roasted grain	Boiled grain	slurry
Lysine	7.84^{e}±0.05	6.87^{d}±0.07	6.571^{a}±0.11	$6. 53^{a}$±0.1	7.46^{c}±0.1
Histidine	1.98^{b}±0.1	1.72^{a}±0.05	1.67^{a}±0.06	1.62^{a}±0.05	1.98^{b}±0.04
Threonine	4.93^{d}±0.05	4.61^{c}±0.09	4.12^{a}±0.09	4.31^{b}±0.05	4.93^{d}±0.05
Methionine	2.71^{b}±0.1	2.12^{a}±0.11	2.03^{a}±0.07	2.67^{b}±0.13	2.92^{c}±0.05
Isoleucine	3.62^{ab}±0.08	3.54^{a}±0.07	3.49^{a}±0.1	3.51^{a}±0.12	3.78^{b}±0.1
Leucine	6.35^{b}±0.05	5.89^{a}±0.06	5.84^{a}±0.08	5.87^{a}±0.04	6.35^{b}±0.11
Phenylalanine	3.74^{b}±0.05	3.54^{b}±0.14	3.21^{a}±0.11	3.57^{b}±0.13	3.67^{b}±0.02
Aspartic acid	10.81^{d}±0.16	9.58^{b}±0.17	9.09^{a}±0.1	10.12^{c}±0.12	10.93^{d}±0.13
Glutamic acid	17.30^{d}±0.08	16.21^{b}±0.07	15.71^{a}±0.05	16.93^{c}±0.14	17.14^{d}±0.14
Alanine	6.82^{c}±0.15	6.12^{b} ±0.03	5.75^{a}±0.01	6.75^{c}±0.14	6.68^{c}±0.12
Arginine	14.94^{c}±0.14	12.85^{b}±0.07	11.34^{a}±0.05	12.71^{b}±0.08	15.12^{d}±0.14
Cystein	3.43^{c}±0.14	3.12^{b}±0.11	2.77^{a}±0.1	3.31^{c}±0.01	3.41^{c}±0.08
Valine	4.78^{b}±0.09	4.61^{b}±0.08	4.15^{a}±0.03	4.71^{b}±0.11	4.78^{b}±0.14
serine	8.61^{b}±0.14	8.28^{a}±0.03	8.08^{a}±0.1	8.21^{a}±0.14	8.72^{b}±0.12
Tryptophan	1.57^{c}±0.07	1.31^{b}±0.06	1.09^{a}±0.03	1.51^{c}±0.05	1.58^{c}±0.04
Glycine	14.64^{d}±0.05	13.82^{b}±0.12	13.41^{a}±0.07	14.21^{c}±0.09	14.37^{c}±0.09
Tyrosine	0.59^{ab}±0.09	0.47^{a}±0.09	0.48^{a}±0.17	0.45^{a}±0.02	0.63^{b}±0.06

NB: means in rows with the same superscript are not significantly different (P>0.05)

Roasting has significantly (p<0.05) reduced amino acid contents when compared with popping, slurring and boiling. Tovar et al (1989) and Písaříkova (2005) found out lysine, arginine, cystein were partially damaged while aspartic acid, alanine, glutamine and phenylalanine were partially racemized during popping. Slurring has no significant (p>0.05) effect on the amino acid profile.

4.6 Changes of Anti-nutritional factors during processing

Results of ant-nutrient contents of both raw and processed samples of grain amaranth are shown in Figure 6, Figure 7 and Figure 8.Oxalates content (303mg/100g) of raw grain amaranth is relatively higher than what has been reported (178 and 278 mg/100 g)by Gelinas and Seguin (2007) and Kariuki *et al.,* 2013). Oxalates and tannins may be removed from food by cooking in water, although this is not the most effective method. Soaking followed by wet cooking may reduce oxalates and tannins more rapidly when compared with just wet cooking (Fennema, 1996).

Roasting was found to be the least effective method of reducing these anti-nutrients contents. The roasting of grain amaranth decreased the oxalate content by 56.1%.Around 80.9% total oxalates were lost when grain amaranth were slurried compared with being boiled (69%) and popped (66.4%).Although moist heat processing (boiling) proved most effective in terms of the reduction of total oxalates, this is expected to be accompanied by the simultaneous loss of water.

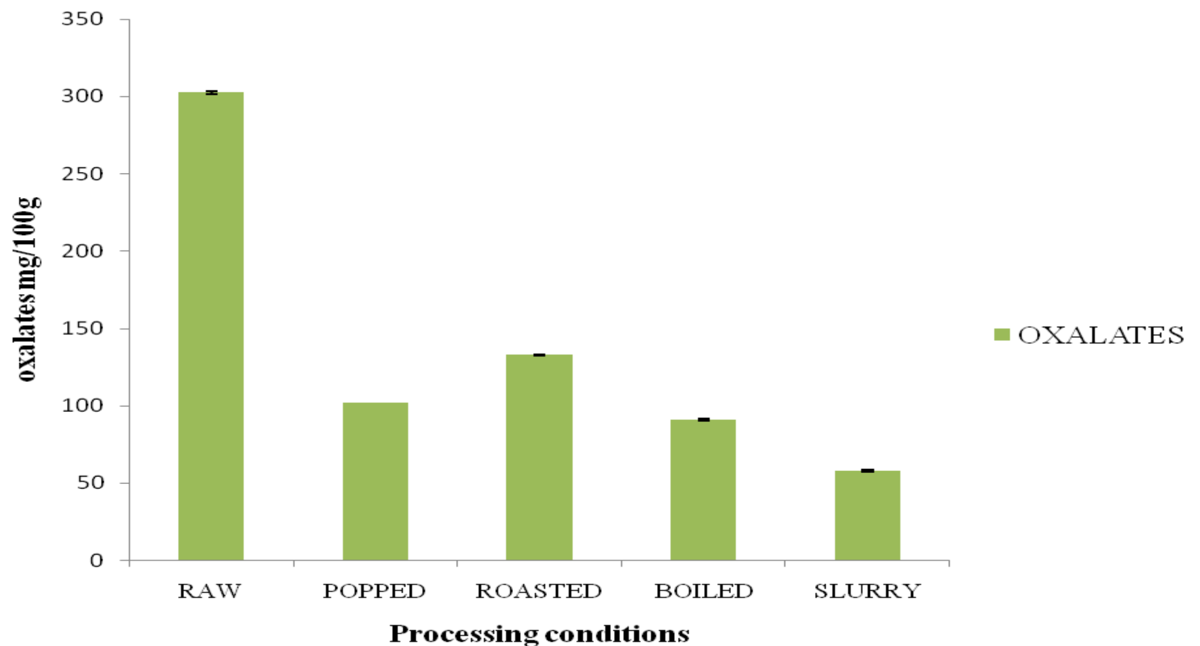

Figure 6: Oxalate contents of raw and processed grain amaranth (mg/100g)

Tannin content (1.4mg/100g) of raw grain amaranth is within the range (1.5-4mg/100g) by Muyonga *et al.,* (2011). The findings here indicate that processing reduced tannin contents which is contrary to the results of Muyonga *et al., (*2011) who found no significant ($p>0.05$) difference of raw and dry heated grains. Heat has been reported to cause reduction in tannins of roasted and popped grain amaranth (Yanez *et al.,*1986); and this reduction may be attributed due to thermal degradation. Griffith and Castell-Perez (1998), found out that moist heat (boiling) reduced tannins significantly, this is possible due to solubilization of tannins in cooking water.

