DETERMINATION OF POLYPHENOL CONTENT, ANTIOXIDANT ACTIVITY, CAFFEINE AND LEVELS OF ZINC AND IRON IN SELECTED COMMERCIALLY AVAILABLE BEVERAGES IN ELDORET TOWN

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DECLARATION

Declaration by the Candidate

This thesis is my original work and has not been submitted for any academic award in any institution; and shall not be reproduced in part or full, or in any format without prior written permision from the author and/or University of Eldoret. Any cited literature is appropriately acknowledged.

Magut K. Hillary Signature……. Date…….

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Declaration by Supervisors

This thesis has been submitted with our approval as University Supervisors.

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Signature Date

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Signature Date

DEDICATION

To my parents Mr and Mrs Bett, my wife Franciscah, son Lenny, my brothers and sisters, where the birth place of inspiration and motivation originates from, I dedicate this thesis.

ABSTRACT

Coffee, tea and soya, are widely consumed beverages in Eldoret town. However there is scanty information available on the level of components of these products in the market. A study was conducted to determine the polyphenol content, antioxidant activity, caffeine levels, and the levels of zinc and iron in selected commercially available coffee, tea, and soya beverages. Three brands of each beverage were sampled from retail outlets within Eldoret town. Sampling was done in triplicate. The samples obtained were analyzed to determine polyphenolic content, antioxidant activity, caffeine, zinc and iron content using Foline Caucultue method, 2,2-diphenyl-2-picrylhydrazyl radical scavenging assay, UV-VIS spectrophotometry and Inductive Coupled Plasma Optical Emmision spectroscopy, respectively. The polyphenol levels was, 7.4 %, 19.6 % and 4.4 % in coffee, tea and soya, respectively. Tea beverage had the highest antioxidant activity (81.4 %) among the three beverages while soya had the lowest (9.0 %). There was a positive linear correlation (r^2=0.68) between polyphenol content and antioxidant activity of the beverages. Coffee had the highest level of caffeine (4.58 mg/g) as compared to tea (0.985 mg/g) and soya (not detected). A multiple comparison analysis indicated that there was significant difference in the amount of caffeine between coffee and soya and between coffee and tea with p values of less than 0.05. There was no significant difference (p=0.946) in the level of Fe in coffee, tea and soya. Zinc content varied significantly in coffee, tea and soya ($p<0.05$), with soya having the highest level (50.57 ppm) whereas tea had the lowest (38.23 ppm). Consumers in Eldoret town can get enough polyphenols, caffeine, Fe and Zn nutrients by consuming the three beverages. The study recommends that all manufacturers should indicate the levels of each components of the beverages for the benefit of consumers.

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ABBREVIATIONS AND ACRONYMS

AAS	Atomic Absorption Spectroscopy
CGA	Chlorogenic Acids
DART	Direct Analysis In Real Time
FAAS	Flame Atomic Absorption Spectrometer
FCR	Folin-Ciocalteu Reagent
FTC	Ferric Thiocyanate Method
HPLC	High Performance Liquid Chromatography
ICP	Inductive Coupled Plasma
RNS	Reactive Nitrogen Species
ROS	Reactive Oxygen Species
SOD	Super Oxide Dismutase
TOFMS	Time-of-Flight Mass Spectrometry
OD	Optical Densities

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CHAPTER ONE

INTRODUCTION

1.1 Background information

Antioxidants are compounds capable of preventing or reducing harmful effects of free radicals (Dillard & German, 2000). Polyphenols are poly hydroxy aromatic compounds and help to quench singlet oxygen, scavenge free radicals, inhibit enzymes and decompose peroxides (Orhan et al., 2003). Caffeine is an alkaloid found abundantly in natural sources such as the seeds of coffee tree (coffee arabica), leaves of tea bush (*Thea sinensis*), nuts of kola tree (*Cola acuminata*) and seeds of cocoa tree (*Theobroma cacao*)(Han et al., 2006). Iron and zinc are among the many elements present in food at trace levels and are reported to be essential to man's wellbeing (Kumar et al, 2007).

Inspite of antioxidants immense benefits, there have been increasing safety concerns over synthetic antioxidants; for instance, butylatedhydroxyanisole (BHA) and butylatedhydroxytoluene (BHT), the two well-known synthetic antioxidants, have been restricted for their DNA damaging and other toxic effects. As a result, natural sources e.g vegetableles, fruits and beverages are increasingly gaining substantial attention as alternative source of biologically active antioxidants. Free radicals are highly reactive oxygen species that are produced as a result of respiration and metabolism at cellular level. If the effects of these radicals are not mitigated, they cause damaging effect to macromolecules of cells like lipids, nucleic acids, proteins and carbohydrates (Kulbacka et al., 2009). Furthermore oxidative stress imposed by excessive accumulation of these free radicals are known to cause a broad range of diseases like inflammation, stroke, heart

disease, diabetes mellitus, cancer, Parkinson's disease and Alzheimer's disease (Mensor et al., 2001; Orhan et al., 2003).

Any food or beverage that acts as an antioxidant plays a critical role in human health. Antioxidant constituents of the plants act as radical scavengers and help in converting the free radicals to less reactive species. Most naturally occurring antioxidants of plants are flavonoids, vitamins, polyphenols, carotenoids and dietary glutathione (Larson, 1988). Manach et al. (1998) have reported that plant derived antioxidants can quench singlet oxygen, scavenge free radicals, inhibit enzymes and decompose peroxides. Therefore flavonoids, tannins, carotenoids and other constituents of plants have attracted great attention of the world as potential antioxidants. These antioxidants can prevent a wide range of diseases including cancer (Record et al., 2001).

Among important antioxidants is Polyphenols. These are a large group of phytochemicals found in plants in significant amounts in form of glucosides of sugar. They are claimed to have great potential to combat chronic diseases. Additionally, they possess antioxidant properties (Scalbert & Williamson, 2000) which impart a significant protective cushion on the body against deleterious effect of free radicals. A number of studies have proved them as potential therapeutic agents (Katalinic et al., 2006; Kumar et al., 2007). Flavonoids are among the plant's polyphenols that possess strong antioxidant properties (Cetkovic et al., 2007). It is reported that there is an inverse relationship between the flavonoids intake and occurrence of heart diseases (Knekt et al., 2002). Additionally, flavonoids intake significantly reduces the total cholesterol and low-density lipoprotein concentration. They possess vasodilatory potential, and stop platelets aggregation (Arai et al., 2000).

Caffeine is an alkaloid, a class of naturally occurring compounds containing nitrogen and having the properties of an organic amine base (alkaline). Tea and coffee are not the only plant sources of caffeine. Others include kola nuts, mate leaves, guarana seeds, and in small amount, cocoa beans (Han et al., 2006).

Many elements present in food at major, minor and trace levels are reported to be essential to man's wellbeing. Their ingestion in excessive amount can however cause severe health problems (Kumar et al., 2005). The optimum concentration needed varies widely depending on the kind of element, age and sex of consumers (Orhan et al., 2003). The body requires both metallic and non-metallic elements for healthy growth, development and proper functioning of the body. The determination of these elements in beverages, water, food, plant and soil is thus of utmost importance and is the subject of many studies by various researchers (WHO, 1998a; b, Saud and Al-Oud, 2003).

The present study determined and compared the levels of polyphenols, antioxidant activity, caffeine and trace elements in selected tea, coffee, and soya drink powder brands sold in major retail shops within Eldoret town.

1.2 Statement of problem

Caffeine (contained in tea and coffee) is the most widely consumed stimulant drug in the world. It is a bioactive compound that when consumed in moderation can have some beneficial effects in the body; it increases alertness, serves as a bronchial dilator, stimulates metabolism and contributes to an increase in dopamine levels in the blood, which improves mood and relieves stress. However, in unmonitored state, especially products from industry heading to market, intake of high level of caffeine in partuclar

cause restlessness, insomnia and anxiety. This requires regular monitoring (Singh,1998). Although a substantial number of studies have been conducted to ascertain the level of caffeine on coffee, tea and soya, scanty information is available on the level of components of the products in the market, more particularly in Eldoret town.

Polyphenol content is used as an indicator of tea, coffee and soya drink quality by the manufacturers. Polyphenols act as antioxidant against free radicals and are very important to the human body (Kamunya & Wachira, 2006). However, to the best of my knowledge, limited research has been done on polyphenolic content and antioxidant activity of tea, coffee and soya beverages in kenya.

It is known that tea, coffee and soy drinks contain iron and zinc as trace elements. These elements are very important for the normal functioning of the human body (Shukla et al., 2007). When the beverage contains high amounts of these elements, it poses a great danger to the consumer. The maximum allowed daily intake of iron and zinc is 45 mg, and 400 µg for adults and 40 mg, 45 µg for children, respectively (WHO, 1998b). In addition, dietary intake of soybean products are known to decrease the risk of cancer, including breast, colon and prostate cancers, and osteoporosis and cardiovascular diseases (Kumar et al., 2005). In Kenya and other developing countries, it is not a normal routine to determine the level of these trace elements in the beverages at consumer level and therefore consumption of these beverages potentially pose harmful effects to the consumer`s health. Manufacturers of most of the beverages in the market do not specify the quantity of caffeine, polyphenols and trace elements on their product label. Therefore there is a need for analysis of different products for total phenolic, antioxidant activity, caffeine and trace elements content so that people can make informed choices.

