FORMS AND BIOAVAILABILITY OF ZINC AND COPPER ESSENTIAL ELEMENTS IN PARTS OF WATERMELONS FROM NGARA AND MWEA MARKETS, KENYA

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DECLARATION

This project is my original work and has not been presented in any other institution of learning for any other award.

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Declaration by supervisors

This work has been submitted with our approval as the University supervisors.

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DEDICATION

I wish to dedicate this work to my beloved wife Agatta Nyambura, children Samuel Ngatia and Vincent Karanja for their love, patience and understanding during the study period when I was away from them. Special dedication goes to my parents Samuel Ngatia and Hannah Mwihaki for their words of encouragements, moral support and blessings which made me endure to the end.

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ABBREVIATIONS AND ACRONYMS

AAS	Atomic Absorption Spectroscopy
AES	Atomic Emission Spectroscopy
AFS	Atomic Fluorescency Spectroscopy
ANOVA	Analysis of Variance
CE	Capillary Electrophoresis
CG	Charleston Grey
CS	Crimson Sweet
DE	Dichloromethane Extract
DNA	Deoxyribonucleic Acid
DTPA	Diethylenetriaminepentaacetic Acid
EDTA	Ethylenediaminetetraacetic Acid
EE	Ethanol Extract
FAAS	Flame Atomic Absorption Spectroscopy
GC	Gas Chromatography
GCMS	Gas Chromatography Mass Spectroscopy
HE	Hexane Extract
HIV & AIDS	Human Immunodeficiency Virus and Acquired Immune Deficiency Syndrome
ICPMS	Inductively Coupled Plasma - Mass Spectroscopy
JKUAT	Jomo Kenyatta University of Agriculture and Technology
KIRDI	Kenya Industrial Research and Development Institute
KU	Kenyatta University
LC	Liquid Chromatography
MT	Metallothioneins
PA	Phytic Acid
RDA	Recommended Daily Allowance
RE	Residual
RNA	Ribonucleic Acid
SB	Sugar baby
SFC	Supercritical Fluid Chromatography
SOD	Superoxide Dismutase
SPSS	Statistical Package for Social Science
USA	United State of America
WE	Water Extract
WHO	World Health Organization

ABSTRACT

During the last few decades analytical chemists and nutrition scientists have increasingly realized that total concentrations of chemical elements cannot give, in general, information about mobility, bioavailability, and the eventual impact of elements on biological organisms. Only the knowledge of the chemical species of the elements can provide an understanding of chemical and biochemical reactions, bioavailability, and subsequent paths of metabolism, thus leading to more information about essentiality or toxicity. This stresses the necessity of speciation analysis to determine the species of an element in a specific matrix. There is need therefore to determine the species of essential elements in different types of fruits, vegetables and other foods. One fruit that is increasingly being consumed and which is available throughout the year and has essential elements is watermelon (*Citrullus lanatus* (Thunb.) Matsum and Nakai). There is need to determine the form in which the essential elements occur in the seed, red flesh, white flesh and peel in a watermelon to avoid deficiency when used as food to encourage use of thrown away parts. The study therefore aimed at providing information on bioavailability and forms of essential elements in the peel, white flesh, red flesh and seed parts of watermelons. Water and ethanol extractable elements are more available for absorption than those extracted by other solvents. Watermelons selected for the study are widely consumed and were purchased from Mwea and Ngara markets in Kenya. They were separated to peel, seed, white and red flesh and dried. Each of the watermelon part was sequentially extracted with hexane, dichloromethane, ethanol, water and lastly residue digested and Flame Atomic Absorption Spectrometry (FAAS) was used to determine the levels of each element in various fractions. Free Zn^{2+} and Cu^{2+} ions and their organic acid complexes in the extracts constitute the bioavailable forms. The data of this study were analysed through One Way Analysis of Variance. The results of this study showed that there were variations in extracted mean levels of Zn and Cu minerals in the parts and varieties of watermelon. Significantly high water extracts mean levels ($p < 0.001$) of 0.46±0.01 mg/100 g, (46%) Cu was recorded in peel of sugar baby from Ngara market compared to the other watermelon samples. Crimson sweet watermelon samples bought from Ngara market recorded the highest Zn water extracts mean levels ($p < 0.001$) of 4.84±0.04 mg/100 g, (52%) in red flesh compared to the other watermelon varieties in the market. Charleston grey watermelon samples from Mwea market significantly recorded a high Zn water extracts mean levels ($p < 0.001$) of 5.96±0.01 mg/100 g, (61%) in white flesh than was recorded in the other parts of the watermelon varieties. Presence of hexadecanoic and octadecanoic acids which are complexing agents that enhance bioavailability of Zn and Cu were obtained in the watermelon extracts through GC - MS analysis. The bioavailable species of Zn and Cu could have been either as free ions or their organic acid complexes. Since Zn and Cu minerals were bioavailable in the four parts of the watermelon samples, people should eat all the parts of watermelon either when dry or fresh as a source of these nutrients. Result from this study will be used to guide people on nutrition aspect of a watermelon which will in turn enhance human health.

CHAPTER ONE

INTRODUCTION

1.1 Background to the study

In the last three decades, scientific interest in the minerals focused on quantitative analysis to determine the amount of elements in a plant sample. Measurement of this gross metal content of plant material has a valuable place in characterizing the properties of individual plant material and is a measure of the ability to act as a sink for metal contaminants. However, for the past few decades there has been recognition that the uptake and bioavailability impact of the minerals is determined not only by the total concentration but their physical and chemical form within an environmental system. The physico - chemical form or speciation governs the behaviour of the metal in aqueous, terrestrial and biological systems (Serife *et al.*, 2003). For example speciation measurements of metal species in fruits and vegetables are necessary for an estimation of the toxicity, bioavailability and bioaccumulation of a particular element with respect to biological organisms and for an understanding of the trace-metal transport in plant products (Gethner and Kastenholz, 1998).

It is a proven fact that the mobility, bioavailability, toxicity or essentiality and fate of an element depends on the form in which it occurs, rather than its total concentration. Exposure to some forms of an element may be harmless, while other species of the same element may be toxic, carcinogenic or mutagenic. Chromium (VI) is more toxic and taken up more readily by organisms than chromium (III). The oxidation state of species is important for the bioavailability, as to a large extent governs acid-base chemistry, charge, solubility and ligand reactivity and hence absorption, transport over biological membranes, excretion and toxicity (Ida, 2011). It is recognized that

the extent of the toxic effects caused by essential elements for example Zn, Cu, Fe, Cr and V is not governed by their total concentration but it is regulated by the forms of the metals that can efficiently interact with biologically active ligands (Trajce and Irina, 2009). When certain foods are heat treated, some essential elements form complexes that are resistant to hydrolysis thereby lowering absorption (Akpata and Ezeanyika, 2011).

The chemical form of an element in a plant product determines the mode of absorption in the intestine and the subsequent metabolism processes that may occur. The bioavailability of a species in food may also be measured and is defined as a measure of the proportion of the total amount of a nutrient that is utilised for normal body functions (Ali, 2016). Watermelon (*Citrullus lanatus* (Thunb.) Matsum and Nakai) is used as a multipurpose vegetable (Aminata *et al*., 2012). It can also serve as food and water source during drought prone periods (Van der Vossen *et al*., 2004). The average moisture content in watermelon is 92% (Yau *et al*., 2010). Watermelons have four distinct parts namely; the peel, the seed, the red/pink/yellow and white flesh parts which are largely enriched with minerals (Murray *et al.*, 2005). Watermelon rind or peel (Drishti and Maha, 2017) has a higher carotenoid content than pulp and seed (Johnson *et al.*, 2013). Watermelon is the richest known source of citrulline. Red flesh watermelons contain less citrulline compared to yellow or orange flesh watermelons. The rind contains more citrulline than flesh on a dry weight basis (Wenge *et al.*, 2010). Seed may serve as a source of protein, fat and carbohydrates as well as minerals (Loukou *et al*., 2007). Red flesh was used as thirst quencher and to make wine (Paulino *et al*., 2011).

1.2 Watermelon parts

The parts of watermelon where extraction of selected essential elements was done are shown in plate1.1.

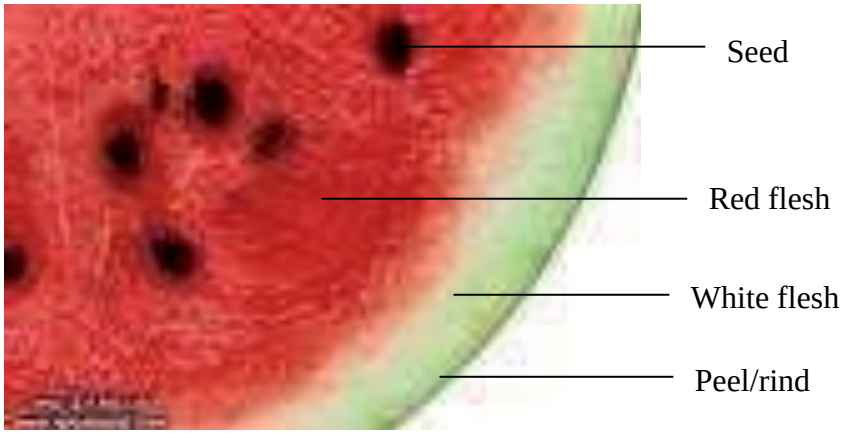

Plate 1.1: Watermelon parts

Source: Author

The white flesh and peel parts of watermelon that are thrown away after eating red flesh are shown in plate 1.2.

Plate 1.2: Watermelon peel and white flesh

Source: Author

1.3 Statement of the problem and justification

Knowledge of essential elements speciation in fruits, vegetables and food is necessary for understanding their nutrition and bioavailability. Speciation information may be used to determine the fate of essential element species in a biological system. Studies have shown that all parts of a watermelon contain substantial amounts of micro and macro - nutrients such as Ca, Cr, Mg, Mo, Fe, V, Zn, Cu among others yet majority of people consume the red flesh part only (Nthiga *et al*., 2013). The study was done on Zn and Cu as both elements are co-factor of more than 200 enzymes in the body such as superoxide dismutase (SOD), compound that have antioxidant activity which can contribute benefits to the treatment of arthritis amongst other diseases (Kojo, 2003).

The levels of Zn and Cu in parts of watermelons are provided in table 1.1 by Mathew *et al*. (2014).

Table 1.1: Mean levels of Zn and Cu in watermelon parts

Amount in mg/100 g		
Watermelon part	Zn	Cu
Peel	7.92	6.22
White flesh	6.39	6.19
Red flesh	5.23	5.21
Seed	5.52	12.25

Source: Mathew *et al*., 2014

Sara (2006) reported 39 % bioavailable Zn in pumpkin seeds while Annan *et al*. (2014) reported (35 - 50) % bioavailable Zn and Cu in muskmelon fruit. Brian (2008) proposed that the predominants forms of Zn and Cu in fruits are low molecular weight complexes, storage metalloproteins and free ions.

The peel, white flesh and seed parts of watermelon that are thrown away, could be a source of minerals. The forms of these elements in the different parts of watermelons have not been determined, although it is well known that bioavailability and toxicity

of elements depend on forms of the elements, for example, organically bound mercury is more toxic than other forms (Cristina *et al*., 2008). Thus there was need to determine the forms in which the individual essential elements exist in the different parts of watermelons so that if they are not toxic the whole fruit can be recommended for use to supply nutrients to the ever increasing human population. The aim of the study was therefore to provide information on forms and levels of Zn and Cu essential elements in seeds, white flesh, red flesh and peel parts of a watermelon bought from Ngara and Mwea markets in Kenya by sequential extraction procedure. Effect of drying on bioavailability of essential elements was determined since the sequential extraction was done on flesh and dried watermelon parts. Levels of the essential elements were also determined on the seeds, red flesh, white flesh and peel parts of watermelons.

1.4 Hypotheses

i. There is significant difference in total concentration and percentage bioavailable essential elements in different parts of watermelon and between different fresh and dried watermelon parts, respectively.
ii. There is significant difference in percentage bioavailable essential elements in watermelon from different places.
iii. There is significant difference in bioavailability of essential elements in different parts of fresh and dried watermelons.

1.5 Objectives

1.5.1 General objective

The study aimed at quantifying amounts and percentages of bioavailable Zn and Cu minerals in different parts of watermelon.

1.5.2 Specific objectives

i. To determine total and bioavailable amounts of Zn and Cu nutrients in peels, white flesh, red flesh and seeds of fresh and dried Charleston grey, crimson sweet and sugar baby varieties of watermelon from Ngara and Mwea markets in Kenya using FAAS.

ii. To determine bioavailable forms of Zn and Cu nutrients in the peels, white flesh, red flesh and seed parts of fresh and dry Charleston grey, crimson sweet and sugar baby varieties of watermelon from Ngara and Mwea markets in Kenya by sequential extraction and GC - MS analysis.

iii. To determine effect of drying peels, white flesh, red flesh and seeds parts of Charleston grey, crimson sweet and sugar baby on bioavailability of Zn and Cu nutrients.

1.6 Significance of the study

The study provided valuable information on the forms of Zn and Cu in four parts of Charleston grey, crimson sweet and sugar baby varieties of watermelon. The information will be used to encourage the general public to grow watermelon in their home gardens and to eat them for enhancing their health. It will help in determining parts of plants that are likely to provide the necessary nutrients to the body since some forms are not easily absorbed. The result of the study will be forwarded to the relevant authorities for appropriate action.

1.7 Scope and limitations of study

Watermelons are sold in many markets in Kenya; however, the samples were obtained from Ngara and Mwea markets only. The study analyzed total concentrations of Zn and Cu and their forms in various parts of Charleston grey, crimson sweet and sugar baby varieties of watermelons grown in Kenya. Soil origins, source, use of fertilizers

and pesticides and seasonal variations were not considered. Biosorption study was not done.

CHAPTER TWO

LITERATURE REVIEW

2.1 Watermelons

Watermelon (*Citrullus lanatus* (Thunb.) Matsum and Nakai) is one of the most widely cultivated crops in the world (Huh *et al.*, 2008). There are over 1,200 varieties of watermelons worldwide (Gichimu *et al*., 2009) and a wide variety of watermelons have been cultivated in Africa (Zohary and Hopf, 2000). Several of these varieties have been recommended for Kenyan range of climate (Tindall, 1983). These include sugarbaby, crimson sweet, Charleston grey, chilean black, congo fairfax and 'tom watson' (Tindall, 1983). However, among these six cultivars, only the first three are available in Kenyan markets with 'sugarbaby' being the most popular (Gichimu *et al*., 2009). Watermelons contain nutrients essential for good health. These nutrients are briefly discussed in the following section.

2.2 Micro and macro nutrients

The minerals are integral part of the normal physiology. The minerals essential to human nutrition are accumulated in different parts of plants. Generally, accumulation of a particular metal in plant is the function of its uptake capacity and intracellular binding states (Clemens *et al*., 2002). Trace elements regarded as nutritionally essential for humans are Co, Cr^{3+}, Cu, Fe, Mn, Mo, Se and Zn and must be recognized as such in the regulatory process (Fraga, 2005).

Essential elements play an important role in the functioning of life on our planet. Some essential elements can become toxic at higher doses (Nath, 2000; Singh, 2004 and Ugwuja *et al*., 2010). These effects depend strongly on the particular form in which the elements occur in the system. The various chemical forms of a given

element or its compounds are known as species (Templeton *et al*., 2000). Essential elements are chemical nutrients needed by the body in large or tiny amounts and are important for growth and development for example, Zn, Cr, Mn and Fe. Elements such as Zn and Cu are known to be components of enzymes which play key roles in the growth of both plants and animals. Hormones need minerals to function properly, therefore lack of minerals especially essential trace elements lead to immobilization of hormones resulting in inefficient control (Underwood, 1977). Most of the essential trace elements have unfilled d - orbitals and readily form coordination complexes with electron rich elements like N, O and S thus competing with each other for such ligands (Brantley *et al*., 2001).

Zinc is a versatile trace element required as a co-factor by more than 200 enzymes (Grodner *et al*., 2000). It is essential for protein, DNA and RNA synthesis and helps in the formation of insulin that is responsible for maintaining blood glucose level. It reduces the incidence of opportunistic infections for example pneumonia in HIV and AIDS patients by up to 45 % (Kabi, 2004). It is important for blood stability since it maintains the proper concentration of vitamin E in the blood and in maintenance of body´s alkaline balance. It is the key mineral required for foetus growth in the womb. It interacts with platelets in blood clotting and also affects thyroid hormone function. It is essential to wound healing and taste perception. Its deficiency can result in poor learning ability in children as a result of impaired neuropsychological function (Grodner *et al*., 2000). Zinc is known to be involved in most metabolic pathways in humans and its deficiency can lead to loss of appetite, growth retardation, skin changes and immunological abnormalities (Ozgur *et al*., 2009).

Adult humans total body Zn is about 1.5 - 2.5 g present in muscle, bone and liver amongst other organs. Absorption of Zn which occurs in intestinal lumen largely depends on its solubility which is influenced by its chemical form, presence of specific inhibitors and enhancers of absorption. Richest food sources of bioavailable Zn include organs and flesh of mammals, fowl, fish, crustaceans, eggs and dairy products unlike cereals and legumes which have low bioavailable Zn due to presence of phytates. Vegetables and fruits are not rich sources of Zn in most of their parts but some like *Citrullus lanatus* have fairly high levels of Zn (Nthiga *et al*., 2013) though bioavailability is uncertain (Kenneth *et al*., 2001).

A recommended dietary allowance (RDAs) for Zn in men is 11 mg/day and 8 mg/day for women and above this level Zn causes adverse health effects (Harlal *et al.*, 2005). However, an RDA of 15 mg/day of Zn was reported (Santos *et al*., 2004) while Biego *et al*. (1998) reported 9.0 - 18.0 mg/day. Oral exposure of 2 - 8 mg/kg/day to zinc chloride and zinc sulphate causes nausea, vomiting, abdominal cramps and diarrhoea in several cases with blood (Babcock *et al*., 1982). Ingestion of zinc chloride by human being causes pharyngitis, esophagitis, hypocalcemia and elevated levels of amylase suggesting acute pancreatitis (Roney *et al*., 2005). It is thus important to know the forms in which Zn occurs.

Copper is required in human body for the conversion of the body´s iron to haemoglobin. It also makes tyrosine (amino acid) usable, allowing it to work as a pigmentation factor for hair and skin. Copper serves as a protein cofactor in fundamental redox reactions involving enzymes such as cytochrome oxidase, superoxide dismutase (SOD), dopamine β-hydroxylase, lysyl oxidase and ceruloplasmin. Copper is essential in cellular respiration, free radical defense,

neurotransmitter function, connective tissue biosynthesis and cellular iron metabolism (Harris and Jonathan, 1996). Copper is involved in myelination of nervous tissue (Douglas, 2008). In humans, Cu is necessary for the development of connective tissue, nerve coverings and bone. It also participates in energy metabolism (Cesar, 2005).

Food Cu concentrations are highly variable. Richest sources of bioavailable Cu in diet include oysters, organ meat and chocolate (Mertz, 1990). Milk from human and cows are poor sources of Cu (Lonnerdal, 1989) though breast milk bioavailable Cu content is higher than from cow milk. Bioavailable Cu concentration in breast milk decreases with time of lactation (Casey *et al*., 1989).

The average daily recommended uptake of Cu should be 0.9 mg, where its deficiency leads to an increase of cholesterol level and coronary diseases (Woolhouse and Walker, 1981). According to Rusjan (2012), WHO suggested that concentration higher than 11.0 $mgkg^{-1}$ leads to Cu toxicity in human and the average daily uptake of Cu should be from 0.4 mg for children upto 1.2 mg for adults. Copper is part of ceruplasmin and superoxide dismutase (SOD), compounds that have antioxidant activity which can contribute benefits to the treatments of arthritis. Copper converts amino acids to tyrosine in the production of the skin pigment melanin. It is essential for synthesis of phospholipids, myelin shealth component that surrounds nerves. Absorption of Cu which takes place in the stomach and upper intestine is increased by acids and inhibited by calcium (Kojo, 2003). It is thus important to know the forms in which Cu occurs.

2.3 Levels of Zn and Cu in watermelon parts, vegetables and other fruits

Depending on area where watermelon plant has been grown, soil pH and weather different researchers have reported varying amounts of Zn and Cu in watermelon parts. The total levels of Zn and Cu in different watermelon parts are tabulated in table 2.1 as reported by different researchers.

Table 2.1: Levels of zn and Cu in watermelon parts

Amount in mg/100 g		
Watermelon parts	Zn	Cu
peel	0.74 - 1.83	0.20
White flesh	0.38 - 2.41	0.22
Red flesh	0.11	0.05 - 1.93
Seed	5.52 - 6.18	1.50 - 12.25

Source: Tarek, 2001; Iyaka, 2007; Elezuo *et al*., 2011; Tarazona and Aguayo, 2012; Nthiga *et al*., 2013; Aremu and Ibrahim, 2014; Luana and Edira, 2014; Mathew *et al*., 2014; Matilda *et al*., 2014 and Paul *et al*., 2014

The levels of Zn and Cu in watermelons and other foods are tabulated in tables 2.2 and 2.3 by Jean and Rachel (2010). and Ismael *et al*. (2011)., respectively.

Table 2.2: Zinc and Cu minerals present in vegetables and watermelon fruit

Amount in mg/100 g		
Vegetables	Zn	Cu
Watermelon	0.39	0.21
Cabbage	0.36	0.04
Lettuce	0.27	0.05
Legumes	1.07	0.49
Bulbs	0.46	0.61

Source: Jean and Rachel, 2010

Table 2.3: Zinc and Cu minerals present in watermelon and other fruits

Amount in mg/100 g		
Fruit	Zn	Cu
Watermelon(seed)	0.24	0.40
Guava	0.28	0.50
Mango	0.13	0.90
Banana	0.11	0.11

Source: Ismail *et al*., 2011

2.4 Effect of drying raw food matrix on bioavailability of essential elements

Heating of food samples containing essential elements such as Zn at high temperatures can cause changes in the matrix structure which can influence their

absorption. Thermal heating of food samples can result in structural changes in inter- and intra- molecular ionic strength thereby causing differences in bioavailability of essential elements (Luana and Edira, 2014). Thermal treatment of food reduces nutrient bioavailability (including that of Zn and Cu) by inducing chemical changes (Snedeker and Greger, 2006). Thermal treatment of food samples can lead to significant tissue structure damage resulting in nutrient loss depending on temperature applied (Diane and Beate, 2012). In drying food matrix there occurs mineral loses such as those of Zn and Cu (Masumi *et al*., 2002).

2.5 Speciation

The term speciation has been defined in different ways such as the distribution of species, transformation of species, the analytical activity to identify, determine the concentrations of species and measuring their distribution (Ure and Davidson, 2002). The term can be used to indicate that a method gives more information on the form in which the element is present than other more commonly applied techniques such as measuring distinct organomercury compounds as opposed to a total mercury determination (Templeton *et al.,* 2000). Element speciation determines the different forms a chemical element within a given compound occurs, enabling chemists to predict possible ramifications for the environment and human health (Martine *et al*., 2005). Speciation of an element is the distribution of an element amongst defined chemical species in a system (Templeton *et al.,* 2000). The geochemistry and bioavailability of trace elements are strongly influenced by speciation.

There are two mechanisms by which trace metals can be assimilated by organisms. Ionic species may form surface complexes with carrier proteins used in transport and be transported across cellular membranes. Non - ionic species may be transported

across biological membranes by passive diffusion. An understanding of the speciation of trace metals is critical to an evaluation of bioavailability (Simkiss and Taylor, 1989). In living systems, organometallic species may be converted to alkylated compounds where distinction can be made between biomethylation of the compound or the addition of a longer alkyl chain. Arsenic and Se can undergo both. For some metals such as Hg, methylation leads to increased toxicity. Methylation in some metals such as As and Se leads to detoxication (Dumont, 2006).

Depending on the plant species, between 58 % and 91 % of the Zn in a plant can be in a water-soluble form (low molecular weight complexes and free ions) (Brown *et al*., 1993; Meyer and Rausch, 2008). An example of this is the group of phytochelatins (cysteine, glutamic acid and glycine) which have been identified in a wide range of species and are synthesized in response to exposure to excess concentrations of Cd, Zn and also Hg. Indigestible plant ligands such as phytate, dietary fibers, lignin and products of non-enzymatic maillard browning formed during cooking inhibit intestinal Zn absorption, hence removal of Zn-binding ligands. Milling or fermentation improves Zn bioavailability. Zinc form insoluble complexes with phytate at pH values usually found in foods. Zinc complexes in various vegetables (kohlrabi, Chinese cabbage, chard, leek, spinach, Jerusalem artichokes) are similar and contain low molecular weight species and are anionic at pH 8 (Andrej, 2010).

After uptake from soil, Zn in plants does not undergo valency changes and its predominant forms might be low molecular weight complexes, storage metalloproteins, free ions and insoluble forms associated with the cell walls (Brown *et al*., 1993). According to Walker and Welch (1987) and Robson (1993), reducing sugars, amino acids and compounds which contain S are possible Zn ligands.

Pumpkin seeds contain phytates which accumulate in the seeds during the ripening period and binds Zn^{2+}, Ca^{2+}, Mg^{2+}, Mn^{2+} and Cu^{2+} impairing their bioaccessibility to humans. Phytate is considered as an antinutrient because of its capacity to immobilize certain elements from the diet. Phytate reduces the Zn bioavailability to people because of the formation of insoluble salts or even co-precipitation of Zn as a zinc-calcium phytate complex, where the stability and solubility of the complexes depend on the pH value, the phytate-to-zinc molar ratio and the presence of other compounds (Andrej *et al*., 2011).

It is proposed that Zn in Kohlrabi and Chinese cabbage is bound to an unknown glutamic acid derivative, possibly a malic acid ester. Zinc, Cu and Mg in aqueous leachates of apple and carrot samples elute as low molecular mass complexes with non - carbohydrate compounds in the water-soluble fraction. Zinc in several vegetable samples is predominantly present as species with a molecular mass lower than 5000 Da. Large fraction of the Zn in plants and animals is incorporated into organic compounds like metalloenzymes and other Zn-containing proteins. Some of the Zn may also be present in an inorganic form, especially in plant foodstuffs. Zinc is incorporated in many macromolecules, especially in proteins, where it plays also a structural role and forms strong complexes with polar groups containing O, N and sulphur. In some cases zinc is bound so tightly that it can be removed only with severe chemical treatment. Phytate complexes are largely insoluble at the pH of the small intestines and therefore formation of Zn-phytate complexes increases fecal losses of indigenous Zn. Zinc complexes in various vegetables (kohlrabi, Chinese cabbage, chard, leek, spinach, Jerusalem artichokes) are similar and contain low

molecular weight species and are anionic at pH 8. They show similar elution behaviour in gel permeation and anion exchange chromatography (Andrej, 2010).

Phytic acid which is myoinositol (1, 2, 3, 4, 5, 6) hexakisphosphoric acid has a unique structure which strongly chelates with cations such as Ca, Mg, Zn, Cu, Fe and K to form insoluble salts. Phytate accumulates in the seeds during the ripening period and is the main storage form of both phosphate and inositol in plant seeds and grains. Phosphorus, in this form is not utilized by human beings, dogs, pigs, birds or agastric animals because they lack the intestinal digestive enzyme phytase.

Figure 2.1 represents phytic acid which strongly chelates some cations such as Zn making them unavailable to human beings.

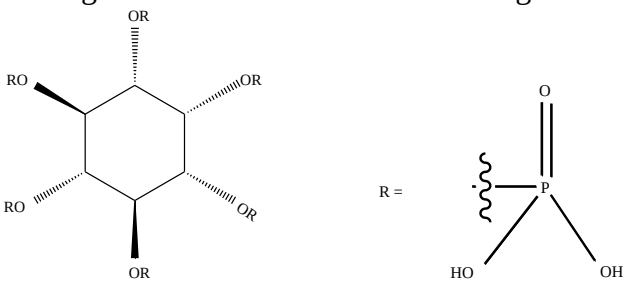

Figure 2.1: Phytic acids

Source: Kumar *et al*., 2010

The small intestines of the human is devoid of phytate-degrading enzyme and also the microbial population in the upper part of the digestive tract is limited. This makes phytate-mineral complex remains partially hydrolysed in the human gut. The pH is an important factor influencing the solubility of phytate, it being more soluble at lower than at higher pH values. Calcium ion, Cd^{2+}, Zn^{2+} and Cu^{2+} salts tend to be soluble at pH lower than 4 - 5 whereas Mg-phytate is soluble at acid pH up to pH 7.5 (Kumar *et al*., 2010).