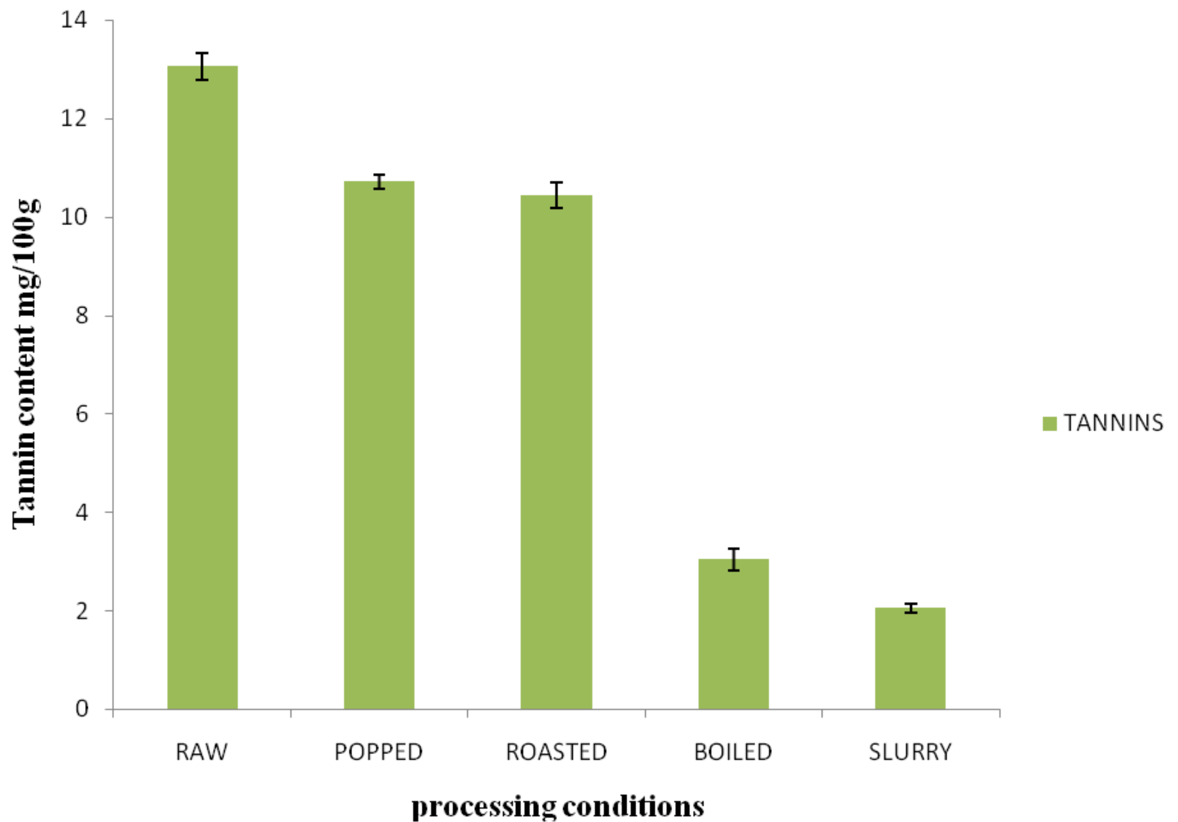

Figure 7: Tannin contents of raw and processed grain amaranth (mg/100g)

As shown in Figure 4, raw grains had the phytates content amounting to around 0.796mg/100g. The phytates content of the raw grain is in the range (0.29-7.92mg/g) that has been reported by Whittaker and Ologunde (1990);Ruiz and Bressani (1990) and Matz (1991. Generally, all the processing techniques reduced the phytates contents in grain amaranth. Roasting and popping had slight reduction of phytates content, while boiling and slurring had great reduction of phytates

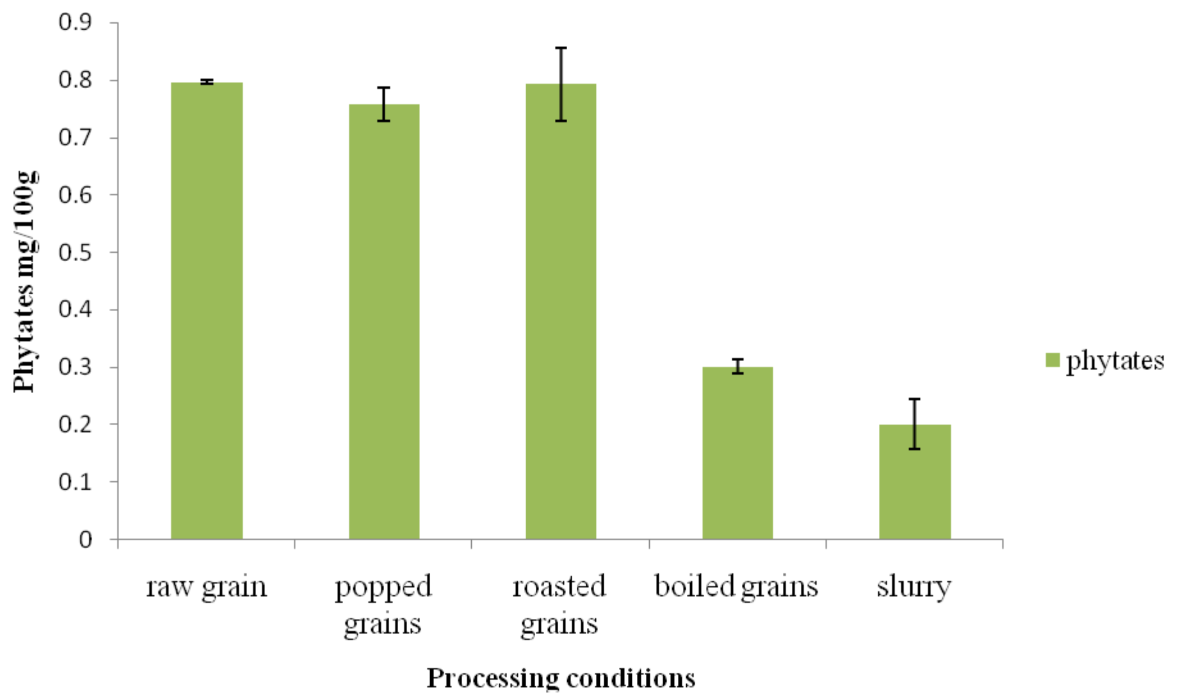

Figure 8: Phytate contents of raw and processed grain amaranth (mg/100g)

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4.7 Protein digestibility

The protein digestibility of raw and processed grain amaranth varied with the processing method applied (Figure 9).Raw grain had a protein digestibility of 74.8%. Popping, boiling and slurring improved protein digestibility (3.24%, 11.39% and 17.75%) respectively while roasting led to a marked reduction by 8.30%.

The results were in disagreement with an earlier work (Písaříkova *et al*. 2005) that reported a reduction in-vitro protein digestibility from 68.1 to 50.6 as a result of popping while Arendt, Morrissey, Moore and Dal Bello (2008) reported increased of protein digestibility probably which occurred due to better availability of amino acids following heat treatment.

In vitro digestibility of 61–76% has previously been reported for raw grain amaranth proteins (Correa *et al.,*1996).The relatively low protein digestibility of raw grain amaranth may be attributed due to the influence of anti-nutrients such as enzyme inhibitors, lectins, phytates, tannins and dietary fiber, which inhibits protein digestion(Nestares *et al.,*1993). Also the protein structures in the raw samples are not denatured and hence less protein are accessible for enzyme digestion (Fennema, 1996).

The in vitro protein digestibility values recorded from grain amaranth were higher than reported digestibility values for whole raw maize (66.6%) and sorghum (55.8–59.1%) (Duodu *et al*.,2002).The reduction in protein digestibility resulting from roasting of grain amaranth might be attributed by amino acid degradation, formation of intramolecular disulfide bonds and maillard reaction, changes associated with dry heat processing(Nestares *et al.,*1993).

The lower digestibility for roasted seeds as compared to popped ones point to more pronounced protein changes roasting. There was prolonged time during roasting when compared with popping. The nature of the change in protein digestibility resulting from heat treatment seems to relate partly to the extent of formation of complexes between proteins and other grain components and the level of matrix disintegration, which impacts the access of proteolytic enzymes (Owusu-Apenten, 2002).

