ASSESSMENT OF CATECHINS AND OTHER POLYPHENOLS AS PARAMETERS OF TEA QUALITY IN SELECTED ECOLOGICAL ZONES IN KENYA

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DECLARATION

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

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DEDICATION

This thesis is dedicated to my beloved daughter Jemimah Moraa Matara Mangenya.

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LIST OF ABBREVIATIONS AND ACRONYMS

EC	Epicathecin
ECG	Epicatechin gallate
EGC	Epigallocatechin gallate
EGCG	Epigallocatechin- 3 - gallate
CTC	Cut, Tear and Curl
TFs	Theaflavins
TRs	Thearubigins
IBMK	Isobutylmethylketone (4-methylpenta-2-one)
TBK	Tea Board of Kenya
ITC	International Tea Committee
HPLC	High Performance Liquid Chromatography
ORAC	Oxygen Radical Absorbance Capacity
TBARS	Thiobarbituric Acid Reactive Substance
DPPH	2, 2-Diphenyl-2-picrylhydrazyl
TRI	Tea Research Institute
CRF	Coffee Research Foundation of Kenya
KTDA	Kenya Tea Development Agency
ANOVA	Analysis of Variance
GMP	Good Manufacturing Practices
KTGA	Kenya Tea Growers Association
TBEA	Tea Brokers of East Africa

ABSTRACT

Kenya is the third largest producer of tea in the world after China and India and specializes in the processing and export of black CTC tea. Over 90 % of Kenyan teas are sold to the world market through an auction based system that is dependent on the quality of tea on offer. In the tea trade, quality is used to indicate the presence of special desirable attributes in the tea liquor which are detected by visual, smell and taste. The chemical composition of tea is very complex and is currently a subject of broad medicinal and toxicological scientific studies. Previous studies have extensively shown that catechins together with their oxidation products including theaflavins and thearubigins are responsible for the sensory characteristics associated with black tea liquors; astringency or briskness, colour, strength and brightness. This study determined the biochemical profiles of tea grown by small scale farmers in Kisii, Murang'a and Meru ecological zones in Kenya. The levels of catechins were quantified using reversed phase HPLC while total polyphenols content were determined by spectrophotometric analysis. Theaflavins and thearubigin contents were determined by the Flavognost, Roberts and Smith methods respectively. Antioxidant activity of the tea samples was determined using the method of Brand-Williams. This study found that teas grown in the three ecological zones differed significantly in their polyphenols, catechins, theaflavins and thearubigins contents ($P < 0.05$). Interestingly, tea samples analyzed exhibited high antioxidant activity which differed significantly ($p < 0.05$) across the zones suggesting potential use of the black teas for medicinal use. Similarities between biochemical profiles and organoleptic evaluation at level $P < 0.01$ and at $p < 0.05$ depicted positive correlation. This study sets a precedent for the characterization of biochemical profiles of tea from all the tea growing areas in Kenya

CHAPTER ONE

INTRODUCTION

1.1 Background to the study

Tea, *Camellia sinensis* (L) *Kuntze* is an evergreen shrub that belongs to the genus *Camellia* that includes over 200 species (Banerjee, 1992) and plant family *Theacea*. Of all the *Camellia* spp., tea is the most important both commercially and taxonomically. The two main cultivated varieties of tea are *Camellia sinensis* var. *assamica* which has relatively large leaves and *Camellia sinensis* var. *sinensi*s with small semi-erect leaves. The tea plant originated from the forests of South-East Asia and has over time been introduced to many areas of the world including Europe, North and South America, Africa and Australia. It is cultivated commercially for use both as a beverage and medicinal purposes (Tanmoy & Bhagat, 2010). As a beverage, tea is the oldest, most popular non-alcoholic drink globally (Chen *et al.,* 2003; Gulati *et al.,* 2003; Yanagimoto, *et al.,* 2003; Sand, 2003; Seenivasan *et al.,* 2008; Gebretsadik & Bhagwan, 2010) after water (Wheeler & Wheeler, 2004; Thangaphazham *et al.,* 2007; Fwu–Ming & Hong-Wen, 2008; Zerabruk *et al.,* 2010).

Depending on the variety, the tea plant can thrive in a wide variety of geological and climatic conditions (Bonheure, 1990) and naturally grows as tall as 15 m if unpruned (Mondal *et al.,* 2004; Yemane *et al.,* 2008), though when under cultivation it is kept at a low level to enable the young shoots from which the tea is made to be plucked. Tea is a perennial crop that for economic production requires deep well drained soils (Othieno, 1992), with sufficient rainfall distributed throughout the year and the pH value of soils should be between 4 and 6. However studies by the United Planters Association of Southern India, (UPASI, 1984), revealed that the upper acidity limit varies with the nature of the soil, especially the organic matter content and is considered as 5.6 in East Africa and 6.0 in Southern India.

Kenya's germplasm is predominantly of Assam type and many of the commercial clones are genealogically related (Wachira, 2001). This has led to further introduction of genetic resource based on mutually negotiated germplasm exchange agreements to broaden the genetic base of Kenyan tea (Wachira, 2002). Under good management practices (GMP), the tea bush can remain productive for more than 100 years producing between 2.5 to 5 kg of green leaves per annum. The growth and development of the tea plant can be described by four stages; the seedling or cutting, the formation, the mature or commercial and the degraded or moribund stages (Zeiss &Denbraber, 2001; TRFK 2002). The duration of each stage is determined by the genotype and growing conditions.

China, India, Sri Lanka, Kenya, Indonesia, Turkey, Iran, Georgia, Japan, Vietnam, Bangladesh, Argentina, Malawi, Uganda and Tanzania are the main tea producing countries. Tea was first introduced in Kenya from India by a colonial white settler G. W. Cainne in 1903 (Wilson, 1962) who imported the dark leafed "Manipuri" hybrid seed from Assam and established a plantation at Limuru near Nairobi. Its expansion in designated areas was gradual but commercial cultivation began in 1924 (Matherson, 1950). The planting expanded rapidly and by 1920, there were 216 ha of tea in Kenya (Greenway, 1945). By 1963, the acreage was 21000 ha, and 172,000 ha by the year 2011 (TBK, 2011). Kenya is the third largest producer of tea in the world after China and India and specializes in the processing and export of black CTC tea (International Tea Committee (ITC), 2006). Currently tea is the leading foreign exchange earner and export commodity (Gebretsadik & Bhagwan, 2010; TBK 2012) for the country. For instance in 2012, Kenya produced 377 million kilograms of processed black tea from which the export earnings amounted to KShs.109 billion. This was much higher than the total earnings of KShs.97 billion recorded in 2010 or KShs.69 billion in 2009 (TBK 2012). The major importers of Kenyan tea in 2011 were Pakistan (80.8 million kilograms) and Egypt (79.9 million kilograms). Other significant export destinations were the United

Kingdom (UK), Afghanistan and Sudan. Generally the export earnings have doubled in the last five years (TBK 2012).

1.1.1 Tea production in Kenya

Kenya is a tropical East African country with varied climatical and geographical regions (Gesimba *et al.,* 2005). The Kenya tea industry is a rural based enterprise where over 62 % of the crop is produced by small scale farmers.

According to the tea board of Kenya (TBK, 2007), the Kenyan tea industry is generally structured into two sub-sectors: large estate plantations and smallholder subsector. The large estate subsector is composed mainly of transnational companies as well as some local companies with medium to large plantations of over 20 to 700 ha. The small holder subsector consists of individual farmers with an average holding of about 0.1 to 20 ha (KTDA, 2003). The small holder subsector accounts for over 62 % of Kenya's tea output and is currently managed by the Kenya Tea Development Agency (KTDA) which started in 1964 after Kenya gained her independence. On the other hand the large estate subsector, which accounts for the rest of the 38 % of Kenya tea, is represented by the Kenya Tea Growers Association (KTGA) and the Nyayo Tea Zones Co-operation (NTZC). The large plantation subsector has plantations which are managed by trained personnel whereas the farmers in the small subsector rely on agricultural extension officers who offer advisory service in managing their fields (Ogola & Kibuku, 2004).

The tea growing areas in Kenya are divided into two regions delineated by the Great Rift Valley namely; East of the Rift Valley that comprises of the cool Aberdare Highlands, the snow capped Mt. Kenya region, the Nyambene Hills and which hold Meru, Embu, Kirinyaga, Murang'a, Nyeri and Kiambu counties. The West of the Rift Valley block comprises of the highlands of the Mau escarpment around Kericho, Nandi Hills, Mt Elgon and the Kisii highlands which encompasses the Kericho, Bomet, Kisii, Nyamira,

Nandi, Kitale and Vihiga counties (Fig 1). It is on the slopes of these highlands within altitudes 1500 and 3100 m above mean sea level that tea is grown (TRFK, 2002).

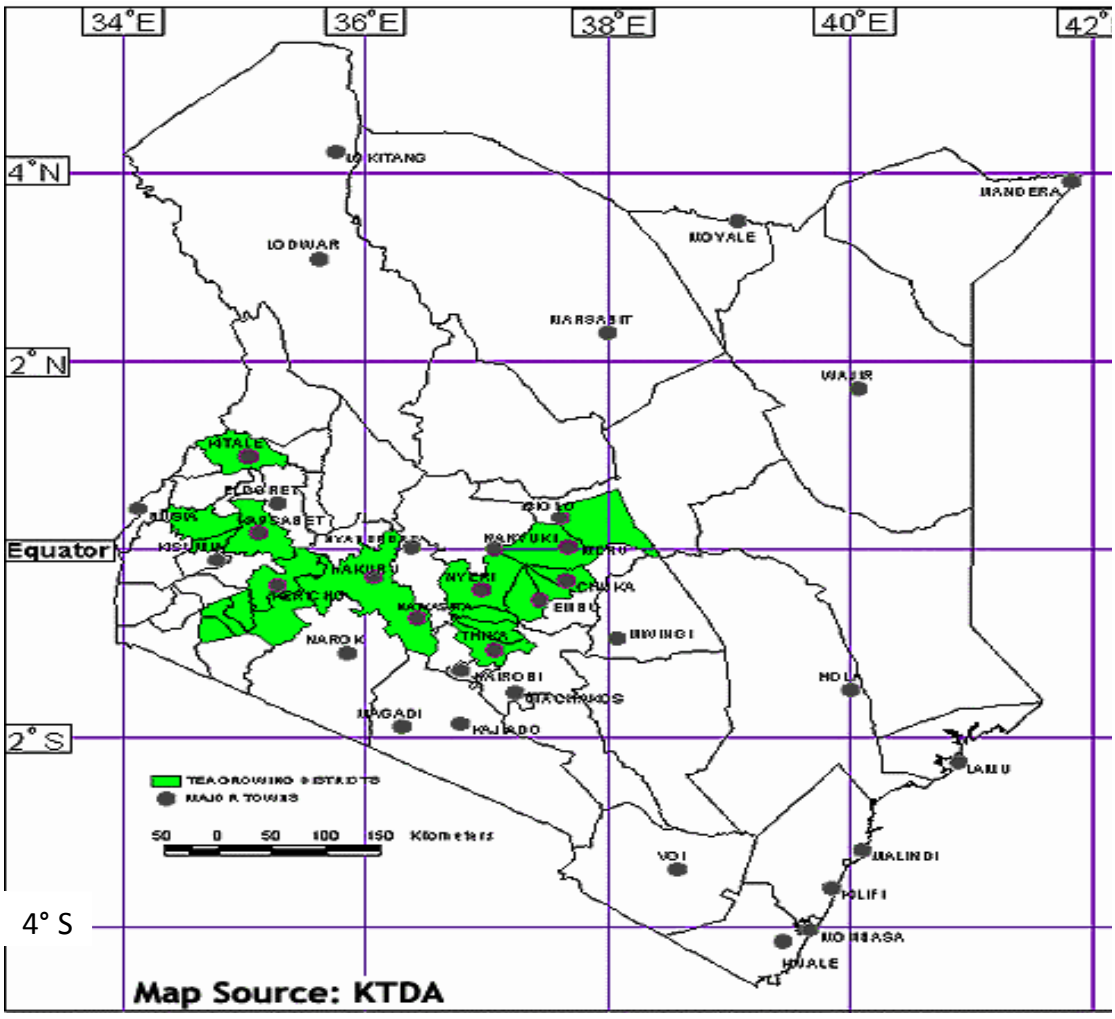


Figure1.1: Map of tea growing areas of Kenya (source: KTDA, 2012)

There has been a marked rise in the Kenya tea production over the years with notable replacement of the pioneer seedling tea with improved clonal cultivars that are better yielding, of good quality and that take about one year from propagation to planting unlike seed plants that take up to three years. These new varieties are said to have better attributes like tolerance to drought, pests and diseases (Wachira, 2002; Owuor and

Obanda, 2001; Owuor *et al.,* 2007). Kenyan black teas have been shown to have between 7 to 27 % more polyphenols when compared with teas from China, Japan and Taiwan (Wachira & Kamunya, 2005). The Kenyan tea germplasm has also been observed to be diverse in its polyphenol composition and content (Magoma *et al.,* 2000) and therefore provides raw material for production of different types of tea products including health drinks. According to the International Tea Committee, ITC (2009) and the Kenya Tea Development Agency, KTDA (2011), Kenya mainly produces black Cut, Tear and Curl (CTC) tea. This type of tea has the advantage of quicker brewing, makes more cups per kilogram and a large proportion of it is sold in bulk in the export market (KTDA, 2011). However in the recent years, the world tea prices have declined due to slowed consumer demand as the costs of production continue to rise. This has resulted in diminishing returns to the farmers and other stakeholders, creating a major challenge that needs to be addressed in a multidisciplinary approach by all stakeholders. In response to the challenge tea industry stakeholders currently are championing value addition initiatives and product diversification as strategies to enable Kenyan tea to compete favorably in the global tea market.

1.1.2 Economic importance of tea

The economic importance of *Camellia sinensis* is primarily due to its use as a beverage and has received much attention in the entire world because of its attractive aroma and pleasant taste. These have lately been augmented with numerous medicinal benefit claims (Tanmoy & Bhagat, 2010). Tea has been socially and habitually consumed by people since 300 BC (Lin *et al.,* 2003). It is served as a diet drink for two thirds of the world population (Muktar *et al.,* 2000). Drinking tea is a special tradition common in many countries such as Japan, China and Vietnam. Tea also cannot be absent in cultural events such as circumcisions, weddings and funerals in the Eastern African region.