Foods of vegetable origin rich in fibre, minerals and vitamins, also bring substances to the diet that although, not well understood nor classified as nutrients, display potent anti - carcinogenic and curative effects on a variety of diseases and illness, these substances are known as phytochemicals. *Citrullus lanatus* contains anti-nutritional components such as saponin, alkaloid, hydrocyanic acid, phenols, oxalate, tannins and phytates in varying concentration (Forester, 2006). Saponins have bitter taste and can reduce plant palatability, some of which are toxic to cold blooded animals at a particular concentration. An intake of 4 - 9 mg/100 g of phytic acid is said to decrease iron absorption by 4 - 5 folds in humans and the lethal level of oxalate in man is 3 - 5 g (Munro and Bassir, 2010). A daily intake of 450 mg of oxalic acid interferes with various metabolic processes (Amaowo *et al*., 2000). Tannins are known to affect the digestive tracts and their metabolites are toxic (Johnson *et al.*, 2012).

Because of its high density of negatively charged phosphate groups, phytic acid (PA) forms very stable complexes with mineral ions rendering them unavailable for intestinal uptake. The carrier proteins found in duodenum are subject to competition among the ions for their binding and consequential absorption. Copper is preferentially bound to transferring, the protein transport molecule in the mucosa, when competing with iron. A metal that activates a specific enzyme can be replaced by another metal that may either block or accelerate that particular enzymatic activity. Carboxypeptide enzyme is activated by Zn which can be replaced by cobalt which causes a decrease in the enzyme activity. The availability of a metal ion for mucosal absorption is generally dependent upon the pH of the intestinal lumen. A mineral must remain in ionic state for it to be absorbed in the intestine (Walter *et al*., 2002).

Dietary proteins can potentially facilitate Zn absorption even in the presence of phytic acid. Proteins prevent the precipitation of zinc in the intestinal lumen and amino acids such as cysteine or peptides which facilitate Zn uptake by the mucosal cells. Consuming fermented foods leads to enhanced Zn absorption due to the presence of organic acids (acetic, citric, lactic or malic acids) which form soluble ligands with Zn, thereby preventing the formation of insoluble Zn phytates. Zinc in foods is relatively easily solubilized at gastric pH, where it binds to organic components at higher pH. Small molecular weight ligands such as organic acids have the potential to increase solubility and facilitate absorption of zinc. Phytic acid enhances Cu absorption because of its ability to bind Zn, thus counteracting its capacity to compete with Ca at the intestinal absorption sites. Fermentation of non digestible carbohydrates such as resistant starch, pentosans or fructans by flora results in the production of short chain fatty acids particularly acetate, propionate and butyrate. These acids form complexes with ions with very low charges able to cross the cellular membrane (Walter *et al*., 2002).

Zinc absorption involves saturable carrier mediated components and non specific unsaturated diffusion controlled process. The complex Zn containing species is broken down by digestive process into free zinc after taking food and then complexed by ligands from pancreas, food and other endogenous factors like citric acid, picolinic acid, histidine and cysteine. The micro villus transport complexed Zn metal into hypothetical 'zinc pool' where it is transferred to liver by albumin and ferritin. Zinc is stored, processed and bound to metallothionen for redistribution in the liver. Metal-free albumin available at the basolateral membrane regulates the amount of Zn that enters the body while metallothionen controls serum Zn level. Absorption of Zn is

also controlled by transmembrane polypeptide and Zn transfer proteins found in the crypts and lower villi. Over absorption of Zn is prevented by sloughing off the remaining Zn in the intestine cells with some cells and excreted (Lusi *et al*., 2011).

All mineral content present in a diet may not be available for absorption and/or utilization for normal health and physiological functions of humans. The proportion of total mineral content in the food utilized for normal body functions is determined by bioavailability in a more general way (Kulkarni *et al*., 2006). Chemical speciation is of interest in environmental analytical chemistry because the behaviour of trace elements in natural systems depends on the forms, as well as the amounts which are present. Since the behaviour of the elements in a soil-water-plant system depends on their forms, the determination of trace elements in plant material is often performed by single or sequential extraction. The procedures involve subjecting a ground plant material to successive attacks with reagents possessing different chemical properties (acidity, redox potential or complexing properties) in which each extract includes a part of the trace elements associated with the sample (Serife *et al*., 2003). Solubility is one of the major factors influencing bioavailability and absorption of metal and metal compounds. The solubility of a metal compound depends on its chemical species, on the pH of its medium (H^+ ions) and on the presence of other chemical species in the medium. Also particle size influences absorption of poorly soluble compounds: fine particles are usually more soluble (Foulkes, 2000). Bioavailability is the proportion of total metals that are available for incorporation into biota (bioaccumulation). Total metal concentrations do not necessarily correspond with metal bioavailability (Davis *et al*., 1994).

Total Zn content and the level of other constituents in the diet that affect Zn bioavailability determine importance of a given foodstuff as a source of dietary Zn. Molar ratios of phytate to Zn in the diet greater than 20 are associated with biochemical and/or clinical evidence of Zn deficiency (Akindahunsi and Oboh, 1999). Elements that have a similar coordination number and a similar configuration in water solution compete for absorptive pathways. Zinc and Cu can interact where an excess of one element induces a deficiency of the other. Also a deficiency of one element may affect metabolism of another element. Copper deficiency causes iron-deficiency anemia. Copper is a component of ceruloplasmin (ferroxidase 1) where its deficiency decreases ferroxidase activity thereby preventing mobilization of Fe from stores (by being oxidized from +2 to +3 and its incorporation into haemoglobin. Insoluble cereal and vegetable fibres exacerbate the adverse effect of phytate on Zn absorption especially when protein intakes are low. Amount and type of protein can affect Zn bioavailability. Inclusion of small amounts of animal and fish protein increases the apparent absorption of Zn and counteracts the negative effect of phytic acid. Phytate degradation by microbial phytase is less in high-tanning cereals such as brown sorghum and finger millet, possibly because of the inhibitory effect of polyphenols on phytase activity (Adrianne *et al*., 1996).

Ceruloplasmin, albumin and other quantitatively less important Cu binders mediate the distribution of Cu in the body. Males have lower plasma Cu concentrations than females. If fructose is the predominant carbohydrate in the diet Cu status is deteriorated. Amino acids are proposed to be mandatory ligands for the uptake of Cu by the brush border membrane. Cysteine is an effective chelating agent for copper, but in addition it produces a reduction in bioavailability due to the reduction of Cu

from divalent to monovalent state. Glutathione forms an intermediary complex with Cu in enterocyte before transporting cation to other protein, namely superoxide dismutase and metallothionein. Absorption of Cu is lowered after copper interacts with ascorbic acid due to reduction of cupric to cuprous copper. When ascorbate is administered during post absorptive period, Cu tissue utilization is enhanced. Ascorbate reacts with ceruloplasmin in tissues facilitating transfer of the element across certain membranes. Monovalent Cu that results from ascorbate electron transfer is the form that gets absorbed from ceruloplasmin by select types of cells. Presence of organic acids such as citric, lactic, acetic and malic acids in foods leads to solubilization of Cu thereby increasing its bioavailability. When Zn intake is high and protein consumption is low it can lead to marginal Cu deficiency (Raul, 1998).

Organic Zn complexes especially from oysters are more readily absorbed than inorganic Zn salts. The organic Zn form appears to be less affected by absorption modifiers than the inorganic forms. Metal ions such as Zn, Fe and Ca are chelated by phytate, in the gastrointestinal tract rendering them unavailable for absorption. *Myo* - inositol phosphates with fewer than five phosphate groups do not have a negative effect on Zn absorption. Like phytate, polyphenols form insoluble complexes with metal cations that inhibit intestinal absorption of nonheme iron, perhaps Cu and Zn, but not Ca or manganese. Inhibitory effect of bioavailability of trace elements such as Fe and Zn by oxalic acid is less clear. Inhibitory effect of Cu absorption by vitamin C is still uncertain. Organic acids (citric, lactic, acetic, butyric, propionic and formic acids) which are produced as a result of fermentation of vegetables have the potential to form soluble ligands with trace minerals in the gastrointestinal tract facilitating absorption of nonheme Fe and Zn (Rosalind, 2007).

The risk of deficiencies and attendant pathologies of minerals from fruits and vegetables depends on factors such as the daily dietary intake, the chemical form of the minerals in the food consumed, technological treatment of the products, presence of substances that limit or increase the bioavailability of minerals and the physiological state and overall health of the consumer. The contents of trace elements in plants are low, are critical in terms of biological activity and when incorporated into mineral complexes, their ability is enhanced (Faik *et al.,* 2001). Absorption depends on nutrient appropriate form (for instance charged, complexed), necessary absorptive transport systems are in place in the gut (this depends in part on the individual's nutritional status), presence in the food matrix of inhibitory or promotive substances (Michael, 1999). Zinc bioavailability in a vegetarian diet is lower because of high intakes of phytic acid and fibre. Vegetarians' diets may influence Cu utilization or distribution without affecting Cu absorption. Copper absorption is not affected by presence of phytic acid and cellulose (Janet *et al.,* 1998).

Phytate is considered to be the most potent dietary inhibitor of Zn bioavailability where oxalate, fibre, EDTA and polyphenols such as tannins are other inhibitors. Zinc inhibitors can be reduced through appropriate food processing technologies adopted in households. Through fermentation of food, phytases are produced which breakdown phytates increasing the amount of available Zn. Amino acids such as histidine and cysteine are promoters of Zn absorption. Diets with a phytate-zinc molar ratio greater than 15 have relatively low Zn bioavailability, those with phytate-zinc molar ratio between 5 and 15 have medium Zn bioavailability, and those with a phytate - zinc molar ratio less than 5 have relatively good Zn bioavailability. Release of amino-acid and cysteine-containing peptides during digestion of cellular animal proteins,

enhances Zn absorption. Soaking cereals such as oat followed by sprouting reduces phytate content and doubles the amount of absorbed Zn in comparison with untreated oats. Leavening products improves Zn content. Soaking and germination enhances enzymatic hydrolysis of phytates. The initial enzymatic changes that precede germination breaks down the higher carbohydrates and other storage molecules such as Ca, Mg and phytate. Fermented products are low in soluble fibre and high in insoluble fibre. Organic acids produced during fermentation such as citric, formic, butyric, lactic and acetic acids potentiate Zn absorption by forming ligands with Zn. Absorption of other minerals such as Ca and Fe is enhanced by reduction of phytates in the diet (Walingo, 2009).

Organic acids promote bioaccessibility of mineral fortificants in fruit beverages. Calcium, casein and polyphenols inhibit bioaccessibility of mineral fortificants in fruit beverages. The interaction of minerals such as Zn and Fe which have similar electronic configurations and share absorptive pathways may lower bioavailability of both minerals. Organic acids in fruit juices yield low-molecular weight soluble chelates with Zn, thereby promoting Zn solubility (Antonia *et al.*, 2009). Presence of oxalate in food causes irritation in the mouth and interferes with absorption of divalent minerals particularly calcium by forming insoluble salts with them. Phytate in food binds some essential mineral nutrients strongly rendering them unavailable for absorption (Muhammad *et al.*, 2011). Bioavailability and absorption of Zn may be inhibited by certain dietary constituents that are abundant in some vegetarian diets such as oxalates in vegetables and phytates in cereals and legumes, tannins in tea and coffee and possibly soy protein. Zinc from animal sources is more bioavailable than Zn from plant foods. Insoluble fibre, phytate, protein and some minerals such as Fe,

Ca and P can reduce Zn absorption. Food preparation techniques such as leavening bread, soaking and sprouting beans, grains and seeds, could reduce binding of Zn by phytate and increase Zn bioavailability (Duo, 2011).

Zinc and Cu absorption is enhanced by an increase in total protein where the enhancing effect is even greater if the protein is from cellular animal sources (Bjorn and Hallberg, 1979). The absorption of nonheme Fe and Zn from plant based foods is enhanced by flesh foods (meat, poultry, fish and sea food). Excess zinc (25 or 50 mg/day) decreases biochemical indices of copper status (Fischer *et al*., 1984; Yadrick *et al*., 1989). Iron supplements inhibit Zn (but not Cu) absorption by both uptake and transfer through the intestinal cell. Germination reduces polyphenol content of some legumes as a result of complexation with proteins and gradual degradation of oligosaccharides. Reduced gastric acid secretion can affect negatively absorption of Fe, Ca and Zn because gastric acid affects the solubilization of these inorganic nutrients (Rosalind, 2007).

Trace metals may exist in a form where they are reversibly bound to inorganic anions as Cl^-, F^-, CO_3^{2-} and ^-OH. Trace metals may also be bound to organic ligands such as amino acids. In order to comprehend the environmental chemistry of an element it would be necessary to characterize in full the proportions and chemistries of all its various forms under the diverse range of conditions possible in natural systems. Whilst this is clearly impracticable, speciation science seeks to characterize, at least some of the most important forms of an element and finally infer from such information the likely consequences to the environment (Kolodynska, 2011). In the last two decade analytical scientists have understood the mechanisms that control the

mobility, distribution and bioavailability of metals. The fate, transport, toxicity and biological activity of trace metals and the counter ions in plants are critically controlled by chemical and physical associations that they undergo in natural systems, that is, in their speciation (Ure and Davidson, 1995).

It is common knowledge that depending on a particular environmental system, a high total metal concentration may be less toxic than another with a lower metal concentration. It is also known that the more stable the metal complex, the lower is its toxicity. Metal complexation with certain ligands therefore, has important consequences with regard to their toxicity. This is because complexed forms are known to be less toxic than the free metal ions. A good example is Cu where the hydroxyl complexes are less toxic than the free copper ions (Bowen, 1979). Stable isotope composition is important from both theoretical point of view and in physical and environmental chemistry but is generally of minimal importance in risk assessment concerning human health. Elemental speciation at the macromolecular level has biological significance in physiology, biochemistry and nutrition. Organic complexation is of intermediate importance as most chelates are labile relative to covalent complexes; they influence bioavailability and cellular uptake. Valence state and inorganic and covalent organometallic speciation are of great importance in determining the toxicity of elements (Apostoli *et al.*, 2006). The toxicity of compounds varies in relation to the compound for example for Sn, mono- and dialkylated species are less toxic than trialkylated ones. The toxicity of organometallic species also varies with the organism monitored. For example, trimethyltin is more toxic for insects, triethyltin for mammals and tributyltin for fish, fungi and bivalves such as shellfish (Agata and Jacek, 2000).

Specific proteins bind Cu in foods of plant or animal origin while a small proportion is chelated by numerous small nutrient molecules particularly amino acids such as histidine. Copper tends to be in the cupric state in biological systems though it also exists as Cu^{1+} (Maria and Maryam, 1996). The predominant forms of Zn in plants are low molecular weight complexes, storage metalloproteins, free ions and insoluble forms associated with cell walls (Brian, 2008). The water soluble Zn in fruits occurs mainly as an anionic compound possibly associated with amino acids. The water insoluble forms of Zn forms strong complexes with radicals of polar groups containing sulphur, nitrogen and oxygen (Brown *et al*., 1993). The highest elemental concentrations in the water soluble fraction (49 - 75) % for Zn and Cu from plant matrix are complexes with polypeptide ligands (phytochelatins and metallothioneins). Low molecular weight, soluble anionic Zn complexes comprise the majority of the naturally occurring forms of Zn in edible portions of food crops soluble in polar solvents (Joanna *et al*., 1999). The forms of Zn present in plant tissues depend on plant species, tissues studied and concentration of Zn in that tissue (Kupper *et al*., 2004; Sarret *et al*., 2009; Monsant *et al*., 2011). In watermelon fruit (20 -50) % of the Zn is present as Zn^{2+}, (40 - 99) % is associated with vacuolar carboxylic acids such as malate, oxalate and citrate. Upto 45 % is associated with histidine and remainder is largely bound to phosphate groups and cell wall components (Salt *et al*., 1999; Kupper *et al*., 2004; Sarret *et al*., 2009; Monsant *et al*., 2011; Philip and Martin, 2011).

Sara (2006) working on untreated pumpkin seeds reported 39 % bioavailable Zn. Carbanaro *et al*. (2001) working on cooked beans reported 15 % bioavailable Cu

which they attributed to formation of an insoluble complex of polyphenol proteins under heat treatment. Vitali *et al*. (2008) reported (46.7 - 69.1) % bioavailable Cu in tea leaves and attributed the bioaccessibility of an element to largely depend on food matrix components of a given fruit or vegetable. About (35 - 50) % bioavailable Zn and Cu was found by Anna *et al*. (2014) working on muskmelon fruit juices. Konieczynski and Wesolowski (2013) found 14 % and 40 % bioavailable Zn in *Calendulae* herbal fruit and *Calundulae* herbal leaves respectively. In addition Konieczynski and Wesolowski (2013) reported 30 % and 12 % bioavailable Cu in *Calendulae* herbal fruit and *Calundulae* herbal leaves, respectively.

Copper exists in four oxidation states; Cu^{0}, Cu^{+1}, Cu^{+2} and Cu^{+3}. Cuprous copper (Cu^{+1}) exists only in water solution when complexed, usually in a tetrahedral form with affinity for sulphur and nitrogen ligands. Cuprous copper is unstable in aerated aqueous solution over the pH range 6 to 8 and undergoes auto oxidation reduction into elemental copper (Cu^{0}) and cupric ion (Cu^{+2}). The cupric ion (Cu^{+2}) is the one generally encountered in water. Cupric ion is the dominant toxic copper species at pH levels less than 6 (Eisler, 1997). The Cu^{+3} occur as a complex stabilized by electropositive element (Moira, 2005).

High concentrations of metals such as Zn and Cu in a plant lead to non - synthesis of amino acids which promotes metal absorption in human beings (Ashraf *et al*., 2011). Although the amounts of diethylenetriaminepentaacetic acid (DTPA) - extractable metals do not absolutely represent the actual quantities of soil metals that can be taken up by plants, they do appear to be good indicators of the potentially bioavailable quantity (Wang *et al*., 2006). It is likely that most protein-bound/ionic metals in plant

tissues, together with a proportion of metals bound to ion exchange sites on inorganic soil particles could become readily available for assimilation (Hunter *et al*., 1987).

Metallothioneins (MT) are low molecular weight proteins (<10000 Da) that confer a remarkable ability to bind significant numbers of mono- or divalent metal ions. In invitro process any given MT binds Cu^{1+} more strongly than Zn^{2+}. The number of metal ions that can be bound to a MT, no matter from which species or phylum, depends on the number of thiolate sulfurs (or in some cases other metal binding residues, particularly histidines), the preferred coordination number and geometry of the respective metal ion, and also the protein fold. For each MT, there are an ideal number of metal ions which results in a well structured protein with all cysteine thiolates bound to at least one metal ion greatly reducing susceptibility towards air oxidation of a thiol. Metal speciation depends on how well a protein fold can accommodate a respective metal thiolate clusters (Oksana *et al*., 2013). Toxicity of an element occurs due to presence of a toxic species or when recommended daily allowance (RDA) of an element is exceeded. In this study, we shall treat toxicity on exceedence of RDA of an element and not presence of a toxic species.

2.6 Methods for separation of species

Classical sequential extraction methods are based on the principle of selective dissolution of predefined phases and analyzing the released metals with an element specific detector. These schemes which are equilibrium based normally take (8 - 16) hours and suffer generally the problems of non-selectivity of reagents used; re-adsorption and re-distribution. Continuous extraction methods are more suitable for risk assessment studies since the environmental processes they simulate are also dynamic in nature (Von and Modupeola, 2006). The ‘bioavailability’ has been used to

compare trace elements extracted from plant materials by some live organism, as plants and biota (Giuliano, 2005). A variety of separation techniques have been employed in speciation analyses including: gas chromatography (GC) (Ali, 2016); liquid chromatography (LC), capillary electrophoresis (CE) and supercritical fluid chromatography (SFC). The LC, however, has emerged as one of the most popular separation techniques for elemental speciation analysis (Jules *et al.*, 2014; Ali, 2016). This study used gas chromatography due to its availability, ability of separating and identifying organic compounds complexing to Zn and Cu after being interfaced with mass spectroscopy.

Sequential extraction method is employed to extract the plant powders using solvent of different polarity, from non polar to polar (Pathmanathan *et al*, 2010). Solvent extraction of plant material results in the mass transfer of soluble active principle to the solvent, and this takes place in a concentration gradient. The rate of mass transfer decreases as the concentration of active principle in the solvent increases, until equilibrium is reached (Handa *et al.*, 2008).

2.7 Method of trace elements analysis

The element - selective detection techniques generally used for speciation purposes include; atomic absorption spectroscopy (AAS) (Ali, 2016); atomic emission spectroscopy (AES); atomic fluorescence spectroscopy (AFS) and inductively coupled plasma mass spectroscopy (ICP - MS) (Jules *et al.*, 2014; Ali, 2016). In comparison with AES and ICP - MS, AAS is the more popular detection method (Ali, 2016). For this study, the AAS was used due to its availability, reproducibility and time efficiency.

2.8 Principle of atomic absorption spectrophotometry (AAS)

The AAS is a method of elemental analysis that works on the principle of absorption of radiation energy by free atoms. Each metal absorbs characteristic energy corresponding to specific wavelength. The characteristic wavelengths are element specific and accurate to (0.01 - 0.1) nm. The AAS instrument looks for a particular metal by focusing a beam of UV light at a specific wavelength through a flame and into a detector. The sample of interest is aspirated into the flame which converts the metal compounds in the sample into gaseous atoms of the metal that absorbs some of the light, thus reducing its (transmission) intensity. The transmitted radiation is converted into an absorbance (Garcia *et al.*, 2009). The AAS instrument has a source of radiation, atomizer, monochromator, detector and read out system. Figure 2.2 shows a schematic diagram of AAS.

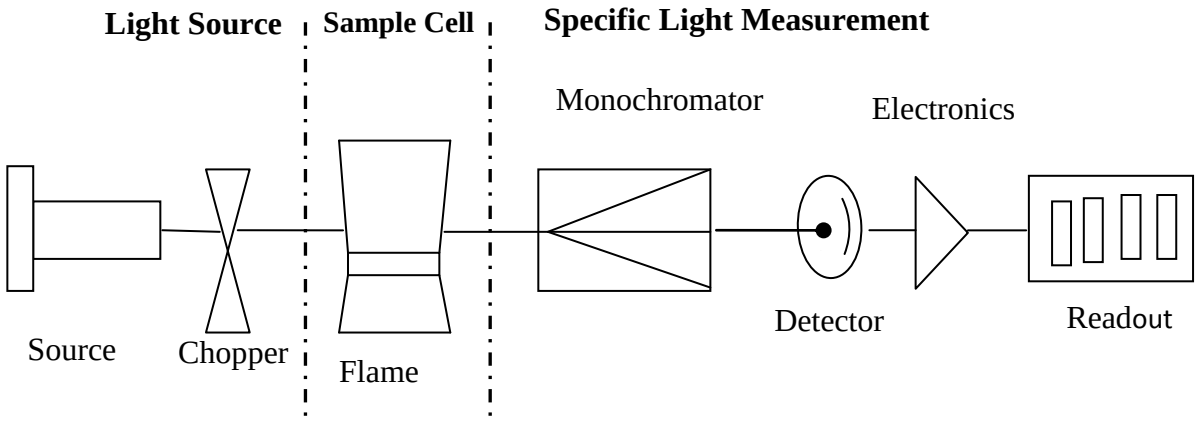

Figure 2.2: Schematic diagram of AAS

Source: Skoog and Leary, 1992

2.8.1 Sources of radiation

Sources of radiation used in AAS can be categorized as line or continuous depending on band width of their wavelength. Continuous sources of radiation which are less sensitive emit light over a broad spectrum of wavelength. Xenon arc, deuterium lamps and mercury lamps are examples of continuous sources of radiation. Line sources of radiation are more sensitive than continuous sources as they have bandwidths that are narrower than absorption peaks. Hollow cathode lamps, electrodeless discharge lamps and laser beams are examples of line sources of radiation.

2.8.2 Atomisers for atomic spectroscopy

Atomisers provide high temperature source for disolvating and vapourising sample to obtain free atoms for spectroscopic analysis. Flame atomiser and electrothermal atomiser are commonly used in atomic absorption spectroscopy. Laser atomiser is only used for specialized studies in research laboratory.

2.8.3 Monochromators

Monochromators disperse or separate radiations so that the selected wavelength corresponding to a particular energy of interest is transmitted. Monochromator has lenses or mirrors that focus radiation, entrance and exit slit to restrict unwanted radiation and dispersing medium to separate wavelengths. Prism and diffraction grating are two types of dispersing elements.

2.8.4 Detectors

Detectors convert radiant energy into electrical signal. Phototube, photomultiplier tube and photo diode arrays are common types of detectors (Skoog and Leary, 1992).

2.8.5 Read out system

The read out system is digitized and interfaced with microprocessors which allow programming of various operations leading to simplicity. The AAS was used due to availability, sensitivity, selectivity and reproducibility.

2.9 Principle of gas chromatography mass spectroscopy (GC - MS)

The GC - MS is a technique performed on a sample identification and quantification according to mass/charge ratio and works on the principle that a mixture separates into individual gaseous substance when heated. These gaseous compounds are separated by GC and then characterized and quantified by the MS (Bowers *et al.*, 2002). Figure 2.3 shows a schematic diagram of GC - MS.

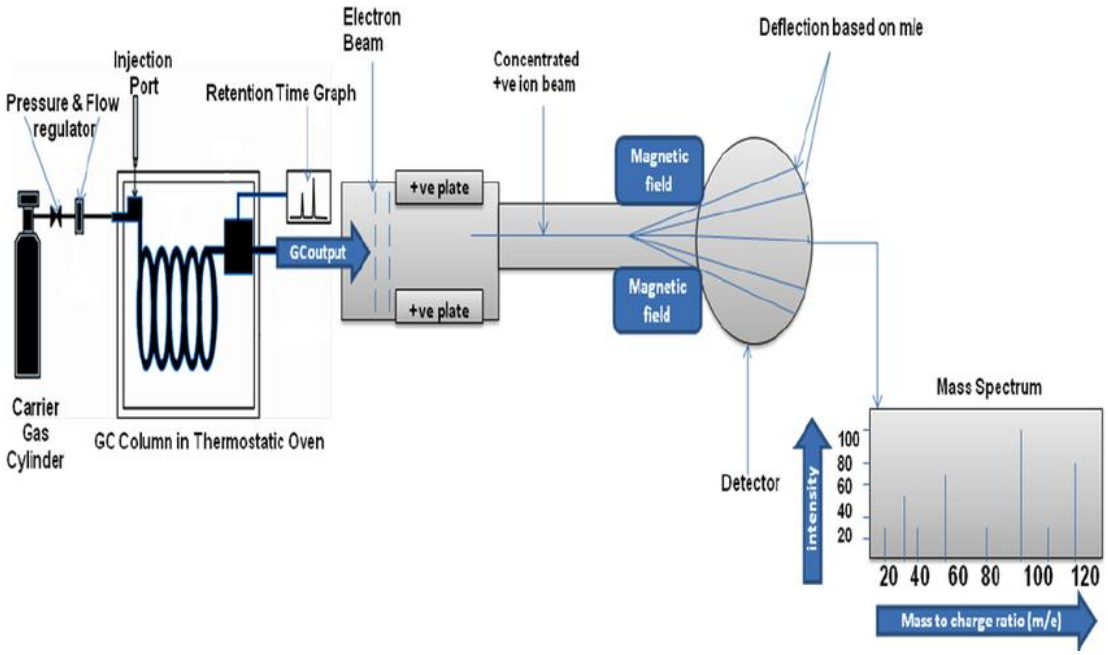

Figure 2.3: Schematic diagram of GC - MS

Source: Zenzen *et al*., 2010

CHAPTER THREE

MATERIALS AND METHODS

3.1 Research design

The study used experimental design which involved sampling of Charleston grey, crimson sweet and sugar baby varieties of watermelon from Ngara and Mwea markets in Kenya. Each fruit was separated into peels, seeds, red flesh and white flesh. Each part of watermelon was digested using nitric acid and also sequentially extracted using various solvents to determine levels and bioavailable forms of Zn and Cu, respectively. Levels of Zn and Cu were analysed using AAS and forms of Zn and Cu determined through GC - MS analysis.