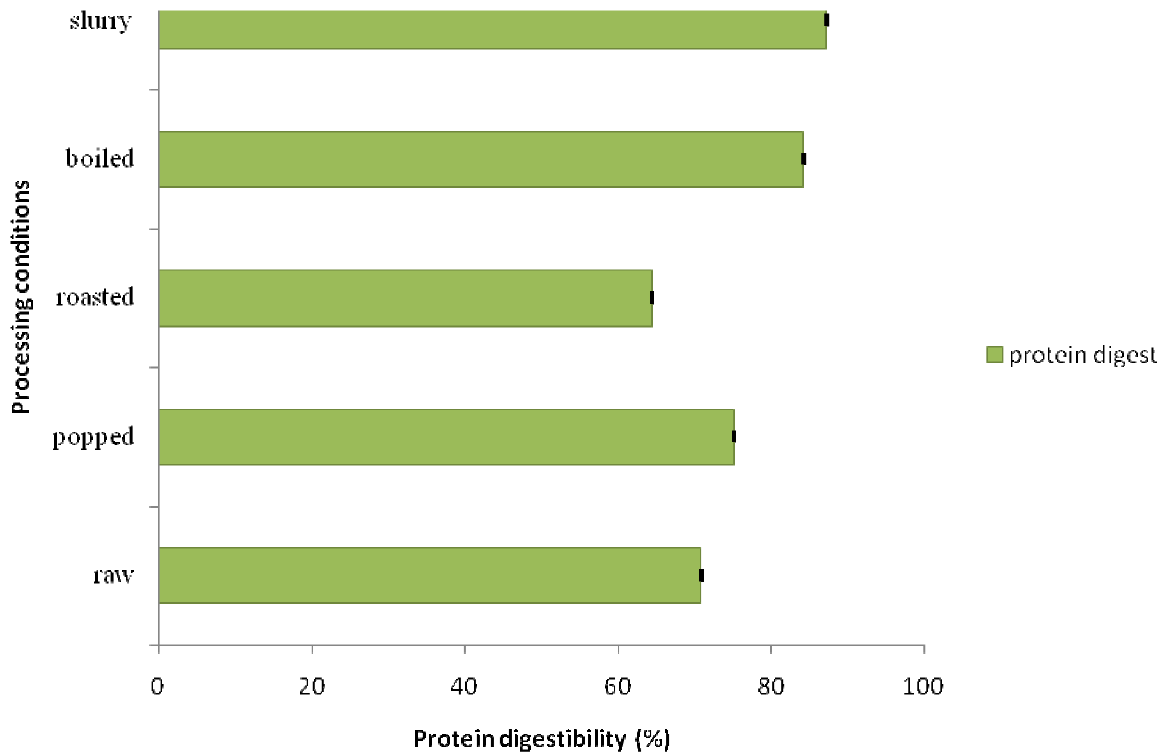


Figure 9: Protein digestibility (%) of raw and processed grain amaranth

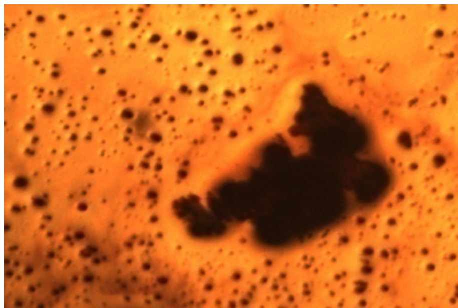
4.8 Starch gelatinization

The results of degree of starch gelatinization of the raw and processed grains are shown on Figure 10.Due to high levels of amylopectin of amaranth starch (waxy starch), the pictorial colour appeared brown. The iodine solution in the raw sample is still clogged in the granule which shows no gelatinization at all.

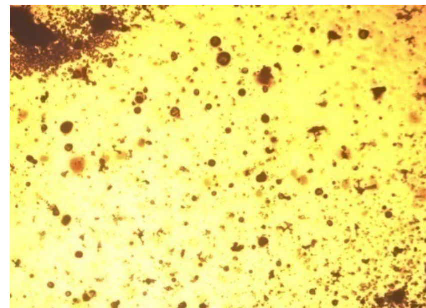
For the popped and roasted samples, the solution tends to have slight mobility which signifies that gelatinization took place although it was partial. The solution mobility was more pronounced in both boiled and slurried samples which imply that there is high degree of starch gelatinized.

Most starches will gelatinize upon heating to above $80^{0}C$ in excess water. Gelatinization markedly increases susceptibility for amylolytic degradation due to loss of crystalline structure which is clearly demonstrated by moist processing techniques(Wianecki & Kołakowski, 2007).

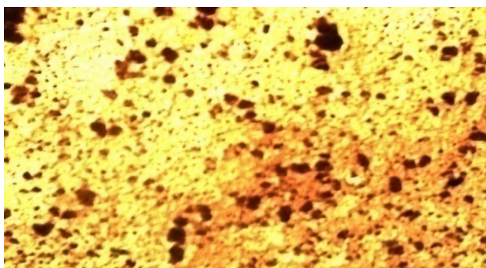
Doesthale (2002) reported that during milling some starch granules (usually 5-10%) are partially damaged by grinding action of roller mills and increase their water binding capacity whose effect is shown during slurring.



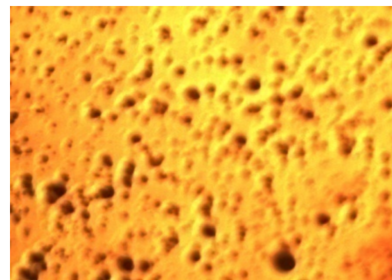
Raw grain



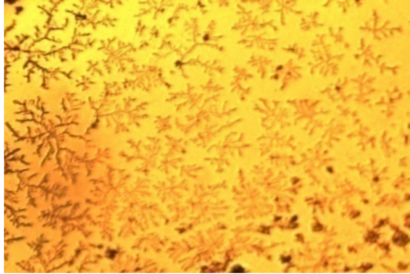
Roasted grains



Popped grains



Boiled grains



Slurried sample

Figure 10: Degree of starch gelatinized at different processing techniques

Dry heat (popping and roasting) changes starch to dextrin, a form of sugar that is very soluble (Fennema, 1996). Popping is achieved by rapid, intense heating of grain; it makes water expand all at once; thereby expanding the grain. As expansion takes place, some of the granules are gelatinized resulting in the grain being much more available to digestive enzymes. In roasting, water is lost without the expansion of the grain; this is because grains are heated at a much slower rate than the popping. This resulted to partial gelatinization of starch.

During dry heat, there is partial breakdown of starch that lead to formation of dextrin .On heating dextrin polymerise to form longer chains, and become brown coloured substances called pyrodextrins**.** Pyrodextrins contribute to the brown colour and the characteristic taste and texture of many foods including toast and bread crust (Fennema, 1996).

CHAPTER FIVE

5.0 CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusion

Grain amaranth was found to contain high levels of nutrients particularly protein, minerals. The minerals of importance are calcium, iron and zinc. It has high levels of unsaturated fatty acids particularly linoleic and oleic acids. Grain amaranth has been found to contain antinutrients like tannins, phytate and oxalate, which have negative effects on mineral absorption and digestion of ingested food.

The protein amino acid composition of grain amaranth (*A.albus)* is most favorable for human demands. The lysine and sulfur amino acids (methionine, cystein, and cystine) contents are high. The relatively high content of essential amino acids indicates that amaranth grain can be used in poor protein foods.

In conclusion, the study has shown that popping, roasting; boiling and slurring have varying effect on nutritional composition and level of anti-nutrient content. Popping and roasting causes significant reduction of unsaturated fats acids when compared to boiling and slurring.

Processing by popping, boiling and slurring improved the protein digestibility while roasting decreased protein digestibility. Wet heat processing was better in gelatinizing starch in grain amaranth as compared to dry heat processing.

5.2: Recommendation

From the study the following are the recommendations:

1. Determine the effect of other processing techniques on the nutrients and ant-nutrient content of grain amaranth as well as other conventional cereals for instance flaking, steaming, mechanical processing.
2. Identify the best temperature and time combination especially in roasting in order to maintain maximum nutrients retention and subsequent reduction of antinutrients components of the processed grain.
3. Carry out *in vivo* tests in order to determine the bioavailability of nutrients after the grain amaranth has been subjected to named processing techniques.