Tea is an important source of revenue for tea producing countries (Grahan, 1992). In Kenya, tea contributes about 4.0 % of its GDP and about 26% of all foreign exchange

income, earning the country KShs. 110 billion in 2012; KShs. 116 billion in 2013 (TBK, 2013). At the household level, the tea plant is called the crop of the poor especially in tropical mountainous areas. Even with minimal investment, tea can be planted and the two leaves and a bud from the mature bush harvested on a ten day period. Tea is grown on hard and sloping soils where other food crops or cash crops do not grow well (Vo Thai, 2006). The planting of tea on remote mountain areas is also considered an effective method to cover the sloping lands thus providing a means of soil conservation and environmental conservation. Cultivation of tea in the remote areas also provides many jobs to rural communities and certainly contributes to the development of local infrastructure and consequently poverty eradication.

It also contributes to environmental conservation through enhanced water infiltration; reduced surface erosion and mitigation of global warming through carbon sequestration. The tea plant absorbs carbon from the environment. Tea farms can therefore generate revenue for the farmers from carbon credits in the future once registered with relevant carbon trading agencies. Factory companies who register their operations as clean development mechanism (CDM) projects stand to gain revenue through carbon credits and also safeguard the environment by embracing friendly and sustainable operations.

Green tea leaves are also used as vegetables such as "lappet tea" in Burma and "Meing tea" in Thailand (Vo Thai, 2006). Tea branches removed during the pruning stage can be harnessed as a source of energy to light up "jikos 'and hence could be a source of alternate energy for use in villages. Pruned tea branches not used for energy supply are used in situ to provide organic matter to the soil.

1.1.3 Chemical constituents of tea

The tea leaf is composed of a number of different micronutrients and compounds all of which are interconnected with the chemical reaction of its processing. Inorganic components (mainly found as salts in the cell sap) include; K, Ca, P, Mg, Mn, Fe, S, Al,

Na, Si, Zn, and Cu. Nitrogen compounds also play a major role in the tea leaf. Three-quarters of the nitrogen in the leaf is in amino acids like theanine, which is unique to tea. Theanine is an amino acid found only in tea leaves, which imparts a pleasantly sweet taste to tea. It is degraded to glutamic acid and has relaxation effects in humans, making them feel calm, alert and focused. The rest comes from the alkaloid caffeine, a natural component of all teas. Although a serving of tea usually contains less than half the caffeine of coffee, actual caffeine levels are dependent on specific blends and the brew strength. In general green tea contains 3 to 6 % caffeine and black tea contains 2 to 4 % caffeine on a dry weight basis. Caffeine increases alertness, aids in tasks requiring focusing ability and vigilance. The leaf also contains carbohydrates which are mostly pectins and very small amounts of sugars and starches, pigments derived from chlorophyll and flavones, and vitamins B and C. The most important parts of the leaf in terms of its chemical reactions are the enzymes and polyphenols. The most important enzymes are polyphenol oxidase (PPO) and Peroxidase (PO). The polyphenols occurring in the tea plant are derivatives of gallic acid and catechins. Tea also contains phenolic acids, mainly caffeic, quinic and Gallic catechins and other polyphenols such as quercitin, myricitin and kaempferol.

1.2 Statement of the problem

Kenyan teas are grown in different regions that differ in both their soil characteristics and environmental conditions. The influences of these factors on biochemical composition and thus plain tea quality parameters are not well understood. In recent times, tea prices have tended to remain below USD 3 per kilogram. But increased costs of production and farm inputs have resulted in decreased profitability for the tea producers. Currently the small holder tea farmers in different tea growing zones in the country face financial constraints that in the long term shall threaten the tea farming business. The fact that within the same sector farmers get differing rates of income or returns from tea sales may make those regions with low rates to start contemplating going into alternative activities. In order to offer an objective solution to those small

scale farmers suffering low prices in different ecological zones due to the quality of teas from these areas, there is need first to assess and ascertain the variations in the biochemical compositions in the teas from different ecological zones and illustrate how they affect organoleptic quality and possibly pricing.This study is the first of its kind in Kenya and aims to profile biochemical attributes of tea from the different ecological zones and the effect of environmental conditions on biochemical composition and subsequently on quality and prices.

1.3 Objectives

1.3.1 General objective

To elucidate the differences in the quality attributes of teas grown and processed by small scale farmers in three ecological zones represented by Murang'a, Meru and Kisii tea growing catchment areas based on biochemical analysis.

1.3.2 Specific objectives

i. To determine the variations in plucking leaf quality standards of harvested leaf processed in three ecological zones represented by Muranga, Meru and Kisii.
ii. To quantitatively determine the levels of catechins, caffeine, total polyphenols and inorganic nutrient content in clonal green tea leaf.
iii. To determine the levels of theaflavins, thearubigins and total polyphenols, liquor brightness, total colour responsible for black tea quality of leaf processed
iv. To determine the radical scavenging properties (antioxidant capacity) of processed black tea using DPPH radical scavenging technique.
v. To evaluate a correlation between biochemical components of black teas from different ecological zones and their organoleptic quality.

1.4 Null hypothesis

There are no significant differences in the plucking standards of green leaf, individual catechins, polyphenols, plain quality parameters, antioxidant potential and correlation in black teas processed in three ecological zones represented by Muranga, Meru and Kisii

1.5 Justification

Kenya is a major producer of black tea. All consumers globally including tea consumers are keen about the biochemical composition, food safety standards, and health benefits accrued from the products they consume (Moseti, 2013; Moseti *et al.*, 2013). In this regard, tea from Kenya being grown in highlands is thus expected to experience slow growth conditions leading to high polyphenol and catechin contents. In the tea trade, these growing characteristics are associated with high quality (Owour *et al.*, 1990). For this reason, Kenyan tea is usually blended with low catechin content teas from other tea producing nations to enhance their quality. Though Kenyan teas are on average of high quality, the quality of tea from the different tea growing regions still vary due to myriad of reasons among them; the various stages of production, soil and fertilizers, climate and environmental conditions (Robertson,1983; Owour,1995).

Screening of tea for the levels of catechins, caffeine, total polyphenols, TFs and TRs is aimed at obtaining data on green tea leaf composition and changes during processing at the factory level that lead to variations in tea quality arising from different growing zones amongst small scale farmers in Kenya. The knowledge acquired from this study will add to the body of knowledge necessary in developing an objective way of assessing and marketing of tea from various ecological zones in Kenya. In addition, it may offer invaluable information on how variations in ecological conditions of altitude, soils, and ambient conditions of growth lead to variations in production quality parameters of leaf harvesting intervals, leaf standards, wither, duration of fermentation conditions and temperature and the organoleptic quality of the final product.

This study will a help in quantifying constituents in teas from different zones that are unique to them and the development of specific quality attributes of black tea. The data obtained may provide insight into the processing conditions necessary for production of quality teas irrespective of ecological zone and growth conditions. In this way, higher returns may be realized by the individual tea factories which hitherto have tended to produce less than high quality black tea.

Information obtained from this study will be useful in highlighting the best regions to obtain tea with desirable levels of polyphenols, caffeine and catechins for value addition of tea products. Branding may involve stating the types of biochemical composition present in the teas and in what concentration. Buyers will then be able to source Kenyan teas from specific regions as per catechins and phenolic contents.

CHAPTER TWO

LITERATURE REVIEW

2.1 The tea plant

Botanically, the tea plant has three main varieties based on leaf features such as size, pose and growth habits (Sealy, 1958). These are the China, the Assam and the Cambodia or Indo-China tea: *Camellia sinensis* variety *sinensis, Camellia* variety *Assamica* and *Camellia* variety *Cambodia*, respectively. The three main taxa can also be differentiated by foliar, floral and growth features (Hadfield, 1974; Sealy, 1958) and by biochemical affinities (Hazarika *et al.,* 1984, Magoma *et al.,* 2000; Owuor *et al.,* 1986, Ozowa *et al.,* 1969; Roberts *et al.,* 1958; Sanderson, 1963; Takeo, 1983). These tea varieties are present in all the tea growing zones in Kenya.

2.2 Types of commercial teas

Nowadays hundreds of different types of commercial teas are produced. The different types of tea products are based on the method of manufacture and consequently their chemical composition (Reeves *et al.,* 1987). Commercial teas can generally be classified into three main categories; the non-fermented green teas, the partially fermented oolong and paochong teas, and the fully fermented black and pu-erh (red) teas (Lin *et al.,* 1998). In Kenya, the majority of processed teas are categorized as black. The nature and quality of a given tea product irrespective of its category is mainly dependent on the chemical composition of the unprocessed tea and the reactions they undergo during the manufacture process. The cutting technique of manufacture of the mentioned tea products (green, oolong and black) may be orthodox or non-orthodox and vary considerably in their impact on the formative and degradative reaction patterns of the various cellular components (Mahanta & Hemanta, 1992; Wilson & Clifford, 1992). In the world market, black tea represents approximately 78 % of the global tea production and is the most common type of tea in the United States of America and Europe (Jian-

Min, Yuan, 2007). Though most of the tea produced in the world can be classified as non-aerated, green and aerated, black tea (Owuor *et al.,* 1986), processing has diversified leading to production of speciality tea products such as purple, white flavored, organic, decaffeinated, herbal and various other types of tea. In this present study, black Cut, Tear, Curl (CTC) tea was used as it is the main product processed, marketed and consumed in the study area (Kenya).

2.3 Factors influencing tea quality

Many studies have compared the biochemical composition of black teas produced in different countries. These studies have shown that the composition of the volatile flavor compounds in black tea (Wickremasinghe *et al.,* 1973; Yamanishi *et al*., 1968); black tea aroma (Aisaka *et al.,* 1978; Owuor *et al.,* 1996) and black tea plain quality parameters (Owuor *et al.,* 1986b) vary with geographical area of production. However it is also known that the biochemical and quality variations occur due to the variation in the genetic make-up of the plants (Magoma *et al.,* 2000; Owuor *et al.,* 1995; 1998) and green leaf quality (Owuor et *al.,* 1987, 1990). Other factors constituting important influences on the composition of tea include species, seasons, age of the leaves (plucking position), climate, horticultural conditions (soil,water, minerals and fertilizers) (Lin *et al.,* 1996) and type of processing.

2.3.1 Green leaf plucking standards

It has been well demonstrated that agronomic practices affect the quality of black tea from *Camellia Sinensis* (L) *O.Kuntze*. One such agronomic/cultural practice reported to affect black tea quality is plucking standard. Coarse plucking reduces black tea quality (Owuor *et al.*,1987, 1990; Owuor,1989,1990) due to the decline in catechin levels (Forrest and Bendall,1960) and changes in polyphenol oxidase isoenzyme composition and activity (Takeo & Baker,1973; Thanaraj & Seshadri,1990; Obanda & Owuor.,1992) leading to a general decline in total TF levels (Owuor *et al.*,1987; Owuor,1990). There is

concomitant increase in unsaturated fatty acids (Owuor *et al.*, 1990) leading to production of less aromatic black teas (Owuor *et al.*, 1987).

Although the recommended plucking standard in Kenya is two leaves and a bud (Othieno, 1988), KTDAs minimum leaf quality standard is 75% good quality for its factories though some farmers are known to pluck coarser leaf. Such a practice is erroneously thought to lead to higher crop volume, even though it sacrifices quality. The proponents of the practice argue that the extra crop, obtained by coarse plucking per plucking round, more than compensates for loss in quality. However, recently it was demonstrated that, provided plucking rounds are shortened, fine plucking can be carried out at shorter interval, resulting in improved yields (Odhiambo, 1989; Owuor & Odhiambo, 1984) and quality (Owuor *et al.*, 1990).

Despite the knowledge that green leaf catechin levels and composition (Forrest & Bendall, 1966; Obanda & Owuor, 1992), and /or polyphenol oxidase activity (Takeo & Baker, 1973; Thanaraj &Seshadri,1990) decline with coarse plucking standards, black tea manufacturers normally set the same fermentation time, with changes only being instituted to take into account the variations in ambient temperatures. Thus fermentation times are shortened during hot conditions and lengthened during cold conditions. Variations do not normally take into account the standard of plucking of the leaf. This is so as fermentation conditions of time, temperature, and humidity have been documented to cause changes in the chemical compositions and hence quality of black tea at two leaves and a bud plucking standard (Cloughley, 1979; Owour & Reeves, 1986). As a result fermentation is normally very closely monitored during black tea processing.

In this study which compared variations in biochemical compositions of tea from three ecological zones, an attempt was made to relate whether the variations in plucking standards in the zones contribute to the quality of black teas emanating from them.

2.3.2 Tea processing

Tea is obtained from the processing of young shoots of the tea plant. The young plants are raised from cuttings obtained from mother bushes and are carefully tendered in nursery beds until they are 12 to 15 months old. They are then planted out in tea fields with a spacing of between 1.0 to 1.5 m (KTDA, 2011). This practice of raising tea is standard across the smallholder sector in Kenya. Unlike most herbs that only need to be dried, commercial tea must be "processed". Tea is harvested manually or mechanically, with each pluck taking only the flush (youngest two leaves and a bud), and these tender and succulent fresh growth is the raw material from which a number of tea products are processed. Plucking youngest two leaves and a bud represents standard or fine plucking, the first step towards superior quality tea manufacture. The interval between plucking and delivery is kept as short as possible and great care is taken when transporting green leaf to the factory (KTDA, 2012).

Figure 2 is a summary of the general steps in the manufacture of different types of tea. The steps involved in the manufacture of black CTC teas, the principle product in the study area are highlighted below:-

2.3.2.1 Withering

This is the first stage in tea processing where harvested tea leaves are loaded into troughs fitted with powerful exhaust fans that draw moisture and carry the humid air out. This stage takes between 8 to 20 hours during which time the moisture content of the fresh leaves are reduced to between 65 and 67 %, making them amenable to subsequent processing steps (KTDA, 2011). During withering diverse biochemical changes occur (Robinson & Owuor, 1992; Costa *et al.*, 2002) including changes in proteins, caffeine, sugars, organic acids, polyphenol oxidase(PPO) activity, chlorophyll, minerals, volatile compounds and permeability of the cell membrane (Dev Chouldhury & Bajaj, 1980).