3.2 Sampling sites

The main criterion for selection of sampling sites was the availability of watermelon varieties. Ngara market was considered because of its nearness to Nairobi and the fact that it receives watermelons throughout the year from all over the country and other neighbouring countries. Watermelons are grown throughout the year at Mwea by irrigation. Sampling was done once where all the available species were obtained from each market. Samples were obtained from three vendors and three watermelons of each variety were collected per station giving a total of fifty four watermelon samples.

3.3 Cleaning of apparatus

All plastic and glass ware apparatus were washed with liquid detergent in hot water followed by soaking overnight in 10 % analytical grade nitric acid. Finally the wares

were rinsed with distilled water and later dried in an oven while the plastic bottles were dried in open racks.

3.4 Chemicals and solvents

All reagents used in this study were of analytical grade with > 98 % purity. The dichloromethane was purchased from Sarabhai M. chemicals in India, manufactured in 2012. Ethanol was from Scharlau chemicals in Spain manufactured in 2012. Nitric acid was from Rankem Molychem in India, manufactured in 2011. Hexane was from Molychem in India, manufactured in 2011. Hydrogen peroxide was from Merck chemicals in Germany, manufactured in 2011. Zinc nitrate and copper nitrate were both from Merck chemicals in Germany, manufactured in 2011.

3.5 Equipments and atomic absorption spectrophotometer (AAS) operating conditions

The equipments used in this study included shaking water bath (Model DKZ - 1, made in 2000), analytical balance (Model AAA, Adam Co Ltd, made in 2008), Gas Chromatography (Model G3243A, made in 2005) and Varian Atomic Absorption Spectrophotometer (AAS) (Model AA - 6300 Shimadzu, made in 2002). The operating conditions for the AAS are given in table 3.1

Table 3.1: The AAS operating conditions

Operating parameters	Zn	Cu
Wavelength (nm)	213.9	324.7
Slit width (nm)	0.5	0.5
Flame type	Air/acetylene	Air/acetylene
Oxidant flow rate (L/min)	0.9	4.5
Sensitivity (ppm)	0.01	0.01
Lamp current (mA)	75	20
Optimum working range (ppm)	2.0 - 8.0	2.0 - 8.0

3.6 Preparation of standards

Stock solutions were prepared from salts of high purity (> 98 %). Each metal and salt were first dried at 105 °C followed by cooling in desiccator prior to weighing and transferred into 1 litre volumetric flasks. Stock solutions containing 1000 mg/L of the analytes were prepared from nitrate salts of Zn and Cu in 1 % of HNO_3 followed by serial dilutions.

3.7 Sample collection and pre - treatment

Samples obtained were placed in separate labeled plastics to maintain their freshness and then were transported to KU, Department of Botany for identification and then taken to chemistry laboratory. Each of the watermelons was cleaned using distilled water, cut and the four parts (seed, peel, red and white flesh) separated. The four parts were weighed and then dried at 105 °C in a gravity oven for at least 24 hours to constant weight for complete drying. The powdered samples were ground using an agate mortar and pestle to reduce them to homogeneous fine powder and sieved (600 µm mesh) for removing large undesired particles. To avoid contamination aluminium foil and containers were used to hold samples in the oven. The ground samples were stored in well labeled plastic bags awaiting extraction and analysis.

3.8 Preparation of samples for atomic absorption spectrophotometer (AAS)

Total concentration was determined by digesting one gram of the dried watermelon part with 7 mL of HNO_3 (65 %) and 1 mL of H_2O_2 (30 %) in a beaker on a hot plate at 200 °C for 15 minutes and then cooling. Filtration was done and filtrates put in 25 mL volumetric flask and made up to the mark with distilled water. Ground samples were re-dried at 105 °C in an oven for an hour then cooled in desiccators. Thirty seven and half grams (37.5 g) of the powdered dried watermelon part were extracted following

the method adopted by Loiy *et al.* (2010). Extraction of each part was performed sequentially using hexane, dichloromethane, ethanol, water and the remaining residue digested using concentrated nitric acid. The various watermelon parts were extracted at the beginning with 112.5 mL hexane for 24 h in conical flask using cool maceration with occasional shaking. Extracts were filtered using filter paper and the extraction procedure was repeated thrice. The filtrates were combined and concentrated using rotary evaporator (model 210 VGP made in 2007) to 25 mL. The resulting plant residue was dried using the rotary evaporator and extracted with 112.5 mL dichloromethane, 112.5 mL ethanol (90 %), 112.5 mL distilled water, respectively, following the same methods as done previously for hexane.

One gram of the remaining watermelon residue part and 5 mL of the concentrated extracts were separately digested by addition of 7 mL of HNO_3 (65 %) and 1 mL of H_2O_2 (30 %) to each in a beaker on a hot plate at 200 °C for 15 minutes and then cooled. Filtration was done and filtrates put in 25 mL volumetric flask and made up to the mark with distilled water. Concentrated extracts were kept at 4 °C in desiccators for further assays which were done in triplicate (Loiy *et al.*, 2010). The samples were then analyzed in triplicate by flame atomic absorption spectrophotometer (Model AA - 6300 Shimadzu, made in 2002). For the extract that showed presence of Zn and Cu the remaining extracts were analysed for possible function groups that could be complexing with the metals using GC - MS.

3.9 Elemental species analysis and method validation

3.9.1 Elemental species analysis

Analysis of Zn and Cu was done in triplicates using computerized Varian Atomic Absorption Spectrometer model AA - 6300 Shimadzu made in 2002. The samples,

standards and blanks were analyzed in triplicates. For precision, standards were measured before and after the sample solution. The calibration of the instrument using standards and blank was frequently done between samples to ensure stability of the base line. Different fractions were analyzed to determine presence of the essential elements using AAS. The GC (Model G3243A, made in 2005) was used to separate and determine possible functional groups that could be complexing to the metals.

3.9.2 Method validation

To validate the procedure the instrument was calibrated by analyzing calibration solutions that produced responses of 0.000 absorbance and standards of known concentration for each element. Quality checks were performed on the instrument by checking the absorbance after every fifteen sample runs (Fagbote and Olanipekun, 2010). Flame - AAS method was calibrated by running standards and the absorbances obtained were used to calculate correlation coefficient (R^2) values. Regression analysis was used to calculate the linearity of the calibration curves.

A recovery test was conducted to determine the accuracy of the analytical procedure. The recovery test was investigated by spiking a suitable known amount of the analyte elements into a test portion of the sample having a known concentration of the analyte and analyzing the spiked test portion along with the original sample. The precision of the method was expressed as a percent relative standard deviation (% RSD) of the triplicate analyses. When the test solutions did not agree with the recommended range of (90 - 110) % recovery and ± 5 relative differences (Hight, 1998), the test solutions were again prepared and re-calibration of instrument done to analyze the test solutions. Recovery test was done by spiking 10 mL aliquot of 5 µg/mL Zn and 20

µg/mL Cu into separate conical flask containing 1 g of dried watermelon samples. Same digestion procedure was followed for non-spiked and spiked watermelon samples side by side. Each sample was analyzed for their respective spiked metals by atomic absorption spectrophotometer (AAS). The concentration of the unspiked sample was subtracted from the concentration of the spiked sample to determine the amount recovered. The recovered amount was divided by the concentration of the known standard added (spiked) and multiplied by 100 and expressed as a percentage recovery.

3.10 Data analysis

The mean values that were obtained for Zn and Cu analyses in the four parts of watermelon varieties samples were compared by one-way analysis of variance (ANOVA) at 95 % level using statistical package for social science (SPSS 11.0). The student t - test was used to compare the means in levels of the elements between the two markets.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Introduction

Bioavailabilities mean levels of Zn and Cu from selected samples of peel, white flesh, red flesh and seeds of Charleston grey, crimson sweet and sugar baby watermelons by sequential extraction using hexane, dichloromethane, ethanol and water on dry and fresh watermelons were determined in triplicates using AAS. Signal responses of AAS obtained are discussed in the following sub sections.

4.2 Method validation

4.2.1 Regression analysis and detection limit

The correlation coefficients (r) were calculated using absorbance readings and concentration of ideal standards. A plot of absorbance readings (y - axis) against the corresponding concentration (x - axis) of standards gave the calibration curves. The established calibration curves were used to evaluate the linearity of AAS through regression analysis. The detection limits, the correlation coefficients, the equations of the calibration curves and the range of standards used to establish them are represented in table 4.1.

Table 4.1: Detection limits, correlation coefficients and equations of the calibration curves for the determination of essential elements in watermelon samples by AAS

Element	Method detection Limit µg/ml	Concentration range of standards µg/ml	Correlation coefficient of calibration curve	Equations for calibration curve
Zn	0.015	0.0 - 8.0	0.996	Y = 0.143x
Cu	0.0090	0.0 - 8.0	0.999	Y = 0.190x

x - Concentration, y - absorbance

From Table 4.1 there was a very good correlation between concentration and absorbance as the correlation coefficients of all calibration curves were ≥ 0.996 (Gareth, 2011). The method detection limits for all the metals were < 0.1 µg/mL suggesting the method was applicable in determining essential elements at trace levels.

4.2.2 Recovery test

The recovery test for watermelon samples was performed in triplicates and the results are indicated in table 4.2.

Table 4.2: Recovery test results for the metals (percentage)

Elements	Concentration (µg/mL)			Mean % recovery	% RSD
	Un-spiked sample mean ± SE	Standard added to the sample	Spiked sample mean ± SE		
Zn	3.56 ± 0.02	5.00	8.57 ± 0.01	100.2 ± 0.57	0.99
Cu	0.42 ± 0.01	20.00	20.41 ± 0.04	99.95 ± 2.37	4.1

Table 4.2 results indicate that the percentage recovery lies within the range (99.91 - 100.2) % which is within the acceptable range for the percentage recovery of (90 - 110) % and RSD (0.99 - 4.1) and within the acceptable range for all elements ± 5 % (Hight, 1998). This confirms that the method was of good precision and accuracy and therefore the results presented in this thesis are valid.

4.3 Levels of Zn in watermelons

Total mean levels of Zn in various varieties of watermelons are discussed in the following sub sections.

4.3.1 Total mean levels of zinc in parts of Charleston grey (CG), crimson sweet (CS) and sugar baby (SB) watermelons

The results for comparison of total mean levels of Zn in the same parts of CG, CS and SB watermelons samples obtained from Ngara and Mwea markets are discussed in the following sub sections. The comparison of the total mean levels of Zn in dry peel, white flesh, red flesh and seeds of CG, CS and SB watermelons purchased from Ngara and Mwea markets were calculated and compared at $\alpha = 0.05$ level as shown in table 4.3.

Table 4.3: Comparison of total mean levels of Zn (mg/100 g) in the same part of dried CG, CS and SB watermelons from Ngara and Mwea markets, n = 18

market	part	Charleston grey	crimson sweet	sugar baby	P - value
Ngara	peel	7.59 ± 0.08^{Aa}	7.27 ± 0.03^{Aa}	9.48 ± 0.03^{Bb}	< 0.001
Mwea		9.03 ± 0.03^{Bc}	7.27 ± 0.03^{Ab}	4.74 ± 0.02^{Aa}	< 0.001
P - value		< 0.001	1.000	< 0.001	
Ngara	White flesh	7.86 ± 0.03^{Ab}	7.50 ± 0.03^{Ba}	7.95 ± 0.01^{Bc}	< 0.001
Mwea		9.71 ± 0.02^{Bc}	6.96 ± 0.03^{Ab}	5.56 ± 0.01^{Aa}	< 0.001
P - value		< 0.001	< 0.001	< 0.001	
Ngara	Red flesh	6.11 ± 0.02^{Aa}	9.11 ± 0.01^{Bb}	9.22 ± 0.08^{Bc}	< 0.001
Mwea		6.55 ± 0.03^{Aa}	6.52 ± 0.02^{Aa}	7.19 ± 0.03^{Ab}	< 0.001
P - value		< 0.001	< 0.001	< 0.001	
Ngara	seed	7.45 ± 0.01^{Ac}	6.13 ± 0.03^{Aa}	7.15 ± 0.03^{Ab}	< 0.001
Mwea		7.35 ± 0.03^{Ab}	6.02 ± 0.01^{Aa}	7.56 ± 0.02^{Ac}	< 0.001
P - value		0.016	0.010	< 0.001	

Mean levels of Zn compared at $\alpha = 0.05$. Means with same small and capital letters in a given row and column respectively are not significantly different (one way ANOVA, SNK test).

4.3.1.1 Total mean levels of Zn in dried peel of Charleston grey (CG), crimson sweet (CS) and sugar baby (SB) watermelons

From Table 4.3, total mean levels of Zn in peels from Ngara market ranged from 9.48 ± 0.03 mg/100 g to 7.27 ± 0.03 mg/100 g in the order SB > CG > CS with that of SB

differing significantly from those of CG and CS which were statistically equivalent. Total mean levels of Zn in peels from the three watermelons varieties from Mwea market ranged from 9.03 ± 0.03 mg/100 g to 4.74 ± 0.02 mg/100 g with CG having the highest mean values and SB having the least mean values and differed significantly. The total Zn mean levels in peel in this study was higher than what other workers have reported, 1.83 ± 0.01 mg/100 g (Tarazano and Aguayo, 2012); 0.74 ± 0.04 mg/100 g (Paul *et al*., 2014). The variation of total Zn mean levels in the same part of a watermelon variety grown in different areas can occur due to seasonal variations (Mathew *et al*., 2014). During wet seasons, Zn is mobile and a large extent dissolve in water, thus becoming available for uptake by plants. In dry spells, Zn is immobile and exist in insoluble forms and thus unavailable for plant uptake (Honorata *et al*., 2016).

4.3.1.2 Total mean levels of Zn in dried white flesh of Charleston grey (CG), crimson sweet (CS) and sugar baby (SB) watermelons

As shown in Table 4.3, white flesh from Ngara market had total Zn mean levels ranging from 7.95 ± 0.01 mg/100 g to 7.5 ± 0.03 mg/100 g in the order SB > CG > CS and differed significantly. Total mean levels of Zn in white flesh from Mwea market ranged from 9.71 ± 0.02 mg/100 g to 5.56 ± 0.01 mg/100 g in the order CG > CS > SB and differed significantly. The total Zn mean levels in white flesh in this study is higher than what other researchers have reported, 1.05 ± 0.08 mg/100 g (Mohamed and Ahmed, 2006) and 0.38 mg/100 g (Iyaka, 2007). The variation of total Zn mean levels in the same part of a watermelon variety grown in different areas can occur due to differences in the level of minerals in the soil (Matilda *et al*., 2014).

4.3.1.3 Total mean levels of Zn in red flesh of dried Charleston grey (CG), crimson sweet (CS) and sugar baby (SB) watermelons

As tabulated in Table 4.3, total mean levels of Zn in red flesh from Ngara market ranged from 9.22 ± 0.08 mg/100 g to 6.11 ± 0.02 mg/100 g in the order SB > CS > CG and differed significantly. The total mean levels of Zn from Mwea market ranged from 7.19 ± 0.03 mg/100 g to 6.52 ± 0.02 mg/100 g in the order SB > CG > CS and differed significantly. The total Zn mean levels in red flesh in this study is higher than what other workers have reported, 0.11 mg/100 g (Nthiga *et al*., 2013); 2.43 mg/100 g (Luana and Edira, 2014) and 3.64 mg/100 g (Aremu and Ibrahimu, 2014). The variation of total Zn mean levels in the same part of a watermelon variety grown in different areas can occur due to differences in the level of minerals in the soil (Mathew *et al*., 2014).

4.3.1.4 Total mean levels of Zn in seed of dried Charleston grey (CG), crimson sweet (CS) and sugar baby (SB) watermelons

From the results in Table 4.3, it can be seen that seeds from Ngara market had total Zn mean levels ranging from 7.45 ± 0.01 mg/100 g to 6.13 ± 0.03 mg/100 g in the order CG > SB > CS and differed significantly. The total mean levels of Zn in seeds from Mwea market was in the order SB > CG > CS and ranged from 7.56 ± 0.02 mg/100 g to 6.02 ±0.01 mg/100 g and differed significantly. The total Zn mean levels of seed in this study is lower than what has been reported by other researchers, 10.6 ± 0.34 mg/100 g (Tarek, 2001) and within what other researchers have found, 7.5 mg/100 g (Torrens, 2015), 6.18 mg/100 g (Elezuo *et al*., 2011). However, the total Zn mean levels of seed in this study is higher than what has been reported by other workers 5.10 ± 0.02 mg/100 g (Matilda *et al*., 2014) and 5.52 ± 0.20 mg/100 g (Mathew *et al*., 2014). The variation of total Zn mean levels in the same part of

different watermelon variety grown in the same area can occur due to differences in the stage of harvest of the fruit (Matilda *et al*., 2014).

4.3.1.5 Comparison of total mean levels of Zn in parts of dried Charleston grey (CG) watermelons from Ngara and Mwea markets

As can be seen from Table 4.3, the results show that CG watermelons from Ngara market had total Zn mean levels ranging from 6.11 ± 0.02 mg/100 g to 7.86 ± 0.03 mg/100 g with white flesh having the highest levels and red flesh having the least levels. A similar trend was observed in CG watermelons from Mwea market which had a total Zn mean levels ranging from 6.55 ± 0.03 mg/100 g to 9.71 ± 0.02 mg/100 g. There was significant difference in levels of Zn in Ngara and Mwea markets CG watermelons in all parts. The variation in total Zn mean levels for watermelon grown from different region can occur due to differences in the levels of Zn in the soils and pH levels of soil (Mathew *et al*., 2014). At a soil with a low pH, organic acids that form from decomposition of soil organic matter could increase mobility of Zn by forming stable metal ligand complexes that are easily absorbed by plants. At higher pH values of soil, Zn may exist in insoluble complexes or organic acids may decrease mobility of Zn by co - adsorbing on soil surfaces and forming soil organic acid metal bridges that are not easily absorbed by plants (Ding *et al*., 2014).

4.3.1.6 Comparison of total mean levels of Zn in parts of dried crimson sweet (CS) watermelons from Ngara and Mwea markets

From Table 4.3, the results show that CS watermelons from Ngara market had total Zn mean levels ranging from 6.13 ± 0.03 mg/100 g to 9.11 ± 0.01 mg/100 g with red flesh having highest levels and seed having least levels. For CS watermelons from Mwea market, the total Zn mean levels ranged from 6.02 ± 0.01 mg/100 g to 7.27 ±

0.03 mg/100 g with peel having the highest levels and seed having the least levels. There was significant difference in levels of Zn in various parts except in the peel of CS watermelons from Ngara and Mwea markets. The variation of total Zn mean levels for watermelon grown in different regions can occur due to differences in pH levels of soil (Matilda *et al*., 2014). As pH levels of the soil decreases, more free ions of Zn occur and thus are available for plant uptake. As pH levels of soil increases, Zn occur in insoluble complexes that are not available for plant uptake (Honorata *et al*., 2016).

4.3.1.7 Comparison of total mean levels of Zn in parts of dried sugar baby (SB) watermelons from Ngara and Mwea markets

From Table 4.3, the results show that SB watermelons from Ngara market had total Zn mean levels ranging from 7.15 ± 0.03 mg/100 g to 9.48 ± 0.03 mg/100 g with peel having highest levels and seed having least levels. Sugar baby watermelons from Mwea market had total Zn mean levels ranging from 4.74 ± 0.02 mg/100 g to 7.56 ± 0.02 mg/100 g with seed having the highest levels and peel having the least levels. There was significant difference in levels of Zn in Ngara and Mwea markets SB watermelons in all parts. Seasonal variations and maturity of the fruit can make watermelons from different regions to have different levels of Zn (Mathew *et al*., 2014).

4.3.2 Mean levels of Zn species in various fractions and parts of dried Charleston grey (CG) watermelons

The results for mean levels of Zn species in the parts of CG watermelons samples obtained from Ngara and Mwea markets are discussed in the following subsections. The results of the mean levels of Zn species in dried peel, white flesh, red flesh and

seeds of CG purchased from Ngara and Mwea markets were calculated and compared at $\alpha = 0.05$ level as shown in **tables** 4.4 and 4.5.

Table 4.4: Mean levels of Zn species (mg/100 g) in various fractions and parts of dried CG watermelons from Ngara and Mwea Markets, n = 18

Samplig site	Watermelon part	Mean concentration (mg/100 g ± SD)					
		Water extract	Ethanol extract	dichloromethane extract	Hexane extract	Residual	P - value
Ngara market, n = 9	Peel	3.83 ± 0.01^{Cd}	0.61 ± 0.01^{Ab}	0.57 ± 0.01^{Ad}	0.54 ± 0.01^{Aa}	2.08 ± 0.06^{Bd}	< 0.001
	White flesh	3.59 ± 0.01^{Ec}	1.23 ± 0.01^{Cc}	0.52 ± 0.01^{Ac}	0.83 ± 0.01^{Bc}	1.72 ± 0.01^{Da}	< 0.001
	Red flesh	1.91 ± 0.01^{Da}	1.29 ± 0.01^{Cd}	0.50 ± 0.01^{Ab}	0.60 ± 0.01^{Bb}	1.88 ± 0.01^{Db}	< 0.001
	Seed	2.67 ± 0.01^{Db}	0.57 ± 0.01^{Ba}	0.49 ± 0.01^{Aa}	0.86 ± 0.01^{Cd}	2.86 ± 0.01^{Ed}	< 0.001
	P - value	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	
Mwea market, n = 9	Peel	4.56 ± 0.01^{Ec}	1.12 ± 0.01^{Cc}	0.73 ± 0.01^{Bd}	0.51 ± 0.01^{Ab}	2.11 ± 0.02^{Db}	< 0.001
	White flesh	5.96 ± 0.01^{Ed}	1.05 ± 0.01^{Cb}	0.56 ± 0.01^{Ba}	0.45 ± 0.01^{Aa}	1.69 ± 0.01^{Da}	< 0.001
	Red flesh	2.03 ± 0.01^{Da}	1.17 ± 0.02^{Cd}	0.58 ± 0.01^{Bb}	0.45 ± 0.01^{Aa}	2.31 ± 0.02^{Ec}	< 0.001
	Seed	2.85 ± 0.01^{Cb}	0.47 ± 0.01^{Aa}	0.61 ± 0.01^{Bc}	0.47 ± 0.01^{Aa}	2.95 ± 0.01^{Dd}	< 0.001
	P - value	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	

Mean values with the different small letter(s) within the same column are significantly different and mean values with different capital letter within same row are significantly different ($\alpha = 0.05$, $P < 0.001$, one-way ANOVA, SNK-test)

Table 4.5: Comparison of extracted mean levels of Zn species (mg/100 g) from various solvents and parts of dried CG watermelons from Ngara and Mwea markets

Solvent	Part	Ngara	Mwea	P - value
Water	peel	3.83 ± 0.01	4.56 ± 0.01	< 0.001
	White flesh	3.59 ± 0.01	5.96 ± 0.01	< 0.001
	Red flesh	1.91 ± 0.01	2.03 ± 0.01	< 0.001
	seed	2.67 ± 0.01	2.85 ± 0.01	< 0.001
Ethanol	peel	0.61 ± 0.01	1.12 ± 0.01	< 0.001
	White flesh	1.23 ± 0.01	1.05 ± 0.01	< 0.001
	Red flesh	1.29 ± 0.01	1.17 ± 0.02	< 0.001
	seed	0.57 ± 0.01	0.47 ± 0.01	< 0.001
Dichloromethane	peel	0.57 ± 0.01	0.73 ± 0.01	< 0.001
	White flesh	0.52 ± 0.01	0.56 ± 0.01	0.002
	Red flesh	0.50 ± 0.01	0.58 ± 0.01	< 0.001
	seed	0.49 ± 0.01	0.61 ± 0.01	< 0.001
Hexane	peel	0.54 ± 0.01	0.51 ± 0.01	0.008
	White flesh	0.83 ± 0.01	0.45 ± 0.01	< 0.001
	Red flesh	0.60 ± 0.01	0.45 ± 0.01	< 0.001
	seed	0.86 ± 0.01	0.47 ± 0.01	< 0.001
Residue	peel	2.08 ± 0.06	2.11 ± 0.02	0.438
	White flesh	1.72 ± 0.01	1.69 ± 0.01	0.008
	Red flesh	1.88 ± 0.01	2.31 ± 0.02	< 0.001
	seed	2.86 ± 0.01	2.95 ± 0.01	< 0.001

Mean levels of Zn compared at $\alpha = 0.05$

The results presented in Tables 4.4 and 4.5 are discussed in the following sub sections.

4.3.2.1 Mean levels of Zn species (mg/100g) in peels of various fractions in dried Charleston grey (CG) watermelons and their comparison from Ngara and Mwea markets

From Table 4.4, mean levels of Zn species extracted by various solvents in dried CG peels

from Ngara market ranged from 3.83 ± 0.01 mg/100 g (50.2 %) to 0.54 ± 0.01 mg/100 g (7.1 %) in the order water extract (WE) > residual (RE) > ethanol extract (EE) > dichlomethane extract (DE) > hexane extract (HE) and differed significantly. The same pattern was obtained in Mwea market peels with values ranging from 4.56 ±

0.01 mg/100 g (50.5 %) to 0.51 ± 0.01 mg/100 g (5.6 %) and amount of Zn species extracted by various solvents differed significantly. Upon comparison as tabulated in Table 4.5, significantly different amounts of Zn species were extracted from peels by all the extracting solvents with WE having the highest levels and HE having the least levels both from Mwea market. Zinc species that remained in residue did not differ significantly in Ngara and Mwea markets.

4.3.2.2 Mean levels of Zn species (mg/100 g) in white flesh of various fractions and their comparison in dried Charleston grey (CG) watermelons from Ngara and Mwea markets

The results in table 4.4 show that the mean amount of Zn species extracted by various solvents from CG white flesh from Ngara market ranged from 3.59 ± 0.01 mg/100 g (45.5 %) to 0.52 ± 0.01 mg/100 g (6.6 %) in the order WE > RE > EE > HE > DE and differed significantly. From Mwea market, mean levels of Zn species extracted by various solvents from CG white flesh ranged from 5.96 ± 0.01 mg/100 g (61.4 %) to 0.45 ± 0.01 mg/100 g (4.6 %) in the order WE > RE > EE > DE > HE and differed significantly. Upon comparison as shown in Table 4.5, significantly different amounts of Zn species were extracted by all the extracting solvents from white flesh part of CG watermelons with WE having the highest levels and HE having the least levels both from Mwea market. Significantly different amount of Zn species was left in the CG watermelons residue from Ngara and Mwea markets.

4.3.2.3 Mean levels of Zn species (mg/100 g) in red flesh extracted by various solvents from dried Charleston grey (CG) watermelons and their comparison in Ngara and Mwea markets

As seen from table 4.4, the mean amount of Zn species extracted by various solvents from Ngara market CG red flesh ranged from 1.91 ± 0.01 mg/100 g (31.1 %) to 0.50

± 0.01 mg/100 g (7.5 %) in the order WE > RE > EE > HE > DE and differed significantly. Mean levels of Zn species extracted by various solvents in CG red flesh from Mwea market ranged from 2.31 ± 0.02 mg/100 g (35.3 %) to 0.45 ± 0.01 mg/100 g (6.9 %) in the order RE > WE > EE > DE > HE and differed significantly. Upon comparison as can be observed in Table 4.5, significantly different amounts of Zn species were extracted by all the extracting solvents from red flesh part of CG watermelons with WE having the highest levels and HE having the least levels both from Mwea market. Significantly different amount of Zn species was left in the residue of red flesh part of CG watermelons from Ngara and Mwea markets.