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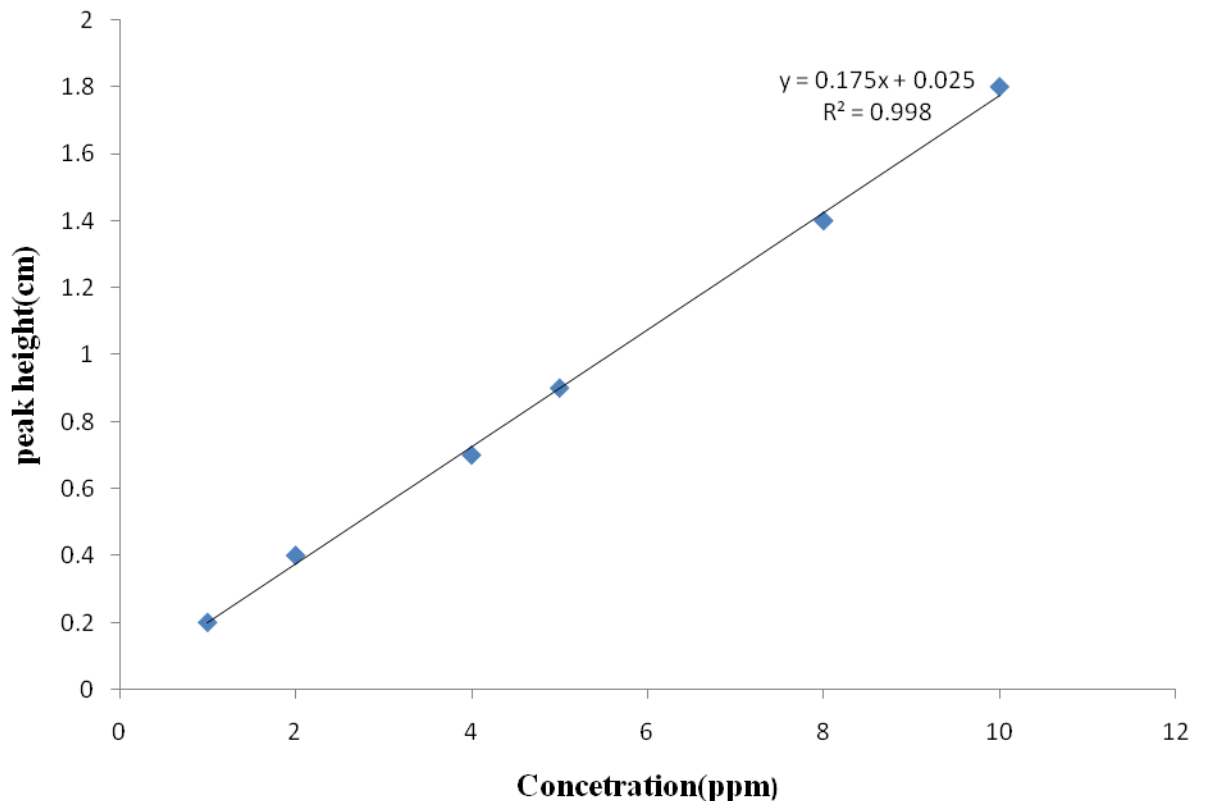
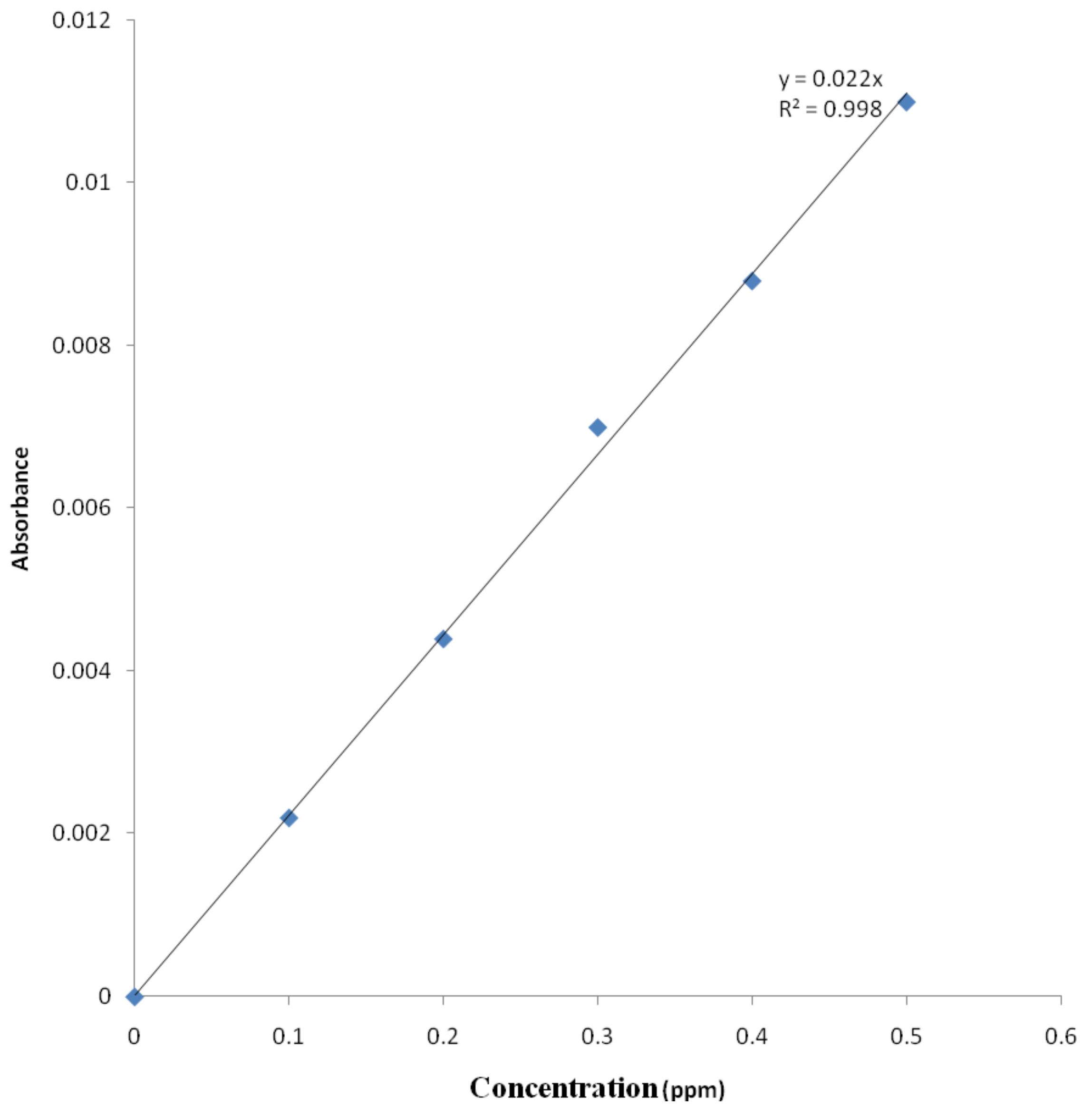
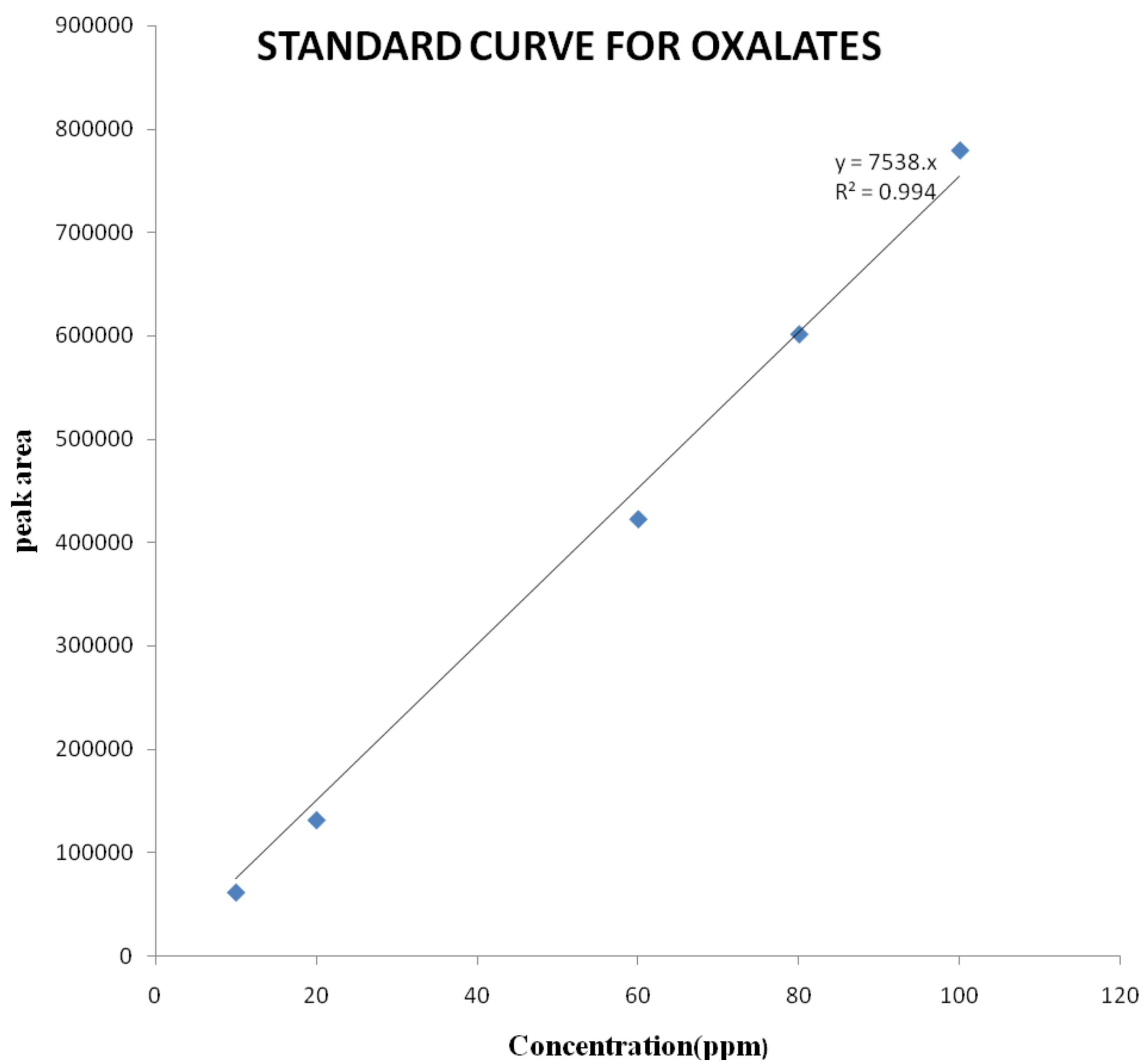
APPENDICES