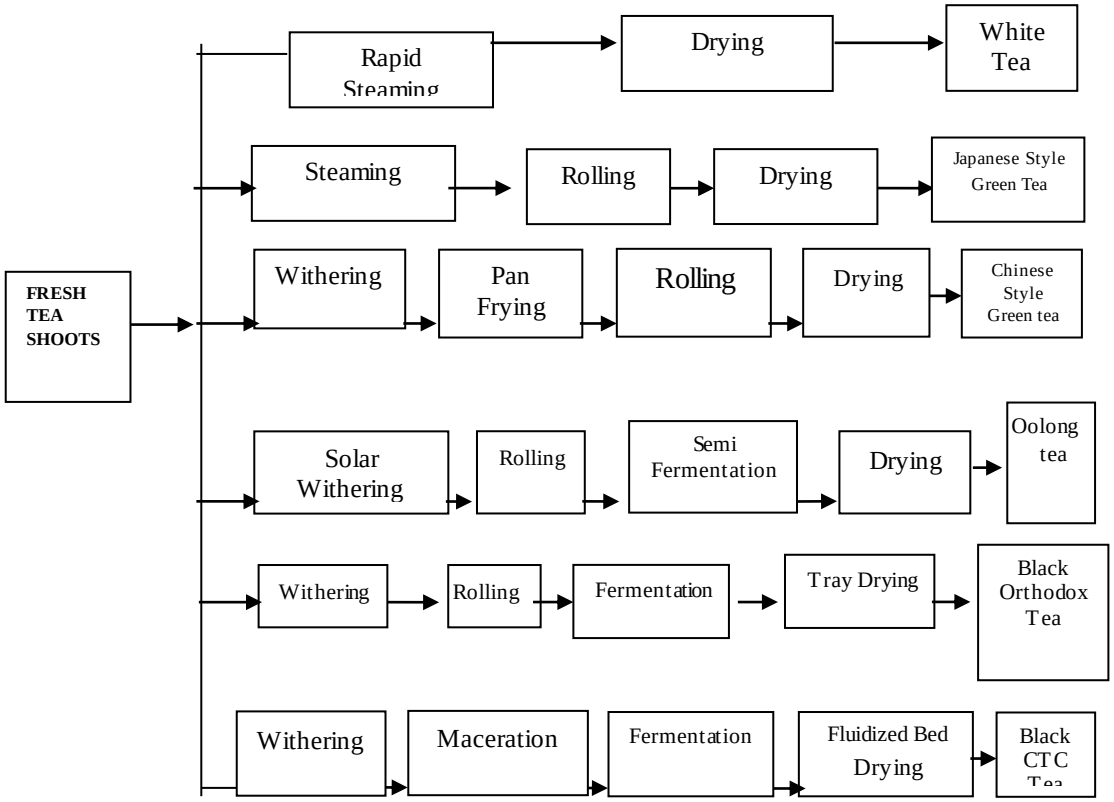


Figure2.1: Schematic diagrams on major steps in tea manufacture and corresponding types of tea: (Source: Hilal and Engelhardt, 2007).

2.3.2.2 Maceration

This step is also referred to as rolling and is accomplished by the radicon gear box, and CTC machines where the cell structures are disrupted exposing the cell contents to atmospheric oxygen (KTDA, 2011) bringing various enzymes into intimate contact with their substrates, in this case the polyphenols. The chemical and biochemical reactions initiated during withering proceed at an accelerated rate during and after rolling (Hara *et al.*, 1995).

2.3.2.3 Fermentation or aeration

The important reactions that occur during this stage are the development of colour, strength and flavour quality of tea by the production of non-volatile compounds through the enzymatic oxidation of catechins and their gallates and the production of volatile compounds responsible for characteristic aroma of black tea (Tombs &Mashingadze, 1997). These chemical and biochemical reactions make fermentation a most critical step in black tea manufacture (KTDA, 2011).

2.3.2.4 Drying or firing

This step is primarily intended to cause cessation of enzymic activity and reduce the moisture content to about 3 % of the dry mass (KTDA, 2011). However other changes other than removal of moisture that occur during this step include a significant loss of volatile compounds, an increase in the levels of amino acids and the binding of polyphenols to other tea components and an increase in carboxylic acids and maillard reactions (Hara *et al.,* 1995). This stage is also critical in the tea processing as it contributes to the keeping quality of tea once in the value chain

2.3.2.5 Grading or sizing

After drying the teas, they are then sorted into primary and secondary grades; the criteria being the size of the leaves and their fiber content, where whole, large tea leaf particles gain a higher grading (KTDA, 2012). The dry tea is exposed to static electricity charged PVC rollers that pick up the fibres and the open leaf. Grading facilitates the international trade in tea and is the central component in attaching a monetary value to the various grades of tea. Also it is an important tool for tea experts in making evaluations and comparisons between the different varieties of tea grown and manufactured world over. Tea is graded into seven grades that are further classified into primary grade teas that includes Broken Pekoe (BPI), Pekoe Fannings(PF1), Pekoe Dust (PD) and Dust 1 (D1) and secondary grade teas that include Fanning's, Dust and Broken Mixed Fibres (BMF).

2.4 Bioactive compounds of tea

The chemical composition of fresh tea leaves is complex and includes polyphenolic compounds, carbohydrates, proteins, lignin, mineral ash, amino acids, lipids, organic acids, chlorophyll as well as carotenoids and volatile substances (Cabrela *et al.*, 2003).

2.4.1 Tea catechins

The six catechin-derived polyphenols in tea leaves, that is, catechin (+ C), epicatechin (EC), epicatechin gallate (ECG), gallocatechin (GC), epigallocatechin (EGC) and epigallocatechin gallate (EGCG), are oxidized by the enzyme Polyphenol oxidase (PPO). The catechins first form intermediate compounds called orthoquinones that are very unstable and reactive. The orthoquinones then combine in pairs in a series of condensation reactions. They can function as either hydrogen acceptors or donors. The combinations can happen through C-O or C-C bonds. The compounds formed are called theaflavins (TFs), which are larger molecules and are unique in chemistry.TFs are brighter and brisker than others and as such, the quality of tea depends not just on the polyphenol count, but the composition of the catechins and the availability of PPO. TFs are unstable and further oxidize through the action of PPO to form thearubigins (TRs) that are much larger and more complex.Their chemical structure is yet to be known.The compounds though, have a high complexation affinity with metals like aluminium (Al) and manganese (Mn) and alkaloids like caffeine.TRs are largely responsible for the flavor, aroma and colour of the liquor; some make it brighter and brisk, others dull.

Catechins are the main bioactive molecules in tea and are the most frequent (Cabrela *et al.*, 2003; Yilmaz, 2006). Apart from tea, catechins are found in a variety of foods such as wine, fruits and chocolates (Yilmaz, 2006). The most important catechins in tea are; epigallocatechin gallate (EGCG), epigallocatechin (EGC), epicatechin gallate (ECG) and epicatechin (EC) (Fig 3) . Also present in smaller amounts are catechin (+C), gallocatechin (GC), and gallocatechin gallates. Catechins that contain three hydroxyl

groups in the B-ring (position 3', 4'and 5') are called gallocatechins while gallic acid substitution in position 3 of the aliphatic ring is characteristic of gallated catechins (Pellilo *et al.*, 2002) (Fig 3). Catechins differ in chemical structure, reduction potentials (Bajaj *et al.*, 1987) and contribution to the astringent taste of tea (Ding *et al.*, 1992; Kuhr & Engelhardt, 1991) and can be categorized according to the number of hydroxyl groups on the B-ring or as gallated or nongallated catechins. Catechins in tea can be used to predict plain black tea quality potential of green and black tea (Owuor & Obanda, 2001). Catechins account for 6 - 12 % of the dry weight of green tea leaves with EGCG constituting 10 to 50 % and being the most bio active due to its degree of gallation and hydroxylation (Stewart *et al.*, 2004). Besides, catechins together with phenolic acids such as gallic acid constitute up to 30 % of the dry weight of the tea leaf and contribute significantly to the taste of tea (Forrest & Bendal, 1969; Grahan, 1992; Roberts, 1992). It's noteworthy that EC, EGC and +C are simple, non gallated tea catechins while ECG and EGCG are gallated catechins (Sanderson, 1972; Sanderson & Graham, 1973) (Fig 3).

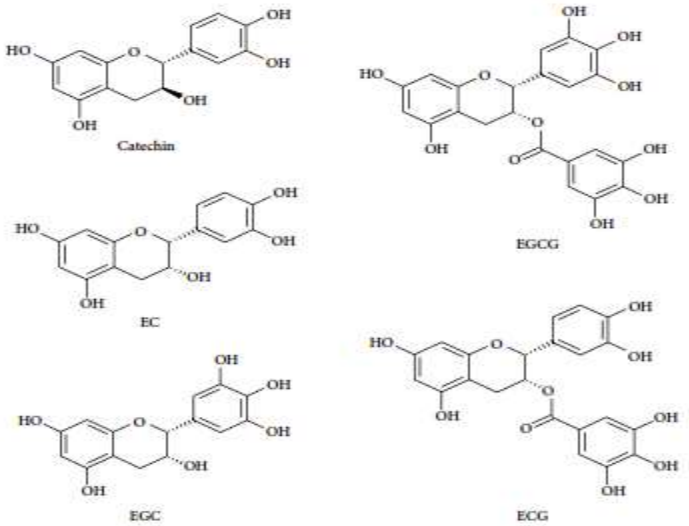


Figure2.2: Chemical structures of the major catechins present in tea (Zhao *et al.*, 2014)

The simple non gallated tea catechins EC, EGC and +C are not as astringent as the gallated catechins ECG and EGCG. Apart from their role in tea quality, catechins are increasingly being identified with a number of diverse properties of benefit to human health (Obanda, Owuor & Taylor, 1996; Sano *et al*., 1991). At present it is agreed that catechins, the major polyphenols in the green tea shoots together with their oxidation products are responsible for most of the sensory characteristics associated with black tea liquors (Biswas *et al.,* 1973; Roberts & Smith, 1963; Sanderson *et al.,* 1976). Flavanol glycosides which are also present in the green tea leaf are believed to contribute substantially to tea liquor colour (McDowell *et al*., 1990).

Climatic and agronomic factors have been found to affect both the catechin and other phenolic contents of the green tea shoot and the composition of the resultant black tea liquors (Hilton *et al.,* 1973; Owuor *et al*., 1995; Ramaswoy, 1994). Studies carried out have shown that the tea germplasm with low catechin and total polyphenol content were likely to produce low quality black teas (Obanda *et al.,* 1992). However Kenyan tea germplasm spread throughout the ecological zones under study has been shown to have between 7 and 27 % more total polyphenols than germplasm from China, Japan and Taiwan traditionally used for green and oolong tea production (Wachira and Kamunya, 2005). This indicates that Kenyan teas irrespective of ecological zone are rich in antioxidants as they have high levels of total polyphenols. Studies on catechin expression in Kenyan teas have revealed that ECG, EGCG, EC and +C range between 0.52 – 5.95, 0.42 – 1.95, 0.00 – 2.19 and 0.00 – 1.55 mmol/g of dry matter respectively (Magoma *et al.,* 2001).

2.4.2 Theaflavins and thearubigins

During the processing of black tea at the fermentation stage, tea catechins usually undergo coupled oxidation by the enzymatic action of polyphenol oxidase (PPO) (Joseph *et al.,* 2005). They are oxidized to homogeneous substances called theaflavins (TFs), which give a bright/orange, yellow/ red colouration in fermented black tea and

contribute to the brightness and briskness of the tea liquor. Theaflavins further act as oxidizing agents for gallic acid to produce epitheaflavic acid which in turn combine with the TFs to produce chemically heterogenous substances called thearubigins (TRs) which tend to be brownish-red (Brown *et al.*, 1966; 1965; Deb & Ullah, 1968; Takino *et al.*, 1964). These thearubigins are responsible for the colour, body and taste of teas (Obanda *et al.*, 2001, 2004). Theaflavins and thearubigins are found in black and oolong teas. The processing of green tea does not include an oxidation step hence catechins remain intact in the processed product. Together these oxidation products and flavonol glycosides (McDowell, *et al*., 1990), give black tea liquors most of its taste and colour. Theaflavins have astringent tastes and contribute to the briskness of black tea (Deb & Ullah, 1968). Pure theaflavins have a taste of fierce astringency thereby inducing a mouth drying and rough astringent oral sensation. The taste thresholds of TFs are much lower than their precursor catechins, though theaflavin digallate (TFDG) possess the strongest astringency among the TFs and as a consequence, precursor substances for TFs and TRs which are the catechins in fresh young shoots. They have been found to positively correlate with black tea quality parameters (Obanda *et al.*, 1997). The manufacture of quality black tea is a highly controlled process that optimizes all process variables to ensure that the right ratios of TFs and TRs are achieved. The chemical structure of Theaflavins (TFs) and thearubigins (TRs) are shown in Figure 4 below. Theaflavins consist mainly of four major compounds, which are normally termed Theaflavin (TF1), Theaflavins-3-gallate (TF2A), Theaflavin-3-gallate (TF2B) and Theaflavins -3, 3'-digallate (TF3). Although the structure of theaflavins is very complex, they have the same hydroxyl substituted benzotropolone ring which is a characteristic structure of Theaflavins. Theaflavins can also be synthesized through condensation of catechins between di and tri-hydroxylates B rings of catechins. The reaction of condensation involves the oxidation of the B-ring of Catechins to the quinones, followed by addition of the gallocatechin quinine to the catechins quinone, prior to carboxyl addition across the ring and subsequent decarboxylation.