1.3 Rationale of study

According to dos Santos & de Oliveira (2001), tea is the most popular non-alcoholic beverage in the world after coffee which is consumed by millions of people around the world. The consumption of soya and soy products is increasing worldwide mainly due to acclaimed health benefits. However, bioactive compounds present in soybeans vary greatly with the cultivar, weather and geographical sowing location (Seguin et al., 2004). These non alcoholic beverages (tea, coffee and soya) have shown antioxidant activity and have been known to exhibit numerous potentially beneficial medicinal properties including inhibition of carcinogenesis, tumorigenesis and mutagenesis as well as the inhibition of tumour growth and metastasis; all these attributable to the presence of antioxidants. In addition these bioflavonoids have antibacterial and anti-allergic properties, and have been demonstrated to induce apoptosis in human leukemia cells, inhibit platelet aggregation, and inhibit human immunodeficiency virus (HIV) and reverse transcriptase (dos Santos & de Oliveira 2001, Seguin et al., 2004). In as much as their importance is known, little research has been published on polyphenol, caffeine and trace element levels of tea, coffee and soya in Kenya.

Logically, it is then expected that the availability of data on the different levels of caffeine, polyphenol content, antioxidant activity and trace element in the selected locally consumed beverages will be of great benefit to the consumer. The findings will also set a foundation for further study on the specific phenols present in the beverages.

1.4 Overall Objective

- To determine and compare the polyphenol content, antioxidant activity, caffeine, zinc and iron of different selected brands of tea, coffee and soya beverages sold in Eldoret town.

1.4.1 Specific objectives

- To determine and compare polyphenol content and antioxidant activity in three selected brands of tea, coffee and soya drink, using Folin-Ciocalteu method and 2,2-diphenyl-2-picrylhydrazyl (DPPH) free-radical scavenging assay respectively.
- To determine and compare caffeine levels in selected brands of tea, coffee and soya drink.
- To determine and compare the levels of Zn and Fe in selected brands of tea, coffee and soya drinks using ICP-OES.

CHAPTER TWO

LITERATURE REVIEW

2.1 Introduction

Polyphenols are a widely distributed and important class of plant secondary metabolites, which possess aromatic ring with one or more hydroxyl substituents. Polyphenolic compounds are mostly water soluble since they are frequently occurring in combination with sugars as glycosides (Cetkovic et al., 2007). Plant polyphenols are very important for growth development and play a key role in defense against microbial activities, and infections as well. They provide oxidative stabilities to the plants in case of injuries, and act as antioxidants *in Vitro* by sequestering metal ions and scavenging reactive oxygen and nitrogen species (Frei & Higdon 2003).

The general structure of polyphenol is shown in the Figure 2.1 below.

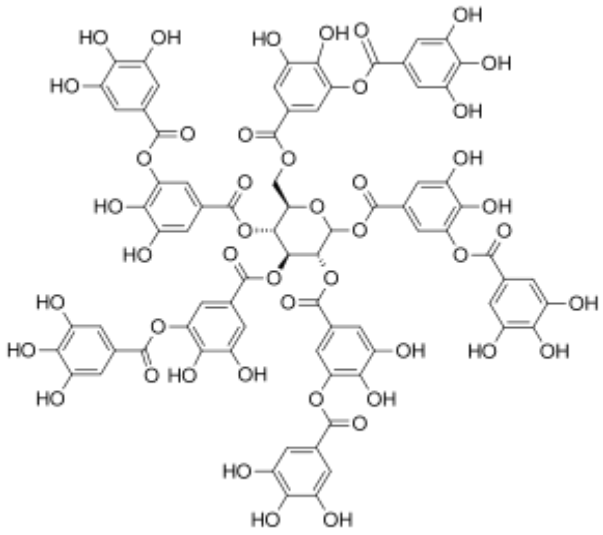

Figure 2.3 General structure of polyphenols (Frei & Higdon, 2003)

Caffeine also known as methyltheobromine; guaranine; 1,3,7-trimethylxanthine; 1,3,7-trimethyl-2,6-dioxopurine ($C_8H_{10}N_4O_2$), is a chemical substance naturally found in tea and coffee and added to colas. It is a stimulant and diuretic. Caffeine is highly soluble in supercritical fluid carbon dioxide and in water.

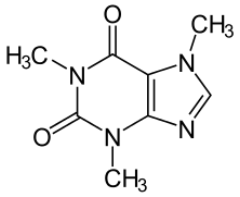

Figure 2.4 The structure of caffeine (Bowen, 1966)

A trace element is an element in a sample that has an average concentration of less than 100 parts per million measured in atomic count or less than 100 micrograms per gram. Trace elements can be found in tea, coffee and soya beverages. Nine elements (K, Ca, Cr, Mn, Fe, Cu, Zn Sr and Rb) are known to be available in tea, coffee and soya at different levels (Bowen, 1966; Marcos et al., 1996; Fernandez-Caceres et al., 2001).

2.2 The polyphenol content of tea, coffee and soya drink

Polyphenols attract great attention due to their antioxidant activity. Pharmacological activities of many plants, fruits and vegetables are closely related to the presence of natural antioxidants especially, phenolic acids and flavonoids. These compounds have great importance for their ability to prevent oxidation and are used as major ingredients in food preservation. Antioxidants significantly decrease the adverse effect of reactive species and at the same time, antioxidant therapy has great impact in the treatment of many other diseases (Kumar et al., 2005).

Polyphenol contents are currently being used as indicators of black/green tea, coffee and soya quality (Kamunya & Wachira, 2006). The polyphenols are primarily responsible for the beneficial healthful properties of the beverages (Sharangi, 2009). The quality of tea mainly depends on the standard of the green leaf, and this is affected by agronomic practices among other factors. According to Rachael et al. (2014), the nutritional requirements for tea are dependent on the type of clone and the geographical location.

2.2.1 Polyphenolic content of Tea

A number of studies have been done to determine polyphenolic content of tea and tea products both locally and internationally. Among the recent researches is a study that determined the polyphenol content and antioxidant capacity of commercially available tea in Argentine market. The findings showed significant variation in polyphenol content between black and green tea. Twelve samples of eight brands were analyzed. The antioxidant capacity was determined by the ferric thiocyanate method (FTC) and 2,2-diphenyl-2-picrylhydrazyl (DPPH) free-radical scavenging assay. Green tea showed a higher polyphenol content than black tea. The total polyphenol concentration in green tea was found to vary from 21.02±1.54 to 14.32 ±0.45 % of gallic acid equivalents (GAE), whereas in black tea, the polyphenol content ranged from 17.62±0.42 to 8.42±0.55 % of GAE ($P<0.05$) (Claudia et al., 2008).

A study was done in Egerton University on polyphenols and free radical scavenging properties of Kenyan tea seed oil cake. The study determined the polyphenol content and antioxidant activity of six selected tea cultivars of Kenya and employed the use of UV-Visible spectrometer with gallic acid as the standard. Free radical scavenging capacity was measured by UV-Visible spectrophotometer a using 2,2-diphenyl-1-picrylhydrazyl

(DPPH) as a free stable radical. The study revealed that tea seed oil cake contained polyphenols in the range of 1.03-2.60 %. The antioxidant activity of tea seed cake was evaluated by scoring the percent free radical scavenging activity which ranged from 8-16 % (Moseti, 2013)

Karori et al. (2007) conducted a similar study at Egerton University, Kenya, on the antioxidant capacity of different types of tea samples. Twelve types of commercial tea samples were assayed to determine their phenolic composition and antioxidant activity. The results showed that total polyphenols, total catechins and antioxidant activity were significantly ($P<0.05$) different in the commercial tea samples. Green tea had the highest levels of catechins, polyphenols and antioxidant activity. Green, black and white tea products processed from Kenyan tea cultivars had significantly ($P<0.05$) higher antioxidant activity than green tea processed from tea cultivars from Japan and China (Karori et al., 2007).

2.2.2 Polyphenolic content of Coffee

Beneficial health effects of coffee are usually attributed to its high phenolic and antioxidant activity (ability to inhibit the process of oxidation). Many publications provide comparison of the antioxidant activity in such popular beverages as coffee and tea (Richelle et. al., 2001; Rawel & Kulling, 2007). Antioxidant activity of coffee is related to chlorogenic, ferulic, caffeic, and *n*-coumaric acids contained in it (Nicoli et al., 1997).

A research done in Ethiopia on coffee phenols indicated that Chlorogenic acids (CGA) are the main phenolic compounds in coffee. In that study, the levels of CGA in coffee brands; Arabica Jimma (rJM), Arabic a Nekemit (ArNK), Arabica Sidamo (ArSD),

Arabica Jimma (ArJM) raw, and Arabica Jimma (ArJM) Husk were determined using High Performance Liquid Chromatography (HPLC). The order of CGA concentration (mg/g) in the coffee samples was as follows: ArJM raw>ArJM>ArSD>ArNK>ArJM Husk. Generally, Arabica Jimma raw (46.144 mg/g) had the highest while Arabica Jimma husk (0.981 mg/g) had the least concentration of CGA (Abebe and Kebba, 2013).

In Latvia, a similar research was done to investigate the polyphenols in coffee available in local markets. Eight coffee varieties were analyzed spectrophotometrically for polyphenol content. The results indicated that the polyphenol content ranged from 1300 to 1700 mg gallic acid equivalent per 100 g of sample. However, the polyphenol content did not vary significantly between coffee varieties (Dutta et al., 2013).

A similar study was done in Romania to determine the polyphenol content of four types of coffee; Arabica instant, Arabica ground, Arabica premium and Arabica powder bulk using Folin Ciocalteu method. Arabica premium had the highest concentration of polyphenols (0.196 mg gallic equivalent) while Arabica ground had the lowest concentration (0.177 mg gallic equivalent) of polyphenols (Delia et al, 2014).

2.2.3 Polyphenolic content of Soya drink

Soybean is a legume that is consumed worldwide. Soybean is a complex food matrix containing low or no starch, about 20 % oil and 40 % high-quality protein in addition to several important bioactive compounds, including lunasin, trypsin inhibitors, isoflavones, and saponins (Božanić, 2006). Polyphenol concentration of the ranges 1–3 mg/g in the mature soya bean seeds have been reported (Malenčić et al.,2007).