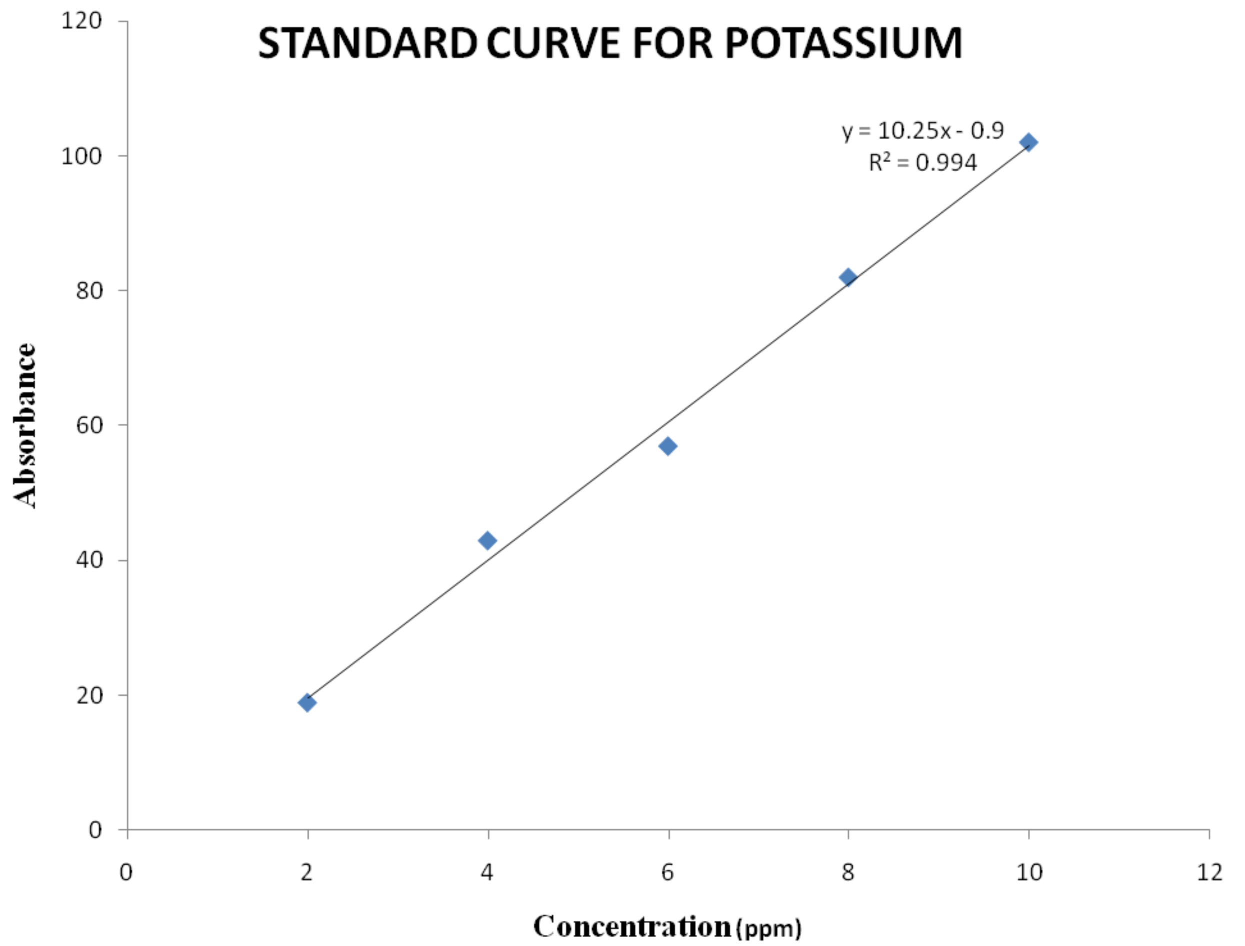
Appendix 0-1: Standard curves for anti-nutrients