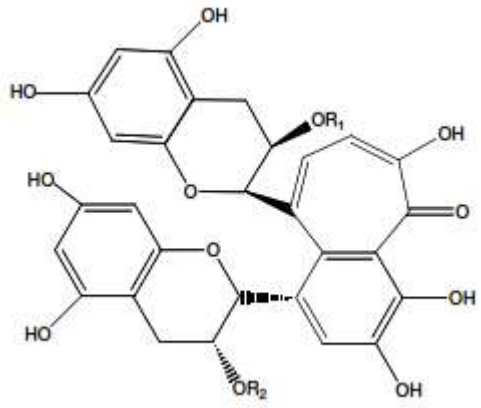


Figure2.3: Structure and general scheme for the formation of individual theaflavins

Simple theaflavins R_1 = R_2 = H; Theaflavin - 3 - gallate (TF - 3 - g) R_1 = 3,4,5 trihydroxybenzoyl and R_2 = H; Theaflavin - 3' - gallate (TF - 3' - g) R_1 = H and R_2 = 3,4,5 trihydroxybenzoyl; Theaflavin - 3, 3' -digallate (TF - 3, 3' -dg) R_1 = R_2 = 3,4,5 trihydroxybenzoyl.(**Obanda** *et al.*,2006)

2.4.3 Tea alkaloids

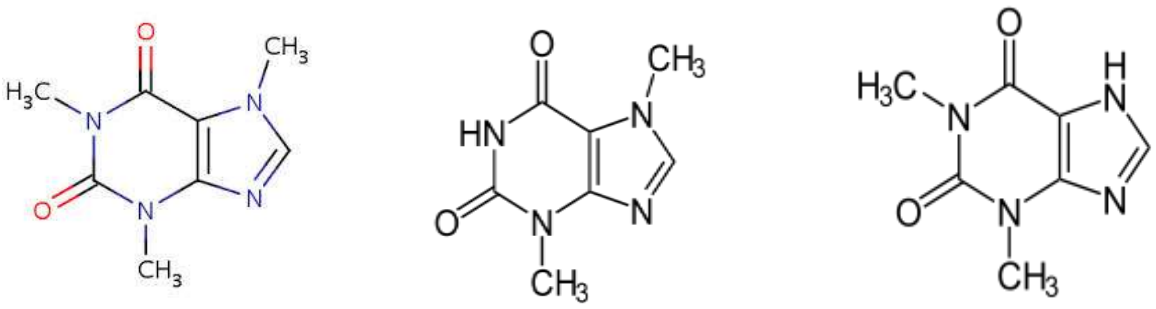
Caffeine, theophylline and theobromine are the main methylxanthines found in tea. These tea alkaloids are significant factors in the quality of tea. Caffeine, one of the major alkaloids gives tea its briskness and the creamy characteristics usually observed for black tea (Milin *et al.,* 1969). On average, tea leaves contain 3 % caffeine by weight although this can range from 1.4 % to 4.5 %. Many factors determine the caffeine content in the dry leaf, such as soils, the altitude in which the tea is planted, type of tea plant (clone), position of the leaf on the tea bush and cultivation practices (i.e. agronomic practices in a tea field). Apparently, caffeine level is not affected in any way by the level of oxidation during tea manufacture. Green, oolong, black and white teas all contain caffeine. A standard cup of tea will contain an average of about 50 mg of caffeine while a cup of coffee having 65 to 175 mg and a bar of chocolate about 1 to 35 mg (Monique *et al.*, 1996; Chin *et al.*, 2008).

Studies done at the Tea Research Foundation of Kenya (TRFK) have shown that the contents of caffeine in commercialized tea clones in Kenya range from 1.6 to 4.9 %. The most popular clone in that smallholder sector, clone TRF 6/8 has caffeine content of 1.65 %. This clone owes its popularity to production of high quality black tea, which research has shown, is associated with high levels of total polyphenols. Overall most tea clones cultivated in Kenya and their resultant tea products can be described medium to low in caffeine content (3.0 %). Efforts to develop naturally caffeine free tea or tea with trace amount of caffeine are on-going.

Chemically, caffeine (1, 3, 7-trimethylxanthine) is an alkaloid and a member of the xanthine family. It is odorless, non-toxic, has a bitter taste and is highly soluble in water. It occurs naturally in coffee, tea, coca, kolanuts and a variety of other plants. Some plant species that contain caffeine such as tea and coffee may also contain trace amounts of another alkaloid called theophylline (Nathanson, 1984). Unlike caffeine, theophylline has only two methyl groups. Theophylline has a stronger effect than caffeine on the heart and breathing. Some species related to tea, also contain another alkaloid called theobromine (Nathanson, 1984). This alkaloid is largely found in cocoa where its concentration is about 7 times that of caffeine. Theobromine has a weaker stimulating effect than caffeine (Fig 5). Caffeine is bioactive and in moderation, it has beneficial effects on 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 (Ker *et al.*, 2010). However, at high levels it can cause restlessness, insomnia and anxiety (Snel *et al.*, 2011). It can also exert some mild withdraw effects such as transient but persistent headache and inability to concentrate and can be addictive (Juliano *et al.*, 2004). Caffeine is the most widely consumed stimulant drug in the world (Nehling *et al.*, 1992; Arnand, 2011)

Research has shown that caffeine is rapidly absorbed following oral consumption. Peak blood (plasma) levels are achieved usually within 30 minutes. It is metabolized in the liver. It has a very short half-life of only about 3 to 5 hours in adults and is easily

excreted in urine. Because of its short half-life in the body one needs to keep coming back for more (Arnand, 2011). It causes stimulation by antagonizing the effect of adenosine (which causes a calming effect). Because of it pharmacological properties, caffeine is used in the pharmaceutical industry and is often a component of several over the counter analgesics (Arnand, 2011). It is also added to several types of commercial drinks including cola and pepper soft drinks, energy drinks, frozen desserts, chocolates and candies (Chin *et al.*, 2008).



Caffeine Theobromine Theophylline

Figure2.4: Structure of caffeine and other alkaloids (Ashihara *et al.*, 2008**)**

2.4.4 Tea minerals

Tea contains fluoride, minerals and trace elements such as Fe, Ca, K, Mn, Cr, N and Zn which are essential to human health (Xie *et al.*, 1998; Cabrela *et al.*, 2003). Some of these minerals have been found to be adequate in distinguishing teas of different geographical origin (Marcos *et al.*, 1998; Fernandez *et al.*, 2001, 2004). The main sources of the heavy metals in plants including tea are the growth media i.e. soil. Other sources include agro inputs such as insecticides, herbicides and fertilizers that may be absorbed through the leaves, roots and the barks of the plant (Fwu-Ming & Hong-Wen, 2008) as well as rainfall in atmospheric polluted areas due to high traffic density and

industrialization (Lozak *et al.*,2002; Sobukola *et al.*, 2008) and substandard machinery during transportation and processing.

Research has also indicated that the content of essential elements in plants is conditional, the content being mainly affected by the characteristics of the soil and the ability of plants to selectively accumulate some metals (Divrikli *et al.*, 2006). Also most heavy metals are not biodegradable, have long biological half-lives and thus persist in different body organs a phenomenon called bioaccumulation where they eventually lead to unwanted side effects (Jarup, 2003; Sathawara *et al.*, 2004). Such elements include Pb and Cd and are toxic to humans even at low concentration and have been associated with the etiology of a number of diseases especially cardiovascular, kidney, nervous as well as bone disease (WHO, 1996; Steenland & Boffetta, 2000; Jarup, 2003). Tea has been found to have different levels of the heavy metals such as iron (Fe), zinc (Zn), copper (Cu), lead (Pb) and cadmium (Cd) to name just a few. Their levels have been found to vary across the zones where tea is grown (Moseti, 2013; Moseti *et al.*, 2013). As explained above, some of these metals have varying biological importance and toxicological relevance to humans.

2.4.5 Tea amino acids

Amino acids found in tea are attributed to aroma and cream formation and are less cited in the quality of tea (Davies *et al.*, 1997). Tea contains theanine (ɣ-glutamylethylamide) whose many biological uses have been claimed (Ozawa *et al.*, 1969). For instance, it has been reported that theanine decreases the level of nor epinephrine and serotonin in the brain (Chu *et al.*, 1997) and in naturally hypertensive rats its administration results in decreased blood pressure. Recently, cooperative effects of antitumor agents and theanine have been reported (Sugiyama *et al.*, 1999). Another amino acid of interest contained in tea is ɣ-aminobutyric acid (GABA). Normal green tea contains less than 0.1 % of this aminoacid, while the anaerobic treatment of fresh leaves before the normal manufacturing process increases the content enormously. The continuous drinking of

anaerobically treated tea has been shown to produce a decline in blood pressure (Hakamata, 1990). And such specially processed teas are sold in Japan to people suffering from hypertension. Amino acids have been described as sweet and sour (Nelson *et al.*, 1998).

2.5 Tea health benefits

Based on extensive animal experiments and available epidemiological data, the medical community recognizes tea as a beverage that may offer several health benefits (Jha *et al.*, 1996; Moreda-Pineiro *et al.,* 2003; Naithan & Kakkar, 2005). Some of the health benefits that have been ascribed to regular consumption of tea include; the reduction of serum cholesterol (Hans *et al.*, 2007), decreased risk of cancers and cardiovascular diseases (Jankun *et al.*, 1997; Hakim *et al.*, 2004; Zuo *et al.,* 2002; Chan and Han, 2000; Chung *et al.,* 2003), prevention of a number of diseases including skin cancer (Katharine, 2001; Lambert *et al.*, 2005), Parkinson's disease (Richard, 2001), Myocardial infarction (Cheng, 2003) and coronary artery disease (Hirano *et al.,* 2003).

Due to its polyphenols, tea has been considered medicinal since the ancient times. Research on the effects of tea on human health continues to be fuelled by the growing need to provide natural health diets that include plant derived polyphenols. The health benefits in tea have been attributed to the strong antioxidant activity of catechins and other phenolic compounds (Zuo *et al.,* 2002; Karori *et al.*, 2007) that protect the body against free radical induced oxidative stress (Pourmorad *et al.,* 2006). In addition, tea polyphenol have been associated with a amelioration of inflammation (Karori *et al.*, 2008; Paola *et al.,* 2005); inhibition of diabetes (Vinson *et al.,* 2001; Sabu *et al.,* 2002), prevention of intestinal damage and anti-diarrhea properties (Astar *et al.,* 2003), enhancement of oral health (Wu & Wei, 2002) and the potential to improved spatial cognitive learning ability (Hague *et al.,* 2006). Other benefits include anti allergic action (Yamamoto *et al.,* 2004), anti-hypertensive activities (Henry *et al.,* 1981) antifungal and antibacterial activities (Clarke *et al.*, 1998; Ann *et al.*, 2004; Koech *et al.*, 2013). Green

tea catechins (EGCG and ECG) have also been found to inhibit plasmodium falciparum growth in vivo, a parasite that has been implicated in causing malaria, the killer disease in Africa and Asia (Sanella *et al.,* 2007).

The upsurge in the interest in the therapeutic potential of plant polyphenols, especially tea polyphenols means that human beings are more serious on their health than previously.

2.5.1 Antioxidant capacity of tea

Tea catechins provide beneficial health effects by protecting the body from the damaging effects of free radicals (Amie *et al.,* 2003). Free radicals are unstable molecules that arise from the hydrogen atom, nitric oxide (NO) and molecular oxygen (reactive oxygen species). Free radicals contribute to chronic and aging diseases such as cancer, heart diseases, stroke, rheumatoid arthritis, diabetic cataracts, Alzheimer's diseases, central nervous system injury and Acquired Immune Deficiency syndrome (AIDS) (Pourmorad *et al.,* 2006; Rao *et al.,* 2006).

Antioxidants inhibit oxidation reactions caused by free radicals by preventing or delaying damage to cells or tissues (Paquay, 2001). Tea has one of the highest total polyphenol content of all the plants at 6 to 12 % of the leaf by dry weight (Lakebrink, 2000) and it is a major source of the dietary flavonoids which are highly antioxidant.

Tea flavonoids are antioxidant *in vitro* and many common flavonoids are several times more potent than vitamin C and E (Vinson, 1995). Currently, the available synthetic antioxidants are Butylated Hydroxyl Anisole (BHA), Butylated Hydroxyl Toluene (BHT) and gallic acid esters. These are potentially carcinogenic and hence the need to substitute them with naturally occurring antioxidants (Pouramorad *et al.,* 2006). The antioxidant activity of tea flavonoids may account for the results of a number of epidemiological studies suggesting their protective roles mentioned above. Further studies have demonstrated that co-administration of drugs with EC and EGCG inhibits

glucoronidation and sulfation of orally administerd drugs thereby increasing the bioavailability of such drugs in the body (Hang *et al.*, 2003; Lambert, 2005). EGCG has also been reported to be a powerful antagonistic of the human immunodeficiency virus reverse transcriptase (Nance & Shearer, 2003). TRs were found to suppress the transcription of HIV in the cell and the gallic acid moiety of TRs can enhance suppressive activity (Nakane *et al.*, 1994).

2.6 Techniques for analyzing bioactive compounds in tea

There are a number of assays that are used to analyse bioactive compounds present in tea. These assays include assaying the antioxidant capacity in tea, and analyzing other bioactive compounds using chromatographic techniques, spectrophotometric analysis and organoleptic evaluation amongst other methods.

2.6.1 Determination of antioxidant activity in tea.

There are a number of assays that can be used to measure the antioxidant capacity of foods including tea. These include the Oxygen Radical Absorbance Capacity (ORAC); Thiobarbituric Acid Reactive Substance (TBARS) and the DPPH method. Among these, the DPPH method is the most widely used because it is rapid, simple and inexpensive (Koleva *et al.*, 2001). The assay is used to test the ability of compounds to act as free radical scavengers and more recently it has also been used to quantify antioxidants in complex biological systems (Villano *et al.*, 2006). The trend in antioxidant activities obtained using the DPPH method is comparable to trends found using the other methods (Koleva *et al.*, 2001). The DPPH method can also be used for solid samples without prior extraction and concentration hence saving time. The DPPH system is a stable radical generating procedure since it can accommodate a large number of samples within a short time and is sensitive to detect samples at low concentration. The antioxidant process of DPPH reaction is thought to occur in two stages (Brand-Williams *et al.*, 1995).

DPPH + Flav-H ⟶ DPPH-H + FLAV'…… (1)

Antioxidant Antioxidant radical

Flav' + X ⟶ Flav-X…….. (II)

Antioxidant radical Non radical product

Flav' + Flav' ⟶ Flavonoid (Non-radical)

Using different oxidants can result in the formation of different oxidants products from the catechins and the main site of anti-oxidant action depends on the oxidant used and the structures of the catechins.

2.6.2 Chromatographic methods of analyzing key compounds found in tea.

Several studies determining tea catechins and alkaloids separately (Dalluge *et al.*, 1998; Finger *et al.,* 1992) and simultaneously (Lin *et al.,* 1998; Fernandez *et al.,* 2000) have been carried out. Though several authors propose capillary electrophoresis as the technique to be used (Horie *et al.*, 1997; Horie *et al.*, 1998; Arce *et al.*, 1998), the analytical method commonly used for the determination of these compounds is HPLC (Bailey *et al.,* 1990; Kerio *et al.,* 2011), which currently constitutes the most convenient approach for routine analysis.