4.3.2.4 Mean levels of Zn species (mg/100 g) in seeds extracted by various solvents from dried Charleston grey (CG) watermelons and their comparison from Ngara and Mwea markets

As shown in table 4.4, the mean amount of Zn species extracted by various solvents from seeds in CG watermelons from Ngara market ranged from 2.86 ± 0.01 mg/100 g (38.3 %) to 0.49 ± 0.01 mg/100 g (6.8 %) in the order RE > WE > HE > EE > DE and differed significantly. In Mwea market the Zn species mean levels extracted from seeds by various solvents ranged from 2.95 ± 0.01 mg/100 g (40.1 %) to 0.47 ± 0.01 mg/100 g (6.4 %) in the order RE > WE > DE > EE ≥ HE and differed significantly. Upon comparison as can be seen in Table 4.5, significantly different amount of Zn species were extracted by all the extracting solvents with WE having the highest levels while EE and HE jointly had the least levels. Significantly different amounts of Zn species were left in CG watermelons residue from Ngara and Mwea markets. The variation of mean levels of Zn species in various extracts of the watermelon can occur due to phytate - Zn complex ratio, protein and anti - nutritional components in the plant matrix (Akindahunsi and Oboh, 1999).

The Zn species extracted by water and a fraction of ethanol depending on pH would be available for absorption in human digestive system while one extracted by dichloromethane, hexane and left in residual would not be available for absorption in human digestive system. The bioavailable forms of Zn in CG watermelons from Ngara market ranged from 43 % to 61 % with water extracts in peel having the highest levels and ethanol extracts in seed having the least levels. The bioavailable forms of Zn in CG watermelons from Mwea market ranged between (44 - 72) % with WE in white flesh having the highest levels and EE in seed having the least levels. Peel and white flesh parts should be eaten as they have more bioavailable Zn than the red flesh part commonly eaten.

All parts of CG watermelons fruit combined contained a lower concentration of bioavailable Zn species than the recommended daily allowance (RDA) of Zn set at 18 mg (Biego *et al*., 1998; Santos *et al*., 2004; Harlal *et al*., 2005; Ozgur *et al*., 2009; Kiran *et al*., 2012) which agrees with similar studies performed on cabbages (Andrej, 2010) and on leaves and fruits of herbal teas (Konieczynski and Wesolowski, 2013). In his study Andrej (2010) reported (31 - 87) % water extractable Zn species in white cabbage which is slightly more than (31 - 61) % obtained in CG in this study. In their study Konieczynski and Wesolowski (2013) reported water extractable Zn species of 14 % in fruits and 40 % in leaves of the herbal teas which is lower than (31 - 61) % water extractable Zn species in CG obtained in this study. White flesh part of CG watermelons from Ngara market would provide Zn levels of 4.82 mg/100 g (dry weight) with 60 % of Zn being bioavailable while that from Mwea market would provide Zn levels of 7.01 mg/100 g (dry weight) with 61 % biovailable Zn.

The variation in Zn mean levels could be attributed to factors such as plant constituents in each part (oxalates, phytates, tanins, insoluble fibre) and competing minerals which reduce Zn absorption (Duo, 2011). In addition the levels differ with plant part and species (Kiran *et al*., 2012). Ondo *et al*. (2012) working on *Amaranthus cruentus* plant found higher concentration of Cu and Zn in roots than in leaves and stems. When these workers supplemented the plant with these metals, Cu was easily translocated to leaves than Zn (Kabata-Pendias, 2011).

4.3.3 Mean levels of Zn species in various fractions and parts of dried crimson sweet (CS) watermelons

The results for mean levels of Zn species in the parts of CS watermelon samples obtained from Mwea and Ngara markets are discussed in the following sub sections. The results of the mean levels of Zn species and their comparison in dried peel, white flesh, red flesh and seeds of CS purchased from Mwea and Ngara markets were calculated and compared at $\alpha = 0.05$ level as shown in tables 4.6 and 4.7. The results presented in Tables 4.6 and 4.7 are discussed in the following sub sections.

4.3.3.1 Mean levels of Zn species (mg/100 g) in peels extracted by various solvents from dried crimson sweet (CS) watermelons and their comparison from Ngara and Mwea markets

From the results in Table 4.6, the mean levels of Zn species in various fractions in peels of CS watermelons from Ngara market ranged from 3.57 ± 0.01 mg/100 g (49.2 %) to 0.50 ± 0.01 mg/100 g (6.9 %) in the order WE > RE > HE > DE > EE and were significantly different. In Mwea market, the mean levels of Zn species from CS peels ranged from 4.21 ± 0.01 mg/100 g (57.9 %) to 0.53 ± 0.01 mg/100 g (7.3 %) in the order WE > RE > DE > EE > HE and amount of Zn species extracted by various solvents differed significantly. As tabulated in table 4.7 significantly different amounts of Zn species were extracted from peels by all the solvents and the ones left in the residue with WE for watermelons from Mwea market having the highest levels and EE for watermelons from Ngara market having the least levels.

Table 4.6: Mean levels of Zn species (mg/100 g) in various fractions and parts of dried crimson sweet (CS) watermelons from Ngara and Mwea Markets, n = 18

Sampling site	Watermelon part	Mean concentration (mg/100 g ± SD)					
		Water extract	Ethanol extract	dichloromethane extract	Hexane extract	Residual	p - value
Ngara market, n = 9	Peel	3.57 ± 0.01^{Eb}	0.50 ± 0.01^{Aa}	0.56 ± 0.01^{Bc}	0.84 ± 0.01^{Cd}	1.79 ± 0.01^{Db}	< 0.001
	White flesh	3.78 ± 0.01^{Ec}	0.95 ± 0.02^{Cc}	0.53 ± 0.01^{Ab}	0.65 ± 0.01^{Bb}	1.63 ± 0.01^{Da}	< 0.001
	Red flesh	4.84 ± 0.04^{Ed}	1.42 ± 0.01^{Cd}	0.51 ± 0.01^{Aa}	0.63 ± 0.01^{Ba}	1.86 ± 0.01^{Dc}	< 0.001
	Seed	1.51 ± 0.01^{Da}	0.79 ± 0.01^{Bb}	0.50 ± 0.01^{Aa}	0.82 ± 0.01^{Cc}	2.54 ± 0.01^{Ed}	< 0.001
	p - value	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	
Mwea market, n = 9	Peel	4.21 ± 0.01^{Ed}	0.57 ± 0.01^{Bb}	0.74 ± 0.01^{Cc}	0.53 ± 0.01^{Ad}	1.22 ± 0.01^{Da}	< 0.001
	White flesh	3.57 ± 0.01^{Ec}	0.89 ± 0.01^{Cc}	0.53 ± 0.01^{Ba}	0.44 ± 0.01^{Ab}	1.53 ± 0.01^{Dc}	< 0.001
	Red flesh	2.98 ± 0.01^{Eb}	1.25 ± 0.01^{Cd}	0.59 ± 0.01^{Bb}	0.42 ± 0.01^{Aa}	1.28 ± 0.02^{Db}	< 0.001
	Seed	1.86 ± 0.01^{Da}	0.50 ± 0.01^{Ba}	0.60 ± 0.01^{Cb}	0.46 ± 0.01^{Ac}	2.60 ± 0.02^{Ed}	< 0.001
	p - value	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	

Mean values with the different small letter(s) within the same column are significantly different and mean values with different capital letter within same row are significantly different ($\alpha = 0.05$, $P < 0.001$, one-way ANOVA, SNK-test)

Table 4.7: Comparison of extracted mean levels of Zn species (mg/100 g) in various fractions and parts of dried CS watermelons from Ngara and Mwea markets

Solvent	Part	Ngara	Mwea	P - value
Water	peel	3.57 ± 0.01	4.21 ± 0.01	< 0.001
	White flesh	3.78 ± 0.01	3.57 ± 0.01	< 0.001
	Red flesh	4.84 ± 0.04	2.98 ± 0.01	< 0.001
	seed	1.51 ± 0.01	1.86 ± 0.01	< 0.001
Ethanol	peel	0.50 ± 0.01	0.57 ± 0.01	< 0.001
	White flesh	0.95 ± 0.02	0.89 ± 0.01	0.002
	Red flesh	1.42 ± 0.01	1.25 ± 0.01	< 0.001
	seed	0.79 ± 0.01	0.50 ± 0.01	< 0.001
Dichloromethane	peel	0.56 ± 0.01	0.74 ± 0.01	< 0.001
	White flesh	0.53 ± 0.01	0.53 ± 0.01	1.000
	Red flesh	0.51 ± 0.01	0.59 ± 0.01	< 0.001
	seed	0.50 ± 0.01	0.60 ± 0.01	< 0.001
Hexane	peel	0.84 ± 0.01	0.53 ± 0.01	< 0.001
	White flesh	0.65 ± 0.01	0.44 ± 0.01	< 0.001
	Red flesh	0.63 ± 0.01	0.42 ± 0.01	< 0.001
	seed	0.82 ± 0.01	0.46 ± 0.01	< 0.001
Residue	peel	1.79 ± 0.01	1.22 ± 0.01	< 0.001
	White flesh	1.63 ± 0.01	1.53 ± 0.01	< 0.001
	Red flesh	1.86 ± 0.01	1.28 ± 0.02	< 0.001
	seed	2.54 ± 0.01	2.60 ± 0.02	0.002

Mean levels of Zn compared at $\alpha = 0.05$

4.3.3.2 Mean levels of Zn species (mg/100 g) in white flesh extracted by various solvents from dried crimson sweet (CS) watermelons and their comparison from Ngara and Mwea markets

As shown in Table 4.6, CS watermelons white flesh part from Ngara market had Zn species mean levels ranging from 3.78 ± 0.01 mg/100 g (50.3 %) to 0.53 ± 0.01 mg/100 g (6.7 %) and followed the order WE > RE > EE > HE > DE, differing significantly. Mwea market extracted Zn species mean levels of CS white flesh ranged from 3.57 ± 0.01 mg/100 g (51.3 %) to 0.44 ± 0.01 mg/100 g (6.3 %) in similar order apart from HE and DE and the amount of Zn species extracted by various solvents differed significantly. As can be observed from Table 4.7,

significantly different amounts of Zn species were extracted by all the extracting solvents apart from DE with WE from watermelons from Ngara market having the highest levels and HE for watermelons from Mwea market having the least levels. Significantly different amounts of Zn species were left in the residue of CS watermelons from Ngara and Mwea markets.

4.3.3.3 Mean levels of Zn species (mg/100 g) in red flesh extracted by various solvents from dried crimson sweet (CS) watermelons and their comparison from Ngara and Mwea markets

As tabulated in Table 4.6, the amount of Zn species extracted in various solvents of CS watermelons in red flesh ranged from 4.84 ± 0.04 mg/100 g (52.6 %) to 0.51 ± 0.01 mg/100 g (4.9 %) in the order WE > RE > EE > HE > DE and differed significantly. Mwea market mean levels of extracted Zn species in CS watermelons ranged from 2.98 ± 0.01 mg/100 g (45.7 %) to 0.42 ± 0.01 mg/100 g (6.4 %) in a similar order apart from HE and DE and differed significantly. Upon comparison as can be observed from table 4.7, significantly different amounts of Zn species were extracted by all the extracting solvents and the ones left in the residue with WE for CS watermelons from Ngara market having the highest levels while HE for CS watermelons from Mwea market having the least levels.

4.3.3.4 Mean levels of Zn species (mg/100 g) in seeds extracted by various solvents from dried crimson sweet (CS) watermelons and their comparison from Ngara and Mwea markets

The results in Table 4.6 show that the mean amount of Zn species extracted by various solvents from the seeds of CS watermelons from Ngara market ranged from 2.54 ± 0.01 mg/100 g (41.4 %) to 0.50 ± 0.01 mg/100 g (7.8 %) in the order RE > WE > HE > EE > DE while CS watermelons from Mwea market had mean levels for Zn

species ranging from 2.60 ± 0.02 mg/100 g (43.2 %) to 0.46 ± 0.01 mg/100 g.(7.6 %) in the order RE > WE > DE > EE > HE. In each market there was significant difference in amount of Zn species extracted from seed by various solvents. As can be seen from table 4.7, significantly different amounts of Zn species were extracted by all the extracting solvents with WE having the highest levels while HE had the least levels both from Mwea market. Significantly different amount of Zn species was left in the residue of CS watermelons from Ngara and Mwea markets. The variation of mean levels of Zn species in various extracts of the watermelon can occur due to incomplete extraction (Vogel-Mikus *et al*., 2009).

The bioavailable forms of Zn from Ngara market ranged from 37 % to 68 % with WE in red flesh having the highest levels and EE in peel having the least levels. The bioavailable forms of Zn from Mwea market CS watermelons ranged between 39 % to 65 % with peel WE having the highest and peel EE having the least.

From the results in table 4.6, peel and white flesh parts should be eaten as they have substantial amount of bioavailable Zn that can supplement the one present in the eaten red flesh part. All parts of CS watermelon fruit combined contained a lower concentration of bioavailable Zn than the RDA of zinc set at 18 mg (Biego *et al*., 1998; Santos *et al*., 2004; Harlal *et al*., 2005; Ozgur *et al*., 2009; Kiran *et al*., 2012). Thus even if all parts of the fruit are eaten cannot cause adverse health effects based on Zn mean levels as long as one eats about 100 g per day. In a similar study by Devi *et al*. (2013) a slightly higher bioavailable Zn species of 3.2 mg/100 g to 8.2 mg/100 g was recorded in a medicinal plant. These investigators proposed that variation of concentration of elements in different plants is observed even if they were collected from the same area. This is due to the absorption of different elemental concentration

from the soil by different plants and storing them in different parts of the plant. Red flesh part of the CS watermelons from Ngara market would provide Zn levels of 6.26 mg/100 g with 68 % of Zn being bioavailable while peel part of the CS watermelons from Mwea market would provide Zn levels of 4.78 mg/100 g (dry weight) with 66 % of Zn being bioavailable.

The absorption of metals in the alimentally canal depends on chemical forms of the metals, concentration and pH medium of the mixture (Lusi *et al*., 2011). The levels of Zn obtained in this study were lower compared to those obtained in a similar study by Olabanji *et al*. (2013) who reported that the uptake of metal by plants roots depends on the form the metals exist in the soil and the nature of the soil and the plant species. The variation in the bioavailable Zn mean levels in part of the same variety of watermelon could also be as a result of difference in stage of maturity, phytate-Zn molar ratio and presence of strong polar groups containing O, N and S which form strong complexes with Zn (Andrej, 2010).

4.3.4 Mean levels of Zn species in various fractions and parts of dried Sugar baby (SB) watermelons

The results for mean levels of Zn species in the parts of SB watermelon samples obtained from Mwea and Ngara markets are discussed in the following sub sections. The results of the mean levels of Zn species in dried peel, white flesh, red flesh and seeds of SB watermelons bought from Mwea and Ngara markets were calculated and compared at $\alpha = 0.05$ level as shown in tables 4.8 and 4.9.

Table 4.8: Mean levels of Zn species (mg/100 g) in various fractions and parts of dried sugar baby (SB) watermelons from Ngara and Mwea markets, n = 18

Sampling site	Watermelon part	Mean concentration (mg/100 g ± SD)					
		Water extract	Ethanol extract	dichloromethane extract	Hexane extract	Residual	p - value
Ngara market n = 9	Peel	4.53 ± 0.01^{Dc}	1.61 ± 0.01^{Bb}	0.56 ± 0.01^{Ab}	0.64 ± 0.01^{Ab}	2.17 ± 0.16^{Cb}	< 0.001
	White flesh	2.59 ± 0.01^{Ea}	1.82 ± 0.01^{Cc}	0.54 ± 0.01^{Ab}	0.65 ± 0.01^{Bb}	2.34 ± 0.01^{Dc}	< 0.001
	Red flesh	4.70 ± 0.02^{Dd}	1.94 ± 0.01^{Cd}	0.51 ± 0.08^{Aa}	0.45 ± 0.01^{Aa}	1.56 ± 0.01^{Ba}	< 0.001
	Seed	2.68 ± 0.01^{Db}	0.45 ± 0.01^{Aa}	0.51 ± 0.01^{Ba}	0.80 ± 0.01^{Cc}	2.69 ± 0.01^{Dd}	< 0.001
	P - value	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	
Mwea market n = 9	Peel	1.32 ± 0.01^{Da}	1.00 ± 0.01^{Ba}	0.70 ± 0.01^{Bc}	0.53 ± 0.01^{Ab}	1.19 ± 0.02^{Cb}	< 0.001
	White flesh	2.46 ± 0.01^{Eb}	1.03 ± 0.01^{Cb}	0.54 ± 0.01^{Ba}	0.45 ± 0.01^{Aa}	1.09 ± 0.01^{Da}	< 0.001
	Red flesh	3.22 ± 0.01^{Ed}	1.37 ± 0.01^{Cc}	0.54 ± 0.01^{Ba}	0.43 ± 0.01^{Aa}	1.62 ± 0.01^{Dc}	< 0.001
	Seed	2.51 ± 0.01^{Dc}	1.39 ± 0.01^{Cd}	0.60 ± 0.01^{Bb}	0.45 ± 0.01^{Aa}	2.62 ± 0.02^{Ed}	< 0.001
	P - value	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	

Mean values with the different small letter(s) within the same column are significantly different and mean values with different capital letter within same row are significantly different ($\alpha = 0.05$, $P < 0.001$, one-way ANOVA, SNK-test)

Table 4.9: Comparison of extracted mean levels of Zn species (mg/100 g) in various solvents of dried SB watermelons parts from Ngara and Mwea markets

Solvent	Part	Ngara	Mwea	P – value
Water	peel	4.53 ± 0.01	1.32 ± 0.01	< 0.001
	White flesh	2.59 ± 0.01	2.46 ± 0.01	< 0.001
	Red flesh	4.70 ± 0.02	3.22 ± 0.01	< 0.001
	seed	2.68 ± 0.01	2.51 ± 0.01	< 0.001
Ethanol	peel	1.61 ± 0.01	1.00 ± 0.01	< 0.001
	White flesh	1.82 ± 0.01	1.03 ± 0.01	< 0.001
	Red flesh	1.94 ± 0.01	1.37 ± 0.01	< 0.001
	seed	0.45 ± 0.01	1.39 ± 0.01	< 0.001
Dichloromethane	peel	0.56 ± 0.01	0.70 ± 0.01	< 0.001
	White flesh	0.54 ± 0.01	0.54 ± 0.01	1.000
	Red flesh	0.51 ± 0.08	0.54 ± 0.01	0.540
	seed	0.51 ± 0.01	0.60 ± 0.01	< 0.001
Hexane	peel	0.64 ± 0.01	0.53 ± 0.01	< 0.001
	White flesh	0.65 ± 0.01	0.45 ± 0.01	< 0.001
	Red flesh	0.45 ± 0.01	0.43 ± 0.01	0.044
	seed	0.80 ± 0.01	0.45 ± 0.01	< 0.001
Residue	peel	2.17 ± 0.16	1.19 ± 0.02	< 0.001
	White flesh	2.34 ± 0.01	1.09 ± 0.01	< 0.001
	Red flesh	1.56 ± 0.01	1.62 ± 0.01	< 0.001
	seed	2.69 ± 0.01	2.62 ± 0.02	0.001

Mean levels of Zn compared at $\alpha = 0.05$

The results presented in tables 4.8 and 4.9 are discussed in the following sub sections.

4.3.4.1 Mean levels of Zn species (mg/100 g) in peels extracted by various solvents from dried Sugar baby (SB) watermelons and their comparison from Ngara and Mwea markets

From the results in table 4.8, the Zn species mean levels in various fractions of SB watermelons in peels from Ngara market ranged from 4.53 ± 0.01 mg/100 g (47.6 %) to 0.56 ± 0.01 mg/100 g.(5.9 %) in a decreasing order of WE > RE > EE > HE > DE. Apart from HE and DE a similar order was observed in Mwea market SB watermelon Zn species mean levels which ranged from 1.32 ± 0.01 mg/100 g (27.8 %) to 0.53 ± 0.01 mg/100 g (11.2 %). There was significant difference in SB watermelons mean levels of Zn species in various solvent extracts from each market.

4.3.4.2 Mean levels of Zn species (mg/100 g) in white flesh extracted by various solvents in dried Sugar baby (SB) watermelons and their comparison between Ngara and Mwea markets

As seen from table 4.8, Ngara market mean levels of Zn species in various fractions of SB watermelons in white flesh ranged from 2.59 ± 0.01 mg/100 g (32.4 %) to 0.54 ± 0.01 mg/100 g (7.3 %) in a decreasing order of WE > RE > EE > HE > DE while SB watermelons from Mwea market had Zn species mean levels ranging from 2.46 ± 0.01 mg/100 g (44.2 %) to 0.45 ± 0.01 mg/100 g (8.2 %) in the order WE > RE > EE > DE > HE. In each market there was significant difference in SB watermelons Zn species mean levels in all solvent extracts. As observed in Table 4.9, all the extracting solvents extracted significantly different amounts of Zn species from white flesh part of sugar baby watermelons apart from DE with WE for watermelons from Ngara market having the highest levels while HE for watermelons from Mwea market having the least levels. Significantly different amounts of Zn species were left in the residue of white flesh part of SB watermelons from Ngara and Mwea markets.

4.3.4.3 Mean levels of Zn species (mg/100 g) in red flesh extracted by various solvents from dried Sugar baby (SB) watermelons and their comparison from Ngara and Mwea markets

From table 4.8, mean levels of Zn species extracted by various solvents from red flesh of dried SB watermelons from Ngara market ranged from 4.70 ± 0.02 mg/100 g (50.8 %) to 0.45 ± 0.01 mg/100 g (4.9 %) in a decreasing order of WE > EE > RE > DE > HE while SB watermelons from Mwea market, had Zn species mean levels ranging from 3.22 ± 0.01 mg/100 g (44.9 %) to 0.43 ± 0.01 mg/100 g (6.0 %) in the order WE > RE > EE > DE > HE. In each market there was significant difference in mean levels of extracted Zn species from SB watermelons in all solvent extracts. The results in table 4.9 show that all the extracting solvents extracted significantly different Zn

species from red flesh part of SB watermelons except DE with WE for SB watermelons from Ngara market having the highest amount while HE for watermelons from Mwea market having the least values. Significantly different amounts of Zn species were left in the RE of red flesh part of SB watermelons from Ngara and Mwea markets.

4.3.4.4 Mean levels of Zn species (mg/100 g) in seeds extracted by various solvents from dried Sugar baby (SB) watermelons and their comparison from Ngara and Mwea markets

From the results in table 4.8, it can be seen that Zn species mean levels in dried SB watermelons in seeds from Ngara market ranged from 2.69 ± 0.01 mg/100 g (37.7 %) to 0.45 ± 0.01 mg/100 g.(6.3 %) in a decreasing order of RE > WE > HE > DE > EE and differed significantly. Zinc species mean levels in dried SB watermelons in seeds from Mwea market ranged from 2.62 ± 0.02 mg/100 g (34.6 %) to 0.45 ± 0.01 mg/100 g (5.9 %) in the order RE > WE > EE > DE >HE and differed significantly. Upon comparison as can be seen from table 4.9, significantly different mean levels of Zn species were extracted from seed part of dried SB watermelons with WE for watermelons from Ngara market having the highest levels while HE for watermelons from Mwea market having the least levels. Significantly different mean levels of Zn species remained in the residue of seeds of dried SB watermelons from Ngara and Mwea markets. The variation of mean levels of Zn species in various extracts of the watermelon can occur due to stage of maturity, phytate - Zn molar ratio, presence of strong polar groups containing O, N and S which form strong complexes with Zn (Andrej, 2010).

The bioavailable extraction of Zn species from seeds in dried SB watermelons ranged from 45 % to 72 % with WE in red flesh from Ngara market having the highest levels while EE in seed had the least levels. From Mwea market, WE in red flesh of SB watermelons had the highest levels and EE in peel of SB had the least levels.

From the results of Table 4.8, all parts of SB watermelon fruit combined contained lower concentration of bioavailable Zn species than the RDA of zinc of 18 mg (Biego *et al*., 1998; Santos *et al*., 2004; Harlal *et al*., 2005; Ozgur *et al*., 2009; Kiran *et al*., 2012). Red flesh part of SB watermelons from both market would provide higher amount of Zn with that of Ngara market providing (4.59 mg/100 g, 68 % bioavailable Zn) and that of Mwea market providing (6.64 mg/100 g, 49 % bioavailable Zn) when consumed. Thus even if all parts of the fruit are eaten cannot cause adverse health effects as a result of Zn mean levels as long as one eats about 100 g of each part per day. Zinc in most vegetables predominantly occurs as species with a molecular mass lower than 1 kDa and about (50 - 90) % soluble in water. Andrej (2010) and Tereza *et al*. (2011) did similar work on vegetables and reported water extractable Zn species of (50 - 90) % which is higher than (45 - 72) % of Zn species of this study. The difference could be due to partial extraction and stage of harvest of fruits which were not investigated in this study. In addition different fruit tissues (structures) have highly differing elemental concentrations depending on the tissue origins (*maternal, embryona*), physiology and/or function which can bring variation as reported by Vogel-Mikus *et al*. (2009).

The strong binding of Zn by pectins in citrus juices leaves about (50 - 65) % extractable Zn. Anna *et al*. (2014) extracted about (50 - 60) % Zn from citrus juices which is close to (49 - 64) % obtained in this study though the medium are different.

The difference may arise due to variation in extracting solvent and plant species. The variation in the bioavailable Zn species mean levels in the same variety of watermelons from different parts could be due to Zn - phytate complex ratio, presence of protein and anti-nutritional components and pH value of the soil where these fruits grew (Akindahunsi and Oboh, 1999).

4.4 Levels of copper in watermelons

Total mean levels of Cu in various varieties of watermelon are presented in the following sub sections.

4.4.1 Total mean levels of copper in parts of dried Charleston grey (CG), crimson sweet (CS) and sugar baby (SB) watermelons

The results for total mean levels of Cu in dried parts of CG, CS and SB watermelon samples obtained from Ngara and Mwea markets are discussed in the following sub sections. The results of the total mean levels of Cu in peel, white flesh, red flesh and seeds of CG, CS and SB watermelons bought from Ngara and Mwea markets were calculated and compared at $\alpha = 0.05$ level as shown in table 4.10.

Table 4.10: Comparison of total mean levels of Cu (mg/100 g) in the same part of dried CG, CS and SB watermelons from Ngara and Mwea markets, n = 18

market	part	Charleston grey	crimson sweet	sugar baby	p - value
Ngara	peel	0.65 ± 0.02^{a}	0.66 ± 0.03^{a}	0.99 ± 0.01^{b}	< 0.001
Mwea		0.59 ± 0.01^{a}	0.66 ± 0.01^{b}	1.00 ± 0.02^{c}	< 0.001
p - value		0.031	0.996	0.668	
Ngara	White flesh	0.42 ± 0.03^{a}	0.56 ± 0.04^{c}	0.47 ± 0.04^{b}	< 0.001
Mwea		0.51 ± 0.02	0.46 ± 0.05	0.44 ± 0.03	0.422
p - value		0.041	0.130	0.567	
Ngara	Red flesh	0.47 ± 0.01^{a}	0.81 ± 0.03^{c}	0.65 ± 0.01^{b}	< 0.001
Mwea		0.50 ± 0.01^{a}	0.56 ± 0.05^{b}	0.62 ± 0.04^{c}	< 0.001
p - value		0.072	0.004	0.490	
Ngara	seed	1.80 ± 0.02	1.54 ± 0.02	1.53 ± 0.01	0.422
Mwea		1.85 ± 0.01	1.42 ± 0.06	1.66 ± 0.05	1.000
p - value		0.060	0.100	0.038	

Mean levels of Cu compared at $\alpha = 0.05$. For a given row, means with the same superscript are not significantly different. For a given column in same watermelon part, means where $p \leq 0.05$ are significantly different (one way ANOVA, SNK test).

The results presented in table 4.10 are discussed in the following sub sections.

4.4.1.1 Total mean levels of Cu in peel of dried Charleston grey (CG), crimson sweet (CS) and sugar baby (SB) watermelons

From the results in table 4.10, it can be seen that Ngara market watermelons Cu mean levels in dried peel ranged from 0.99 ± 0.01 mg/100 g to 0.65 ± 0.02 mg/100 g in the order SB > CS > CG and differed significantly while those from Mwea market had Cu mean levels ranging from 1.00 ± 0.02 mg/100 g to 0.59 ± 0.01 mg/100 g in the same order and differed significantly. The total Cu mean levels of peel in this study is higher than 0.20 ± 0.01 mg/100 g (Paul *et al*., 2014). The variation in total Cu mean levels for watermelon grown from different region can occur due to differences in the pH levels and concentration in soil (Mathew *et al*., 2014). At a soil with a low pH, organic acids that form from decomposition of soil organic matter could increase mobility of Cu by forming stable metal ligand complexes that are easily absorbed by plants. At higher pH values of soil, Cu may exist in insoluble complexes or organic acids may decrease mobility of Cu by co - adsorbing on soil surfaces and forming soil organic acid metal bridges that are not easily absorbed by plants (Ding *et al*., 2014).