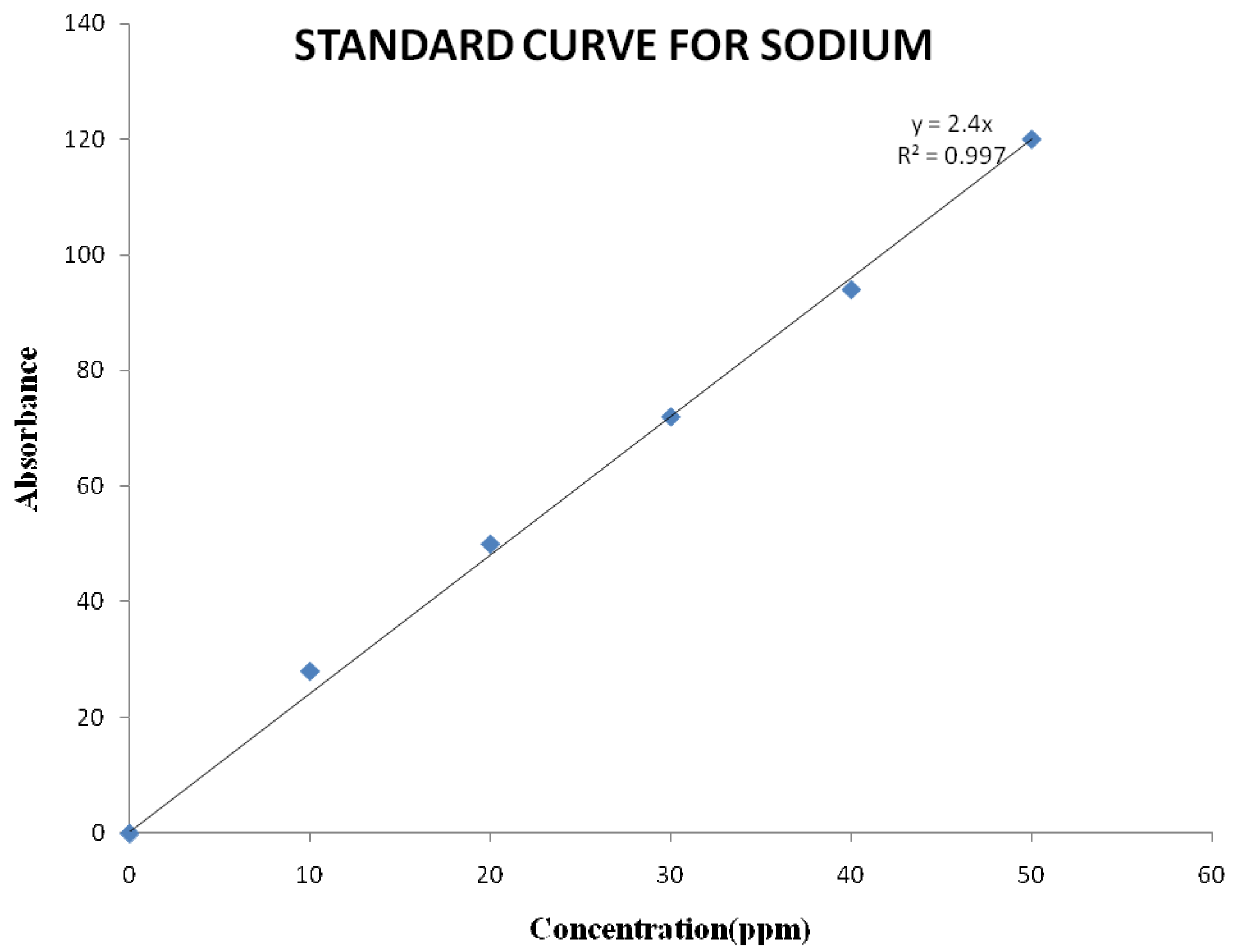


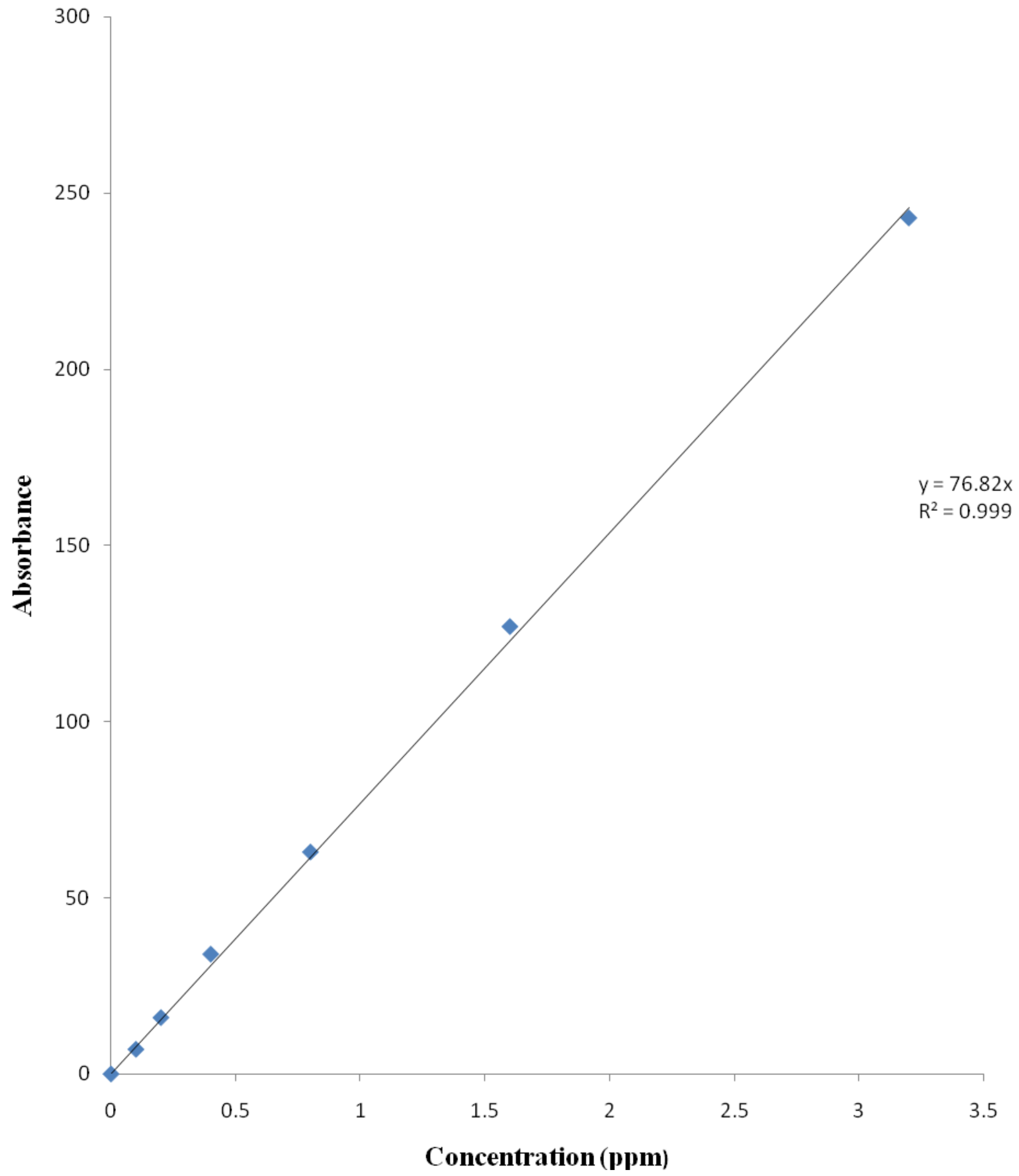

STANDARD CURVE FOR TANNINS
y = 0.022x
R² = 0.998
Absorbance
0.012
0.01
0.008
0.006
0.004
0.002
0
0
0.1
0.2
0.3
0.4
0.5
0.6
Concentration (ppm)