The ISO standard 14502-2 specifies a HPLC method for the determination of the total catechin content. The leaf is ground and extracted with 70 % aqueous methanol at 70 °C. The five most abundant catechins in the extract ((+) catechin, epicatechin, epigallocatechin, epigallocatechin gallate and epicatechin gallate) are determined by HPLC on a phenylbonded column using gradient elution with a UV detector at 278 nm. The use of a diode array detector is recommended for spectra comparison purposes. The

method has been developed to give good separation of the principal components while ensuring a short enough run time. A key consideration for ISO at the time the method was established was to ensure that the method worked on the most readily available fit for purpose equipment, in this case HPLC with UV detection. External catechin standards of defined purity and moisture content can be used directly or alternatively, the much more readily available caffeine may be used as a standard in conjunction with relative response factors (RRFs) for each individual catechin. The RRFs concept was developed as individual catechin standards are not always available or costly, they tend to be unstable and also hygroscopic thus leading to increased errors.These RRFs to caffeine have been established by an ISO international inter-laboratory ring test and overcome many of the shortcomings described in using catechin standards.

In this method of analysis, separation of tea catechins is made using a high resolution silica based 2.2 µm C_{18} column at a wavelength of 278 nm to separate, detect and quantify them. In the case of gradient separation, the concetration of acetonitrile or methanol is gradually increased. Dalluge *et al.*, (1998) compared a variety of stationary phases and elution conditions, and observed that the stationary phases which utilized ultrapore silica and maximized coverage of the silica support improved the separation and that the presence of acid in the mobile phase was essential. According to their results, the separation using a methanol based mobile phase was poor. Chromatographic peaks in samples are identified by comparing retention times and UV spectrum with those of reference standards. Working standard solutions are injected into the HPLC, and peak area responses are obtained. A standard graph for each component is prepared by plotting concentration versus area. Quantification is then carried out from integrated peak areas of the sample and the corresponding standard graph.

2.6.3 Spectrophotometric analysis of tea

The polyphenol content can be determined spectroscopically according to ISO 14502-1-2005 procedure, using the Folin-Ciocalteu phenol reagent as described by Pourmorad *et al.*, (2006). The new ISO standards ISO parts 1 and 2 (ISO, 2005a, b) have been developed to facilitate international trade and provide robust validated analytical tools in cases of dispute. These standards will help to protect consumers and ensure that their expectations are met. The substances characteristic for tea are measured using the two recent standards: ISO 14502-1 measures the total polyphenolic content of tea, and ISO 14502-2 measures the catechin content. Tea is rich in polyphenols, and ISO 14502 parts 1 specifies the method for the determination of the total polyphenol content of leaf or instant tea by a colourimetric assay using Folin–Ciocalteu phenol reagent (Singleton *et al.*, 1999). It is quite clear that most methods for a determination of a group of compounds like polyphenols suffer from response factors of the individual compounds. However, the Folin–Ciocalteu method gives reproducible results. There are almost an unlimited number of ways tea can be brewed therefore a method that ensures consistency in analysis and obtains an accurate level was needed. The ISO methodology provides a standardized method of extraction, the polyphenols are extracted from finely ground leaf using 70 % methanol at 70 °C. For instant teas they are dissolved in hot water containing 10 % acetonitrile. The extracted samples are stabilised with ethylenediaminetetraacetic acid (EDTA) which sequesters the iron ions thus preventing catechin degradation. Ascorbic acid is also in the stabilisation solution to prevent oxidation. The total polyphenol content is determined spectrophotometrically by reaction with the Folin–Ciocalteu reagent, a mixture of phosphor-tungstic acids as oxidants which on reduction by readily oxidized phenolic hydroxyl groups yields a blue colour with an absorption maximum around 765 nm. The use of gallic acid as a calibration standard enables useful total polyphenols data to be obtained. The method is applicable to both green and black teas with the more complex polyphenols in black tea also responsive to the Folin–Ciocalteu reagent.

2.6.4 Organoleptic evaluation of tea

Sensory quality characteristics of black tea are assessed using either sight, smell and /or taste of the beverage (Cloughley, 1981; Ding *et al.*, 1992; Sanderson *et al.*, 1976). Although the use of sensory evaluation is always criticized as being subjective and influenced by market factors such as demand, supply and consumer preferences (Biswas *et al.*, 1973) and personal preferences of the individual tasters, it still remains the fastest and the most practical method of quality assessment in the black tea trade. Several studies have demonstrated linkages between the sensory quality characteristics of the resultant black tea, the chemical composition of the green leaf and the black tea processing parameters (Bendall 1959; Biswas, *et al.*, 1973; Roberts & Smith, 1963). At present, it is agreed that catechins, the major polyphenols in the green tea shoots, together with their oxidation products are responsible for most of the sensory characteristics associated with black tea liquors (Biswas *et al.*, 1973; Roberts & Smith, 1963; Sanderson *et al.*, 1976).

Flavanol glycosides are also present in the green tea leaf and are believed to contribute substantially to tea liquor colour (Mcdowell, Feakes & Gay, 1990). The non volatile compounds are responsible for taste, with some of these compounds also being responsible for the colour. However, the volatile components comprise aroma. Although many aroma compounds are primary tea metabolites, many volatile flavor compounds in tea are secondary metabolites derived from carotenes, aminoacids, unsaturated fatty acids plus other lipids and terpene glycosides during tea processing (Robinson & Owuor, 1992). The composition and concentration of the VFC plays a vital role in the valuation and pricing of black tea (Owuor, 1992; 1988; Wicremasinghe *et al.*, 1973).

2.6.5 Leaf quality plucking determination methods

Though there are two leaf count methods namely leaf count and weighted leaf quality, the method which is mostly preferred at the factories is the leaf count method (KTDA, 2011). In this method, one goes to the withering troughs collects delivered leaf randomly from as many troughs as possible and put them in a quality trough. The collected leaf is then emptied to a leaf count table from whence; one hundred green tea shoots are counted randomly through shuffling. The collected plucked leaf is then sorted into different plucking standards of one leaf and a bud (1 + bud), two leaves and a bud (2 + bud), three leaves and a bud (3 + bud), four leaves and a bud (4 + bud) and all inclusive of the stems. Good plucking quality leaf constitutes one leaf and a bud or banjhi and two leaves and a bud or banjhi and the summation of this should be at least 75 out of the 100 shoots collected from the troughs for leaf quality determination for all KTDA managed tea factories in the study area.

2.7 Food safety standards

Food safety standards are a set of limits that define the Maxima Permittable Concentrations (MPC's) of chemical substances and metal residues that are safe for human consumption. These guidelines are determined by bodies such as the Kenya Bureau of Standards (KEBS), the Food and Agriculture Organization (FAO), the World Health Organization (WHO) among others and their aim is to protect the consumers and ensure fair practices in the food trade. They deal with detailed requirements related to a food or group of foods, the operation and management of production processes and the operation of government regulatory systems for food safety and consumer protection.

The Codex Alimentarius is a collection of standards, codes of practice, guidelines and other recommendations adopted by the Codex Alimentations Commission (CAC) which was established by FAO and WHO in the 1960s. It is the single most important international reference point for development associated with food standards. Table 1,

gives the quality specifications of Kenyan tea as given by the Kenya Bureau of Standards (KEBS), a local certification body. A critical examination of the table clearly reveals that the Kenyan standard for black tea (KS 65: 2009) does not include essential biochemical metabolites such as catechins, caffeine, theaflavins, thearubigins and even antioxidant capacity of Kenyan black tea. Even for essential elements present in tea such as fluoride, there's no minimum percentage concentration set for our Kenyan tea.

Table2.1: Compositional quality requirements limits for Kenyan black tea (adopted from KS 65: 2009)

SL No	Parameters	Requirements/Limits	Test Method
I	Water extract, Percent (m/m) min	32	
Ii	Total Ash, Percent (m/m) on dry matter basis Max Min	 8 4	
Iii	Water soluble ash, of percentage of total ash, min	45	KS2160
Iv	Alkalinity of water soluble ash (as KOH) per cent (m/m) Minimum Maximum	 1.0 3.0	
V	Acid – insoluble ash, % (m/m) max	1.0	
Vi	Crude fibre, per cent (m/m) max	16.5	
Vii	Moisture content % , m/m, max	7.0	
Viii	Arsenic (As), ppm max	0.15	AOAC
Ix	Lead (Pb), ppm max	1.0	
X	Cadmium (Cd), ppm, max	0.1	
Xi	Mercury (Hg) ,ppm max	0.02	
Xii	Zinc (Zn), ppm max	50	
Xiii	Copper (Cu), ppm, max	150	
Xiv	Iron fillings, max	50	K2160
Xv	Yeasts max per g	10^2	KS 220
Xvi	Moulds, Max per g	10^3	
Xvii	*Ecoli*, Max per g	Shall be absent	KS861
Xviii	*Salmonella spp* per 25g	Shall be absent	
Xix	Progenic *Staphylococcus aureus* max per g	Shall be absent	

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study site

The study areas were three ecological zones in Kenya namely Muranga, Kisii and Meru. Processed black tea samples were collected in duplicate from drier mouths of factory managed for small scale farmers in each of the three ecological zones selected to give a good zonal representation. The selected factories from Murang'a in Central Kenya and whose co-ordinates are $0^{o}43^{'}\ 0^{''}$ South $37^{o}9^{'}0''$ East, were Ngere, Kanyenyaini, Gathunguru and Kiru. Kisii ecological zone situated in Nyanza and whose geographical co-ordinates are 0^{0} 41' 0" South, 34^{0} 46' 0" East, the factories selected were Nyankoba, Nyansiongo, Nyamache and Tombe. In Meru situated in Eastern Kenya with co-ordinates 0^{0} 30' 0" North, 37^{0} 39' 0" East, the selected factories were Kinoro, Imenti, Githongo and Kionyo.

Table 3.1: Selected factories from the Murang'a, Meru and Kisii agro-ecological areas

	Ecological area		
	Murang'a	**Meru**	**Kisii**
Factory	1. Ngere	1. Kinoro	1. Nyansiongo
	2. Kanyenya-ini	2. Kionyo	2. Nyankoba
	3. Gathunguru	3. Imenti	3. Tombe
	4. Kiru	4. Githongo	4. Nyamache/Eberege

3.2 Experimental design

In this study a randomized block design approach was used where factories in each area of study were first treated as homogenous units before they were randomly selected and samples taken. Similarly for clonal cultivars, farms in each area of study were stratified into blocks before randomly selecting a block in which a selected clone was plucked and sample collected.

Black CTC (cut, tear, curl) tea was used as it is the main product processed, marketed and consumed in the study area (Kenya).

3.3 Sample collection

The factories were randomly selected from each ecological zone. The CTC black teas collected from these factories were replicated samples picked from different driers in those factories. In each selected factory, two half kilogram of drier mouth black tea was sampled. A total of twelve factories were sampled, four from each ecological zone.

For fresh tea shoots, about 2 kg of young shoots, comprising about 75 % two leaves and a bud and loose leaf were plucked from three clones grown in the three ecological zones. The clones sampled were 31/8, SFS 150 and 301/577. These were the most popular clones in the three ecological zones. Farms were randomly stratified into small blocks within these ecological zones and the fresh shoots plucked from the farms by random sampling of the selected clone.

3.4 Leaf quality determination

Leaf delivered to each factory in this study was subjected to a random leaf quality test to ascertain what quality of leaf was delivered for processing. The leaf count method, which is the most frequently method used for leaf quality determination amongst the KTDA factories, was used (KTDA, 2011). In this method, from the withering troughs,

the delivered leaf randomly collected from as many troughs as possible and put in a quality trough. The collected leaf was then emptied to a table from where; one hundred green tea loose tea leaves are counted randomly through shuffling. The collected plucked leaf from each factory was then sorted into different plucking standards as described earlier. Good quality leaf constitutes one leaf and a bud or banjhi and two leaves and a bud or banjhi and the summation of this should be at least 75 out of the 100 leaves counted from the troughs for quality determination in KTDA managed tea factories.

3.5 Sample pre-treatment

The unprocessed fresh shoots and the black tea samples were placed in clean well labeled paper bags and preserved in a portable cooler to prevent bruising, oxidation and contamination. They were transported to the Coffee Research Foundation (CRF) laboratories in Ruiru, and the Tea Research Foundation of Kenya (TRFK) laboratories in Kericho, Timbilil Estate for screening and analysis. The samples were oven dried at the laboratories (Menment, 854 Schwalech Germany) for 12 hours at 103 ± 2 ^{0}C. Once dry, the samples were finely ground using an electric blending device (Moulinex AR 1043, China) to reduce the particle size and homogenized. Two grams of each sample was weighed to the nearest 0.001g and placed in an infra red moisture determination machine for ten minutes after which each sample was dried to a constant weight and the moisture content of the sample determined automatically.

3.6 Reagents and standard solutions

Formic acid from (Pancreas, Barcelona, Spain), Acetonitrile (Romil, Cambridge, UK) was of HPLC grade. Double distilled water from reputable commercial suppliers was used throughout. Gallic acid, (+) catechin, and epicatechin were purchased from Fluka (Buchs, Switzerland), epigallocatechin and epicatechin gallate were acquired from Sigma (Steinfein, Germany). These reagents were stored at -20 °C. Caffeine was

obtained from Merck Ltd. Stable standard solutions (200 mg/ml) were prepared in acetonitrile and stored at 4 °C. Working standard solutions were prepared weekly from the stock solutions by dilution with acetonitrile. All other chemical reagents such as isobutylmethylketone (IBMK), Flavognost reagent (diphenyl boric acid 2-aminoethylester) were standard items sourced from reputable commercial sources. All solvents used for extraction were of analytical grade while water was double distilled.