4.4.1.2 Total mean levels of Cu in white flesh of dried Charleston grey (CG), crimson sweet (CS) and sugar baby (SB) watermelons

From table 4.10, white flesh part of dried watermelon from Ngara market had Cu mean levels ranging from 0.56 ± 0.04 mg/100 g to 0.42 ± 0.03 mg/100 g with CS having the highest values followed by SB and least CG and differed significantly while those from Mwea market, Cu mean levels in white flesh ranged from 0.51 ± 0.02 mg/100 g to 0.44 ± 0.03 mg/100 g in the order CG > CS > SB and did not differ

significantly. The total Cu mean levels of white flesh in this study is lower than 0.572 ± 0.01 mg/100 g reported by Mohamed and Ahmed (2006) and higher than 0.22 mg/100 g reported by Iyaka (2007). The variation of total Cu mean levels in the same part of a watermelon variety grown in different areas can occur due to seasonal variations (Mathew *et al*., 2014). During wet seasons, Cu is mobile and a large extent dissolve in water, thus becoming available for uptake by plants. In dry spells, Cu is immobile and exist in insoluble forms and thus unavailable for plant uptake (Honorata *et al*., 2016).

4.4.1.3 Total mean levels of Cu in red flesh of dried Charleston grey (CG), crimson sweet (CS) and sugar baby (SB) watermelons

As seen from table 4.10, red flesh part of dried watermelons from Ngara market had Cu mean levels ranging from 0.81 ± 0.03 mg/100 g to 0.47 ± 0.01 mg/100 mg/100 g in the order CS > SB > CG and differed significantly while those from Mwea market had Cu mean levels in red flesh ranging from 0.62 ± 0.04 mg/100 g to 0.50 ± 0.01 mg/100 g in the order SB > CS > CG and differed significantly. The total Cu mean levels of red flesh in this study is higher than 0.05 mg/100 g reported by Nthiga *et al*. (2013) and lower than 1.93 mg/100 g reported by Aremu and Ibrahimu (2014). The variation of total Cu mean levels in the same part of different watermelon variety grown in the same area can occur due to differences in the stage of harvest of the fruit and also soil conditions (Mathew *et al*., 2014).

4.4.1.4 Total mean levels of Cu in seeds of dried Charleston grey (CG), crimson sweet (CS) and sugar baby (SB) watermelons

From table 4.10, the results show that seed part of dried watermelon from Ngara market had Cu mean levels ranging from 1.80 ± 0.02 mg/100 g to 1.53 ± 0.01mg/100

g with CG watermelons having the highest levels followed by CS watermelons and least SB watermelons but did not differ significantly. Mwea market watermelons had Cu mean levels in seeds ranging from 1.85 ± 0.01 mg/100 g to 1.42 ± 0.06 mg/100 g and were statistically equivalent in the three watermelon varieties. The total Cu mean levels of seed in this study is lower than what has been reported by other researchers, 2.1 ± 0.15 mg/100 g (Tarek, 2001); 12.25 ± 0.01 mg/100 g (Mathew *et al*., 2014) and similar to 1.50 ± 0.09 mg/100 g reported by Matilda *et al*. (2014). This can occur due to differences in physical conditions of the soil where the watermelon plant grew (Mohamed and Ahmed, 2006). During wet seasons, Cu is mobile and a large extent dissolve in water, thus becoming available for uptake by plants. In dry spells, Cu is immobile and exist in insoluble forms and thus unavailable for plant uptake (Honorata *et al*., 2016). Copper mean levels in seeds were higher than in the other watermelon parts probably because plants store minerals on different plant parts based on maturity level of plants, the function of each part and vegetation period (Ovca *et al*., 2011).

4.4.1.5 Comparison of total mean levels of Cu in parts of dried Charleston grey (CG) watermelons from Ngara and Mwea markets

As shown in table 4.10, total mean levels of Cu in dried CG watermelons from Ngara market ranged from 1.80 ± 0.02 mg/100 g to 0.42 ± 0.03 mg/100 g in the order seeds > peels > red flesh > white flesh while in Mwea market, the range was from 1.85 ± 0.01 mg/100 g to 0.50 ± 0.01 mg/100 g in the order seeds > peels > white flesh > red flesh, differing significantly in peel and white flesh.

4.4.1.6 Comparison of total mean levels of Cu in parts of dried crimson sweet (CS) watermelons from Ngara and Mwea markets

As tabulated in table 4.10, total mean levels of Cu in dried CS watermelons from Ngara market ranged from 1.54 ± 0.02 mg/100 g to 0.56 ± 0.04 mg/100 g in the order seed > red flesh > peel > white flesh while the range in Cu mean levels in Mwea market was from 1.42 ± 0.06 mg/100 g to 0.46 ± 0.05 mg/100 g and differed significantly only in red flesh part.

4.4.1.7 Comparison of total mean levels of Cu in parts of dried sugar baby (SB) watermelons from Ngara and Mwea markets

From table 4.10, total mean levels of Cu in SB watermelon from Ngara market ranged from 1.53 ± 0.01 mg/100 g to 0.47 ± 0.04 mg/100 g while the range in Cu mean levels in Mwea market was from 1.66 ± 0.05 mg/100 g to 0.44 ± 0.03 mg/100 g with seeds > peels > red flesh > white flesh order followed in both markets and differing significantly only in seed part.

4.4.2 Mean levels of Cu species extracted sequentially using different solvents in various parts of dried Charleston grey (CG) watermelons

The results for mean levels of Cu species in various solvents for various parts of dried CG watermelon samples obtained from Ngara and Mwea markets are discussed in the following sub sections. The results of the mean levels of Cu species in peel, white flesh, red flesh and seeds of CG bought from Ngara and Mwea markets were calculated and compared at $\alpha = 0.05$ level as shown in tables 4.11 and 4.12. The results presented in tables 4.11 and 4.12 are discussed in the following sub sections.

4.4.2.1 Mean levels of Cu species (mg/100 g) in peels extracted by various solvents from dried Charleston grey (CG) watermelons and their comparison from Ngara and Mwea markets

From the results in table 4.11, it can be seen that the mean levels of extracted Cu species in various solvents from peels of dried CG watermelons from Ngara market ranged from 0.34 ± 0.02 mg/100 g (52.5 %) to 0.04 ± 0.01 mg/100 g (6.1 %) in the order RE > EE > WE > DE ≥ HE while peels of CG watermelons from Mwea market had mean levels of extracted Cu species ranging from 0.37 ± 0.01 mg/100 g (61.7 %) to 0.04 ± 0.01 mg/100 g (6.6 %) in the order RE > WE > HE > EE ≥ DE. Watermelons from both markets had significantly different amounts of Cu species

Table 4.11: Mean levels of Cu species (mg/100 g) extracted sequentially using different solvents in various parts of dried CG watermelons from Ngara and Mwea markets, n = 18

Sampling site	Watermelon part	Mean concentration (mg/100 g ± SD)					
		Water extract	Ethanol extract	dichloromethane extract	Hexane extract	Residual	P - value
Ngara market n = 9	Peel	0.11 ± 0.01^{Bc}	0.12 ± 0.01^{Bc}	0.04 ± 0.01^{A}	0.04 ± 0.01^{A}	0.34 ± 0.02^{Cb}	< 0.001
	White flesh	0.05 ± 0.01^{Ab}	0.07 ± 0.01^{Ab}	0.03 ± 0.02^{A}	0.03 ± 0.02^{A}	0.23 ± 0.01^{Ba}	< 0.001
	Red flesh	0.03 ± 0.01^{Aa}	0.04 ± 0.01^{Aa}	0.02 ± 0.01^{A}	0.02 ± 0.01^{A}	0.36 ± 0.01^{Bb}	< 0.001
	Seed	0.54 ± 0.01^{Cd}	0.28 ± 0.01^{Bd}	0.04 ± 0.01^{A}	0.04 ± 0.01^{A}	0.90 ± 0.01^{Dd}	< 0.001
	P - value	< 0.001	< 0.001	0.127	0.193	< 0.001	
Mwea market n = 9	Peel	0.10 ± 0.01^{Bb}	0.04 ± 0.01^{Ab}	0.04 ± 0.01^{A}	0.05 ± 0.01^{A}	0.37 ± 0.01^{Cb}	< 0.001
	White flesh	0.04 ± 0.01^{Aa}	0.06 ± 0.01^{Bb}	0.03 ± 0.01^{A}	0.03 ± 0.01^{A}	0.32 ± 0.01^{Ca}	< 0.001
	Red flesh	0.03 ± 0.01^{Aa}	0.03 ± 0.01^{Aa}	0.02 ± 0.01^{A}	0.03 ± 0.01^{A}	0.36 ± 0.01^{Bb}	< 0.001
	Seed	0.62 ± 0.01^{Cc}	0.26 ± 0.01^{Bc}	0.04 ± 0.01^{A}	0.05 ± 0.01^{A}	0.89 ± 0.01^{Dc}	< 0.001
	P - value	< 0.001	< 0.001	0.112	0.052	< 0.001	

Mean values with the different small letter(s) within the same column are significantly different and mean values with different capital letter within same row are significantly different ($\alpha = 0.05$, $P < 0.001$, one-way ANOVA, SNK-test)

Table 4.12: Comparison of extracted mean levels of Cu species (mg/100 g) in various solvents of dried CG watermelons parts from Ngara and Mwea markets

Solvent	Part	Ngara	Mwea	P - value
Water	peel	0.11 ± 0.01	0.10 ± 0.01	0.260
	White flesh	0.05 ± 0.01	0.04 ± 0.01	0.260
	Red flesh	0.03 ± 0.01	0.03 ± 0.01	1.000
	seed	0.54 ± 0.01	0.62 ± 0.01	< 0.001
Ethanol	peel	0.12 ± 0.01	0.04 ± 0.01	< 0.001
	White flesh	0.07 ± 0.01	0.06 ± 0.01	0.260
	Red flesh	0.04 ± 0.01	0.03 ± 0.01	0.260
	seed	0.28 ± 0.01	0.26 ± 0.01	0.044
Dichloromethane	peel	0.04 ± 0.01	0.04 ± 0.01	1.000
	White flesh	0.03 ± 0.02	0.03 ± 0.01	1.000
	Red flesh	0.02 ± 0.01	0.02 ± 0.01	1.000
	seed	0.04 ± 0.01	0.04 ± 0.01	1.000
Hexane	peel	0.04 ± 0.01	0.05 ± 0.01	0.260
	White flesh	0.03 ± 0.02	0.03 ± 0.01	1.000
	Red flesh	0.02 ± 0.01	0.03 ± 0.01	0.260
	seed	0.04 ± 0.01	0.05 ± 0.01	0.260
Residue	peel	0.34 ± 0.02	0.37 ± 0.01	0.053
	White flesh	0.23 ± 0.01	0.32 ± 0.01	< 0.001
	Red flesh	0.36 ± 0.01	0.36 ± 0.01	1.000
	seed	0.90 ± 0.01	0.89 ± 0.01	0.260

Mean levels of Cu compared at $\alpha = 0.05$

extracted by all extracting solvents and the one that remained in the residual of CG peels. Upon comparison as can be seen in table 4.12 significantly different amounts of Cu species from CG peels were extracted only by ethanol with that from Ngara market being more than that from Mwea market.

4.4.2.2 Mean levels of Cu species (mg/100 g) in white flesh extracted by various solvents from dried Charleston grey (CG) watermelons and their comparison from Ngara and Mwea markets

From table 4.11, the results show that mean levels of Cu species extracted by various solvents from dried CG watermelons from Ngara market in white flesh ranged from 0.23 ± 0.01 mg/100 g (55.4 %) to 0.03 ± 0.02 mg/100 g (7.9 %) in the order RE > EE

> WE > DE ≥ HE while white flesh part of CG watermelons from Mwea market had extracted Cu species mean levels ranging from 0.32 ± 0.01 mg/100 g (66.7 %) to 0.03 ± 0.01 mg/100 g (6.2 %) in the same pattern as those from Ngara market.

In both markets significantly different amounts of Cu species were extracted by all extracting solvents and the one that remained in the residual of CG white flesh. Upon comparison as tabulated in table 4.12 significantly different amounts of Cu species were only left in the residue of CG white flesh with those from Mwea market being more than those from Ngara market.

4.4.2.3 Mean levels of Cu species (mg/100 g) in red flesh extracted by various solvents from dried Charleston grey (CG) watermelons and their comparison from Ngara and Mwea markets

As seen from table 4.11, the mean amount of Cu species extracted by various solvents from red flesh part of dried CG watermelons from Ngara market ranged from 0.36 ± 0.01 mg/100 g (77.1 %) to 0.02 ± 0.01 mg/100 g (3.5 %) in the order RE > EE > WE > DE ≥ HE while CG watermelons from Mwea market had extracted Cu species mean levels ranging from 0.36 ± 0.01 mg/100 g (76.9 %) to 0.02 ± 0.01 mg/100 g (4.3 %) in the order RE > WE ≥ EE ≥ HE > DE. In both markets significantly different amounts of Cu species were extracted by all extracting solvents and the one that remained in the residual of CG red flesh. Upon comparison as can be observed from table 4.12 significantly equivalent amounts of Cu species were extracted by all the extracting solvents from red flesh part of CG watermelons.

4.4.2.4 Mean levels of Cu species (mg/100 g) in seeds extracted by various solvents from dried Charleston grey (CG) watermelons and their comparison from Ngara and Mwea markets

From table 4.11, the mean levels of extracted Cu species by various solvents from seeds of dried CG watermelons from Ngara market ranged from 0.90 ± 0.01 mg/100 g (50.0 %) to 0.04 ± 0.01 mg/100 g (2.2 %) in the order RE > WE > EE > DE ≥ HE while seeds from CG watermelons from Mwea market had extracted Cu species mean levels ranging from 0.89 ± 0.01 mg/100 g (47.9 %) to 0.04 ± 0.01 mg/100 g (2.1 %) in the order RE > WE > EE > HE > DE. In both markets significantly different amounts of Cu species were extracted by all extracting solvents and the one that remained in the residual of CG seeds. Upon comparison as tabulated in Table 4.12 significantly different amounts of Cu species were only extracted from seed part of CG watermelon by water and ethanol with WE from Mwea market having the highest levels while EE still from Mwea market having the least levels. The variation of mean levels of Cu species in various extracts of the watermelon can occur due to presence of organic acids and calcium which promote and inhibit availability of Cu. (Kojo, 2003).

Organic acid apart from ascorbic acid enhance absorption of Cu by forming soluble ligands. Ascorbic acid inhibit Cu absorption by reduction of cupric to cuprous copper which is not easily absorbed (Rosalind, 2007). Calcium promotes Cu absorption after being strongly bound to phytic acid with which they share the same coordination number. Calcium may inhibit Cu absorption during transportation and absorption in the intestine where they share same transferring protein (Walter *et al*., 2002).

There were significantly different amounts of Cu species extracted from various parts of CG watermelon from both Ngara and Mwea markets except in DE and HE. Also different solvents extracted significantly different amounts of Cu species from

watermelons from Ngara and Mwea markets. The Cu species extracted by water and a fraction of ethanol depending on pH would be available for absorption in human digestive system while one extracted by dichloromethane, hexane and left in residual would not be available for absorption in human digestive system.

As shown in table 4.11, peel and white flesh parts should be eaten as they have substantial amount of bioavailable Cu that can add to the one present in red flesh and seeds. The bioavailable extraction of Cu species ranged from 13 % to 47 % with WE in seed and peels having the highest amount from both Mwea and Ngara markets. All parts of CG watermelon fruit combined contained lower bioavailable concentration of Cu than the RDA of copper of (2 - 3) mg (Ozgur *et al*., 2009; Gyamfi *et al*., 2011; Kiran *et al*., 2012). Thus even if all parts of the fruit are eaten cannot cause adverse health effects as a result of Cu mean levels as long as one eats about 100 g per day. Seed part of the watermelon in both markets would provide higher amount of copper with that of Ngara providing (0.82 mg/100 g, 46 % bioavailable Cu) while that from Mwea market providing (0.88 mg/100 g, 37 % bioavailable Cu) when consumed.

Copper has low mobility relative to other elements in plants and most of it appears to remain in root and leaf tissues until they reach maturity. Contrasting results were obtained by Jean *et al*. (2012) who worked on amaranth and roselle plant and reported Cu accumulation on leaves of (2.036 - 3.086) mg/100 g and that of the roots as (0.8 - 1.1) mg/100 g. Variation in the concentration can be brought by where the plant was grown and also the physiological make up of the plants. Also variation in the Cu mean levels in the same variety of watermelons could be attributed to Cu appropriate form (for example charged, complexed) presence in the food matrix of inhibitory or promotive substances (Michael, 1999).

4.4.3 Mean levels of Cu species extracted sequentially using different solvents in parts of dried crimson sweet (CS) watermelons and their comparison from Ngara and Mwea markets

The results for mean levels of Cu species in the parts of dried CS watermelon samples obtained from Ngara and Mwea markets are discussed in the following sub sections. The results of the mean levels of Cu species in dry peel, white flesh, red flesh and seeds of CS bought from Ngara and Mwea markets were calculated and compared at $\alpha = 0.05$ level as shown in tables 4.13 and 4.14. The results presented in tables 4.13 and 4.14 are discussed in the following sub sections.

4.4.3.1 Mean levels of Cu species (mg/100 g) in peels extracted by various solvents from dried crimson sweet (CS) watermelons and their comparison from Ngara and Mwea markets

From the results in table 4.13, mean levels of Cu species of various solvents from peels of dried CS watermelons from Ngara market ranged from 0.33 ± 0.01 mg/100 g (50.0 %) to 0.04 ± 0.01 mg/100 g (6.0 %) and followed a decreasing order of RE > WE > EE > DE ≥ HE while peels of CS from Mwea market had Cu species mean levels ranging from 0.34 ± 0.01 mg/100 g (51.5 %) to 0.04 ± 0.01 mg/100 g (6.1 %) in the order RE > WE > EE > HE > DE. In both markets the amounts of Cu species extracted from CS peels by various solvents were significantly different. Upon comparison as can be observed in table 4.14, significantly different amounts of Cu species were only extracted by water from CS peels with that from Ngara market being more than that from Mwea market.

4.4.3.2 Mean levels of Cu species (mg/100 g) in white flesh extracted by various solvents from dried crimson sweet (CS) watermelons from Ngara and Mwea markets

As tabulated in table 4.13, the mean amount of Cu species extracted by various

Table 4.13: Mean levels of Cu species (mg/100 g) extracted sequentially using different solvents in parts of dried crimson sweet (CS) watermelons from Ngara and Mwea markets, n = 18

Sampling site	Watermelon part	Mean concentration (mg/100 g ± SD)					
		Water extract	Ethanol extract	dichloromethane extract	Hexane extract	Residual	P - value
Ngara market n = 9	Peel	0.18 ± 0.01^{Bb}	0.07 ± 0.01^{Ab}	0.04 ± 0.01^{A}	0.04 ± 0.01^{A}	0.33 ± 0.01^{Cb}	< 0.001
	White flesh	0.15 ± 0.01^{Ba}	0.04 ± 0.01^{Aa}	0.03 ± 0.02^{A}	0.03 ± 0.01^{A}	0.31 ± 0.01^{Ca}	< 0.001
	Red flesh	0.29 ± 0.01^{Cd}	0.08 ± 0.01^{Bb}	0.01 ± 0.01^{A}	0.02 ± 0.01^{A}	0.41 ± 0.01^{Dc}	< 0.001
	Seed	0.26 ± 0.01^{Bc}	0.31 ± 0.01^{Cc}	0.04 ± 0.01^{A}	0.04 ± 0.01^{A}	0.89 ± 0.01^{Dd}	< 0.001
	P - value	< 0.001	< 0.001	0.051	0.112	< 0.001	
Mwea market n = 9	Peel	0.16 ± 0.01^{Cb}	0.07 ± 0.01^{Bb}	0.04 ± 0.01^{A}	0.05 ± 0.01^{A}	0.34 ± 0.01^{Db}	< 0.001
	White flesh	0.04 ± 0.01^{Aa}	0.04 ± 0.01^{Aa}	0.03 ± 0.01^{A}	0.04 ± 0.01^{A}	0.32 ± 0.01^{Ba}	< 0.001
	Red flesh	0.03 ± 0.01^{Aa}	0.06 ± 0.01^{Ab}	0.02 ± 0.01^{A}	0.03 ± 0.01^{A}	0.44 ± 0.01^{Bc}	< 0.001
	Seed	0.26 ± 0.01^{Cc}	0.19 ± 0.01^{Bc}	0.04 ± 0.01^{A}	0.05 ± 0.01^{A}	0.89 ± 0.01^{Dd}	< 0.001
	P - value	< 0.001	< 0.001	0.112	0.112	< 0.001	

Mean values with the different small letter(s) within the same column are significantly different and mean values with different capital letter within same row are significantly different ($\alpha = 0.05$, $P < 0.001$, one-way ANOVA, SNK-test)

Table 4.14: Comparison of mean levels of Cu species (mg/100 g) from various extracts and parts of dried CS watermelons from Ngara and Mwea markets

Solvent	Part	Ngara	Mwea	p - value
Water	peel	0.18 ± 0.01	0.16 ± 0.01	0.044
	White flesh	0.15 ± 0.01	0.04 ± 0.01	< 0.001
	Red flesh	0.29 ± 0.01	0.03 ± 0.01	< 0.001
	seed	0.26 ± 0.01	0.26 ± 0.01	1.000
Ethanol	peel	0.07 ± 0.01	0.07 ± 0.01	1.000
	White flesh	0.04 ± 0.01	0.04 ± 0.01	1.000
	Red flesh	0.08 ± 0.01	0.06 ± 0.01	0.044
	seed	0.31 ± 0.01	0.19 ± 0.01	< 0.001
Dichloromethane	peel	0.04 ± 0.01	0.04 ± 0.01	1.000
	White flesh	0.03 ± 0.02	0.03 ± 0.01	1.000
	Red flesh	0.01 ± 0.01	0.02 ± 0.01	0.260
	seed	0.04 ± 0.01	0.04 ± 0.01	1.000
Hexane	peel	0.04 ± 0.01	0.05 ± 0.01	0.260
	White flesh	0.03 ± 0.01	0.04 ± 0.01	0.260
	Red flesh	0.02 ± 0.01	0.03 ± 0.01	0.260
	seed	0.04 ± 0.01	0.05 ± 0.01	0.260
Residue	peel	0.33 ± 0.01	0.34 ± 0.01	0.260
	White flesh	0.31 ± 0.01	0.32 ± 0.01	0.260
	Red flesh	0.41 ± 0.01	0.44 ± 0.01	0.008
	seed	0.89 ± 0.01	0.89 ± 0.01	1.000

Mean levels of Cu compared at $\alpha = 0.05$

solvents from white flesh part of dried CS watermelons from Ngara market ranged from 0.31 ± 0.01 mg/100 g (55.2 %) to 0.03 ± 0.01 mg/100 g (5.3 %) in the order RE > WE > EE > DE ≥ HE while white flesh of CS watermelons from Mwea market had Cu species mean levels ranging from 0.32 ± 0.01 mg/100 g (68.4 %) to 0.03 ± 0.01 mg/100 g (6.3 %) in the order of RE > WE ≥ EE ≥ HE > DE. In both markets the amounts of Cu species extracted from CS white flesh by various solvents were significantly different. Upon comparison as tabulated in table 4.14, significantly different amounts of Cu species were only extracted by water from CS white flesh with that from Ngara market being more than that from Mwea market.

4.4.3.3 Mean levels of Cu species (mg/100 g) in red flesh extracted by various solvents from dried crimson sweet (CS) watermelons from Ngara and Mwea markets

From the results of table 4.13, the mean levels of extracted Cu species by various solvents from red flesh part of dried CS watermelons from Ngara market ranged from 0.41 ± 0.01 mg/100 g (50.4 %) to 0.01 ± 0.01 mg/100 g (1.6 %) in the order RE > WE > EE > HE > DE while red flesh of CS watermelons from Mwea market had extracted Cu species mean levels ranging from 0.44 ± 0.01 mg/100 g (76.0 %) to 0.02 ± 0.01 mg/100 g (3.4 %) in the order RE > EE > WE ≥ HE > DE. In both markets the amounts of Cu species extracted from CS red flesh by various solvents were significantly different. Upon comparison as shown in table 4.14, significantly different amounts of Cu species were only extracted by water and ethanol from CS red flesh with WE from Ngara market having the highest levels while WE from Mwea market having the least levels. Significantly different amount of Cu species remained in CS residue with that from Mwea market having higher levels than that from Ngara market.

4.4.3.4 Mean levels of Cu species (mg/100 g) in seed extracted by various solvents from dried crimson sweet (CS) watermelons from Ngara and Mwea markets

As shown in table 4.13, the mean levels of extracted Cu species by various solvents from seed part of dried CS watermelons from Ngara market ranged from 0.89 ± 0.01 mg/100 g (57.8 %) to 0.04 ± 0.01 mg/100 g (2.6 %) in the order RE > EE >WE ≥ DE ≥ HE while mean levels of Cu species in seeds of CS watermelons from Mwea market ranged from 0.89 ± 0.01 mg/100 g (62.3 %) to 0.04 ± 0.01 mg/100 g (2.8 %) in the order RE > WE > EE > HE > DE. In both markets the amounts of Cu species extracted from CS seeds by various solvents were significantly different. Upon comparison as observed in table 4.14, significantly different amounts of Cu species

were only extracted by ethanol from CS seed with EE from Ngara market being more than that from Mwea market. The variation of mean levels of Cu species in various extracts of the watermelons can occur due to presence of inhibitory or promotive substances of Cu in a plant matrix (Micheal, 1999).

From table 4.13, the bioavailable fraction of Cu ranged from 15 % to 46 % with EE in seeds of CS watermelons from Ngara market having the highest levels while EE in white flesh having the least levels. In Mwea market, WE in seeds part of CS watermelons had the highest levels while WE in red flesh of CS watermelons had the least levels. All parts of CS watermelon fruit combined contained lower concentration of bioavailable Cu than the RDA of Cu of (2 - 3) mg (Ozgur *et al*., 2009; Gyamfi *et al*., 2011; Kiran *et al*., 2012). Thus even if all parts of the fruit are eaten cannot cause adverse health effects as a result of Cu mean levels as long as 100 g/day of each part is not exceeded. Seed part of CS watermelons in both markets would provide highest amount of Cu with that from Ngara market providing (0.57 mg/100 g, 37 % bioavailable Cu) while that from Mwea market would provide (0.45 mg/100 g, 31 % bioavailable Cu) when consumed. The result of this study obtained a lower bioavailable Cu compared to work done by Anna *et al*. (2014) though the substrates were different. These researchers proposed that due to strong binding of Cu by pectins in citrus juices, only (50 - 65) % of the total concentration become bioavailable. However, Cautela *et al*. (2009) proposed that the free (unbound) fraction of Cu can be lower than 50 % of the total concentration.

Peel and white flesh parts of CS watermelons should be eaten as they have a substantial amount of bioavailable Cu that can add to the one present in red flesh and seeds. The percentage fraction of the bioavailable Cu of this study was higher

compared to (1 - 2) % reported by Haro-Vicente *et al*. (2006) in fruit juices in condition of gastro-intestinal digestion where the contributions toward RDA become very low. The difference in the works is brought by the manner of isolating potentially bioavailable species of Cu. The variation in the Cu species mean levels in the same variety of watermelons could be due to soil condition in the area where they were grown and presence of organic acids and Ca which promote and inhibit availability of Cu from a plant matrix (Kojo, 2003).

Organic acid apart from ascorbic acid enhance absorption of Cu by forming soluble ligands. Ascorbic acid inhibit Cu absorption by reduction of cupric to cuprous copper which is not easily absorbed (Rosalind, 2007). Calcium promotes Cu absorption after being strongly bound to phytic acid with which they share the same coordination number. Calcium may inhibit Cu absorption during transportation in the intestine where they share same transferring protein (Walter *et al*., 2002).