In south Korea, another research was done to determine the phenol content of soybean under different roasting conditions. The roasted soybean exhibited significantly higher antioxidant activity than unroasted using 2,2-diphenyl-1-picrylhdrazyl method (Malenčić et al.,2007)

Kenya is a major producer and exporter of coffee and tea in the world. Although coffee, and soya are a source of phenols that could have potential health benefits, there is limited data on the levels of polyphenols of the these beverages. Therefore there is need to study and quantify the polyphenol content of various beverages in the Kenyan market.

2.3 Antioxidant activity of tea, coffee and soya drink

According to Satish and Dilipkumar (2015**),** polyphenolic compounds present in tea, coffee and soya exhibit antioxidant activity by donating their hydrogen atom to reduce reactive species and the polyphenolic compounds are converted into phenoxy radical (ArO·), which get resonance stability due to delocalization of unpaired electron over aromatic ring.

$ROO^{\bullet} + ArOH \rightarrow ROOH + ArO\cdot$

$HO\cdot + ArOH \rightarrow HOH$

2.3.1 Extraction of Polyphenols

Polyphenolic compounds are mostly stored in vacuoles of the plants, and are commonly extracted in organic solvents. The nature of phenolic compound in extract is dependent on the chemical compounds present in plants, and the extraction method employed. Extraction is carried out with both dried and fresh material. Solvent extraction is a most frequently employed technique for the separation of antioxidant polyphenols. Efficiency

of extraction is dependent on the nature of solvent owing to different polarities of the plant compounds. commmon solvents used for extraction are: aqueous solutions (60 – 80 % v/v) of methanol, ethanol, ethyl acetate, ether, and acetone (Adeneye et al., 2007). Extraction techniques such as mercerization, soxhlet and reflux may be used for preparation of polyphenolic extracts (Rohman et al., 2010). Ultrasound and microwave radiations have also been applied for better extraction of phenolic compounds since time and temperature have great effect on efficacy of extraction (Rafeal et al., 2008). Katalinic et al., (2006) reported that extraction of phenolics increased when time and temperature of infusion is increased and that the yield of extraction with hot solvents is superior than with cold ones.

2.3.2 Polyphenols as antioxidants

Free radicals are reactive species generated in the body as a result of many metabolic processes like respiration and cell mediated immune functions.

(Scalbert & Williamson 2000). Free radicals are also introduced through exogenous sources such as environmental pollution, pesticides and exposure to radiations (Adedapo et al., 2009). They are categorized as reactive oxygen species (ROS), including free radicals like super oxide anion (O^{2-}), hydroxyl radical ($OH^{\bullet}$), and non-radical species like hydrogen peroxide (H_2O_2) and singlet oxygen. Reactive nitrogen species (RNS) including $NO^{\bullet}$, $NO_2^{\bullet}$ as free radicals and HNO_2, N_2O_4 as non radicals.

Different environmental factors and aging elevate the level of free radicals and cells become unable to work efficiently against the free radicals leading to accumulation of radicals and oxidative stress which results in cellular damage (Nagulendran et al., 2007). Reactive oxygen species and reactive nitrogen species deteriorate many biological

molecules like fatty acid, lipids, proteins and DNA, and become a major cause of heart diseases, diabetes, cancer, inflammations and weak immune system. The $OH^{\bullet}$ reacts with the sugar moiety of DNA by abstracting an H-atom from C5carbon atom. One unique reaction of the C5-centered radical of the sugar moiety in DNA is the addition to the C8-position of the purine ring in the same nucleoside (e.g. guanine). This intramolecular cyclization results in the formation of the 8,5-cyclopurine-20-deoxynucleosides. The reactions of carbon-centered sugar radicals result in the DNA strand breaks and base-freesites (Sharififar et al., 2007).

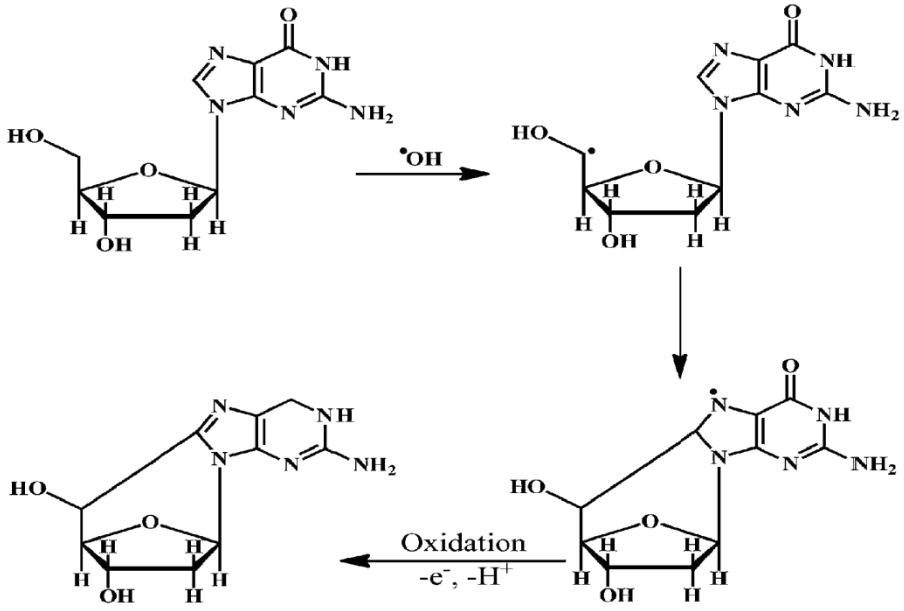

Figure 2.3; Reaction of hydroxyl radical with the sugar moiety of DNA (Satish & Dilipkumar, 2015)

Nature has gifted the defense system to protect the body from injurious effects of free radicals. Through enzymatic defense systems like superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT) and the non-enzymatic defense systems such as vitamins (A, C, E) and polyphenols (Sharififar et al., 2007). All of these act as antioxidants and maintain the level of free radicals in the body. Their mode of action varies and the most common are; reduction, scavenging of free radicals and singlet oxygen and formation of complexes with pro oxidant metals (Osman et al., 2009). The balance between antioxidants and oxidation is believed to be very essential for a healthy

biological system (Katalinic et al., 2006). For the last few years, interest in studying and quantifying the antioxidant components of fruits, vegetable and medicinal plants has increased due to their potential health benefits. Polyphenolic antioxidants stop oxidation in the food system as well as in the human body and defend them from the detrimental effects of free radicals. Polyphenols are the bounteous antioxidants in the diet. Their total consumption as diet could be much higher than other groups of phytochemicals and recognized nutritional antioxidants like vitamin C, E and carotenoids (Scalbert & Williamson, 2000).

2.4 Caffeine levels of tea, coffee and soya

2.4.1 Caffeine content of Tea

Generally tea contains several compounds. In one analysis of black tea, it was found to contain 2.5 % caffeine (Han et al., 2006). In India a study was done to compare the level of caffeine in various brands of tea powders and green tea using UV/VIS spectrophotometer. The study revealed that the concentration of caffeine among seven brands were as follows: Red tea 2.4 gms/50 gms, Taj mahal 1.6 gms/50 gms, Chakra Gold 0.96 gms/50 gms, Gemini 1.7 gms/50 gms, 3 Roses 1.3 gms/ 50 gms, Tetley green tea 0.72 gms/50 gms and Tata tea 2.6 gms/50 gms (Kalra et al., 2011).

In Oman, a study was done to determine the caffeine content of various brands of tea marketed in that country. The study employed UV/VIS spectrophotometric method. The results from the study gave caffeine levels as; Twinnings tea 0.43 gms, Red label tea 0.41 gms, Lipton tea 0.415 gms, Tata tea 0.42 gms, Kanan tea 0.43 gms and Mumtaz 0.41 gms in a 50 gram tea packet (Vastag, 1998).

In 2010, a research was done at Jomo Kenyatta University in Kenya to determine the levels of caffeine in selected tea brands. The study revealed the levels of caffeine to vary

in the following order; mara moja>finlay premium>Kerichogold>sasini. Generally the higher concentration of caffeine in the sample was realized using UV-VIS spectrophotometric method as compared to HPLC method. The amount of caffeine in tea and coffee samples ranged between 1.64 ± 0.01 % to 7.36 ± 0.98 % by HPLC method and 5.74 ± 0.02 % to 25.57 ± 0.09 % by UV/ Vis spectrophotometric method. The study concluded that the acidified water used in UV/VIS method was a better extractor than the pure water used in HPLC (Wanyika et al., 2010).

2.4.2 Caffeine content of Coffee

Coffee plant is the major source of natural caffeine. The caffeine content in coffee plant varies between 1 and 4 % by dry weight (Illy & Viani, 2005). In a study, 10 decaffeinated samples were collected from different coffee establishments and caffeine content determined to confirm if indeed they have no caffeine. The samples contained caffeine in the range of 0-13.9 mg/16-oz serving. The caffeine content for the Starbucks espresso and the Starbucks brewed samples collected from the same outlet were 3.0-15.8 mg/shot and 12.0-13.4 mg/16-oz serving, respectively (Powell et al., 1998).

According to Danhelova et al (2012), who analyzed the content of caffeine in instant and roasted coffee using a direct analysis in real time (DART) ion source coupled to high-resolution time-of-flight mass spectrometry (TOFMS), the study concluded that the quantity of caffeine in analyzed roasted ground coffee samples was in the range of 16.9–26.9 mg/g while in instant coffee samples in the range of 22.4–51.7 mg/g.

Singh, (1998) did a study to determine caffeine content in coffee using fourier transform infra-red spectroscopy in combination with attenuated total reflectance technique. The

study revealed that 8.3 mg caffeine per g of coffee was extracted in aqueous solution and decaffeinated coffee from the same national brand revealed only 0.38 mg caffeine per gram of coffee, against the manufacturer's suggested amount of 0.53 mg caffeine per gram of coffee.