3.7 Extraction of polyphenols from the samples

The polyphenols were extracted using the following procedure (Zuo *et al.*, 2002) and as per ISO 14502-2-2005E. A sample of milled tea (0.2gms) was weighed and extracted with 100 ml of warm methanol. 5.0 ml of methanol were added to each sample and the mixture warmed in a water bath at 70 °C for 10 minutes with constant stirring using a vortex mixer. The mixture was then cooled for five minutes and centrifuged at 3500 rpm for ten minutes after which it was decanted and the sediment extracted again using the 70 % methanol at 70 °C, centrifuged again at 3500 rpm for ten minutes and then added to the upper solution layer which was then topped up to ten ml with methanol. 1.0 ml of this extract was put in a 100 ml conical flask and diluted to volume. Aliquots of 10.0 ml of this solution were transferred to a 25 ml flask. Portions of this solution were filtered through a disposal 0.45 µm filter unit into a vial ready for HPLC analysis. This extraction procedure was carried out at room temperature to prevent possible degradation (Suematsu *et al.,* 1995). Since the results were to be expressed on a dry matter basis, the moisture of the tea samples was determined before the analysis.

3.8 Analysis of tea leaf extracts

3.8.1 Chromatographic determination of catechins in tea samples

The catechins in the dried non processed tea shoots were analyzed quantitatively by using high performance liquid chromatography (HPLC) with a reverse phase two solvent gradient elution (Zuo *et al.,* 2002; Fernandez *et al.,* 2000) systems and as per ISO

14502 procedure (ISO, 14502-2- 200 5E). A Knaer Hitachi (Darmstadt, Germany) HPLC chromatograph, equipped with a L – 7100 pump, a Rheodyne (CATATI, CA) 7725I injection valve with a 20 µL sample loop, and a diode array detector (DAD) L-7455 operating at 278 nm was used for the catechins determination. All the modules were controlled by a personal computer equipped with a Knaer Hitachi D-7000 interface and HPLC system manager software (Merck Hitachi). A 25 cm x 4 mm Lichro CART RP – 18 5 µm column, 4.0 x 46 mm i.d. was used for the separation.

The mobile phase A comprised of acetonitrile/acetic acid/distilled water (8:2:90) v/v/v and mobile phase B was acetonitrile/acetic acid/distilled water (80:2:18) v/v/v. The flow rate was one microlitre/minute and injection volume twenty microlitres. The column (Gemin C_{18}-005 column, 4.0 mm × 46 mm i.d) was operated at 35 °C and UV detection peaks were read at 278 nm. 1.0 ml of the sample was pipetted into separate tubes and diluted to 5.0 ml with stabilizing solution, filtered and loaded into vials. The identification of individual catechins was carried out by comparing the retention times of sample peaks with peaks obtained from the mixed known catechin standards (+C, EC, EGC, ECG, EGCG) under the same conditions Quantification of the catechins was at 278 nm using external calibration curves, R^2=0. 9984 together with the consensus relative response factors (RFS) in respect to caffeine calculated on dry matter basis. The precision of this method was evaluated by carrying out replicate analysis of a standard solution on different days.

$$\% \text{ Total catechins} = \% \{ECG + \% EC + \% EGCG + \% (+C) + \%EGC\}$$

3.8.2 Spectrophotometric determination of total polyphenols

The polyphenol content was determined according to ISO 14502-1-2005 procedure, using the Folin–Ciocalteu Phenol reagent as described by Pourmorad *et al.*, (2006). Measurements of optical densities of the tea samples were carried out using a Shimadzu UV-1800 Series Spectrophotometer.

The reagent was used because it contains phosphotungistic acid as oxidant total polyphenol. About 0.2 grams of each black tea sample collected from each of the different catchment areas was weighed and extracted using the water/methanol extraction procedure (Pourmorad *et al.*, 2006). 1.0 ml of the extract was transferred to a 100 ml volumetric flask and distilled water added to the mark and mixed. 1.0 ml of the diluted extract was then transferred in duplicate into separate tubes. 5.0 ml of ten per cent (v/v) diluted Folin-Ciocalteu was then pipette into each tube and mixed. Within three to eight minutes after addition of the Folin-Ciocalteu Phenol reagent, four milliliter of 7.5 % w/w sodium carbonate solution was added to each tube stoppered and mixed well. The mixture was then allowed to stand at room temperature for 60 minutes and then optical densities (OD) measured in one ml cell each using a Shimadzu UV-1800 series Spectrophotometer at 765 nm. A calibration curve was obtained using gallic acid over a concentration range of 10 to 50 μgml^{-1}. Gallic acid standard solution was prepared by transferring 1, 2, 3, 4 and 5 ml corresponding to 10, 20, 30, 40 and 50 mg anhydrous gallic acid into a 100 ml volumetric flask and filled to the mark with distilled water. The optical density readings of the test samples were referenced to the calibration curve to determine the total polyphenol content of the tea samples. The amounts of the total polyphenols was determined from a standard curve generated using gallic acid as a standard and expressed as the amount of gallic acid equivalent. The total polyphenol content was expressed as a percentage by mass dry matter following ISO standards (Karori *et al.*, 2007; Robert and Smith, 1963; 1972).

$$M = \frac{M_O \times V \times W_{Dm\ std} \times 10000}{100 \times 100}$$

Where, M_o – is the standard mass in grams of gallic acid monohydrate used to prepare the stock solution

V – Is the volume in millilitres of Gallic stock standard solution used to prepare the standard solution A, B, C, D and E.

$W_{DM\ std}$ - is the dry matter content expressed as a mass fraction % of the gallic acid. The total polyphenol content expressed as a percentage by mass on sample dry matter basis was calculated using the formula.

$$W_T = \frac{(D_{sample} - D_{intercept}) \times V_{sample} \times D \times 100}{S_{std} \times M_{sample} \times 10{,}000 \times W_{Dmsample}}$$

Where D_{sample} - is the optical density obtained for the sample test solution

$D_{intercept}$ - is the optical density at point of the last fit linear calibration

M_{sample} - is the mass (g) of the sample test solution

V_{sample} - is the sample extraction volume in milliliters (10 ml for leaf tea)

D - Is the dilution factor used prior to the calorimetric determination?

$W_{DM\ Sample}$ - is the dry matter (expressed as mass fraction in percent) of the test sample.

3.8.3 Determination of total theaflavins content in the tea samples.

The total theaflavin content of the tea samples were assayed for by using the Flavognost method of Hilton and Palmer Jones (1973). Nine grams of the tea samples was weighed and infused with 375 ml of boiling water added from an overhead boiler into a tarred flask. The flask was then agitated in a mechanical shaker for fifty minutes. The infusion was then filtered through rough cotton wool and allowed to cool to room temperature. 10 ml of the tea infusion was then pipetted into 10 ml of IBMK. The mixture was then shaken for ten minutes and allowed to stand until layers separated. 2.0 ml of the upper

layer was pipette into a test tube, followed by 4.0 ml of ethanol and 2.0 ml of Flavognost reagent (2g diphenylboric acid-2-amino ethylester) dissolved in 100 ml of ethanol. The contents were mixed and colour allowed developing for fifteen minutes. The absorbance at 625 nm was read against a 1BMK/ethanol (1:1 v/v blank).

Total theaflavins was then calculated by the formulae;

$TF\ (\mu mol/gm) = A_{625} \times 47.9 \times 100/_{DM}$

Where A_{625} is the absorbance at 625 nm and DM is the dry matter of the sample.

3.8.4 Determination of liquor total colour

Five milliliters of filtered standard tea infusion for theaflavin analysis was pipette into forty five milliliters of distilled water in a 100 ml conical flask. The solution was shaken well to ensure thorough mixing. The absorbance of this solution at 460 nm was read against distilled water. The result was corrected for dry matter content of the black tea samples. Total colour was then calculated by the formulae;

$$TC = E_{460} \times 10 \div (DM \div 100)$$

Where E_{460} is the absorbance at 460 nm and DM is the dry matter of the tea sample.

3.8.5 Determination of liquor brightness

Five milliliters of filtered standard tea infusion for theaflavin analysis was pipette into forty five milliliters of distilled water in a 100 ml conical flask. The solution was shaken well to ensure thorough mixing. The absorbance of this solution at 460 nm was read against distilled water. The result was corrected for dry matter content of the black tea samples. Total liquor brightness was calculated using the formulae;

$$\text{Brightness } \% = 100 \times E_3 \div (E_1 + 2\ E_2)$$

Where E_3, E_1 and E_2, are absorbances of the solutions at 460 nm wavelength of the UV - 1800 Shimadzu spectrophotometer.

3.8.6 Determination of total thearubigins in tea samples

The total thearubigins was determined as described by the methods of Roberts and Smith, (1963). 6.0 ml of 1 % v/v aqueous solution of anhydrous disodium hydrogen orthophosphate was added to the 6.0 ml of cooled tea infusion. The resulting mixture was extracted with ten milliliters of ethyl acetate by vigorous shaking for one minute. The mixture was allowed to settle and the aqueous layer drained off. 5.0 ml of ethyl acetate was then added to the ethyl acetate extract containing the theaflavin fraction in the separating funnel. 10 ml of the ethyl acetate extract was then diluted to 25 ml with methanol in 25 ml volumetric solution (solution E_1). 1.0 ml of the tea infusion was mixed with 9.0 ml of distilled water and made to 25 ml in a volumetric flask with methanol (solution E_2). 1.0 ml of saturated aqueous 10 % oxalic acid was then added to 1.0 ml of tea infusion and 8.0 ml of distilled water and made to 25 ml with methanol (solution E_3). The absorbance of solutions E_1, E_2 and E_3 were obtained at 380 nm and 460 nm respectively using a Shimadzu UV-1800 series spectrophotometer with distilled water as the blank. Each sample was extracted in duplicate for the determination of theaflavin (TFs), thearubigin (TRs) fractions and brightness levels. The percent TFs and TRs values were then calculated using the formulae;

$$TFs\% = 2.25 \times E_1 \times DM$$

$$TRs\% = 7.06 \times (4E_3 - E_1) \div DM\ \%$$

3.9 Analysis of antioxidant activity of tea sample

The radical scavenging activity of the tea extracts from leaf obtained from each zone was determined by the 2, 2-diphenyl-2-picrylhydrazyl (DPPH) radical using a modified method of Brand-Williams *et al.,* 1995. The assay is based on the measurement of the

scavenging ability of antioxidants towards the stable DPPH radical (Moreno *et al.*, 2002). 5.0 g of tea was infused in 100 ml of boiling doubled distilled water followed by stirring with a magnetic stirrer and additional steeping for 30 minutes at room temperature. The extracts were strained through a nylon mesh (120 µm) followed by a filter paper (Whatman No. 54). Aliquots of the extract were kept frozen at -18 ^{0}C until further use. The soluble solid extracts were standardized to give stock solution of 50 mg soluble solids per 100 ml of 50 % methanol. A 50 µl methanolic solution of tea sample was place in a curvette and two milliliter of 6.0 X 10^{-5} M of 80 % methanolic solution of DPPH added. The decrease in absorbance at 517 nm was determined using a Shimadzu UV-1800 series spectrophotometer until the absorbance stabilized. Readings were done between 15 and 30 minutes interval before the reaction reached a plateau phase. The DPPH solution was prepared afresh and kept in the dark to minimize the loss of free radical stock solution. All determinations were performed in triplicate. The percentage inhibition of the DPPH radical was calculated for the absorbance rate according to the method of Yen and Duh (1994).

$$\% \text{ inhibition against DPPH} = [AB - AA] \div [AB \times 100]$$

Where AB was absorbance of the blank sample (50 ml double distilled water and 2 ml DPPH) and AA is the absorbance of the tested sample after 15 minutes.

3.10 Ranking of teas as per tasters' organoleptic evaluation

The black tea samples were randomly numbered and subjected to organoleptic evaluation by two experienced tea tasters at the tasting room of Tea Brokers East Africa (TBEA), Mombasa. The tasters had expert knowledge of black teas, which they auction regularly. The tasters evaluated liquor briskness, brightness, strength and Aroma. The scales used to rank the levels of these parameters in the tea liquor were (Obanda *et al.*, 2001):

Very bright -11, bright -9, fairly bright - 7, a little bright -5, dull -3, very dull -1

Very brisk -11, brisk -9, fairly brisk – 7 a little brisk – 5, soft -3, very soft – 1

Very strong - 11, strong -9, fairly strong -7, a little strong -5, weak -3, poor – 1

Pungent - 11, Flavourly-9, aromatic - 7, a little aromatic -5, soft -3, watery -1

The result of this taster's evaluation enabled the tea samples to be ranked according to organoleptic quality. Ranking would be from the highest scores at a maximum of 44 and lowest at a possible minimum of a score 4. A correlation analysis was then carried out to relate the taste characteristic to the different biochemical components inherent in the teas from the different zones.

3.11 Analysis of inorganic nutrients in black CTC teas

Fe, Zn and Cu contents of the tea samples were determined using Flame Atomic Absorption Spectrophotome- try (FAAS), following the method described by Moseti et al., (2013). To quantify K, P, Ca and Mn in the teas, 0.25 g of each of the finely ground oven-dried tea samples were accurately weighed using an analytical balance (BL-3200 HL, Shimadzu, Japan) into separate, clean and dry specimen tubes and ashed in a muffle furnace (Gallenkamp, England) at 460 ± 2 ºC for 4.5 hours. The ashed samples obtained were allowed to cool followed by wet digestion using two parts of a 1:1 mixture of concentrated HNO_3 (69 - 70.5 %) and concentrated HCl (37 %) to three parts of H_2O_2 under reflux to near dryness (Sitienei *et al.,* 2013). Each sample residue obtained was then dissolved in 25 ml of 0.05 M HCl solution. The clear solution was shaken thoroughly and allowed to stand for 4 hours prior to analysis. Ca and Mn contents of the digests were then quantified using an Atomic Absorption Spectrophotometer (Varian, SPPA-880, and EL98083516). Phosphorus,P was quantified colorimetrically using a UV-Vis Spectrophotometer (UV-1800, Shimadzu, ENG240V, SOFT) fitted with a Shimadzu ASC-5 auto sample changer at 400 nm after complexing with a mixture of ammonium molybdite and ammonium metavanadate whereas K was quantified by flame emission using a flame photometer (Corning, 400, 2604) (Thomas *et al.,* 1967; Spencer,

1950). For quality assurance, two reference tea samples (A and B) were ashed, digested and analysed for all the inorganic nutrients of interest alongside every batch of samples daily throughout the analysis. Reagent and sam- ple blanks treated in a similar way as the samples were also included. Moreover, to check the efficiency of the analytical methods, the reference samples A and B were spiked with known concentrations of the respective analytes and recovery tests performed.