4.4.4 Mean levels of Cu species in various solvents and parts of dried sugar baby (SB) watermelons

The results for mean levels of Cu species in the parts of dried SB watermelon samples obtained from Ngara and Mwea markets are discussed in the following sub sections. The results of the mean levels of Cu species in dry peel, white flesh, red flesh and seeds of SB watermelons bought from Ngara and Mwea markets were calculated and compared at $\alpha = 0.05$ level as shown in tables 4.15 and 4.16.

Table 4.15: Mean levels of Cu species (mg/100 g) in various fractions and parts of dried sugar baby (SB) watermelons from Ngara and Mwea Markets, n = 18

Samplig site	Watermelon part	Mean concentration (mg/100 g ± SD)					
		Water extract	Ethanol extract	dichloromethane extract	Hexane extract	Residual	P - value
Ngara market, n=9	Peel	0.46 ± 0.01^{Dd}	0.07 ± 0.01^{Bb}	0.04 ± 0.01^{A}	0.04 ± 0.01^{A}	0.38 ± 0.01^{Cb}	< 0.001
	White flesh	0.04 ± 0.01^{Aa}	0.05 ± 0.01^{Aa}	0.03 ± 0.01^{A}	0.04 ± 0.01^{A}	0.30 ± 0.01^{Ba}	< 0.001
	Red flesh	0.10 ± 0.01^{Bb}	0.10 ± 0.01^{Bc}	0.02 ± 0.01^{A}	0.02 ± 0.01^{A}	0.41 ± 0.01^{Cc}	< 0.001
	Seed	0.44 ± 0.01^{Cc}	0.13 ± 0.01^{Bd}	0.04 ± 0.01^{A}	0.04 ± 0.01^{A}	0.88 ± 0.01^{Dd}	< 0.001
	P - value	< 0.001	< 0.001	0.112	0.095	< 0.001	
Mwea market n=9	Peel	0.46 ± 0.01^{Dd}	0.1 ± 0.01^{Bc}	0.03 ± 0.01^{A}	0.04 ± 0.01^{Aab}	0.38 ± 0.01^{Cb}	< 0.001
	White flesh	0.03 ± 0.01^{Aa}	0.04 ± 0.01^{Aa}	0.03 ± 0.01^{A}	0.03 ± 0.01^{Aab}	0.32 ± 0.01^{Ba}	< 0.001
	Red flesh	0.1 ± 0.01^{Bb}	0.07 ± 0.01^{Bb}	0.02 ± 0.01^{A}	0.02 ± 0.01^{Aa}	0.42 ± 0.01^{Cc}	< 0.001
	Seed	0.42 ± 0.01^{Cc}	0.28 ± 0.01^{Bd}	0.04 ± 0.01^{A}	0.05 ± 0.01^{Ab}	0.89 ± 0.01^{Dd}	< 0.001
	P - value	< 0.001	< 0.001	0.193	0.031	< 0.001	

Mean values with the different small letter(s) within the same column are significantly different and mean values with different capital letter within same row are significantly different ($\alpha = 0.05$, $P < 0.001$, one-way ANOVA, SNK-test).

Table 4.16: Comparison of extracted mean levels of Cu species (mg/100 g) in various fractions and parts of dried SB watermelons from Ngara and Mwea markets

Solvent	Part	Ngara	Mwea	P - value
Water	peel	0.46±0.01	0.46±0.01	1.000
	White flesh	0.04±0.01	0.03±0.01	0.260
	Red flesh	0.10±0.01	0.10±0.01	1.000
	seed	0.44±0.01	0.42±0.01	0.044
Ethanol	peel	0.46±0.01	0.1±0.01	< 0.001
	White flesh	0.04±0.01	0.04±0.01	1.000
	Red flesh	0.10±0.01	0.07±0.01	0.008
	seed	0.44±0.01	0.28±0.01	< 0.001
Dichloromethane	peel	0.04±0.01	0.03±0.01	0.260
	White flesh	0.03±0.01	0.03±0.01	1.000
	Red flesh	0.02±0.01	0.02±0.01	1.000
	seed	0.04±0.01	0.04±0.01	1.000
Hexane	peel	0.04±0.01	0.04±0.01	1.000
	White flesh	0.04±0.01	0.03±0.01	0.260
	Red flesh	0.02±0.01	0.02±0.01	1.000
	seed	0.04±0.01	0.05±0.01	0.260
Residue	peel	0.38±0.01	0.38±0.01	1.000
	White flesh	0.30±0.01	0.32±0.01	0.044
	Red flesh	0.41±0.01	0.42±0.01	0.260
	seed	0.88±0.01	0.89±0.01	0.260

Mean levels of Cu compared at $\alpha = 0.05$

The results presented in tables 4.15 and 4.16 are discussed in the following sub sections.

4.4.4.1 Mean levels of Cu species (mg/100 g) in peels extracted by various solvents from dry sugar baby (SB) watermelons from Ngara and Mwea markets

From results of table 4.15, the mean levels of extracted Cu species in various solvents of peels of SB from Ngara market ranged from 0.46 ± 0.01 mg/100 g (46.5 %) to 0.04 ± 0.0 mg/100 g (4.0 %) in the order WE > RE > EE > HE > DE with a similar trend in Mwea market with extracted Cu species values ranging from 0.46 ± 0.01 mg/100 g (45.6 %) to 0.03 ± 0.0 mg/100 g (3.0 %). In both markets the amounts of Cu species extracted from SB peels by various solvents were significantly different. Upon

comparison as shown in table 4.16, significantly different amount of Cu species were extracted from SB peels by only ethanol with EE from Ngara market
having more than that from Mwea market.

4.4.4.2 Mean levels of Cu species (mg/100 g) in white flesh extracted by various solvents from dried sugar baby (SB) watermelons from Ngara and Mwea markets

From table 4.15, mean levels of extracted Cu species by various solvents from white flesh of dried SB watermelons from Ngara market ranged from 0.30 ± 0.01 mg/100 g (65.4 %) to 0.03 ± 0.0 mg/100 g (6.5 %) while that from Mwea market ranged from 0.32 ± 0.01 mg/100 g (71.2 %) to 0.03 ± 0.0 mg/100 g (6.6 %) both in the order RE > EE > WE ≥ HE > DE. In both markets the amounts of Cu species extracted from SB white flesh by various solvents were significantly different. Upon comparison as can be observed in table 4.16, significantly different amount of Cu species only remained in the residue of SB white flesh.

4.4.4.3 Mean levels of Cu species (mg/100 g) in red flesh extracted by various solvents from dried sugar baby (SB) watermelons from Ngara and Mwea markets

From the results in table 4.15, mean levels of Cu species from various solvents extracts from red flesh of dried SB watermelons from Ngara market ranged from 0.41 ± 0.01 mg/100 g (63.1 %) to 0.02 ± 0.0 mg/100 g (3.1 %) in the order RE > WE ≥ EE > DE ≥ HE and apart from DE and HE the same pattern was observed in Mwea market ranging from 0.42 ± 0.01 mg/100 g (66.7 %) to 0.02 ± 0.0 mg/100 g (3.1 %) in the order RE > WE > EE > HE ≥ DE. In both markets significantly different amounts of Cu species were extracted by all the extracting solvents. After comparison as can be observed from Table 4.16, significantly different amounts of Cu species were only extracted by ethanol from red flesh part of SB with that from Ngara market being more than that from Mwea market.

4.4.4.4 Mean levels of Cu species (mg/100 g) in seed extracted by various solvents from dried sugar baby (SB) watermelons from Ngara and Mwea markets

As shown in table 4.15, the mean levels of extracted Cu species by various solvents from seeds of dried SB watermelons from Ngara market ranged from 0.88 ± 0.01 mg/100 g (57.5 %) to 0.04 ± 0.0 mg/100 g (2.6 %) while that from Mwea market ranged from 0.89 ± 0.01 mg/100 g (53.0 %) to 0.04 ± 0.0 mg/100 g (2.4 %) both in the order RE > WE > EE > HE ≥ DE. In both markets the amounts of Cu species extracted from seeds of SB watermelons by various solvents were significantly different.

As shown in table 4.16 after comparison, significantly different amounts of Cu species were extracted only by water and ethanol from SB seeds with WE from Ngara market having the highest levels while EE from Mwea market had the least levels. The variation of mean levels of Cu species in various extracts of the watermelon can occur due to presence of organic acids (citric, lactic, acetic and malic) in plant matrix that leads to solubilization, thereby increasing Cu bioavailability (Raul, 1998).

From table 4.15, peels and white flesh parts of SB watermelons should be eaten as they have a substantial amount of bioavailable Cu that can add to the one present in red flesh and seeds. It is noted that a bigger proportion of Cu remained in residual of seed suggesting presence of inbitors of Cu absorption such as ascorbic acid (Rosalind, 2007). The bioavailable forms of Cu from Ngara market ranged between (19 - 56) % with WE in peel having the highest levels and EE in peel having the least levels while in Mwea market, WE in peel had the highest levels and WE in white flesh had the least levels. All parts of SB watermelon fruit combined contained lower concentration of bioavalable Cu than the RDA of copper of (2 - 3) mg (Ozgur *et al*., 2009; Gyamfi

et al., 2011 and Kiran *et al*., 2012). Thus even if all parts of the fruit are eaten, it cannot cause adverse health effects as a result of Cu mean levels as long as one eats about 100 g of each part per day. In both market, seed part of the watermelon would provide higher amount of Cu with those from Ngara market providing (0.57 mg/100 g, 37 % bioavailable Cu) while those from Mwea market providing (0.7 mg/100 g, 47 % bioavailable Cu) when consumed. Thus results from this study suggest that watermelons can be used to add to RDA of Cu. Nasreddine *et al*. (2010) and Khouzam *et al*. (2011) suggested bread and cereals to be the main contributors to the intake of Cu (486.9 $\mu g day^{-1}$). They further reported that the analyzed food varieties (bread, cheese, cucumbers, squash and apple) contributed 73 % of the Cu RDA (0.66 $mg day^{-1}$ out of 0.9 $mg day^{-1}$ RDA).

The result of the bioavailable Cu in this study has a big range compared to (49.2 - 62.4) % of Cu in bread, (51.9 - 66.5) % of Cu in cheese, (40.7 - 58.9) % of Cu in fruit and vegetables as reported by Khouzam *et al*. (2011) and (46.7 - 69.1) % Cu from tea bisquit reported by Vitali *et al*. (2008). The results of these workers showed that nutrients bioaccessibility (Fe, Zn, Cu, Mo and Mn) varies largely as a function of food matrix components and elements of interest. About (27 - 72) % of Cu is absorbed by small mammals from plants as reported by Hunter *et al*. (1987) which is comparable with the findings of this study. These workers found out that Cu translocated from roots to green stems and leaves are predominantly protein bound or ionic.

It can be inferred that various edible parts of plants are storage banks for metals after being absorbed from soil as reported by Ang and Ng (2000). The variation in the copper mean levels in the same variety of watermelons could be due to presence of chelating agent such as cysteine and ascorbic acid which reduces bioavailability of Cu

by reduction from divalent to monovalent state. In addition presence of organic acids (citric, lactic, acetic and malic) in foods leads to solubilization of Cu increasing bioavailability (Raul, 1998). This is in agreement with Anna *et al*. (2014) who proposed that only carboxylic acids (citric, tartaric and malic acids) can form complexes with elements, making their availability in fruits different from the availability that can be anticipated from total concentrations.

Furthermore other workers (Carbanaro *et al*., 2001; Schumann and Elsenhans, 2002 and Camara *et al*., 2005) reported that Cu-phytate complexes remain soluble at the pH in the intestinal lumen. These researchers proposed that sugars, animal proteins, S-amino acids and histidine inhibits Cu absorption rather than dietary fibres. The variations in the concentration of elements in plants are as a result of quality factors such as mineral composition of soil in which plant grows and absorbability of corresponding elements, age of plant, season in which sample was collected and climatic conditions including atmosphere and pollution as reported by Kiran *et al*. (2012).

4.5 Comparison of mean levels of Zn species in various extracts in parts of dried and fresh CG, CS and SB watermelons

The moisture content of the fresh watermelon parts was taken into consideration when measuring mass of the sample for extraction. Since some forms of Zn may change when flesh watermelon parts are dried, comparison of mean levels of extracted Zn in various solvent and parts of watermelons from Ngara market was done as tabulated in tables 4.17 to 4.20.

Table 4.17: Comparison of mean levels of Zn species (mg/100 g) from water extracts in dried and fresh parts of CG, CS and SB watermelons varieties from Ngara market

Variety	Part	Dry	Fresh	P - value
Charleston grey n = 3	peel	3.83 ± 0.01	3.46 ± 0.02	< 0.001
	white flesh	3.59 ± 0.01	4.13 ± 0.01	< 0.001
	Red flesh	1.91 ± 0.01	0.80 ± 0.01	< 0.001
	seed	2.67 ± 0.01	2.14 ± 0.01	< 0.001
Crimson sweet n = 3	peel	3.57 ± 0.01	2.64 ± 0.01	< 0.001
	white flesh	3.78 ± 0.01	2.77 ± 0.02	< 0.001
	Red flesh	4.84 ± 0.04	0.90 ± 0.01	< 0.001
	seed	1.51 ± 0.01	1.96 ± 0.01	< 0.001
Sugar baby n = 3	peel	4.53 ± 0.01	5.83 ± 0.06	< 0.001
	white flesh	2.59 ± 0.01	3.19 ± 0.01	< 0.001
	Red flesh	4.70 ± 0.02	1.06 ± 0.01	< 0.001
	seed	2.68 ± 0.01	3.96 ± 0.01	< 0.001

Water extracts mean levels of Zn species compared at $\alpha = 0.05$

As tabulated in table 4.17, the mean levels of Zn species in WE in fresh watermelons ranged from 4.13 ± 0.01 mg/100 g (59.9 %) to 0.80 ± 0.01 mg/100 g (11.6 %) with white flesh having the highest levels and red flesh having the least levels. In dried watermelon parts, the mean levels of Zn species ranged from 3.83 ± 0.01 mg/100 g (50.2 %) to 1.91 ± 0.01 mg/100 g (31.1 %) with peels having the highest levels while red flesh had the least levels. Significantly different amounts of Zn species were extracted from the corresponding parts of CG, CS and SB fresh and dry watermelons. Drying increased amounts of Zn species extracted from CG watermelon apart from white flesh. Similar observations were made in CS and SB watermelon varieties, but the increase in amount of Zn species extracted as a result of drying depended on parts and watermelon varieties. Upon drying especially the red flesh part of the watermelons, there was increase in the amount of Zn species extracted. This can be attributed to the increase in concentration of Zn species that occurs after a large proportion of water is lost through drying. Significantly different mean levels of Zn species were extracted by water in corresponding dry and fresh parts of CG, CS and SB watermelon varieties. Drying may reduce phosphate group to less than five in the

phytate thus increasing bioavailability of zinc (Kumar *et al*., 2010). Drying may also reduce free amino acids and available sites for Zn - N bond formation resulting in decrease in organozinc compounds with greater bioavailability (Rosalind, 2007).

Table 4.18: Ethanol extracts mean levels of Zn species (mg/100 g) in parts of dried and fresh watermelon varieties from Ngara market

Variety	Part	Dry	Fresh	P - value
Charleston grey n = 3	peel	0.61 ± 0.01	5.78 ± 0.01	< 0.001
	white flesh	1.23 ± 0.01	6.22 ± 0.01	< 0.001
	Red flesh	1.29 ± 0.01	6.43 ± 0.01	< 0.001
	seed	0.57 ± 0.01	1.00 ± 0.01	< 0.001
Crimson sweet n = 3	peel	0.50 ± 0.01	5.77 ± 0.01	< 0.001
	white flesh	0.95 ± 0.02	6.71 ± 0.01	< 0.001
	Red flesh	1.42 ± 0.01	6.09 ± 0.02	< 0.001
	seed	0.79 ± 0.01	0.89 ± 0.02	< 0.001
Sugar baby n = 3	Peel	1.61 ± 0.01	5.61 ± 0.01	< 0.001
	white flesh	1.82 ± 0.01	5.58 ± 0.01	< 0.001
	Red flesh	1.94 ± 0.01	6.60 ± 0.01	< 0.001
	Seed	0.45 ± 0.01	0.99 ± 0.01	< 0.001

Ethanol extracts mean levels of Zn species compared at $\alpha = 0.05$

As shown in table 4.18, the amounts of extracted Zn in dried and fresh parts of CG watermelons using ethanol decreased from 5.78 ± 0.01 mg/100 g (28.9 %) to 0.61 ± 0.01 mg/100 g (8.0 %) in peel and from 6.22 ± 0.01 mg/100 g (31.1 %) to 1.23 ± 0.01 mg/100 g (15.6 %) in white flesh and was significantly different. A similar trend was observed in red flesh and seed parts of CG watermelon. There was significant decrease in amount of Zn species extracted by ethanol from dried watermelon parts compared to all the fresh parts of CG watermelon. Similar observations were made in all the other extracts from CS and SB watermelons. This can be attributed to the fact that when the watermelon is fresh, it is soluble in the polar ethanol solvent but upon drying many insoluble forms of Zn are formed. Drying may reduce free amino acids and available sites for Zn - N bond formation resulting in decrease in organozinc compounds with greater bioavailability (Rosalind, 2007).

Table 4.19: Dichloromethane extracts mean levels of Zn species (mg/100 g) in parts of dried and fresh watermelon varieties from Ngara market

Variety	Part	Dry	Fresh	P - value
Charleston grey n = 3	peel	0.57 ± 0.01	0.42 ± 0.01	< 0.001
	white flesh	0.52 ± 0.01	0.13 ± 0.01	< 0.001
	Red flesh	0.50 ± 0.01	0.17 ± 0.01	< 0.001
	seed	0.49 ± 0.01	0.58 ± 0.03	0.019
Crimson sweet n = 3	peel	0.56 ± 0.01	0.52 ± 0.01	0.008
	white flesh	0.50 ± 0.01	0.28 ± 0.01	< 0.001
	Red flesh	0.45 ± 0.01	0.07 ± 0.01	< 0.001
	seed	0.48 ± 0.01	0.48 ± 0.02	0.768
Sugar baby n = 3	Peel	0.56 ± 0.01	0.68 ± 0.02	< 0.001
	white flesh	0.58 ± 0.01	0.33 ± 0.01	< 0.001
	Red flesh	0.61 ± 0.01	0.12 ± 0.01	< 0.001
	Seed	0.51 ± 0.01	0.44 ± 0.01	< 0.001

Dichloromethane extracts mean levels of Zn species compared at $\alpha = 0.05$

As seen in table 4.19, the levels of Zn species in fresh CG watermelons ranged from 0.58 ± 0.03 mg/100 g (7.25 %) to 0.13 ± 0.01 mg/100 g (1.6 %) with seed having the highest levels and white flesh the least levels. The levels of Zn species in dried CG watermelons ranged from 0.57 ± 0.01 mg/100 g (7.4 %) to 0.50 ± 0.01 mg/100 g (6.5 %) with peels having the highest levels and red flesh having the least levels. Apart from seed, all the other parts of CG watermelon registered an increase in the amount of Zn species extracted upon drying. The same pattern was observed in CS and SB watermelons. The increase in Zn species extracted upon drying depended on the part and species of the watermelon. There was a significance difference in mean levels of Zn species in dry and fresh samples in all parts of CG, CS and SB watermelons. Drying increased the unavailable forms of Zn in all watermelons parts except CG seeds, CS seeds and SB peels suggesting chelation of Zn with high molecular weight which lowers Zn availability (Antonia *et al.*, 2009).

Table 4.20: Hexane extracts mean levels of Zn species (mg/100 g) in parts of dried and fresh watermelon varieties from Ngara market

Variety	Part	Dry	Fresh	P - value
Charleston grey n = 3	peel	0.54 ± 0.01	1.01 ± 0.01	< 0.001
	white flesh	0.83 ± 0.01	0.79 ± 0.01	0.008
	Red flesh	0.60 ± 0.01	0.85 ± 0.01	< 0.001
	seed	0.86 ± 0.01	1.17 ± 0.01	< 0.001
Crimson sweet n = 3	peel	0.84 ± 0.01	1.10 ± 0.01	< 0.001
	white flesh	0.65 ± 0.01	0.89 ± 0.01	< 0.001
	Red flesh	0.63 ± 0.01	0.86 ± 0.01	< 0.001
	seed	0.82 ± 0.01	1.25 ± 0.01	< 0.001
Sugar baby n = 3	Peel	0.64 ± 0.01	0.96 ± 0.01	< 0.001
	white flesh	0.65 ± 0.01	0.95 ± 0.02	< 0.001
	Red flesh	0.45 ± 0.01	0.88 ± 0.02	< 0.001
	Seed	0.80 ± 0.01	1.19 ± 0.01	< 0.001

Hexane extracts mean levels of Zn species compared at $\alpha = 0.05$

From the results in table 4.20, the levels of Zn species in parts of fresh CG watermelons ranged from 1.17 ± 0.01 mg/100 g (14.6 %) to 0.79 ± 0.01 mg/100 g (9.8 %) with seed having the highest levels and white flesh having the least levels. The levels of Zn species in dried parts of CG watermelons ranged from 0.86 ± 0.01 mg/100 g (11.5 %) to 0.54 ± 0.01 mg/100 g (7.1 %) with seed having the highest levels and peel having the least levels. Apart from white flesh all the other parts of CG watermelon registered a decrease in amount of Zn species extracted by hexane upon drying. The same was observed in CS and SB parts of watermelons though the decrease in amount depended on the watermelon species and watermelon part. There was a significance difference in mean levels of Zn species in corresponding dry and fresh samples in CG, CS and SB watermelons in hexane extracts. Drying decreased the unavailable forms of Zn in watermelon parts suggesting chelation of Zn with high molecular weight which lowers Zn availability (Antonia *et al.,* 2009).

4.6 Comparison of mean levels of Cu species in various extracts in parts of dried and fresh CG, CS and SB watermelons

The moisture content of the fresh watermelon parts was taken into consideration when measuring mass of the sample for extraction. Since some forms of Cu may change when fresh watermelons parts are dried, comparison of mean levels of extracted Cu in various solvent extracts in parts of CG, CS and SB watermelons from Ngara market was done as tabulated in tables 4.21 to 4.24.

Table: 4.21: Water extracts mean levels of Cu species (mg/100 g) in parts of dried and fresh watermelon varieties from Ngara market

Variety	Part	Dry	Fresh	P - value
Charleston grey n = 3	Peel	0.11 ± 0.01	0.10 ± 0.01	0.288
	White flesh	0.05 ± 0.01	0.11 ± 0.01	0.002
	Red flesh	0.03 ± 0.01	0.07 ±0.01	0.008
	Seed	0.54 ± 0.01	0.13 ± 0.01	<0.001
Crimson sweet n = 3	Peel	0.18 ± 0.01	0.12 ± 0.02	0.004
	White flesh	0.15 ± 0.01	0.03 ± 0.01	<0.001
	Red flesh	0.29 ± 0.01	0.09 ± 0.01	<0.001
	Seed	0.26 ± 0.01	0.13 ± 0.01	<0.001
Sugar baby n = 3	Peel	0.46 ± 0.01	0.03 ± 0.01	<0.001
	White flesh	0.04 ± 0.01	0.11 ± 0.02	0.002
	Red flesh	0.1 ± 0.01	0.09 ± 0.01	0.288
	Seed	0.44 ± 0.01	0.13 ± 0.01	<0.001

Water extracts mean levels of Cu compared at $\alpha = 0.05$

As seen from table 4.21, amounts of Cu species from fresh CG watermelon ranged from 0.13 ± 0.01 mg/100 g (7.2 %) to 0.07 ±0.01 mg/100 g (3.8 %) with seeds having the highest levels and red flesh having the least levels. The Cu species levels in dried CG ranged from 0.54 ± 0.01 mg/100 g (30.0 %) to 0.03 ± 0.01 mg/100 g (6.5 %) with seed having the highest levels and red flesh had the least levels. Apart from CG peels, significantly different amounts of Cu species were extracted by water from fresh and dry CG watermelon parts. White flesh and red flesh parts of CG registered a decrease in amount of Cu species extracted upon drying while the seed part recorded an

increase of Cu species extracted upon drying. Apart from SB white flesh all the other parts of SB and CS watermelons registered an increase in amount of Cu species extracted upon drying and were significantly different in fresh and dried watermelons. Amounts of Cu species extracted from SB red flesh in fresh and dried watermelons was statistically equivalent. The increase of the bioavailable mean levels of Cu species upon drying in peels could be attributed to denaturing of ascorbic acid which inhibits Cu availability (Raul, 1998).

The variation in the bioavailable mean levels of Cu species of watermelon parts could be attributed to reduction of free amino acids and available sites for Cu - N bond formation resulting in decrease in organocopper compounds with greater bioavailability. The increase in absorption of Cu species upon drying could suggest that the Cu absorption inhibitors, cystine and ascorbic acid got denatured upon drying thereby increasing Cu availability (Raul,1998). Kabata-Pendias (2010) reported (0.01 - 0.32) mg/100 g of bioavailable Cu in fresh vegetables based on edible parts which is close to the result of this study.

Table 4.22: Ethanol extracts mean levels of Cu species (mg/100 g) in parts of dry and fresh watermelon varieties from Ngara market

Variety	Part	Dry	Fresh	P - value
Charleston grey n = 3	Peel	0.12 ± 0.01	0.06 ± 0.01	0.002
	white flesh	0.07 ± 0.01	0.10 ± 0.01	0.021
	Red flesh	0.04 ± 0.01	0.06 ± 0.01	0.070
	Seed	0.28 ± 0.01	0.13 ± 0.01	<0.001
Crimson sweet n = 3	peel	0.07 ± 0.01	0.13 ± 0.01	0.002
	white flesh	0.04 ± 0.01	0.17 ± 0.01	<0.001
	Red flesh	0.08 ± 0.01	0.37 ± 0.01	<0.001
	seed	0.31 ± 0.01	0.06 ± 0.01	<0.001
Sugar baby n = 3	peel	0.07 ± 0.01	0.13 ± 0.01	0.002
	white flesh	0.05 ± 0.01	0.11 ± 0.01	0.002
	Red flesh	0.1 ± 0.01	0.38 ± 0.01	<0.001
	seed	0.13 ± 0.01	0.1 ± 0.01	0.021

Ethanol extracts mean levels of Cu species compared at $\alpha = 0.05$

As tabulated in table 4.22, the levels of Cu species in fresh and dried parts of CG watermelons extracted using ethanol increased from 0.06 ± 0.01 mg/100 g (9.2 %) to 0.12 ± 0.01 mg/100 g (18.4 %) in peel and from 0.13 ± 0.01 mg/100 g (7.2 %) to 0.28 ± 0.01 mg/100 g (15.6 %) in seeds. The amounts of Cu species in fresh and dried parts of CG watermelons extracted using ethanol decreased in white flesh and that in red flesh was statistically equivalent. Similar observations were made in CS and SB watermelons though the increase and decrease in amount of Cu species depended on the watermelon parts and variety of the watermelon. There was significance decrease in amount of Cu species extracted using ethanol in CS red flesh and SB red flesh upon drying. The variation in the bioavailable Cu mean levels in parts of dried and fresh watermelons could be attributed to denaturing of chelating agents (glycine and glutamic acid) thereby inhibiting absorption of Cu (Raul, 1998). A similar study done by Carbanaro *et al*. (2001) reported that Cu water-soluble fraction from cooked beans did not exceed 15 % which could be due to the formation of an insoluble complex of polyphenol-proteins under heat treatment. Statistically the same nutrition values of Cu species were extracted from peel.