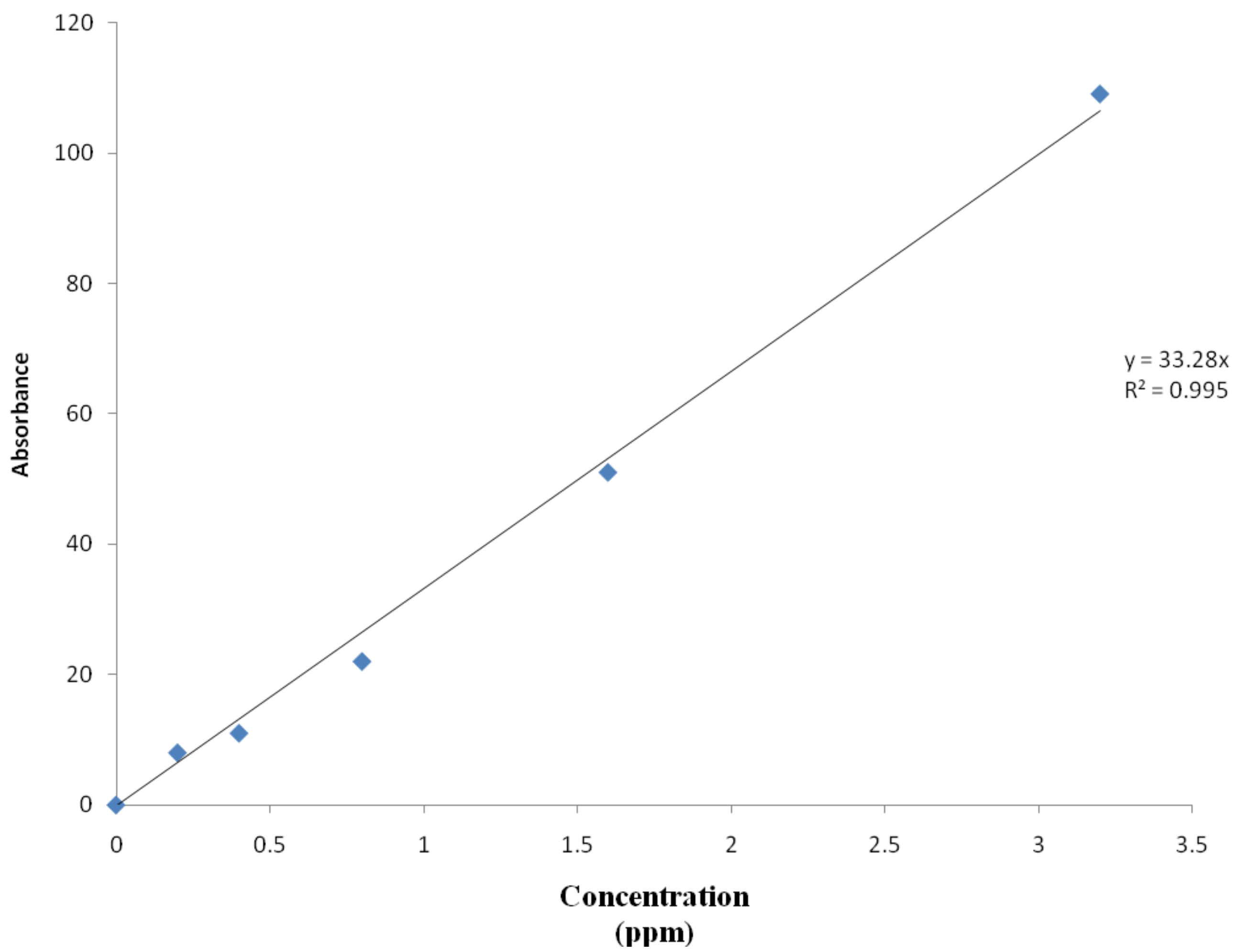

STANDARD CURVE FOR OXALATES
y = 7538.x
R² = 0.994
peak area
Concentration(ppm)
900000
800000
700000
600000
500000
400000
300000
200000
100000
0
0
20
40
60
80
100
120

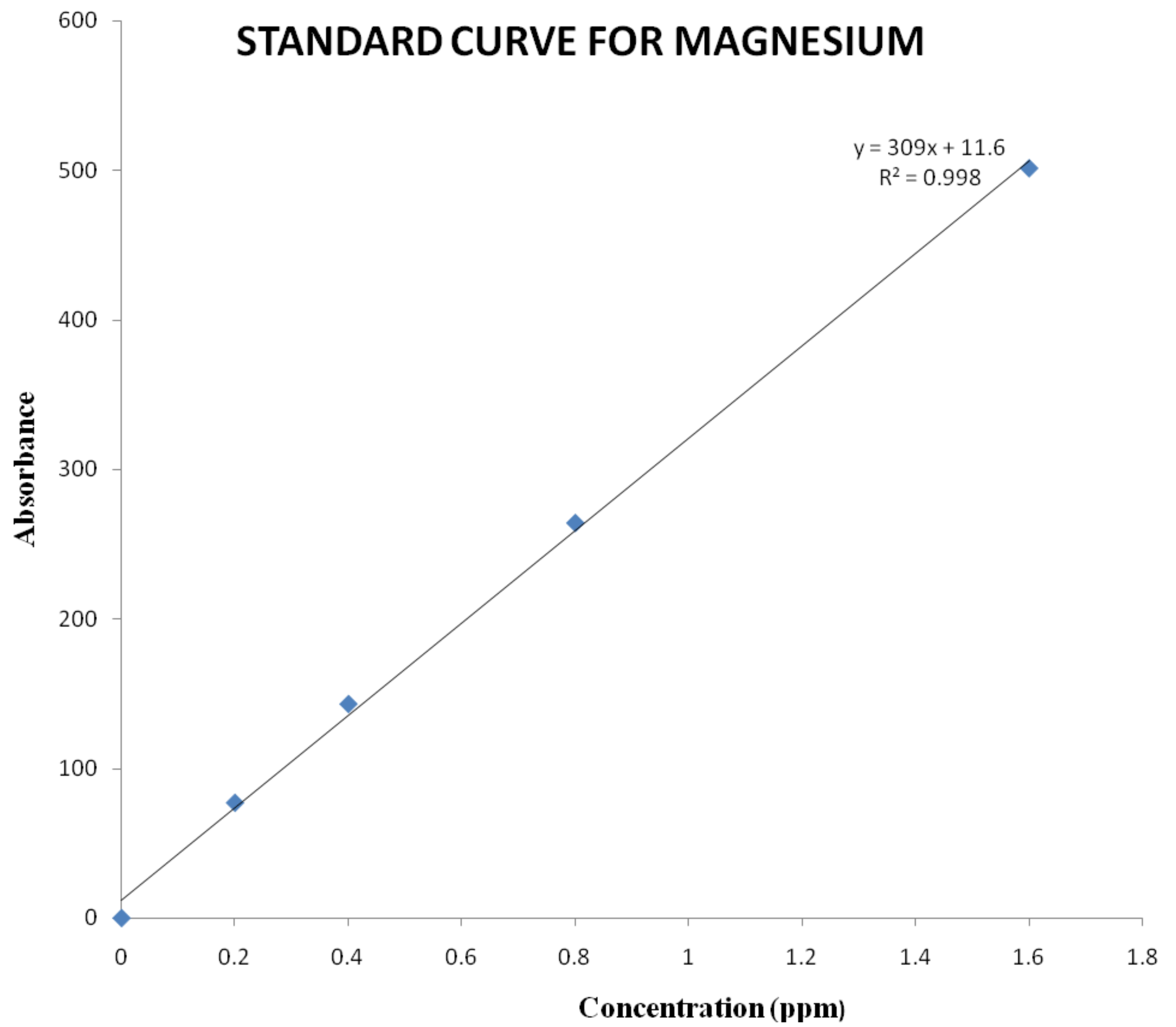
Appendix 1: Standard curves for mineral composition