Furthermore, Smirth (2005), quantified caffeine in beverages and soft drinks using UV-Vis spectroscopic method in; nescafe, tea, coca cola and pepsi cola. The study revealed that the levels of caffeine in nescafe were 64 mg per 2 g sample, Instant tea 40 mg per 2 g bag, Coca Cola 35 mg per 330ml, Pepsi Cola 38 mg per 330 mL and Red Bull 80 mg per 250 mL. The study concluded that the caffeine content per serving was in a decreasing order as follows; Red Bull ⇒Pepsi Cola ⇒ nescafe ⇒Coca Cola ⇒ Instant Tea.

In 2010, a research was done at Jomo Kenyatta University in Kenya to determine the levels of caffeine in three selected coffee brands. The study found the levels of caffeine to vary in the following order Africafe>Nascafe>Dormans (Wanyika et al., 2011).

Caffeine has many effects on the human health. The commonly recognized effects include increased locomotor activity, vigilance, alertness, and arousal as well as sleep disturbance. Furthermore, caffeine can increase blood pressure. It is for these reasons that the current study was done to determine the content of caffeine of the three beverages.

2.5 Trace elements in tea, coffee and soya

Metallic constituents of leaves and seeds are normally different according to the type of plant and geographical sources (Marcos et al., 1996; Fernandez et al., 2001). Zinc is

important in metabolic function and for growth in man. It is found in high concentration in red blood cells as an essential part of the enzyme carbonic anhydrase, which promotes many reactions relating to carbon dioxide metabolism. Zinc also supports normal growth and development during pregnancy, childhood and adolescence. Daily intake of Zn is required because the human body has no specialized Zn storage. High concentration of Zn have been reported to cause diarrhoea, depressed immune function and forms part of the teeth. The human body requires Zn and Fe within certain permissible concentrations for healthy growth and development (Kazi et al., 1999).

Iron is an essential element for most life forms on Earth, including human beings by participating in a wide variety of metabolic processes, including oxygen transport, DNA synthesis, and electron transport. Iron is needed for a number of highly complex processes that continuously take place on a molecular level and that are indispensable to human life, including the transportation of oxygen around our body. Iron is required for the production of red blood cells (haematopoiesis), but it is also part of haemoglobin (that is the pigment of the red blood cells) binding to the oxygen and thus facilitating its transport from the lungs via the arteries to all cells throughout the body. About 70% of the body's iron is bound to hemoglobin in red blood cells (Gupta, 2014).

2.5.1 Trace elements in Tea

The chemical components in black tea have a number of health benefits (Katiyar & Mukhtar 1997; Wheeler & Wheeler 2004). In addition to the organic components, different minerals and trace metals are present in black tea leaves and their infusions (Das et al., 2005). Many elements, in trace amounts, play an important role in metabolic processes and are essential for our health and their nutritional value have also been

associated with the flavoring characteristics of tea (Lambel & Hill 1995). Usually, the tea beverage (infusion) is consumed by mankind. Therefore, the accurate determination of the trace element content of tea infusion is thus very important in assessing any possible implications for health.

From a study in Poland, it was concluded that tea was a rich source of essential elements such as calcium (Ca), phosphorus (P), potassium (K), magnesium (Mg), manganese (Mn), iron (Fe), copper (Cu) and zinc (Zn). The infusions also contained non essential elements such as nickel (Ni), lead (Pb), fluoride (F) and aluminium (Al). The average concentrations in one cup of tea from the Norwegian market contained among other elements average concentrations of 0.6 mg Ca/L, 2.8 mg P/L, 37.1 mg K/L, 2.0 mg Mg/L, 5.9μg Fe/L, 14.1 μg Cu/L, 26.4 μg Zn/L, 0.6 mg Mn/L, 9.9 μg Ni/L, 0.16 μg Pb/L, 0.5 μg Cr/L,1.0 mg F/L and 1.1 mg Al/L (Nardi et al., 2009).

A study was done in Spain to determine the level of trace elements and caffeine in commercially available tea. The study revealed the level of zinc as 78.6 ng/g for Zn and caffeine ranged from 7.5 to 86.6 mg/g. The lower values of caffeine were detected in green and oolong teas (Carmen et al., 2003).

In a study done by Owoeye (2010) on bioaccumulation of heavy metals in tea marketed in Nigeria, four major and most consumed brands of tea were selected for the study. Both aqueous and dry methods were used. Total contents of metal were determined by digesting 1 g of each brand using a mixture (3:1) of concentrated nitric acid (HNO_3) and hypochlorous acid ($HClO_4$). The study showed the level of Fe in the selected samples ranged from 442-1344 mg/kg and the Zn levels ranged from 56.3 to 78.6 ng/g.

2.5.2 Trace elements in Coffee

Coffee is one of the most important agricultural product. Coffee, in terms of international trade fetches billions of Kenya shillings per year, comparable only to petroleum. The determination of mineral nutrients that are contained in it is of great interest due to its large consumption by millions of people around the world (dos Santos & de Oliveira 2001).

A recent evident base study in *Czech Republic* to determine the mineral nutrients and toxic elements (Ca, Cu, Fe, Mg, Zn, Cd, Cr, Mn, Ni, and Pb) in five types of coffee by atomic absorption spectrometry and inductively coupled plasma mass spectrometry, showed that there was no significant differences found between the two used methods (Jarošová et al., 2014).

Alayade et al. (2014) determined the concentrations of Cu, Fe, Zn and the proximate composition of powder and coffee infusions from beans grown under organic or conventional agricultural systems. The study employed a flame atomic absorption spectrometer (FAAS) equipped with a deuterium background corrector to determine the level of the trace elements. The results showed that the levels of Cu, Fe and Zn were higher in conventional coffee powder than in organic powder.

According to Monika et al. (2014) coffee contribute 1% of daily intake of trace elements, iron contribute 124 micrograms of daily intake while Zinc contribute 12.5 micrograms of daily intake.

2.5.3 Trace elements in Soya

Soya drink is a product of processed soya bean seed. Studies have shown that processing does not seem to cause large losses of trace minerals with the exception of silicon, which seems to associate with the adhering soil and dissolves in wash water consequently being lost. Sodium, potassium, magnesium and calcium may also be lost where excessive water for washing and preparation is used and discarded. When soya bean is concentrated with respect to protein, it is found that there is an increase in the iron, zinc, aluminium, strontium, and selenium contents (Asakura et al., 2008).

In the U.S.A, O'Dell (1979) determined trace mineral availability in soybean. The following minor minerals were studied; silicon, iron, zinc, manganese, copper, molybdenum, fluoride, chromium, selenium, cobalt, cadmium, lead, arsenic, mercury, and iodine. Their concentration was found to range from 0.01-140 ppm.

In Thailand, a soybean study was conducted to determine trace elements in soy milk ice cream. It was found that 100 grams of soy milk ice cream consisted of 0.29 mg iron and 0.18 mg zinc (Wiwat, 2008).

In Kenya there is no detailed report on the level of Zn and Fe and other trace elements in soya drink, coffee and tea yet these beverages form a large part of daily beverage intake.

In this study, levels of Zn and Fe in selected tea, coffee and soya brands in Eldoret town were determined and their levels compared. The results are presented in chapter four and discussed in chapter five of this report.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Introduction

This section of the thesis presents the instruments used, reagents and solvents, sample collection and preparation, sample pretreatment sample analysis and procedures for determination of polyphenol content, determination of antioxidant activities, determination of caffeine zinc and iron. This section also presents the method of data analysis used.

3.1.1 Instrumentation

The following instruments were used during the study: Weighing of samples was done using an analytical balance (BL-3200 HL, Shimadzu, Japan). Shimadzu UV-1800 series spectrophotometer was used to analyze the polyphenol content and antioxidant activity and caffeine levels, while ICP-OES-9000 Shimadzu Series was used to analyze zinc and iron levels in the sampled beverages. The ICP-OES system was equipped with a simultaneous solid-state detector allowing measurements from 167 to 785 nm. The sample introduction system consisted of a cyclonic spray chamber and a concentric nebuliser.

3.1.1.1 ICP-OES Operating conditions

The instrument used was Inductive Coupled Plasma Optical Emmision spectrometre (ICPE-OES) – 9000 Shimadzu seriers (Japan). The following parameters were established; instrumental detection limit, precision, optimum background correction

positions, linear dynamic range and interference for each analytical line. The instrument configuration and operating conditions were verified to satisfy the analytical requirements and were as follows:

Table 3.1 ICP-OES Parameters

Parameter	Units
Pressure of gas (pure Argon)	450 KPa
Radio frequency	1.2 KW
Plasma gas flow rate	10.0 L/min
Auxiliary gas	0.6 L/min
Carrier gas flow rate	0.70 L/min
Gas	Pure Argon
Plasma torch	Standard
Premixed standard	0, 0.1, 0.2, 0.5, 1.0, 2.0 ppm
Blank	0.5M HCl

3.1.2 Reagents and solvents used

All the solvents and reagents used were of analytical grade. They were purchased from Sigma Aldrich through the local supplier Kobian Kenya. Distilled water was used as a solvent for solution preparation.

3.2 Cleaning of glass ware

All glassware was washed with soap, soaked in 10 % v/v nitric acid overnight and rinsed with deionised water and dried in an oven at 105°C prior to use.

3.3 Sample collection and preparation

3.3.1 Sample collection

The samples of tea, coffee and soya were purchased from stores within Eldoret town. Three brands each of tea, soya and coffee were selected. The total number of samples were 9 bags each weighing 250 gms. The samples were placed in well labeled paper bags and transported to the Tea Research Foundation of Kenya (TRFK) laboratories situated at Kericho, Timbilil estate (latitude 0˚ 22'S, longitude 35˚ 21'E, altitude 2180m above sea level) where they were stored in a cool dry environment, ready for digestion.