3.12 Data analysis

All various groups (treatments) of data obtained were subjected to Analysis of Variance (ANOVA) using the MSTAT statistical software (Steel *et al.*, 1997) at $p \leq 0.05$. Where statistically significant differences were observed, the Least Significant Difference (LSD) test was used for mean separation. Data was tabulated as means of the triplicate determinations ± standard deviations (SD). Correlation analysis by GraphPad Prism 5.0 for Windows was employed to determine the relationship between the biochemical profiles and sensory quality of the teas.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Green leaf plucking standards

Leaf quality plucking data obtained in the study is presented in Table 3. The mean percent leaf plucking quality for the three agro-ecological areas was 74 ± 1.0 %. The means for each zone were 75 ± 0.7, 74 ± 0.2 and 73 ± 1.1 % for Murang'a, Meru and Kisii zones respectively. This data reveals that farmers in the Murang'a zone deliver better plucked tea shoots followed by Meru and Kisii zones. These differences in mean leaf qualities result in significant differences ($p < 0.05$) in the qualities of leaf processed at the factories in these zones studied (Table 3). Whereas there is not much difference between leaf plucking quality in Murang'a and Meru zones, there are significant variations of leaf in the Kisii factories with leaf standards falling between 72 and 74 % good leaf. It was noted that whereas farmers in the East of the Rift zones, in this case Murang'a and Meru, pluck between three to four rounds per month, farmers in the West of Rift (Kisii) do two plucking rounds per month. This could be the reason contributing to the poor harvested leaf being delivered to the factories in this zone. However, the mean leaf plucking standard delivered to factories within each zone, and interactions thereto were not statistically significant ($p \leq 0.05$) (Table3).

Table4.1: Percent leaf quality of leaf delivered to the factories for black CTC tea manufacture, total catechins, individual catechins fractions, gallic acid and caffeine (Mean ± SD) in black CTC tea from three tea growing agro-ecological areas

Agro-zone	Factory	% LQ	% GA	% EGC	% C	% Caff	% EC	% EGCG	% ECG	% TCat
Murang'a	Ngere	[##a]76±1.5	[a]0.51±0.01	[a]4.80±0.15	[a]0.41±0.05	[cde]3.77±0.14	[abc]0.91±0.22	[a]0.65±0.05	[ab]0.68±0.21	[a]7.45±0.42
	Kenyanya-ini	[abc]75±1.0	[bc]0.34±0.08	[ab]4.66±0.71	[a]0.43±0.01	[c]3.85±0.07	[ab]1.04±0.29	[bc]0.48±0.13	[abc]0.61±0.04	[a]7.22±0.74
	Gatunguru	[ab]75±3.5	[bc]0.33±0.01	[b]4.24±0.03	[bc]0.23±0.01	[cd]3.81±0.00	[a]1.14±0.15	[ab]0.56±0.08	[a]0.72±0.04	[a]6.88±0.28
	Kiru	[abc]74±1.0	[cd]0.25±0.05	[cd]3.34±0.09	[bc]0.22±0.01	[gh]3.40±0.06	[ef]0.60±0.04	[def]0.36±0.02	[def]0.47±0.02	[c]4.95±0.16
	Mean	**75±0.7**	**0.35±0.11**	**4.26±0.66**	**0.32±0.11**	**3.71±0.21**	**0.92±0.23**	**0.51±0.13**	**0.62±0.11**	**6.63±1.14**
Meru	Kinoro	[abc]75±2.6	[b]0.37±0.04	[ab]4.30±0.69	[b]0.28±0.16	[fg]3.58±0.02	[cde]0.74±0.16	[ef]0.31±0.10	[bcde]0.55±0.03	[b]6.18±0.30
	Kionyo	[abc]75±1.2	[b]0.39±0.03	[c]3.67±0.07	[bc]0.27±0.05	[def]3.64±0.10	[bcd]0.85±0.12	[cde]0.41±0.09	[bcd]0.58±0.01	[b]5.78±0.19
	Imenti	[abc]75±1.0	[bc]0.32±0.01	[cde]3.17±0.04	[cd]0.18±0.06	[h]3.27±0.07	[efg]0.51±0.05	[f]0.27±0.01	[cdef]0.49±0.06	[cd]4.63±0.14
	Githongo	[abc]75±1.5	[bc]0.30±0.14	[fg]2.47±0.27	[d]0.12±0.01	[ef]3.63±0.05	[ef]0.58±0.06	[bcd]0.46±0.09	[f]0.39±0.01	[ef]4.02±0.18
	Mean	**74±0.2**	**0.35±0.09**	**3.40±0.77**	**0.21±0.08**	**3.53±0.18**	**0.67±0.15**	**0.36±0.09**	**0.51±0.08**	**5.15±1.00**
Kisii	Nyansiongo	[abc]74±2.9	[bc]0.32±0.02	[def]2.95±0.14	[bcd]0.19±0.07	[bc]3.90±0.20	[def]0.66±0.08	[cde]0.41±0.01	[cdef]0.52±0.06	[c]4.74±0.25
	Nyankoba	[abc]73±1.2	[d]0.21±0.08	[gh]2.25±0.29	[bcd]0.20±0.03	[b]4.06±0.19	[gh]0.31±0.12	[f]0.30±0.05	[ef]0.44±0.02	[fg]3.51±0.44
	Tombe	[bc]72±1.5	[bc]0.32±0.02	[efg]2.68±0.06	[bc]0.23±0.01	[a]4.30±0.06	[fg]0.43±0.01	[f]0.29±0.01	[cdef]0.50±0.02	[de]4.13±0.10
	Eberege	[c]72±2.0	[d]0.17±0.01	[h]1.79±0.12	[bc]0.24±0.04	[i]2.95±0.01	[h]0.17±0.01	[f]0.28±0.00	[bcd]0.58±0.02	[g]3.07±0.09
	Mean	**73±1.1**	**0.27±0.07**	**2.42±0.51**	**0.22±0.02**	**3.80±0.59**	**0.39±0.21**	**0.32±0.06**	**0.51±0.06**	**3.86±0.73**
Pooled mean		**74±1.0**	**0.32±0.09**	**3.36±0.98**	**0.25±0.09**	**3.68±0.36**	**0.66±0.29**	**0.40±0.12**	**0.55±0.10**	**5.21±1.47**
Coefficient of Variation (%)		2.7	17.6	9.4	23.1	2.9	21.5	16.5	18.4	6.5
LSD (p = 0.05)	**Agro-zone**	3.5	0.10	0.55	0.10	0.16	0.24	0.12	0.13	0.59
	Factory	NS	0.03	0.47	0.09	0.16	0.21	0.10	0.11	0.51
	Interactions	NS	0.03	NS	0.12	0.21	0.28	0.13	0.15	0.67

[#] = Standard deviation; [##] = means within a column preceded with the same superscript letter(s) are not statistically significantly different (p > 0.05); NS = not significant; LQ = Leaf quality; GA = Gallic acid; EGC = epigallocatechin gallate; C = Catechin; Caff = Caffeine; EC = Epicatechin; EGCG = Epigallocatechin galllate; ECG = Epicatechin gallate; TCat = Total catechins

Table4.2: Total catechin content in green leaf of the three most popular cultivars of tea in three agro-ecological areas in Kenya (Mean ± SD)

Agro-zone	Clone	% GA	% EGC	% +C	% Caff	% EC	% EGCG	% ECG	% TCat
Murang'a (Kenyenya-ini)	SFS 150	^{a}0.97±0.03	^{bc}4.11±0.87	^{ab}0.60±0.20	^{abc}3.93±0.47	^{ab}1.69±0.58	^{a}9.93±1.85	^{c}2.63±0.29	^{ab}18.96±2.31
	TRFK 31/8	^{b}0.69±0.03	^{cd}3.42±0.31	^{cd}0.42±0.07	^{a}4.30±0.36	^{a}1.95±0.57	^{a}9.90±0.94	^{c}2.56±0.12	^{abc}18.25±1.65
	303/577	^{b}0.69±0.07	^{a}4.99±0.65	^{bcd}0.45±0.06	^{d}2.84±0.32	^{a}1.95±0.34	^{a}8.05±0.15	^{c}2.69±0.26	^{abc}18.13±1.34
	Mean	0.79±0.16	4.17±0.79	0.49±0.10	3.69±0.76	1.86±0.15	9.29±1.08	2.63±0.07	18.45±0.45
Meru (Imenti)	SFS 150	^{b}0.69±0.10	^{d}3.33±0.37	^{e}0.23±0.04	^{ab}4.03±0.37	^{b}1.30±0.03	^{a}8.37±0.57	^{b}3.60±0.04	^{bc}16.84±0.44
	TRFK 31/8	^{a}1.04±0.06	^{cd}3.50±0.16	^{a}0.71±0.12	^{a}4.04±0.06	^{ab}1.47±0.26	^{a}8.07±1.13	^{a}4.10±0.19	^{abc}17.85±1.40
	303/577	^{b}0.63±0.18	^{bc}4.16±0.12	^{de}0.30±0.05	^{bcd}3.11±0.29	^{ab}1.81±0.07	^{ab}7.63±0.62	^{c}2.36±0.29	^{c}16.25±0.44
	Mean	0.79±0.22	3.66±0.44	0.41±0.26	3.73±0.54	1.53±0.26	8.02±0.37	3.35±0.90	16.98±0.81
Kisii (Nyankoba)	SFS 150	^{a}1.04±0.02	^{ab}4.53±0.23	^{cde}0.38±0.04	^{abc}3.80±0.08	^{ab}1.44±0.04	^{a}10.03±0.31	^{b}3.54±0.22	^{a}19.92±0.53
	TRFK 31/8	^{a}1.15±0.19	^{bc}4.12±0.30	^{cde}0.39±0.04	^{a}4.53±0.67	^{ab}1.47±0.05	^{a}9.23±0.26	^{b}3.49±0.09	^{ab}18.69±0.29
	303/577	^{b}0.57±0.12	^{ab}4.70±0.37	^{bc}0.52±0.19	^{cd}3.07±1.10	^{ab}1.57±0.38	^{a}9.09±0.28	^{c}2.55±0.43	^{abc}18.43±1.02
	Mean	0.92±0.31	3.92±0.58	0.43±0.08	3.80±0.73	1.49±0.07	9.45±0.51	3.19±0.56	19.02±0.80
	Pooled mean	**0.82±0.22**	4.09±0.59	0.44±0.15	3.74±0.59	1.63±0.23	8.92±0.92	3.06±0.62	18.15±1.10
Coefficient of Variation (%)		13.5	10.4	23.8	14.4	19.7	19.6	8.2	7.0
LSD (p = 0.05)	**Agro-zone**	0.23	0.86	NS	NS	NS	3.41	0.51	1.91
	Clone	0.23	0.86	NS	1.09	NS	NS	0.51	NS
	Interactions	0.25	NS	0.58	NS	NS	NS	0.57	NS

$^{\#}$ = Standard deviation; $^{\#\#}$ = means within a column preceded with the same superscript letter(s) are not statistically significantly different (p > 0.05); NS = not significant; LQ = Leaf quality; GA = Gallic acid; EGC = epigallocatechin gallate; C = Catechin; Caff = Caffeine; EC = Epicate chin; EGCG = Epigallocatechin galllate; ECG = Epicatechin gallate; TCat = Total catechins

4.2 Chromatographic and spectophotometric analysis of tea samples

4.2.1 The catechins content in clonal tea cultivars grown in the different ecological zones

The study compared the levels of different catechins in three clonal tea cultivars grown by small scale farmers in the three ecological zones in Kenya. The different catechins in the test samples were identified and quantified by HPLC. Authentic catechin standards were used to identify individual catechins fractions in the tea samples for the clones studied as shown in (Table 5) below. Identified catechins in the tea samples were; catechin (+C), epicatechin (EC), epigallocatechin gallate (EGCG), epicatechin gallate (ECG) and epigallocatechin (EGC). The retention times (order of elution) of the catechins, caffeine and gallic acid (GA) were as shown in Table 5. Besides the five peaks identified, several minor peaks were identified which indicated that other unidentified catechin like compounds were present in the tea extracts (Figure 6.). There was however similarity in the HPLC chromatographic patterns among the tea samples from across the ecological zones indicating similarity in catechins profiles.

Table4.3: Retention times for the individual catechins fractions, gallic acid and caffeine

Analyte	Retention time in minutes
Gallic acid (GA)	5.8
Epigallocatechin (EGC)	6.1
Catechin (+C)	6.8
Caffeine (Caff)	7.5
Epicatechin (EC)	9.8
Epigallocatechin gallate (EGCG)	13.9
Epicatechin gallate (ECG)	23.8

The results revealed that the levels of catechins differed significantly ($p \leq 0.05$) across the zones for the different cultivars.