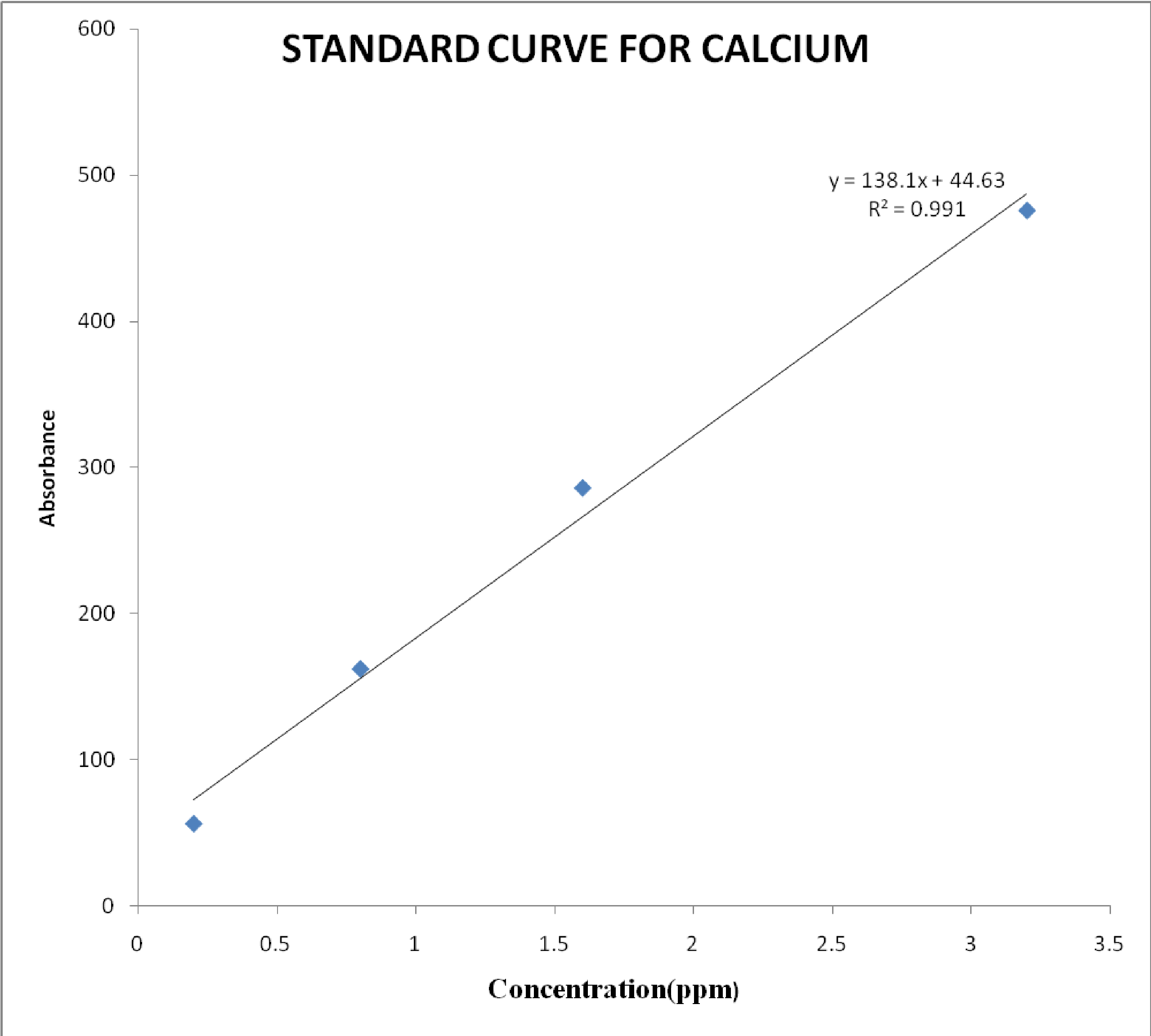


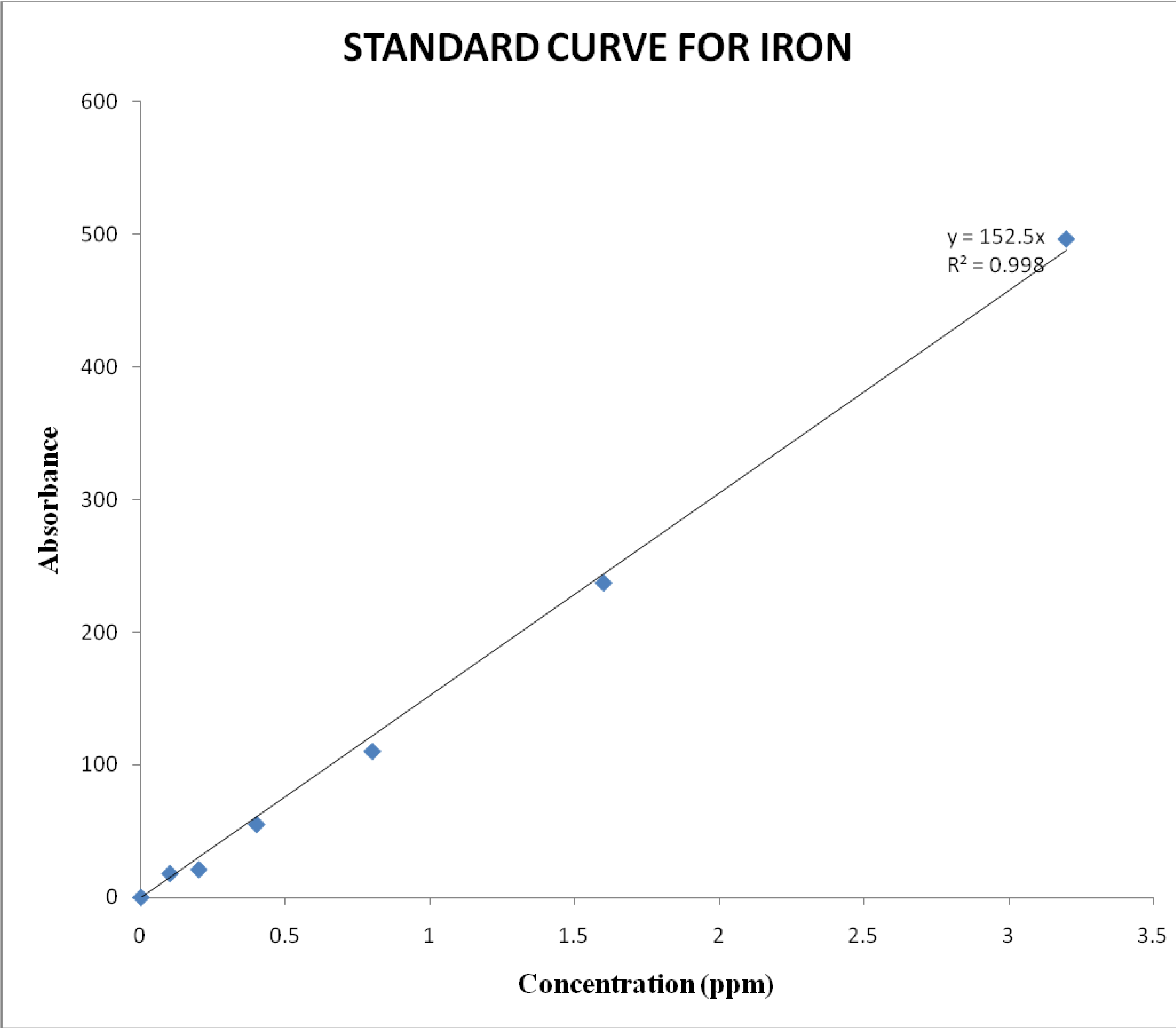

STANDARD CURVE FOR SODIUM
y = 2.4x
R² = 0.997
Absorbance
Concentration(ppm)
0
20
40
60
80
100
120
140
0
10
20
30
40
50
60


STANDARD CURVE FOR ZINC
300
250
200
150
100
50
0
Absorbance
0
0.5
1
1.5
2
2.5
3
3.5
Concentration (ppm)
y = 76.82x
R² = 0.999


STANDARD CURVE OF COPPER
120
100
80
60
40
20
0
Absorbance
0
0.5
1
1.5
2
2.5
3
3.5
Concentration
(ppm)
y = 33.28x
R² = 0.995


STANDARD CURVE FOR MAGNESIUM
y = 309x + 11.6
R² = 0.998
Absorbance
Concentration (ppm)
600
500
400
300
200
100
0
0
0.2
0.4
0.6
0.8
1
1.2
1.4
1.6
1.8


STANDARD CURVE FOR CALCIUM
y = 138.1x + 44.63
R² = 0.991
Absorbance
600
500
400
300
200
100
0
0
0.5
1
1.5
2
2.5
3
3.5
Concentration(ppm)


STANDARD CURVE FOR IRON
y = 152.5x
R² = 0.998
Absorbance
Concentration (ppm)
0
100
200
300
400
500
600
0
0.5
1
1.5
2
2.5
3
3.5