3.3.2 Sample pre-treatment

The acquired samples were crushed using blending device (Moulinex AR1043, China) and then sieved repeatedly to pass through a 2 mm sieve. The powdered samples were stored at room temperature in dry, air-tight containers ready for analysis.

3.4 Sample Analysis

All solutions were prepared using analytical-grade reagents and distilled water.

3.4.1 Determination of polyphenol content

The present study determined the total phenolic content using Folin-Ciocalteu reagent. The method works on the principle that phenolic compounds are oxidized by Folin-Ciocalteu reagent. This reagent is formed from a mixture of phosphortungstic acid, $H_3PW_{12}O_{40}$, and phosphomolybdic acid, $H_3PMo_{12}O_{40}$, which, after oxidation of the phenols, is reduced to a mixture of blue oxides of tungsten, W_8O_{23}, and molybdenum, Mo_8O_{23}. The blue coloration produced has a maximum absorption in the region of 750

nm, and is direct proportional to the total quantity of phenolic compounds originally present.

The Folin-Ciocalteu reagent was prepared as follows: by dissolving 100 g of sodium tungstate, $Na_2WO_4.2H_2O$, and 25 g of sodium molybdate, $Na_2MoO_4.2H_2O$, in 700 mL of distilled water. 50 mL phosphoric acid 85 % and 100 mL of concentrated hydrochloric acid was added and brought to boil and refluxed for 10 hours. Thereafter 150 g of lithium sulfate, $Li_2SO_4.H_2O$, and a few drops of bromine was added and boiled again for 15 minutes, allowed to cool and made up to one liter with distilled water. The resulting mixture was then kept at room temperature for 5 minutes and then 1.5 mL of 6 % (w/v) sodium carbonate added and mixed gently.

3.4.1.1 Treatment of samples for polyphenol analysis

Two grams of the milled samples was placed on pre-weighed moisture free dish and left for 16 hours at 103˚C in the oven to dry. The ground samples (0.2 g) were extracted with five milliliters of 70 % hot methanol/water mixture. Heating of the extraction tube was continued in the water bath for 10 minutes with mixing in the vortex mixer after every 5 minutes. The extraction tubes were then removed from the water bath, allowed to cool to room temperature and stoppers removed. It was then be centrifuged for 10 minutes at 3500 revolutions per minute (rpm). The supernatant was carefully decanted into graduated tubes. Extraction steps was repeated and the extracts combined and made up to 10 mL with cold methanol/water mixture. One milimetre of the sample extract was then transfered to a 100 mL mark volumetric flask and distilled water added to the mark and mixed. One milimetre of the diluted sample extract was then tranfered in duplicate into separate tubes. Five milimetre of ten percent (v/v) diluted Folin-Ciocalteu reagent

was then pipetted into each tube and mixed. Within three to eight minutes after addition of Folin-Ciocalteu reagent, four milimetres of 7.5 % w/w sodium carbonate solution added to each tube, stoppered and mixed using a vortex. The mixture was then allowed to stand for 60 minutes and then optical densities (OD) measured in one milimetre cell each using Shimadzu UV-1800 series spectrophotometre at 765 nm.

A calibration curve was obtained using gallic acid over the concentration range of 10 t0 50 ppm. Monohydrate gallic acid (1000 ppm) was prepared by weighing 0.11 g of gallic acid monohydrate (M.W 188.14) into 100mL volumetric flask and topped up with distilled water. Using pipettes the volume (1,2,3,4,5 mL) of gallic stock solution was transfered into five 100 mL volumetric falsk respectively and diluted to mark with distilled water. The resulting dilutions corresponded to 10, 20, 30, 40 and 50 ppm respectively. The optical density readings of the samples were referenced to the calibration curve to determine the polyphenolic content of the coffee, soya and tea samples. The amounts of the total polyphenols was determined from the standard curve generated using gallic acid as the amount of gallic acid equivalent (GAE). The polyphenol content was expressed as a percentage by mass drying matter (Karori et al., 2007).

A best fit linear calibration graph was constructed from the mass fraction of gallic acid in standard, against the gallic acid optical densities after subtracting the reagent balnk optical density.

The polyphenol content, expressed as apercentage by mass on a sample dry matter basis using the formula;

$$\% \text{ polyphenol} = \frac{(OD_{sample} - OD_{intercept}) \times V \times D \times 100}{Slope_{std} \times m \times 10000 \times DM}$$

Where;

OD_{sample} is the optical densities obtained for the sample test solution

$OD_{intercept}$ is the optical density at the point the best fit linear calibration line intecepts the y-axis

$Slope_{std}$ is the slope obtained from the best fit linear calibration

m is mass of gallic acid monohydrate, in grams, used to prepare the stock solution

V is the volume of gallic acid stock solution , in mL, used to prepare the standard solution

D is the dilution factor volume in mL

DM is the dry matter content, expressed as a mass fraction, in percent, of the sample.

3.4.2 Determination of antioxidant activity

A decrease in absorbance was monitored spectrophotometrically at 517 nm. Five grams of the each sample was infused in 100 ml boiling distilled water for 10 minutes. The extract was the filtered through nylon mesh followed by filter paper (whatman no. 54). The 100 mL aliquots of the extracts were kept frozen (-18 °C). Ten milimetres of the infusion was dried to constant weight at 103 °C in preweighed moisture dishes (12 hours). The weight of the dried soluble solids was takein and expressed as mg/mL.

A methanol solution 50 ppm of antioxidant was mixed with 2 mL DPPH solution (6×10^{-5}M DPPH solution; made with with 80 % methanol) in a cuvette. The absorbance was read in a Shimadzu UV-1800 series spectrophotometre at 515 to 520 nm to show decline in absorbance until 517 nm absorbance. The reading was done at 15 and 30 minutes.

All analysis was performed in triplicates. Inhibition of DPPH radical in term of percentage (%) was calculated using the following formula:

% inhibition of DPPH radical = $[(A_B - A_A)/A_B \ ] X\ 100$

A_B = Absorbance of the blank

A_A = Absorbance of sample tested after 15 minutes

Based on the values of % DPPH remaining, the EC_{50} of each sample was obtained by plotting the % DPPH remaining against antioxidant concentration. The EC_{50} value is the concentration of an antioxidant to quench 50 % radicals in the reaction mixture under the assay condition. The results were expressed as mg of soybean, coffee or tea equivalent per mL of the testing solution. All measurements were conducted in triplicate (Blois, 1958).

3.4.3 Determination of Caffeine

3.4.3.1 Preparation of Standard Solutions for caffeine determination

Standard caffeine powder (50 mg) was weighed and dissolved in 20 mL distilled water. This was made up to 100 mL with distilled water. Ten milliliters of this solution was taken and made up to 100 mL with distilled water to produce a 50 μg/mL working standard. Standard linear calibration curve was run (λmax = 272 nm) to obtain the linear range of sample analysis, correlation factor was with accepted value (0.9916) and the standard calibration curve was linear (y = 0.0344x + 0.2184). The quantitative amount of caffeine in samples (μg/mL) was then determined using the standard curve.

3.4.3.2 Extraction of Caffeine from Beverages

Two grams of sample were weighed and powdered. Two hundred milliliters of distilled water were added to the sample and shaken for 15 minutes using a magnetic stirrer. Sufficient water will be added to produce 250 mL and the solution will then be filtered. To 10 mL of the filtrate, 10 mL of 1M sodium hydroxide (NaOH) was added and extracted immediately with five quantities each of 30 mL of chloroform in a separating funnel. Each extract was washed with 10 mL of water. The chloroform extracts were then combined and filtered through a plug of absorbent cotton wool previously moistened with chloroform. The solution was then evaporated to dryness and the residue dissolved completely in 30 mL of water by warming gently on a water bath. The solution was then cooled and made up to 100 mL mark in volumetric flask.

The absorbance of the resulting sample solution was then measured using a Shimadzu UV-1800 series spectrophotometre at 272 nm. This was done by transfering the solution into 1 cm quartz cuvette and placed into the sample holder of the spectrometer and the spectrum was then taken. These procedure was done in triplicate for each sample.

3.4.4 Determination of zinc and Iron using ICP-OES

3.4.4.1 Reagents for ICP-OES determination of trace elements

All solutions were prepared using analytical-grade reagents and distilled/deionised water. All glassware was washed with neutron soap, soaked in 10 % v/v nitric acid overnight and rinsed with deionised water prior to use. Concentrated nitric and sulfuric acid and hydrogen peroxide 30 % w/w (Merck, Darmstadt, Germany) were used for all digestion.

The multi-element reference solutions containing 50.0 mg/L Fe, and Zn were prepared by dilution in deionised water of 1000 mg/L of mono-element standard stock solutions.

Salts were dried at 105ºC for 1 hr and stored in desiccators before weighing. Deionized water was used for preparing all calibration standards reagents and for dilution. Acid reagents below were used.

i. Hydrochloric acid, HCl, concentrated.

ii. Nitric acid, HNO_3, concentrated.

iii. Nitric acid: 500 mL concentrated HNO_3 was added to 400 mL water and diluted to make 1 L.