From results in table 4.23, the amounts of Cu species in fresh and dried parts of various watermelons extracted using dichloromethane decreased from 0.08 ± 0.02 mg/100 g (14.5 %) to 0.02 ± 0.01 mg/100 g (3.5 %) in CG red flesh and from 0.09 ± 0.02 mg/100 g(14.4 %) to 0.01 ± 0.01 mg/100 g (1.6 %) in CS red flesh and were significantly different. As can be seen from table 4.23, the levels of Cu species in fresh and dried parts of various watermelons extracted using dichloromethane also

Table 4.23: Dichloromethane extracts mean levels of Cu species (mg/100 g) in parts of dry and fresh watermelon varieties from Ngara market

Variety	Part	Dry	Fresh	P - value
Charleston grey n = 3	Peel	0.04 ± 0.01	0.03 ± 0.01	0.288
	white flesh	0.03 ± 0.02	0.03 ± 0.01	0.768
	Red flesh	0.02 ± 0.01	0.08 ± 0.02	0.004
	Seed	0.04 ± 0.01	0.03 ± 0.01	0.116
Crimson sweet n = 3	peel	0.04 ± 0.01	0.04 ± 0.02	0.768
	white flesh	0.03 ± 0.02	0.03 ± 0.01	0.768
	Red flesh	0.01 ± 0.01	0.09 ± 0.02	0.001
	seed	0.04 ± 0.01	0.11 ± 0.02	0.002
Sugar baby n = 3	peel	0.04 ± 0.01	0.03 ± 0.01	0.288
	white flesh	0.03 ± 0.01	0.03 ± 0.01	1.00
	Red flesh	0.02 ± 0.01	0.03 ± 0.01	0.116
	seed	0.04 ± 0.01	0.08 ± 0.02	0.015

Dichloromethane extracts mean levels of Cu compared at $\alpha = 0.05$

decreased from 0.11 ± 0.02 mg/100 g (7.2 %) to 0.04 ± 0.01 mg/100 g (2.6 %) in CS seeds and from 0.08 ± 0.02 mg/100 g (5.2 %) to 0.04 ± 0.01 mg/100 g (2.6 %) in SB seeds and were significantly different. Statistically equivalent mean levels of Cu species were extracted by dichloromethane in the remaining dry and fresh parts of CG, CS and SB watermelons. The decrease in the amounts of Cu species extracted by dichloromethane from watermelon parts could be due to formation of the unavailable forms of Cu upon drying (Raul, 1998).

From the results in table 4.24, the amounts of Cu species in fresh and dried parts of various watermelons extracted using hexane decreased from 0.06 ± 0.01mg/100 g (10.6 %) to 0.03 ± 0.01 mg/100 g (5.3 %) in CS white flesh, 0.06 ± 0.01 mg/100 g (6.0 %) to 0.04 ± 0.01mg/100 g (4.0 %) in SB peel and 0.08 ± 0.02 mg/100 g (5.2 %) to 0.04 ± 0.01 mg/100 g (2.6 %) in SB seeds and were all significantly different. Statistically equivalent mean levels of Cu species were extracted by hexane from dry and fresh parts of the remaining CG, CS and SB watermelons. The decrease in the

Table 4.24: Hexane extracts mean levels of Cu species (mg/100 g) in parts of dry and fresh watermelon varieties from Ngara market

Variety	Part	Dry	Fresh	P - value
Charleston grey n = 3	Peel	0.04 ± 0.01	0.05 ± 0.01	0.561
	white flesh	0.03 ± 0.02	0.05 ± 0.02	0.345
	Red flesh	0.02 ± 0.01	0.03 ± 0.01	0.288
	Seed	0.04 ± 0.01	0.03 ± 0.01	0.288
Crimson sweet n = 3	peel	0.04 ± 0.01	0.03 ± 0.01	0.374
	white flesh	0.03 ± 0.01	0.06 ± 0.01	0.021
	Red flesh	0.02 ± 0.01	0.03 ± 0.01	0.288
	seed	0.04 ± 0.01	0.06 ± 0.01	0.070
Sugar baby n = 3	peel	0.04 ± 0.01	0.06 ± 0.01	0.025
	white flesh	0.04 ± 0.01	0.03 ± 0.01	0.288
	Red flesh	0.02 ± 0.01	0.03 ± 0.01	0.116
	seed	0.04 ± 0.01	0.08 ± 0.02	0.025

Hexanes extract mean levels of Cu compared at $\alpha = 0.05$

amounts of Cu species extracted by hexane from watermelon parts could be due to formation of the unavailable forms of Cu upon drying (Rosalind, 2007).

4.7 GC - MS chromatogram for water extracts

4.7.1 GC - MS chromatogram for water extracts from peel of crimson sweet watermelons

The possible forms of Zn and Cu in the polar ethanol and water extracts are provided in table 4.25 by Snyder and Valdez (2013).

Table 4.25: Bioavailable forms of Zn and Cu in ethanol and water extracts

Forms of Zn	Forms of Cu
Free Zn^{2+} associated with vacuolar carboxylic acids.	Free Cu^{2+} and organic acid molecules.
Mixtures of Zn^{2+}.	Mixtures of $Cu^{2+.}$
Organic acid molecules and Zn - histidine complexes.	Organic acid molecules and Cu- organic acid molecules.
Mixtures of organic - acid complexes and hydroxyl complexes and zinc (II) complexes.	Mixtures of organic - acid complexes and hydroxyl complexes and copper (II) complexes.

Source: Snyder and Valdez, 2013

The water extracts were further subjected to GC - MS analysis to determine possible functional groups that could be complexing to the metals. The samples were spiked into GC - MS where chromatograms formed at different retention time. The mass spectra of the organic compounds that could be complexing to Zn and Cu were matched against those of standards in the machine for identification. The results are given in figure 4.1.

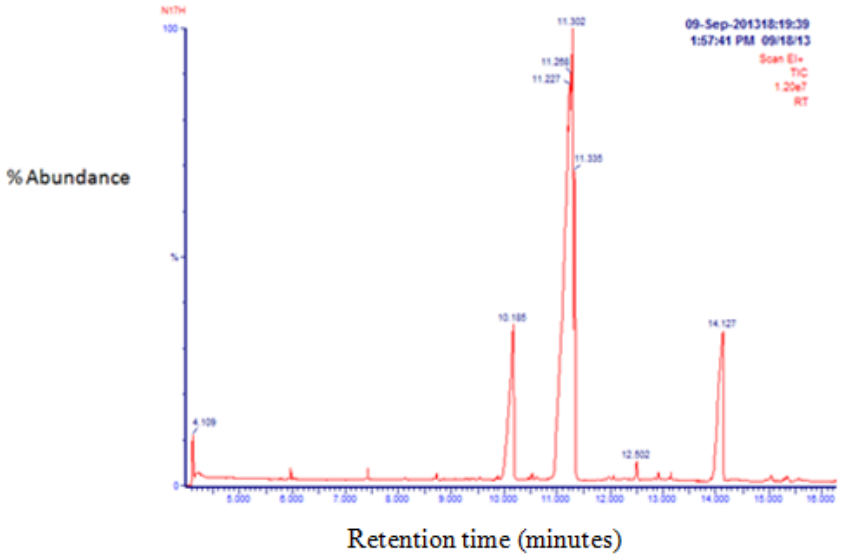

Figure 4.1: GC - MS chromatogram for CS peel water extracts

From Figure 4.1, the possible compound at the peak 14.127 minutes is octadecane, 2,10-dimethyl-3,6,10,16-tetraethyl which does not have any effect on absorption of Zn and Cu (Antonia *et al*., 2009). The possible compound at 12.502 minutes is heptadecane, 2,6,10,15-tetramethyl which does not enhance Zn and Cu absorption (Antonia *et al*., 2009). When the peak at 10.110 minutes was subjected to reverse fit factor of 2.95e5 and compared with the standards of the machine it gave mass spectra shown in Figure 4.2.Figure 4.2 confirms presence of hexadecanoic acid which

enhances Zn and Cu absorption (Lusi *et al*., 2011; Raul, 1998; Rosalind, 2007; Antonia *et al*., 2009). The molecular ion of hexadecanoic acid had a mass of 256. The fragment ion with a mass of 43 could be $CH_3CH_2CH_2^+$ and was the most abundant. The fragment ions with a mass of 59.9 could be $^+CH_2COOH$ while the one with 72.9 could be $CH_2CH_2COOH^+$. The mobile solvent, hexane could also fragment to produce $C_3H_7^+$, $C_2H_4^+$ and CH_3^+ ions that could influence the mass of analyte ions (Marta *et al*., 1999).

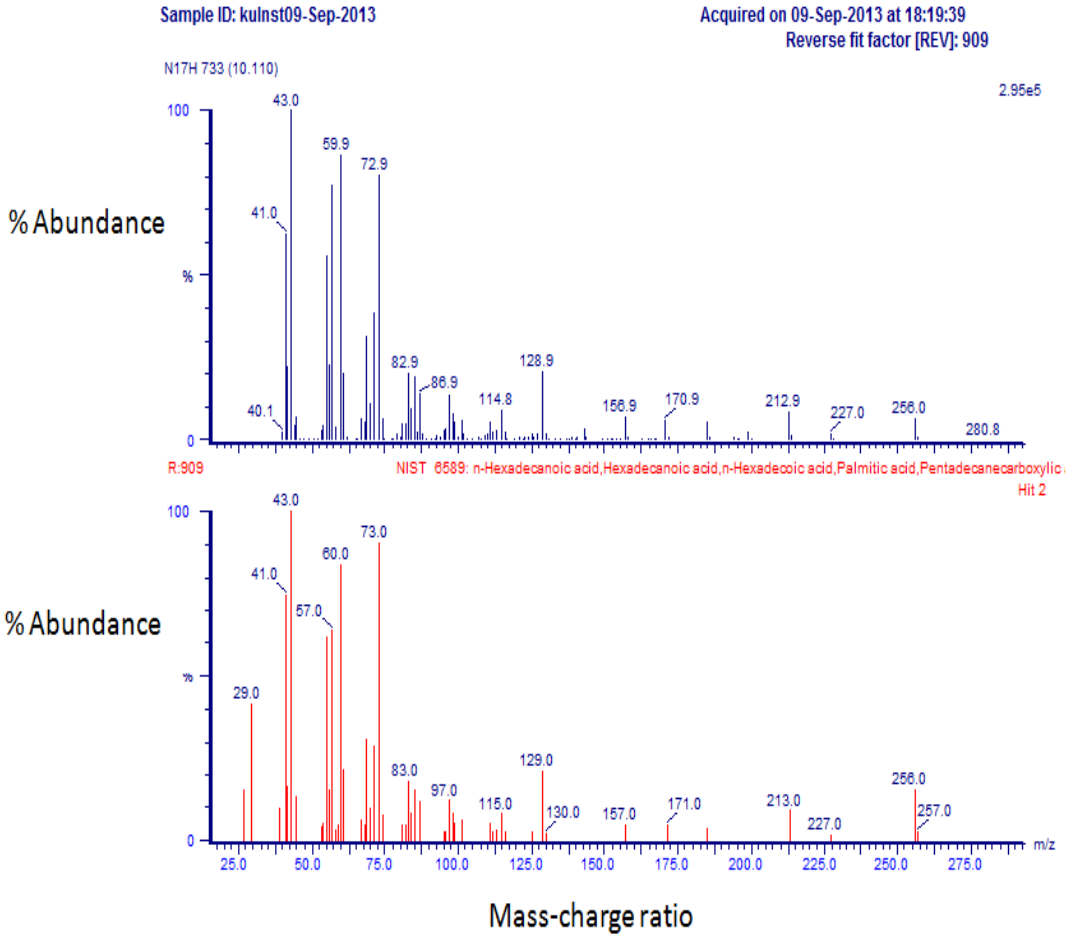

Figure 4.2: Mass spectra for possible organic acids that enhance Zn and Cu absorption

When the peak at 11.243 minutes was subjected to reverse fit factor of 8.73e5 and compared with the standards of the GC - MS machine it gave mass spectra in figure 4.3.

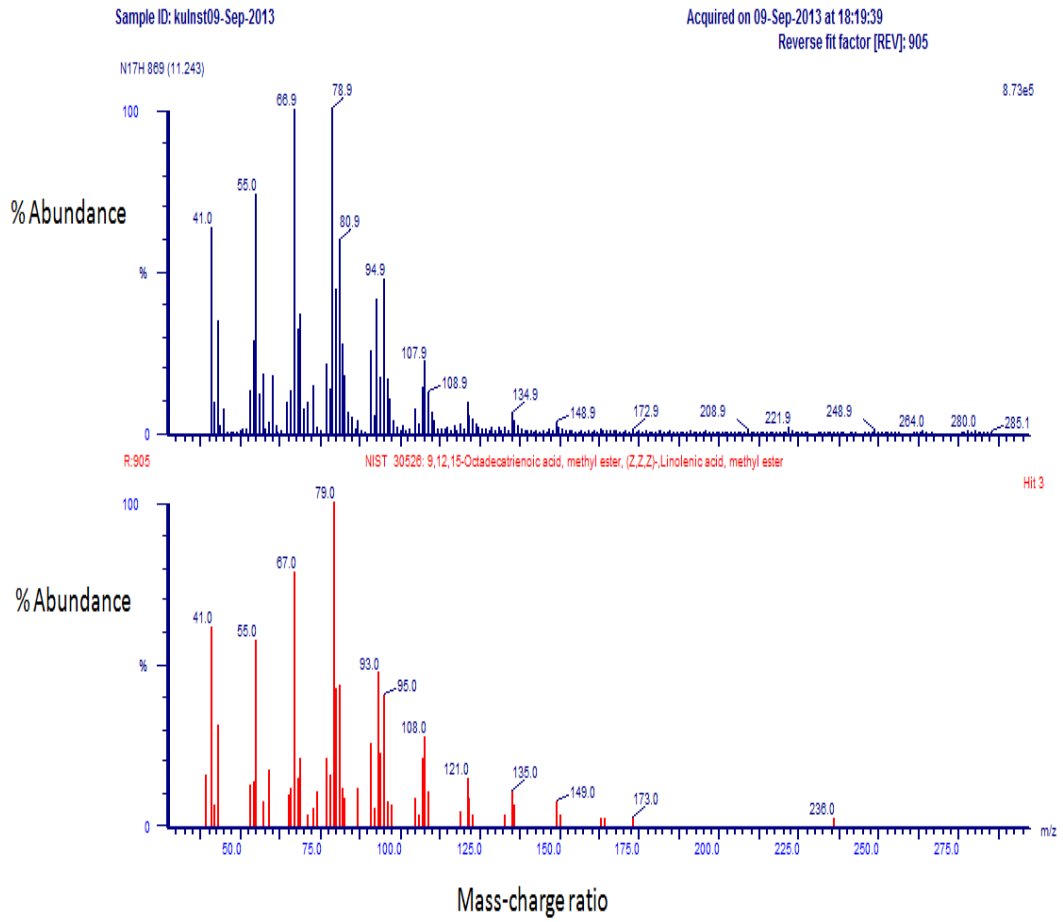

Figure 4.3: Mass spectra for possible organic acids that enhances Zn and Cu absorption

Figure 4.3 confirms presence of octadecanoic acid which promotes absorption of Zn and Cu (Lusi *et al*,. 2011; Raul, 1998; Rosalind, 2007 and Antonia *et al*., 2009). The molecular ion of octadecanoic acid had a mass of 284. The fragment ion with a mass of 41 could be $CH_2CH_2CH_2^+$ while the one at a mass of 55 could be $(CH_2)_3C^+$. The fragment ions with a mass of 69.9 could be $(CH_2)_4C^+$ and was the most abundant. The fragment ion with a mass of 72.9 could be $C(CH_2)_4C^+$. The mobile solvent, hexane could also fragment to produce $C_3H_7^+$, $C_2H_4^+$ and CH_3^+ ions that could influence the mass of analyte ions (Marta *et al*., 1999).

4.7.2. GC - MS chromatogram for red flesh water extracts from sugar baby watermelons

The water extracts were further subjected to GC - MS analysis to determine possible function groups that could be complexing to the metals. The results are given in figure 4.4.

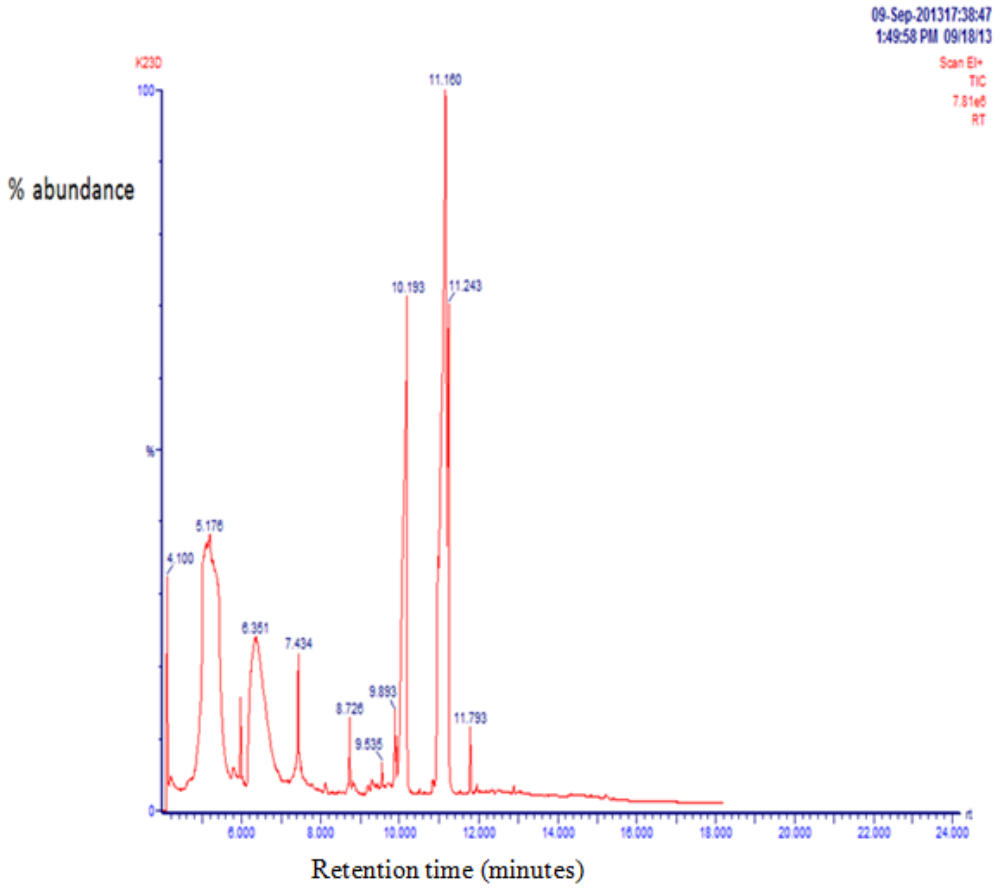

Figure 4.4: GC - MS chromatogram for SB red flesh water extracts

From the results of figure 4.4, possible compounds at 5.178 minutes (4H-pyran-4-one), 6.351 minutes (cyclohexanone), 7.434 minutes (2-Dodecanol) and at 11.243 minutes (octadec-9z-enol) do not solubilize Zn and Cu (Antonia *et al*., 2009). When the fragment at 10.093 minutes was subjected to reverse fit factor of 2.80e5 and compared with the standards of the GC - MS machine, it gave a mass spectra shown in Figure 4.5.

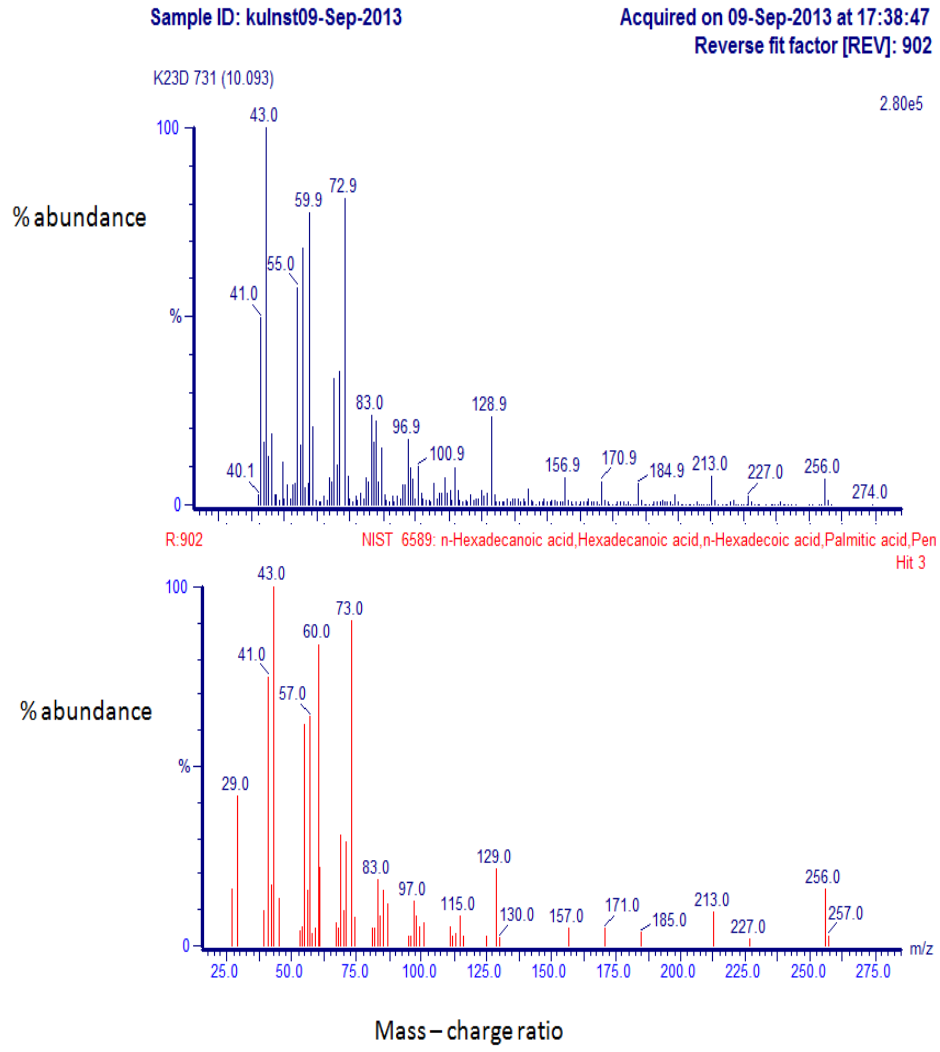

Figure 4.5: Mass spectra for possible organic acids that promotes absorption of Zn and Cu

Figure 4.5 shows presence of hexadecanoic acid which enhances Zn and Cu absorption (Raul, 1998; Rosalind, 2007 and Antonia *et al*., 2009). The mass of the molecular ion for hexadecanoic acid is 256. The fragment ion with a mass of 43 could be $CH_3CH_2CH_2^+$ and was the most abundant. The fragment ions with a mass of 59.9 could be $^+CH_2COOH$ while the one with 72.9 could be $CH_2CH_2COOH^+$. The mobile solvent, hexane could also fragment to produce $C_3H_7^+$, $C_2H_4^+$ and CH_3^+ ions that could influence the mass of analyte ions (Marta *et al*., 1999). Both hexadecanoic and octadecanoic acid were present in the watermelon extracts and probably were in

complexes with Zn^{2+} and Cu^{2+} and were adding to the free bioavailable Zn^{2+} and Cu^{2+} ions. (Snyder and Valdez, 2013).

4.8 Moisture content of different parts of watermelons

Watermelons were separated into peel, white flesh, red flesh and seed and weighed when flesh and upon oven drying in the laboratory to determine percentage moisture content in the watermelons.

4.8.1 Percentage moisture content of watermelons from Mwea market

The bar graph in Figure 4.6 represents moisture content in parts of watermelons from Mwea market.

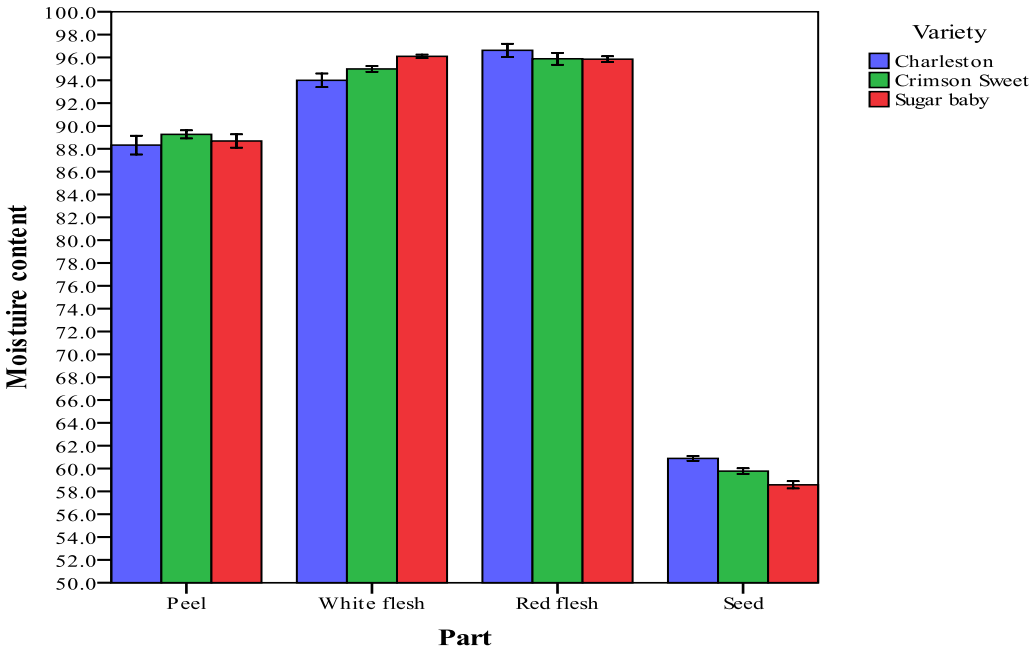

Figure 4.6: Percentage moisture content in parts of watermelons from Mwea market

Results from figure 4.6 indicate that the moisture content of peel from Mwea market ranged from (89.0 - 89.4) % in an increasing order of CG < CS < SB. The white flesh moisture content ranged from (94.7 - 95.9) % in an increasing order of CG < CS <

SB. The moisture content of red flesh followed the order of SB < CG < CS with a range of (95.3 - 96.4) %. The range for moisture content in seed was (59.6 - 60.1) % in an increasing order of CS < SB < CG. Moisture content differed significantly in seed in the three watermelon varieties. The variation in moisture content can be contributed to the interaction of the fruit with environmental factors and their physiological characteristics and also duration of storage after harvesting. Results of this study is within the range of the findings of Ang and Ng (2000) who found a moisture content of (83 - 90) % in mango, guava and papaya fruits.

4.8.2 Percentage moisture content from watermelons from Ngara market

The bar graph in figure 4.7 represents moisture content in parts of watermelons from Ngara market.

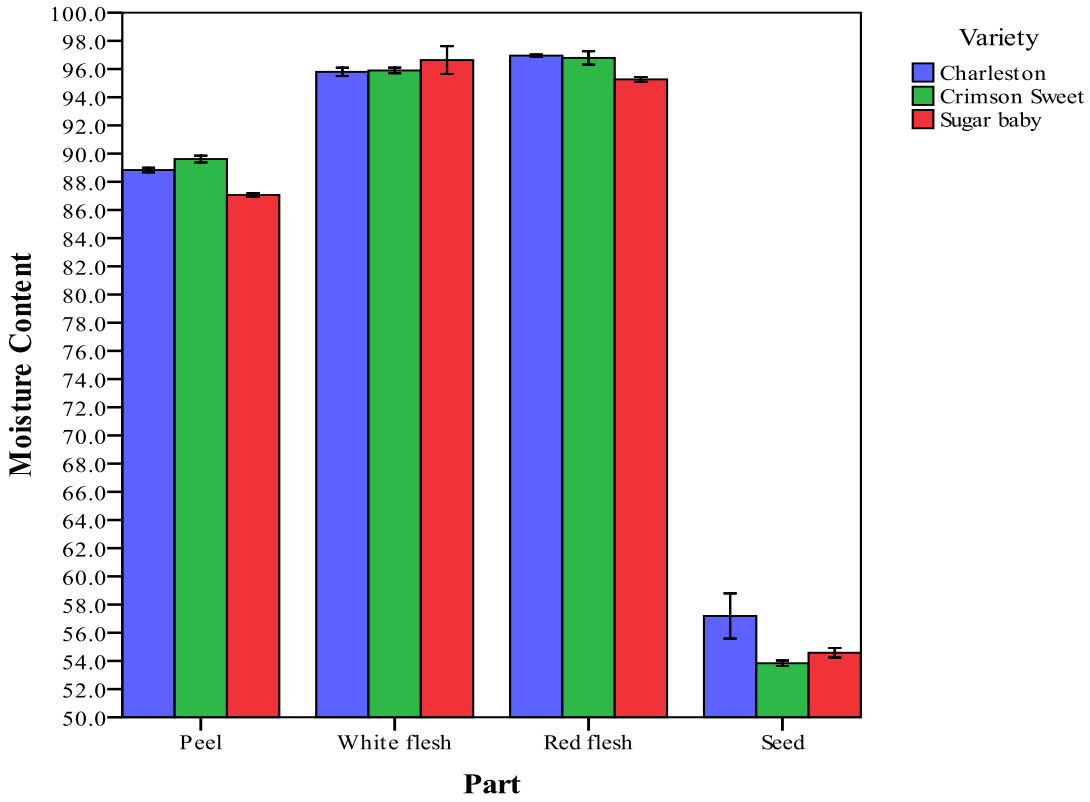

Figure 4.7: Percentage moisture content in parts of watermelons from Ngara market

Results from figure 4.7 indicate that the moisture content of peel from Ngara market ranged from (87.8 - 89.8) % in an increasing order of SB < CS < CG. The white flesh moisture content ranged from (95.4 - 96.0) % in an increasing order of CG < CS < SB. The moisture content of red flesh followed the order of SB < CS < CG with a range of (95.7 - 96.8) %. The range for moisture content in seed was (54.6 - 57.5) % in an increasing order of CS < SB < CG. Moisture content in seed differed significantly. The moisture content of watermelons from Mwea and Ngara markets did not differ significantly. Peel, white flesh and red flesh recorded a moisture content of more than 87 % which is in agreement to the average literature value of 92 % water (Yau *et al*., 2010). The average percentage moisture content of seeds was between 59 % and 61 %.