3.4.4.2 Standard solutions for ICP trace element analysis

Table 3.2: Standard solutions for ICP trace element analysis

Element	**Standard solution for ICP**
Iron	0.100 g iron was dissolved in10 mL concentrated HCl. Five milliliters of concentrated HNO_3 was added the mkixture and diluted to 1000 mL with deionized water; 1.00 mL = 100 μg Fe
Zinc	0.10 g zinc metal was dissolved in 20 mL of HCl and diluted to 1000 mL with water; 1 mL = 100 μg Zn

3.4.4.3 ICP-OES instrumental calibration

The machine was warmed up for 30 minutes. The instrument was then be calibrated using calibration standards and blank. The standard or blank was aspirated for a minimum of 15 seconds after reaching the plasma but before beginning signal integration. The calibration blank was rinsed at least 60 seconds between each standard to eliminate any carryover from the previous standards. Average intensity of multiple integration standards or samples were used to reduce random error. Before analyzing samples, it was necessary to ensure the instrument check so that the concentration values obtained did not deviate from the actual values by more than ± 5 %. The respective wavelength for the two elements analyzed are as shown in table 3.3 below;

Table 3.3: ICP wave lengths for Fe and Zn analysis

Element	Wavelength nm
Iron	259.94
Zinc	213.86

3.4.4.4 Sample digestion procedure for trace element analysis

One gram of sample was transferred to a beaker. Five milliliters of concentrated nitric acid was then be added and also a few boiling chips or glass beads. It was then be boiled at low temperature and evaporated on a hot plate to the lowest volume possible. Heating and addition of HNO_3 was continued as necessary until digestion is complete as shown by

a light-coloured, clear solution. The sample was not allowed to dry during digestion. It was then washed down the beaker walls with water and then filtered. The filtrate was transfer to a 10 mL volumetric flask with two 5 mL portions of water added to rinse the volumetric flask. It was then cooled, diluted to the mark and mixed thoroughly. Portions of this solution were then taken for required metal determinations. .

3.4.4.5 ICP-OES Analysis of samples

Working standard solutions were prepared by serial dilutions of the stock solutions. After the digested samples was cooled, it was filtered and transferred to a 100 mL volumetric flask that had been rinsed with ultrapure water. Three replicate digestions were made for each sample. The average of blank signals was subtracted from analytical signals of digested samples. To express the results on a dry weight basis, the moisture of the samples were removed by keeping them at 60 °C for 6 h.

Samples were analyzed using calibration blank. This permits a check of the sample preparation regents and procedures for contamination. Analysis of samples was done alternatively with analyses of calibration blank. After introduction of each sample, the system was let to equilibrate before starting signal integration. Examination of each analysis of the calibration blank was done to verify that no carryover memory effect occured (Faires et al., 1984).

3.5 Data analysis

During the study, the aquired data was statistically analized using one sample **t** test to determine if level of total iron and zinc differ significantly from the standard values as stipulated. Analysis of variance (ANOVA) was used to test the significant difference in means of polyphenols, antioxidant activity, caffeine, iron and zinc among different

beverages, within and between the groups. In addition, correlation was used to determine whether there was any relation between polyphenol content and the antioxidant activity of the beverages under study. The critical alpha adopted during the study was 0.05 and only an error of less than 5 % was accepted. This means the confidence level was 95 %.

CHAPTER FOUR

RESULTS

This chapter presents the results from the study. Tables and figures have been used to illustrate and summarize all the analyzed data obtained from the study. The tables and figures are serially numbered and have explanatory notes at the bottom of each illustration. The intepretation of the results is in the next chapter.

Table 4.1 shows the level of the polyphenol content and antioxidant activity of the selected brands of coffee, soya and tea in percentages. The data show that the highest polyphenol content among selected coffee was sample CA (8.3 %) while the lowest was CD (6.6 %). In addition among the selected soya beverage, the highest amount of polyphenols recorded during the study was SC (4.5 %) and the lowest was SB and SA both with a percentage of 4.3. On the other hand, tea samples showed that TB and TC had highest polyphenolic content of 19.8 % and TA had the lowest polyphenol amount (19.2). Overally, the data revealed that tea had the highest phenol content in (average of 19.6 %) followed by coffee (average of 7.4 %) and lastly soya (4.4 %).

The present study also determined the Antioxidant activity of coffee, soya and tea. Referring to Table 4.1, the finding revealed that brand CA of coffee had the highest antioxidant activity of 79.3 % and CB had the lowest activity (76 %). With a percentage of 9.4 %, SA brand of soya had the highest antioxidant activity during the study whereas SB had the lowest antioxidant activity of 8.5 %. Finally, brand TA of tea had the highest antioxidant activity of 81.8 and brand TC had the lowest activity of 80.8 %. In Overall,

tea had the highest antioxidant activity among the three beverages while soya had the lowest with percentages of 81.4 % and 9.0 % in that order.

Table 4.1 Percentages of total phenolic and antioxidant activity of the selected beverage

		Sample	TP %	TP % mean	AA%	AA% mean
1	**Coffee**	**CA**	8.3 ± 0.6		79.3 ± 1.6	
2		**CB**	7.4 ± 0.5	7.4 ±0.9	76.0 ± 0.5	77.7 ±1.7
3		**CD**	6.6 ± 0.4		77.9 ± 1.4	
4	**Soya**	**SA**	4.3 ± 0.2		9.4 ± 0.2	
5		**SB**	4.3 ± 0.1	4.4±0.1	8.5 ± 0.6	9.0±0.5
6		**SC**	4.5 ± 0.3		9.1 ± 0.5	
7	**Tea**	**TA**	19.2 ± 0.0		81.8 ± 1.6	
8		**TB**	19.8 ± 0.6	19.6± 0.4	81.7 ± 2.1	81.4±0.6
9		**TC**	19.8 ± 0.5		80.8 ± 2.7	

*CA, CB, CD,SA,SB,SC, TA,TB,TC are code for different brands of coffee, soya and tea respectively

The standard linear calibration curve obtained of spectrophotometric UV/Vis method for the determination of polyphenol content showed a good linear relationship (R^2=0.99) between the absorbance and concentrations of the standard solutions

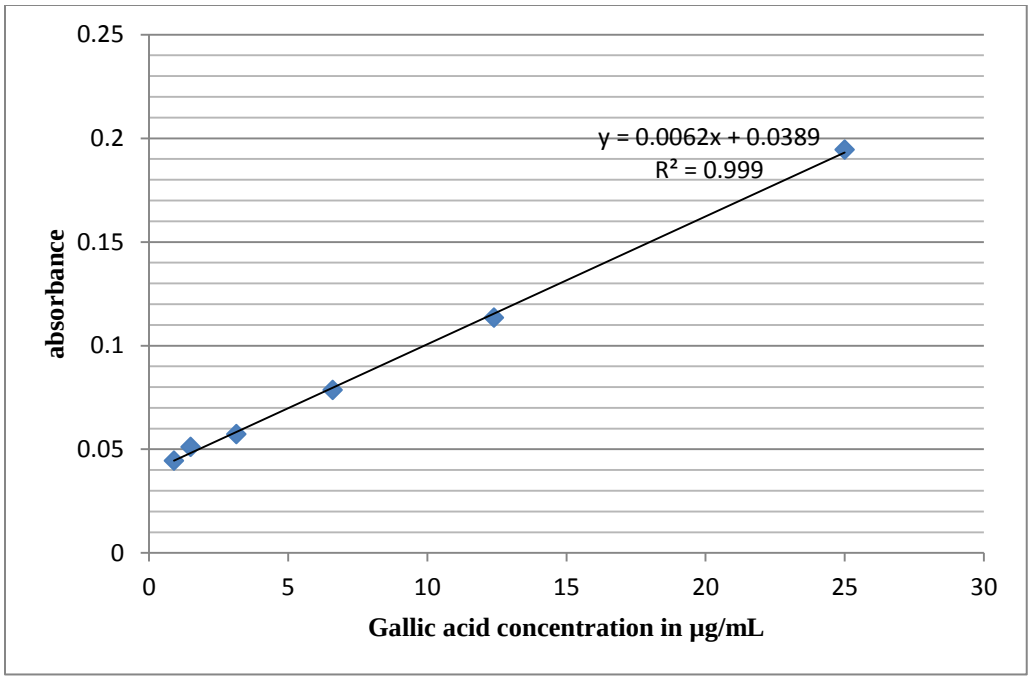

Fig 4.1 Standard Curve of Gallic Acid

Table 4.2 displays a correlation analysis of the data acquired. In general all the three beverages (coffee, soya and tea) commercially available in Eldoret town revealed that their antioxidant activity was directly related to the quantity of polyphenol present in the product. Pearson correlation (rho) was 0.688 and the relationship was significant (p=0.04). Hence as polyphenol level increases the antioxidant activity potential of the beverage also increases.

Table 4.2 Correlation between polyphenols and antioxidant activity of coffee, tea and soya combined

Correlations			
		Total phenol content in %	Antioxidant activity in %
Total phenol content in %	Pearson Correlation	1	.688*
	Sig. (2-tailed)		.040
	N	9	9
Antioxidant activity in %	Pearson Correlation	.688*	1
	Sig. (2-tailed)	.040	
	N	9	9

*. Correlation is significant at the 0.05 level (2-tailed).

In reference to Table 4.3 which shows the relationship between the level of polyphenols and the antioxidant potential of the beverage, it is clearly demonstrated in the present study that as the polyphenol content increases in coffee and soya, the level of activity potential increases while in tea, there is an inverse relationship between phenol content and the antioxidant activity. Though the relation was not significant, the p values for coffee, soya and tea was 0.70, 0.88, and 0.61 respectively

Table 4.3 Correlation of polyphenols and the antioxidant activity of each particular beverage.

Beverage	Pearson Correlation(rho)	P value(Critical alpha 0.05)
Coffee	0.453	0.701
Soya	0.189	0.879
Tea	-0.577	0.609

The standard linear calibration curve obtained of spectrophotometric UV/Vis method for the determination of caffeine showed a good linear relationship (R^2=0.9882) between the absorbance and concentrations of the standard solution.

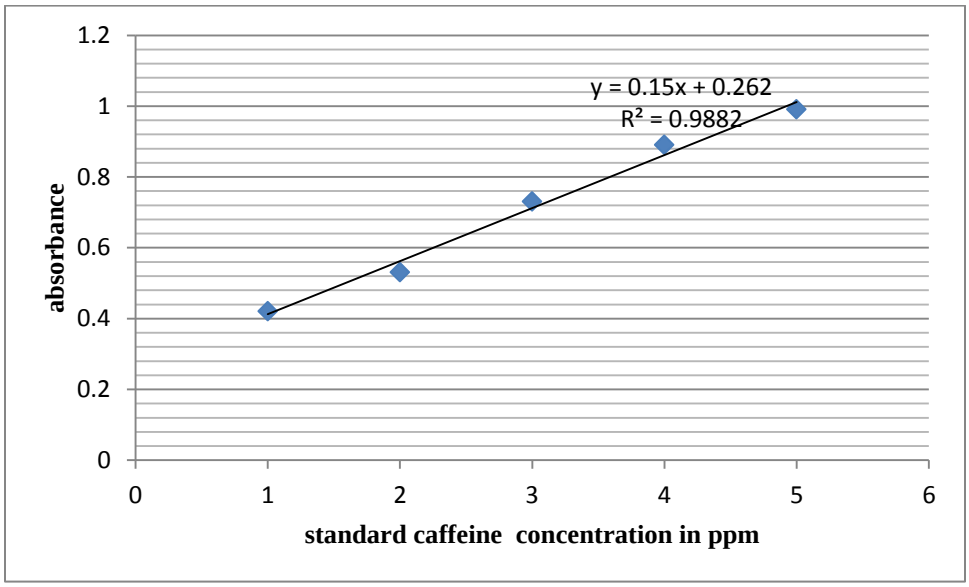

Figure 4.2 A Calibration curve of caffeine for UV-Vis spectrophotometric method.

In Table 4.4, the level of caffeine is presented. The data showed that coffee had the highest level of caffeine (4.58 mg/g) as compared to soya (Not detected) and tea (0.985 mg/g).

Table 4.4 Level of caffeine in coffee soya and tea

	Sample identity	**mg/g**	**Mean (mg/g)**
Coffee	CA	5.738±0.2	
	CB	4.125±0.0	4.58±1.0
	CD	3.875±0.4	
Soya	SA	ND	
	SB	ND	ND
	SC	ND	
Tea	TA	0.800±0.3	
	TB	1.000±0.6	0.985±0.2
	TC	1.156±0.0	

Table 4.5 shows that there was a statistically significant difference($p<0.05$) between and within the groups in the level of caffeine in coffee, soya and tea brands.

Table 4.5 One way ANOVA table for caffeine levels in coffee, tea and soya

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	34858011.556	2	17429005.778	49.592	.000
Within Groups	2108703.333	6	351450.556		
Total	36966714.889	8			

In multiple comparisons analysis as displayed in Table 4.6 there was significant difference in the amount of caffeine between coffee and soya, and between coffee and tea with p values of 0.00 and 0.001 respectively. The data also showed that there was no significant difference in the quantity of caffeine present in tea as compared to absence in soya.

Table 4.6 Multiple Comparison analysis (Tukey HSD)

Multiple Comparisons		
Type of beverage	Type of beverage	Sig.
Coffee	Soya	.000*
	Tea	.001*
Soya	Coffee	.000*
	Tea	.184
Tea	Coffee	.001*
	Soya	.184

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

From Table 4.7 it can be realized that the highest level of iron among coffee brands was in CD (152.0 ppm) and the lowest was CA which recorded 80.0 ppm of Fe content. Furthermore, soya brand SC had the highest level of iron while SA had the lowest content of 173.0 ppm and 78.0 ppm correspondingly. The brand of tea with the highest level of iron during the study was TA (114.0 ppm) whereas TC recorded the lowest level (104.0 ppm). In general soya brand coffee had the highest level of Fe (mean=119.43 ppm) while tea had the lowest (mean=109.8 ppm).

On the other hand, Zinc content of the three beverages was also determined. The findings revealed that among the coffee brands, CB had the highest content at 47 ppm however CA had the lowest level of Zn at 40.7 ppm. Among soya brand analyzed, it was found out that sample SB had the highest amount of Zn (55.3 ppm) and SC (46.7 ppm) was the

lowest. Finally, tea brand TC had the level of Zn at 39 ppm and brand TA recorded lowest at 37.7 ppm. Overall brand soya had the highest quantity of Zn (mean=50.57 ppm) while tea had the lowest (mean=38.23 ppm).

Table 4.7 Levels of Zinc and Iron determined in the selected beverages

		Fe in ppm		Zinc in ppm	
Sample identity		**Mean±SD**	**Mean**	**Mean±SD**	**Mean**
1	CA	80.0±17.0		40.7 ± 6.5	
			119.43±36.5		44.13±3.2
2	CB	126.3±15.3		47.0±7.0	
3	CD	152.0±9.2		44.7 ± 8.5	
4	SA	98.0 ± 6.0		49.7±7.6	
5	SB	173.0 ± 3.0	116.33±50.0	55.3±5.7	50.57±4.4
6	SC	78±13.2		46.7 ± 5.5	
7	TA	114.7±12.5		37.7 ± 5.5	
8	TB	110.7±15.5	109.8±5.4	38.0 ± 7.0	38.23±0.7
9	TC	104.0 ± 14.0		39.0 ± 4.0	

The model summary below (Table 4.8) indicate that there was no significant difference in the level of Fe among coffee, soya and tea, ($p=0.946>0.05$) with an F statistic of 0.056. Zinc content varied significantly between and within the group in coffee, soya and tea ($p=0.009<0.05$) and F statistic is 11.539.

Table 4.8 Model ANOVA Summary for Zn and Fe variation in coffee, soya and tea

Summary model of ANOVA					
Fe content of selected beverages in ppm					
	Sum of Squares	**df**	**Mean Square**	**F**	**Sig.**
Between Groups	145.096	2	72.548	.056	.946
Within Groups	7737.853	6	1289.642		
Total	7882.949	8			
Zn content of selected beverages in ppm					
Between Groups	228.309	2	114.154	11.539.	0.009
Within Groups	59.360	6	9.893		
Total	287.669	8			

To determine superficially the variation in the level of Fe and Zn among the three beverages, a multiple comparison using one way ANOVA post hoc test was conducted. The output is displayed in Table 4.9. Referring to Zn level in the table, the study found out that there is no statistically significant variation in the level of Zn between coffee and soya ($p=0.101>0.05$), between coffee and tea ($p=0.132$) and there is a statistically significant variation in the level of Zn between soya and tea $p=0.007$. Hence soya had the highest quantity of Zn.

The findings also revealed that the quantity of Fe in coffee, soya and tea did not differ significantly. This means that there is no statistically significant difference in amount of Fe among the three beverages since the p value (as shown in the table) are all greater than the study's critical alpha 0.05.

Table 4.9 Multiple comparison on One Way ANOVA (Tukey HSD Version)

Dependent Variable: Zn content of selected beverages in ppm		
		Sig.
Coffee	Soya	.101
	Tea	.132
Soya	Coffee	.101
	Tea	.007*
Tea	Coffee	.132
	Soya	.007*
Dependent Variable: Fe content of selected beverages in ppm		
Coffee	Soya	.994
	Tea	.943
Soya	Coffee	.994
	Tea	.973
Tea	Coffee	.943
	Soya	.973

* . The mean difference is significant at 0.05 level

The acceptable standard level of Fe and Zn for all beverages in Kenya is 50 ppm. The study used its finding to compare the level of Fe and Zn from the standard concentration. One sample t test output (Table 4.10) indicated that the concentration of Zn in soya did

not differ significantly from the acceptable level. However, Zn concentration was significantly lower in coffee and tea, p=0.035 and p=0.00 respectively.

Table 4.10 One sample t test for the variation from the standard concentration of Zn and Fe

One-Sample Statistics. Test value=50ppm (standard conc.)				
Beverage	**N**	**Mean**	**Std. Deviation**	**Sig.**
		Different level of Zn		
Coffee	9	44.1111	6.97217	.035
Soya	9	50.5556	6.67291	.809
Tea	9	38.2222	4.91878	0.000
		Different level of Fe		
Coffee	9	105.000	47.22552	0.008
Soya	9	116.333	44.00284	0.002
Tea	9	109.777	13.03627	0.000

CHAPTER FIVE

DISCUSSION

Results of the present study indicate that tea sample TA had the highest polyphenolic content and also showed the highest antioxidant activity as compared to the other samples of tea, coffee and soya. In overall, tea samples indicated the highest polyphenol content, followed by coffee with soya having the least. Soya sample SB scored the lowest in both total polyphenol and antioxidant activity. Several factors, including genotype, geographical origin, growing conditions (including soil composition and moisture regimes, harvesting time, post-harvest treatments and physical structure of the leaves) have been shown to influence the polyphenolic content and composition of coffee, tea and soya (Cheruiyot et al., 2007; Wachira & Kamunya, 2010). The different process methods in manufacture of the individual brands of coffee, soya and tea could be another reason for the polyphenol and antioxidant activity difference.

There was high radical scavenging activity on DPPH by tea samples as shown in Table 4.1. The high antioxidant activity of the teas is mainly attributed to the presence of high levels of bioactive catechins that have the ability to donate hydrogen ions to stabilise the free radicals. The high antioxidative effect of polyphenols as seen in both tea and coffee samples is due to the presence of phenolic hydroxyl groups in their structures that make them potent free radical scavengers (Wanyika et al., 2010).