CHAPTER FIVE

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

With regard to the results obtained from this study, the following conclusions were made.

i. The levels of Zn and Cu depended on watermelon species and watermelon parts.

ii. Total concentration of Zn in parts of Charleston grey watermelons was in the order white flesh > peel > seed > red flesh while that from crimson sweet and sugar baby did not follow a well defined pattern.

iii. Total mean levels of Cu in Charleston grey, crimson sweet and sugar baby was in the order seed > peel > red flesh > white flesh.

iv. Generally the bioavailable species of Zn ranged between (72 - 37) % with CG white flesh from Mwea market having the highest significant amount (5.96±0.01 mg/100 g) while CS seeds from Ngara market had the least significant amount (1.51±0.01 mg/100 g). The bioavailable species of Cu ranged between (56 - 13) % with SB peels from Ngara market having the highest significant amount (0.46±0.01 mg/100 g) while CG red flesh from Mwea market had the least significant amount (0.03±0.01 mg/100 g).

v. All the solvent extracts from watermelon samples were found to contain varying amounts of Zn and Cu.

vi. Water extracted more Zn and Cu while hexane and dichloromethane which extracted almost to the same extent had the least of the elements in question.

vii. About (30 - 40) % portion of Zn and (44 - 65) % portion of Cu remained bound in the watermelon fruit residue after the sequential extraction due to strong binding.

viii. Drying of watermelon parts increased extraction of Zn and Cu in water and dichloromethane and decreased in ethanol and hexane.

ix. Charleston grey watermelon samples from Mwea market had statistically higher mean levels of Zn than those from Ngara market. Crimson sweet watermelon samples mean levels of Zn from both markets differed significantly. Sugar baby watermelon samples from Ngara market had higher Zn mean levels than from Mwea market and were statistically significant. Watermelon samples from Mwea and Ngara markets had Cu mean levels that were statistically the same.

x. Free Zn^{2+} and Cu^{2+} ions and their organic acid complexes constitute the bioavailable forms of these elements. Both hexadecanoic acid and octadecanoic acid were found in water extracts and probably were existing as complexes with Zn^{2+} and Cu^{2+}.

xi. The bioavailable species of Zn ranged between (40 - 70) % while that of Cu species ranged between (35 - 54) %, thus when these essential elements are consumed cannot lead to toxicity as their recommended daily allowance are not exceeded.

5.2 Recommendations

5.2.1 Recommendations from this study

i. The Kenyan population should be encouraged to eat all the parts of watermelon either as a fruit or vegetable as the essential elements are found across all the four parts and are bioavailable.

ii. Since all parts of watermelon have substantial amount of bioavailable Zn and Cu, people should be encouraged to eat all watermelon parts as a source of these elements to supplement what they get from other food.

5.2.2 Recommendations for further work

i. Bioavailability studies on forms of more essential elements in watermelons should be carried out.

ii. Bioavailability studies on forms of essential elements in other fruits and vegetables should be carried out.

iii. Speciation studies on effect of concentration on extraction of elements having similar coordination and configuration numbers in a given solvent should be carried out.

iv. Analysis of peels and white flesh parts for chemicals that could cause toxicity if eaten before recommending their use.

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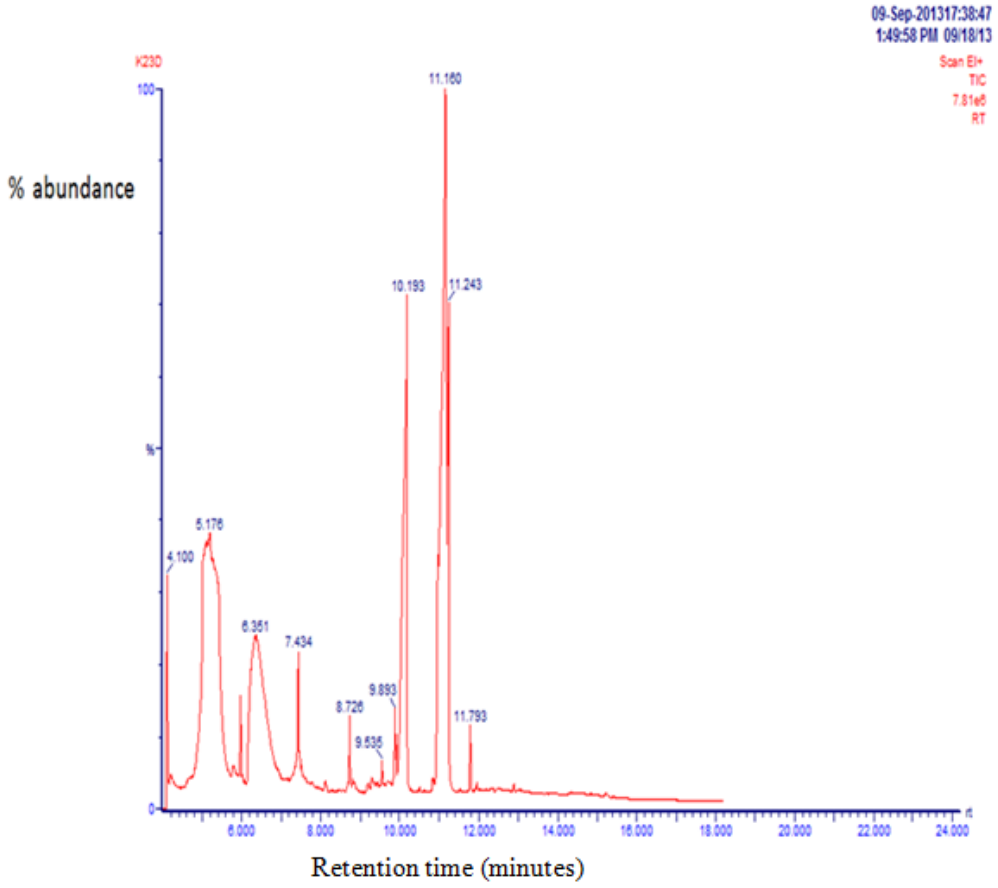
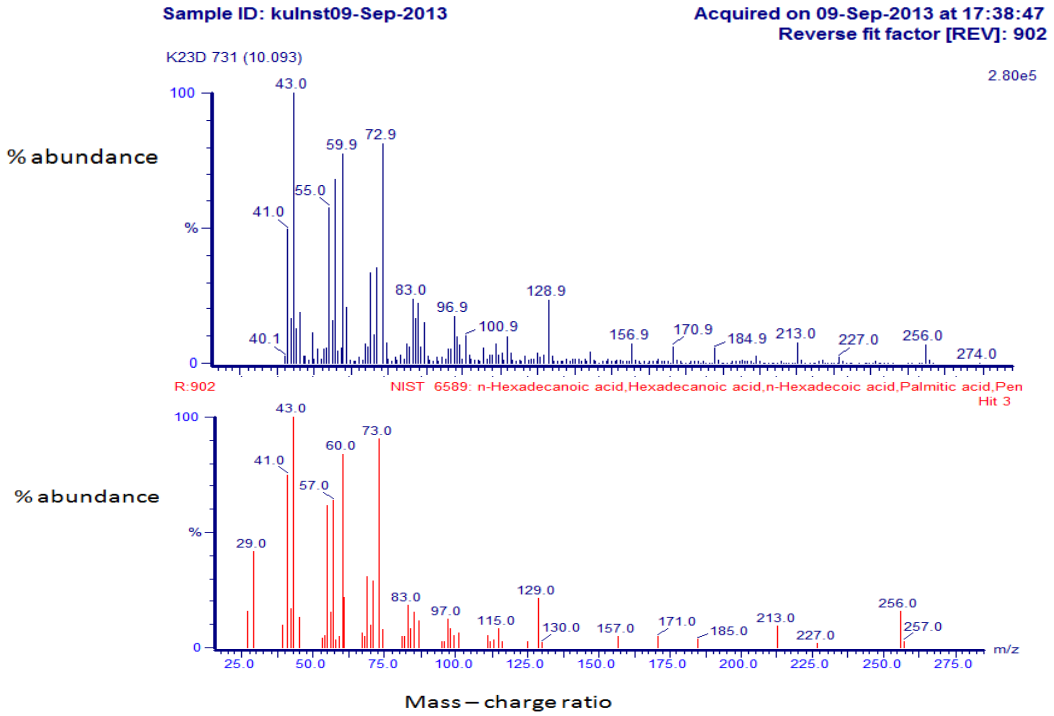
APPENDICES

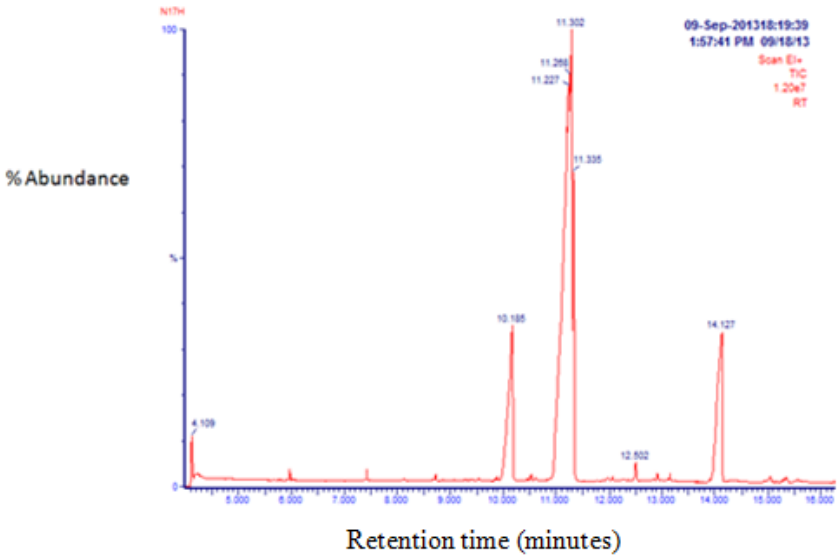
Appendix 1: GC - MS chromatogram for SB red flesh water extracts



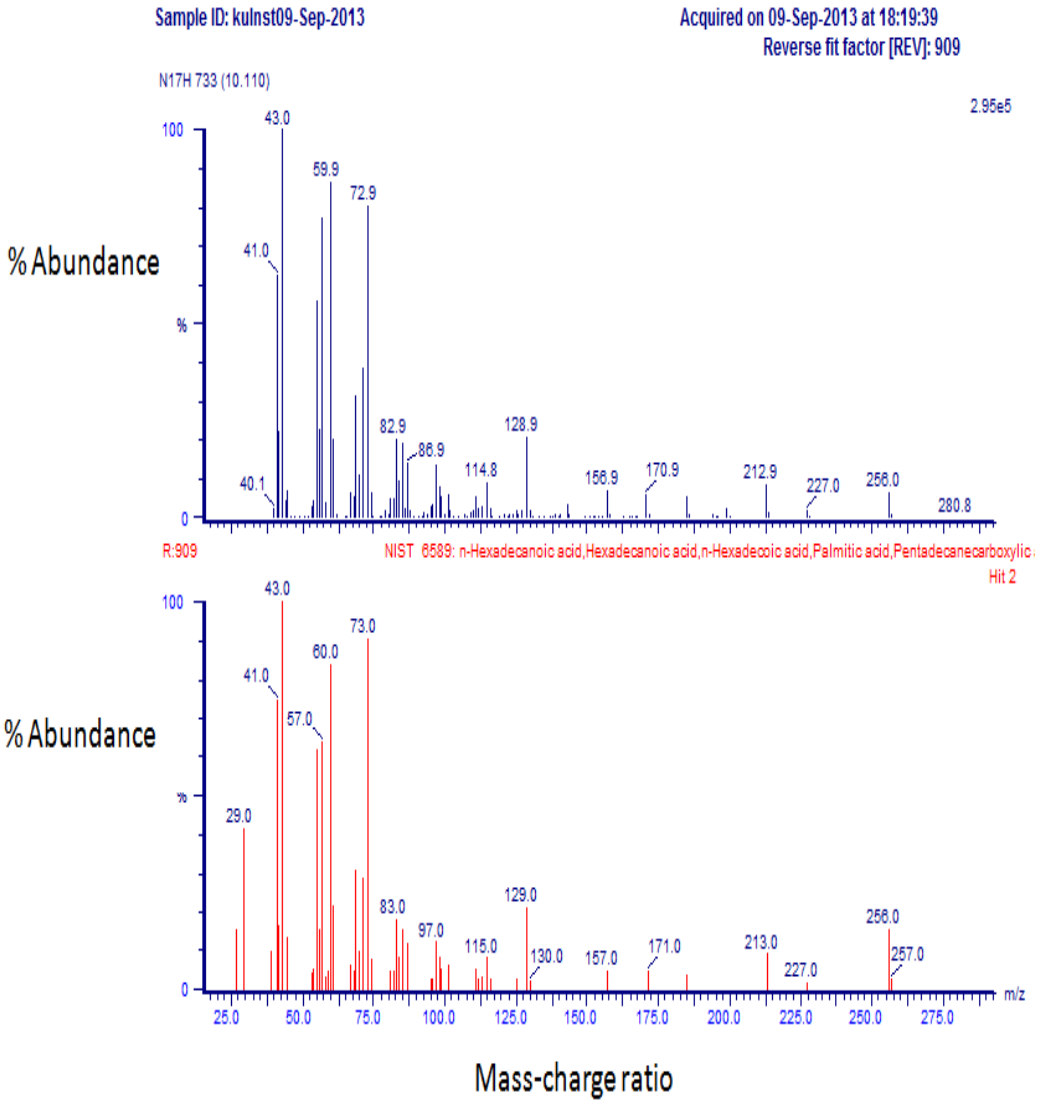
Appendix 2: Mass spectra for possible organic acids that promotes absorption of Zn and Cu



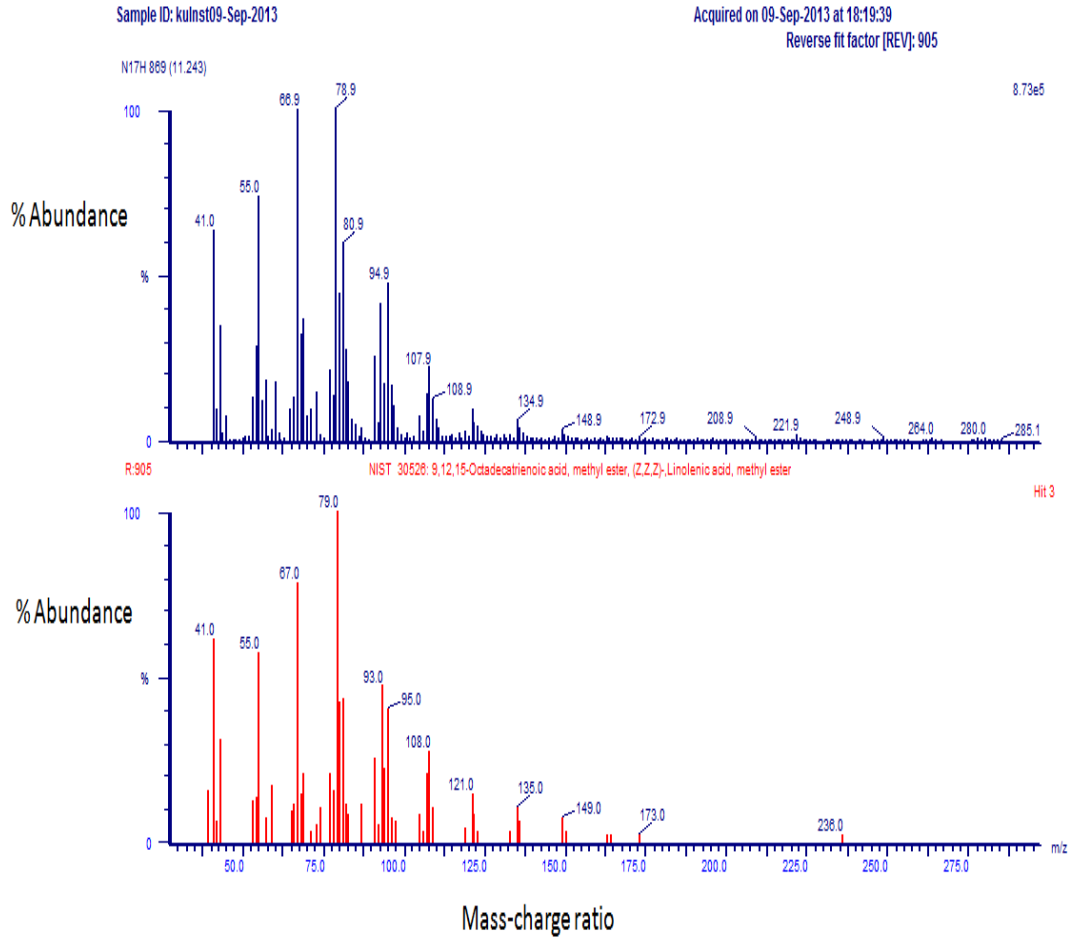
Appendix 3: GC - MS chromatogram for CS peel water extracts



Appendix 4: Mass spectra for possible organic acids that enhance Zn and Cu absorption



Appendix 5: Mass spectra for possible organic acids that enhances Cu and Zn absorption



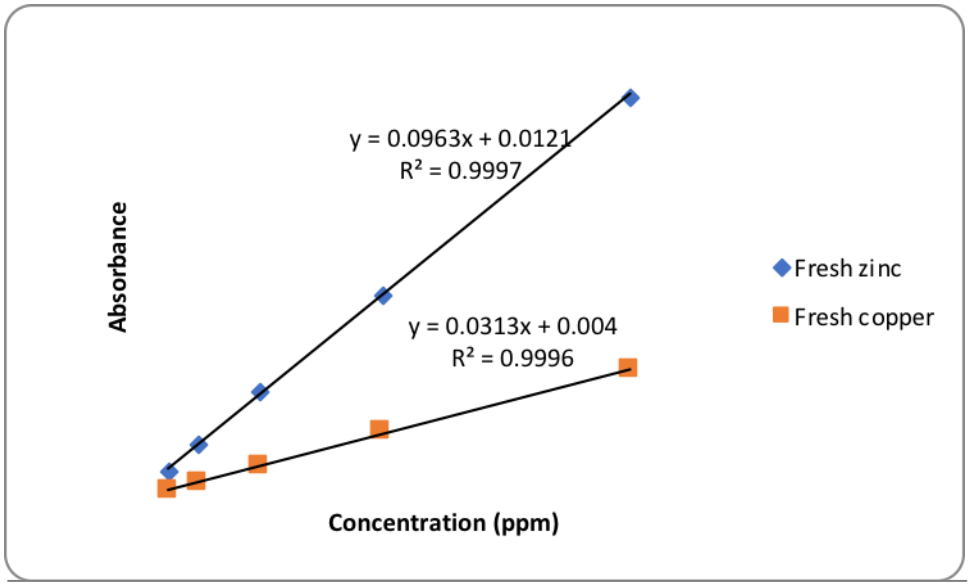
Appendix 6: Absorbance calibration curves data for fresh zinc and copper

Concentration(ppm)	Fresh zinc	Fresh copper
0.5	0.054	0.018
1	0.107	0.036
2	0.213	0.066
4	0.399	0.132
8	0.780	0.253

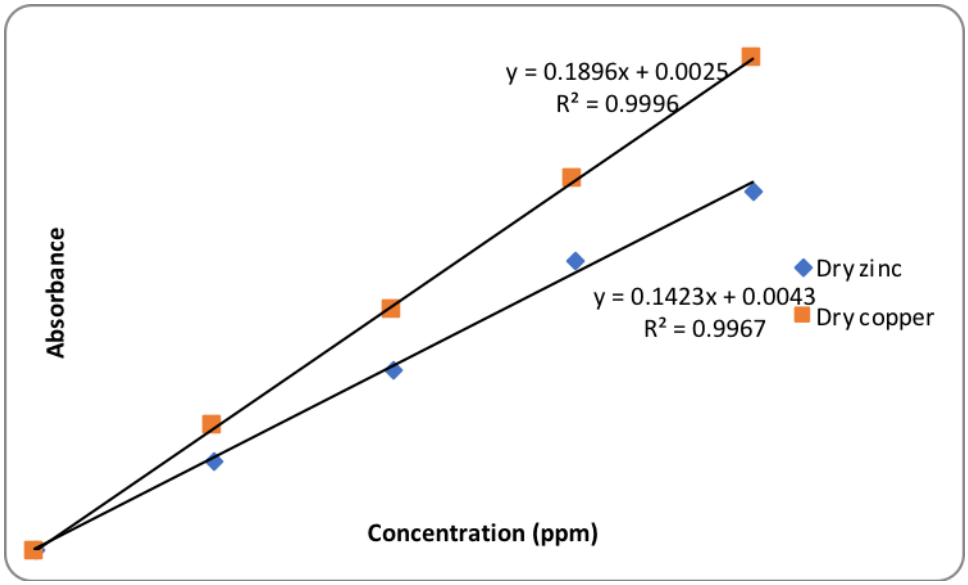
Appendix 7: Absorbance calibration curves data for dry zinc and copper

Concentration (ppm)	Dry zinc	Dry copper
0	0.006	0.0009
2	0.2815	0.3923
4	0.5599	0.7429
6	0.9016	1.1512
8	1.1193	1.5179

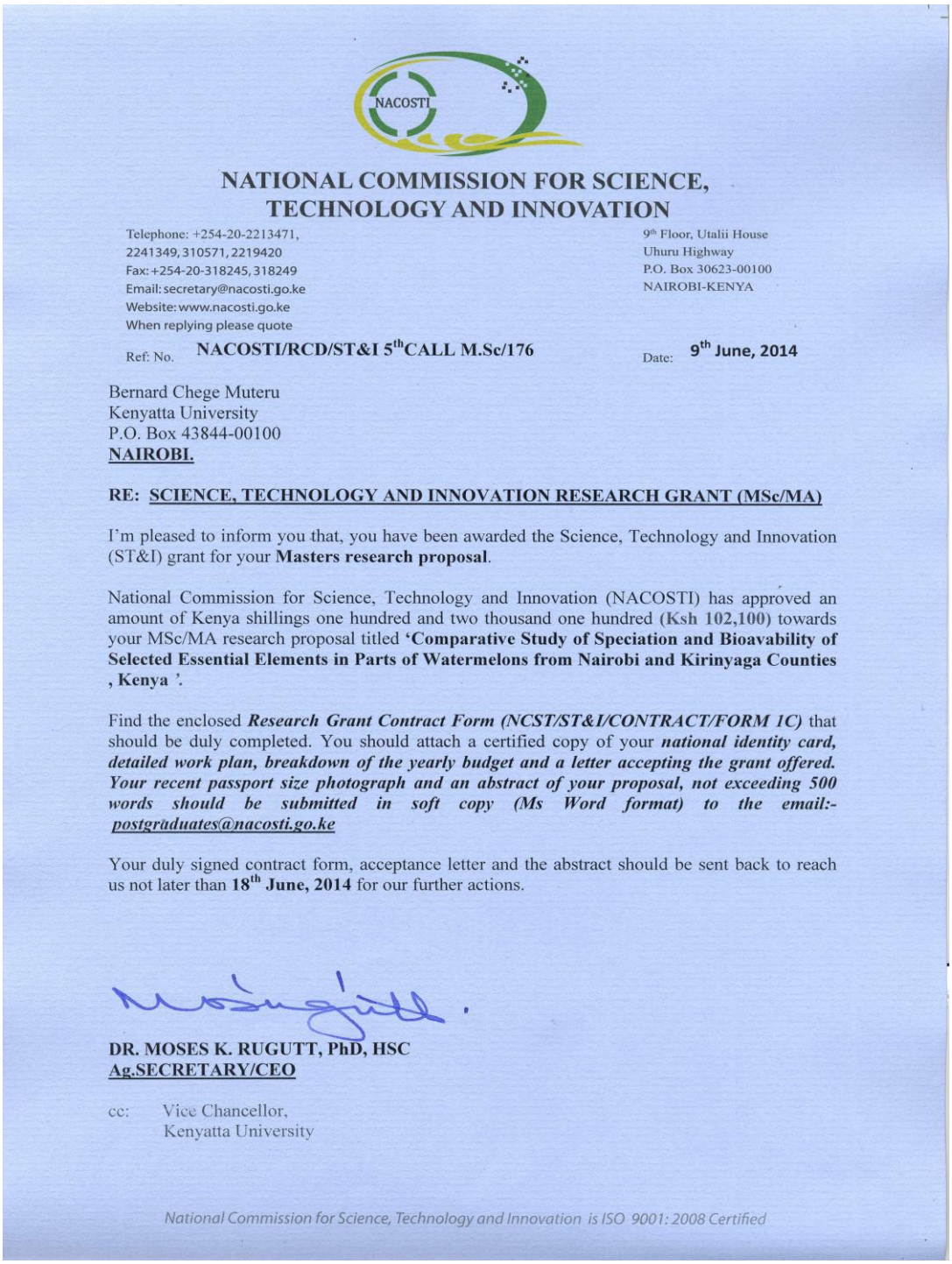
Appendix 8: Calibration curve for fresh Zn and Cu



Appendix 9: Calibration curve for dry zinc and copper



Appendix 10: NACOSTI grant letter



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Ref: No. **NACOSTI/RCD/ST&I 5th CALL M.Sc/176** Date: **9th June, 2014**

Bernard Chege Muteru
Kenyatta University
P.O. Box 43844-00100
NAIROBI.

RE: SCIENCE, TECHNOLOGY AND INNOVATION RESEARCH GRANT (MSc/MA)

I'm pleased to inform you that, you have been awarded the Science, Technology and Innovation (ST&I) grant for your **Masters research proposal**.

National Commission for Science, Technology and Innovation (NACOSTI) has approved an amount of Kenya shillings one hundred and two thousand one hundred **(Ksh 102,100)** towards your MSc/MA research proposal titled **'Comparative Study of Speciation and Bioavability of Selected Essential Elements in Parts of Watermelons from Nairobi and Kirinyaga Counties , Kenya '.**

Find the enclosed ***Research Grant Contract Form (NCST/ST&I/CONTRACT/FORM 1C)*** that should be duly completed. You should attach a certified copy of your ***national identity card, detailed work plan, breakdown of the yearly budget and a letter accepting the grant offered. Your recent passport size photograph and an abstract of your proposal, not exceeding 500 words should be submitted in soft copy (Ms Word format) to the email:- postgraduates@nacosti.go.ke***

Your duly signed contract form, acceptance letter and the abstract should be sent back to reach us not later than **18th June, 2014** for our further actions.

DR. MOSES K. RUGUTT, PhD, HSC
Ag.SECRETARY/CEO

cc: Vice Chancellor,
Kenyatta University

National Commission for Science, Technology and Innovation is ISO 9001: 2008 Certified