A regression analysis was conducted to show the relationship between polyphenol content and antioxidant activity. And the findings revealed that there was a direct strong linear correlation(R^2 = 0.688) between the two. This findings concur with a study done

by Dutta et al. (2013) (r^2 = 0.9287) implying that polyphenols possess antioxidant property that provides protection against oxidative stress. This also suggest that the antioxidant properties of coffee, tea and soya are a consequence of the synergistic activity of beverage. The current study is corroborated by numerous investigations of the antioxidant activity of plant extracts and have confirmed a high linear correlation between the values of phenol concentration and antioxidant activity (Katalinić et al., 2006). Similar findings by Borneo et al. (2008) indicated that polyphenolic content of plants contribute directly to their antioxidant action and recounted a positive correlation between DPPH free radical scavenging activity and total phenolic compounds**.** In addition, Wijngaard et al. (2009) also recorded a strong correlation (r = 0.72) between polyphenols and DPPH radical scavenging capacity of different vegetable and fruit by products.

However in the present study there was a negative correlation in the quantity of polyphenol content and antioxidant activity in tea samples analyzed using DPPH. This fact was brought out by Karori et.al.(2007) that variation in the polyphenolic composition of the different tea brands is attributed to leaf maceration during manufacturing. Maceration which involves rolling and cutting of the tea shoots releases polyphenol oxidase that interacts with phenolic compounds, one simple catechin and one gallocatechin, to produce the aflavins and the arubigins that posses a benzotropolone skeleton. Hence the product could be having antioxidant activity but lack or may have low phenol content.

The current findings demonstrate that coffee contains higher level of caffeine than tea and soya as shown in Table 4.4. This is different from previous work reported by Kalra,

Kumar and Maithani (2011). who found tea to be of high in caffeine levels than coffee. A number of factors influence the variation of caffeine in the two beverges, for example Kaplan et al. (1974) described that processing conditions affect caffeine content and that certain types of tea and coffee contains somewhat more caffeine than others. All the coffee samples in this study had high caffeine levels as compared to tea and soya samples, this is normally because coffee is ground extremely fine and therefore enhance extraction of caffeine.

The significant difference between and within the groups in the level of caffeine (Table 4.5) could be attributed to origin, genetic and environmental variability, harvest time and processing manner of plant material (Athayde et al. 2000). The multiple regression results in Table 4.6, showed clearly that there was significant difference in the amount of caffeine between coffee and soya and between coffee and tea with p values of 0.00 and 0.001, respectively. The amount of caffeine in food products varies according to the type of product and method of preparation. Generally, the coffee bean is higher in caffeine content than tea; hence coffee products are expected to contain more caffeine than tea. Also, dark-roast coffee may contain less caffeine than the lighter product because roasting reduces the caffeine content of the coffee bean (Nicoli et al., 1997).

According to US Food and Drugs Administration on classification of caffeine consumption, caffeine intake of 130 - 300 mg/day is low/moderate, above 400 mg/day is high while above 6,000 mg/day is heavy. Low/moderate consumption is considered safe (U.S. Code of Federal Regulations, 2003). It is difficult to calculate the exact amount of caffeine consumed by individuals because the amount of product used per cup is usually not weighed/measured but dispensed by means of teaspoons. Also, there are a variety of

cup sizes but use of a known amount of product would eliminate the importance of cup size. The amount of caffeine needed to produce effects varies from person to person according to body weight and individual sensitivity. Children for instance, due to their smaller weight, may manifest hyperactivity and other side effects if they regularly consume beverages with moderate caffeine content which are considered safe for adults (Benjamin et al., 1991). Therefore, people who need caffeine restriction (pregnant women, people suffering from hypertension, diabetes, glaucoma, insomnia) should choose products with low caffeine content and avoid regular consumption. Caffeine is an addictive substance, so regular consumption of products that contain even moderate amounts of caffeine may lead to addiction to the products with withdrawal reactions like mood changes and flu-like symptoms. Including; headaches, fatigue, irritability, difficulty in concentrating, depression, nausea/vomiting and muscle ache or stiffness (Juliano and Griffiths, 2004). These reactions may lead to increased intake of caffeine with increased adverse effects. Therefore, the use of caffeine should be regulated with a requirement for its presence in foods and drinks, along with amounts, to be clearly stated on the tables of constituents of such beverages, which is not practiced by Kenyan manufacturers.

Zinc concentration in coffee was significantly lower ($p=0.035$) as compared to tea and soya. This could be attributed to the removal of the outer layer of the coffee bean during the milling which greatly reduces the level of Zn in the bean. This could hold true for the finding of the current study that there is no significant difference in the level of Zn in soya and coffee as both undergo roasting process that removes the Zinc rich coat.

Soya samples had the highest level of Zn as compared to tea and coffee, this is in agreement to the attribute given by Gençcelep et al. (2009) that legumes are known to be zinc accumulators, a family to which soya (soy bean) belongs**.** Zinc has been recognized as a co-factor of the superoxide dismutase enzyme, which is involved in protection against oxidative processes. In addition, Zinc has been shown to promote wound healing, and also play a role in taste, appetite and growth (Alayande et al., 2012). Iron on the other hand is an essential mineral in human health, playing a role in immune function, cardiovascular health and cognitive development (Seena et al., 2006).

Prevous studies have been done on the level of Zn in tea. Srividhya et al., (2011) using Atomic Absorption Spectrometry method recorded 26.26 ppm which is lower than the findings of the present study. Similarly Gebretsadik and Chandravandish (2010) reported Zn levels between 20.2 and 21.6 ppm in commercially available Ethiopian tea using AAS. The lower levels of iron in the samples as observed in soya samples SA and SC could be attributed to processing where the beans are roasted, then the coat is removed before crushing. The removal of bran, or seed coat have been implicated in the reduction of minerals in grains (Olanipekun et al., 2015).

Among the objective of the current study was to determine the level of Fe among the three beverages. The level of Fe in tea samples ranges from 104 ppm to 114.7 ppm as shown in Table 4.7. These values are lower than those obtained by Moseti, (2013) who analyzed the iron level in 24 selected black tea brands from Mombasa tea auction using flame atomic absorption spectroscopy. He found the level of Fe to be in the range of 151 and 369 ppm. The current study findings also differ from Gebretsadik and Chandravandish (2010) who reported Fe levels between 319 and 467 ppm in

commercially available Ethiopian tea using AAS. The variations ($p<0.005$) were evident as samples of tea, coffee and soya had varying levels of Fe, an observation attributed to the difference in processing.

High levels of Fe in tea leaves have been associated with mature leaf (Wanyoko & Njuguna, 1983) implying that the high Fe content in tea sample (TB) may be attributed to rough plucking standards and long plucking interludes among other factors hence the need for strict adherence to Good Agricultural Practices (GAP). In addition, Moseti, et al.,(2013) observed the possibility of introduction of additional Fe along tea processing chain and could be so in coffee and soya processing This is possibly due to wear and tear of the machine parts during the rolling stage in tea manufacture. This observation further underlines the need for strict adherence to good manufacturing practices (GMP) in the tea, coffee and soya industries.

Foods/beverages recognized as good sources of iron tend to also contain zinc. This corroborates the finding of present study as shown in Figure 4.3 Which depict the association between iron content and zinc content in coffee, soya and tea samples (O'Neil et al., 2012).

A number of studies have been done to determine the level of trace element in beverages. A study done in Brazil which showed that in soybean based fruit juices, the iron levels ranged from 0.08 to 1.38 mg/100 mL (average of 0.96 mg/100 mL± 0.29), and the zinc from 0.04 to 0.68 mg/100 mL (average of 0.43 mg/100 mL ± 0.12). In soymilk the same minerals content ranged from 0.38 to 1.738 mg/100 mL (average of 1.08 mg/100 mL± 0.71), 0.25 to 0.38 mg/100 mL (average of 0.29 mg/100 mL ± 0.04), respectively. These results indicated that samples of soymilk contained, on average, more

iron, when compared to soybean based fruit juices. The iron: zinc (Fe: Zn) molar ratio was in average 1.0: 2.6 and 1:4.4 for soybean based fruit juices and soymilk, respectively (Luciula et al., 2012). The maximum allowed level of Zn and Fe in processed products (tea,coffee and soya) by Kenya Bureau of Standards (KEBS) is 50 ppm as indicated in the Kenya Standard KS 65: 2009.

CHAPTER SIX

CONCLUSION AND RECOMMENDATIONS

6.1 Conclusions

The current study sought to determine the total polyphenolic contents, antioxidant activity, caffeine level and trace elements level of different selected brands of coffee, tea and soya available in Eldoret town. According to results obtained from this study, the selected beverages possessed significant amount of polyphenols and showed strong antioxidant potential in Folin-Ciocalteu method and 2,2-diphenyl-2-picrylhydrazyl (DPPH) free-radical scavenging assay respectively. There was a significant relationship ($p=0.04$) between the polyphenol content and antioxidant activity with Pearson correlation (rho) of 0.688. Tea had the highest level of caffeine (0.985 mg/g) while soya had the lowest level (ND). There was statistically significant difference in the amounts of caffeine between coffee and soya, between coffee and tea; p values of less than 0.05.

The level of Fe was highest in coffee and lowest in tea. Soya had the highest level of zinc whereas tea had the lowest. There was no signifant difference in the level of iron in coffee, soya and tea with a p value of 0.95. Zinc content varied significantly in coffee, soya and tea with a p value of 0.009. The levels of zinc among the three sampled beverages did not differ significantly from the standard acceptable level while iron content of the three beverages was significalty higher (coffee, $p=0.008$, tea, $p=0.000$, soya, $p=0.002$) than the acceptable level of 50 ppm.

6.2 Recommendations

6.2.1 Recommendations from the study

1. The study recommends that the manufacturers should indicate the composition of each beverages on the packaging for the benefit of consumers.
2. The findings of the present studies suggest that the selected beverages possess polyphenols with antioxidant activity. The beverages be consumed so as to benefit from their high antioxidant potential.

6.2.2 Recommendations for further studies

1. Speciation study be done on polyphenols and caffeine levels in decafinated beverages.
2. A study be done on the levels and toxicity of other trace elements in tea, coffee and soya.
3. Study be done on the safety of iron present in the beverages

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