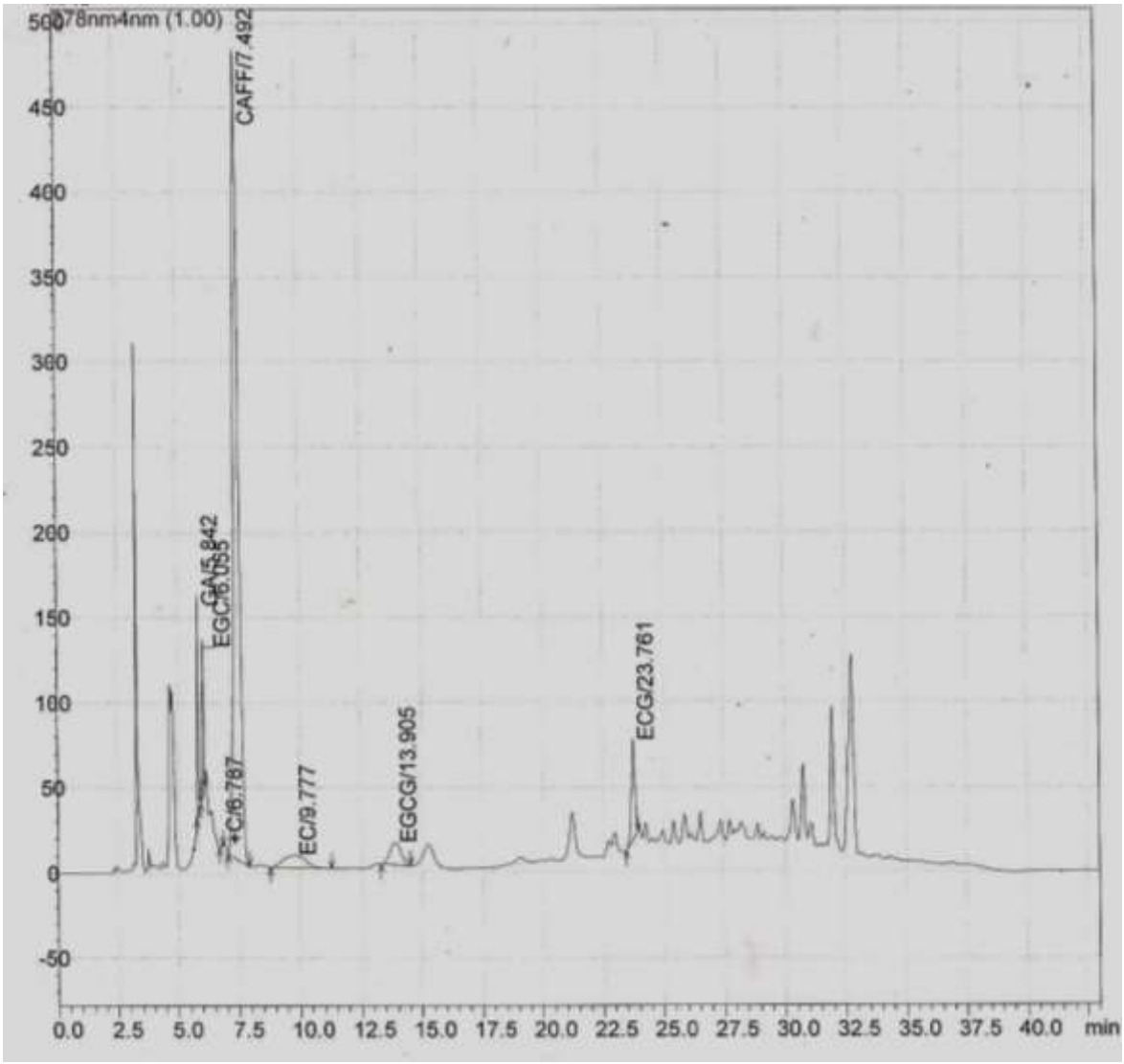


Figure 4.1: A sample HPLC profile of black tea caffeine, gallic acid and individual catechins at 278 nm

Figure 6 gives a sample chromatogram of catechin fractions in black teas from the three agro-ecological areas studied. It's remarkable that peaks corresponding to caffeine were

always higher in all cases. This can be explained by the fact that the amount of caffeine increases during the fermentation process owing to its liberation from catechins gallates (Xie *et al.,* 1998). Caffeine is present in higher amounts in the cases of black CTC teas, showing values between 2.95±0.01 % for Eberege in Kisii to 4.30±0.06 % for Tombe factory catchment in Kisii, while the average zonal variation was 3.80±0.59 % for Kisii zone; 3.71±0.21 % for Muranga zone and 3.53±0.18 % for Meru zone.The pooled mean for the three ecological zone was 3.68±0.36%. The differences in the caffeine levels among the black teas were statistically significant at $p \leq 0.05$ across the three zones, within the factories and in their intereactions thereof. In the non-fermented clonal green/ fresh shoots cultivars, caffeine levels ranged between 2.84 ± 0.32 % for clone 303/577 from Kanyenyaini factory catchment in Muranga to 4.53±0.67 % for cloneTRFK 31/8 from Nyankoba factory catchment in Kisii. The differences in the caffeine levels amongst the tea cultivars were not statistically significant across the agro ecological zones and in their intereactions thereto at $p \leq 0.05$, but they were statistically significant across the three clones studied. This variation in caffeine levels would be due to genetic factors and the different agronomic practices experienced in these ecological zones, some of which have been cited such as difference in plucking rounds, leaf quality and the response to the different ecological zones such as difference in altitudes which confers on the tea plant varying shoot growths.

For black CTC teas, EGC,EC,EGCG, ECG and +C are the major catechins present in , with average contents for the total catechin content being 6.63±1.14% in Muranga Zone , 5.15±1.00 % in Meru Zone, and 3.86±0.73% in Kisii Zone. EGC average contents were 4.26±0.66 %, 3.40±0.77%, and 2.42±0.51 % for Muranga, Meru and Kisii Zones respectively. EC average content values across the zones were 0.92±0.23%, 0.67±0.15, and 0.39±0.21% for Muranga, Meru and Kisii Zone while ECG values across the zones also follow a similar pattern and are 0.62±0.11, 0.51±0.08 and 0.51±0.06. EGCG average contents were 0.51±0.13 % for Muranga, 0.36±0.09 % for Meru and 0.32±0.06 % for Kisii Zone. +C levels also followed a similar pattern across the zones and were

0.32±0.11% for Muranga, 0.21±0.08 for Meru and 0.22±0.02% for Kisii Zone. All the catechins assayed were statistically significant at $p \leq 0.05$ across the agro zones, within the factories and in their interactions thereto (Table 3). Black teas in this study had very low levels of (+) - catechin. This would be due to the fact that (+) - catechin is degraded by oxidation during manufacturing. The data obtained in this study is comparable to those reported by other authors (Balentine, 1992; Grahan, 1992; Hara, 1994).

From the results of these data, we can therefore conclude that the levels of catechins in black teas among the ecological zones were in decreasing order from Muranga ecological Zone, followed by Meru Zone and then Kisii ecological Zone. These differences can be attributed to the fact that the three zones are in different ecological zones that confer on the teas varying conditions resulting into different shoot growth rates.

Among the clonal cultivars fresh green leaf sample, the total catechin content for each cultivar differed significantly across the zones as shown in Table 4. The total catechn content ranged between 16.25±0.44% for clone 303/577 at Imenti in Meru and 19.92±0.53% for clone SFS 150 at Nyankoba in Kisii. The average total catechin content across the zones was 18.45±0.45% for Muranga zone, 18.15±1.10% for Kisii zone and 16.98±0.81% for Meru zone. This zonal variation in total cathechin content was statistically significant at $p \leq 0.05$ across the agro ecological zones but was not statistically significant amongst the clones and in their interactions thereof. The most dominant catechins in the green leaf were EGCG, EGC, ECG, EC and +C in that order. EGCG levels were 9.29±1.08% in Muranga, 8.92±0.92% in Kisii zone and 8.02±0.37% in Meru zone. The variation in EGCG levels was statistically significant across the agro ecological zone at $p \leq 0.05$. EGC levels were 4.17±0.79% ,4.09±0.59% and 3.66±0.44% respectively for Muranga,Kisii and Meru zones.These levels were statistically significant across the zones and between the clones at $p \leq 0.05$ but not significant in their interactions thereof. ECG levels were 2.63±0.07% for Muranga.3.35±0.90% for Meru and 3.06±0.62% for Kisii zone.These variations in ECG levels were statistically

significant at p≤0.05 across the zones , within the clones and in their interactions thereof. EC levels were 1.86±0.15% for Muranga, 1.63±0.23% for Kisii and 1.53±0.26% for Meru zone. These variations were however not significant across the zones, within the clones and in their interactions thereof. +C levels were 0.49±0.10% for Muranga, 0.44±0.15% for Kisii zone and 0.41±0.26% for Meru zone.The variations were not significant across the zones and within the zones but were statistically significant at p≤0.05 in their interactions thereof. The results for the catechin content in the fresh shoots agree with earlier studies who found that catechins account for about 15 % of the plant dry weight and that the major tea catechins are EGCG, EGC, ECG and EC (Balentine, 1992).

4.2.2 Tea polyphenols

This study compared total polyphenol levels of black tea samples processed from tea leaf grown by small scale farmers in three ecological zones in Kenya. The data in Table 6 reveals that polyphenol levels across the three ecological zones fall between 13.9±0.4% for Eberege in Kisii zone and 18.7±0.6% for Ngere in Muranga zone.The average zonal variation in total polyphenol content was 17.8±1.6% for Muranga,16.5±0.7% for Meru zone and 16.1±1.7% for Kisii zone. The pooled mean total polyphenol content for the three zones was 16.8±0.9%. These total polyphenol content of the black teas processed from germplasm in the three zones was statistically significant at p≤0.05 across the agro zones, within the factories and between their interactions thereto.The black teas are rich in polyphenols.This compares favourably with other studies done at the TRFK that established clone 6/8 as a high quality clone with highest polyphenol content of 24.4 and 19.3 % in both green and black processed teas respectively. While total polyphenol content for processed purple coloured leaf cultivars ranged between 16.2 to 18.7 % which is still higher than conventional green teas from Chinese and Japanese cultivars (Koech *et al.*,2013). This finding is also in agreement with data obtained for catechins in the preceding section and confirms zonal variations in polyphenol contents in black tea. Table 6 clearly shows that the polyphenol

contents of black tea vary with the source of the green tea leaf sample.The difference in the polyphenol content across the zones would be attributed to the different agronomic practices in the three zones such as plucking rounds, leaf quality and difference in altitude which are major contributors to the variations in the total polyphenol contents of the teas from the three zones.

Table 4.4: Biochemical composition and characteristics of black CTC tea from three tea growing ecological areas in Kenya (Mean±SD)

Agro-zone	Factory	% TP	% TF	% TR	% TCol	% Brigt	% AC
Murang'a	Ngere	^{a}18.7±0.6	^{abc}1.03±0.09	^{bc}15.1±0.4	^{c}4.14±0.11	^{ab}48.6±4.5	^{a}84.2±3.6
	Kenyanya-ini	^{a}18.5±0.4	^{abcd}1.00±0.08	^{a}18.8±0.6	^{abc}4.68±0.12	^{bc}45.6±2.6	^{ab}83.6±2.4
	Gatunguru	^{a}18.6±0.1	^{a}1.14±0.02	^{a}18.4±4.2	^{abc}4.67±0.09	^{f}31.1±1.8	^{ab}82.3±2.4
	Kiru	^{e}15.5±0.1	^{cd}0.91±0.04	^{abc}16.0±4.2	^{a}5.15±0.28	^{a}50.0±2.9	^{ab}82.1±1.6
	Mean	**17.8±1.6**	**1.02±0.09**	**17.1±1.8**	**4.66±0.41**	**43.8±8.6**	**83.0±1.0**
Meru	Kinoro	^{d}16.4±0.3	^{cd}0.90±0.03	^{ab}16.8±0.7	^{a}4.91±0.51	^{ef}32.7±1.9	^{ab}82.4±1.6
	Kionyo	^{c}17.0±0.1	^{abcd}0.97±0.09	^{ab}17.3±0.3	^{ab}4.72±0.49	^{de}35.2±2.0	^{abc}81.9±3.2
	Imenti	^{e}15.5±0.2	^{d}0.81±0.36	^{ab}18.2±1.8	^{bc}4.25±0.31	^{b}46.3±2.6	^{de}77.3±1.6
	Githongo	^{cd}16.9±0.1	^{abcd}0.98±0.08	^{ab}18.1±1.2	^{abc}4.67±0.36	^{c}42.6±2.4	^{f}71.6±2.8
	Mean	**16.5±0.7**	**0.92±0.08**	**17.6±0.7**	**4.64±0.28**	**39.2±6.3**	**78.3±5.0**
Kisii	Nyansiongo	^{bc}17.2±0.3	^{ab}1.12±0.01	^{abc}15.7±0.3	^{ab}4.78±0.45	^{d}38.3±2.2	^{ef}75.3±2.9
	Nyankoba	^{e}15.6±0.2	^{cd}0.89±0.08	^{a}18.5±0.3	^{a}5.01±0.42	^{e}34.7±2.1	^{bcd}80.2±1.1
	Tombe	^{b}17.7±0.0	^{bcd}0.92±0.08	^{ab}17.5±0.5	^{ab}4.78±0.44	^{g}24.0±1.4	^{cde}78.3±0.8
	Eberege	^{f}13.9±0.4	^{e}0.38±0.02	^{c}13.5±0.7	^{d}3.38±0.31	^{g}24.4±1.4	^{ab}82.3±0.9
	Mean	**16.1±1.7**	**0.83±0.32**	**16.3±2.2**	**4.49±0.75**	**30.3±7.3**	**79.0±3.0**
	Pooled mean	**16.8±0.9**	**0.92±0.10**	**16.9±0.7**	**4.60±0.09**	**37.8±6.9**	**80.1±2.5**
Coefficient of Variation (%)		1.8	13.0	11.3	7.4	5.2	2.7
LSD (p = 0.05)	**Agro-zone**	0.5	0.05	NS	NS	3.5	3.8
	Factory	0.5	0.18	2.9	NS	3.0	3.3
	Interactions	0.6	0.24	NS	0.68	3.9	4.4

$^{\#}$ = Standard deviation; $^{\#\#}$ = means within a column preceded with the same superscript letter(s) are not statistically significantly different (p > 0.05); LSD = Least Significant Difference; NS = not significant; TP = total polyphenols; GLQ =Green leaf quality; TF = Theaflavins; TR = Thearugigins; TCol = Total colour; Bright = Brightness; AC = Antioxidant capacity

4.2.3 Total theaflavins (TFs) and Total thearubigins (TRs) levels of black CTC teas

The data on TFs and TRs is shown in Table 6. TFs levels in the three zones fall between 0.38±0.02% for Eberege in Kisii and 1.14±0.02% for Gatunguru in Muranga zone. The zonal variation was 1.02±0.09% for Muranga, 0.92±0.08% for Meru zone and 0.83±0.32% for Kisii zone. The pooled mean for the three zones was 0.92±0.10%. The difference in the zonal variation was statistically significant at $p \leq 0.05\%$ across the agrozones, between the factories and in their interactions thereto.

TRs levels fall between 13.5±0.7% in Eberege in Kisii and 18.8±0.6% for Kanyenyaini in Muranga. The zonal variation was 17.6±0.7% in Meru, 17.1±1.8% in Muranga and 16.3±2.2% in Kisii zone .The pooled mean for the three zones was 16.9±0.7%. The difference in the TRs was statistically significant between the factories, but was not significant across the agro zones and in their interactions thereto.

The TFs and TRs levels obtained in the current study are comparable with those reported by other researchers elsewhere (Koech *et al.*, 2013). For example in a TRFK study, TRs levels have been found to be between 14.6 to 17.2 %, with black tea from the popular high black tea quality clone TRFK 6/8 showing the highest total TFs and the lowest content of TRs amongst the black tea products(Koech et al.,2013).

4.2.4. Total colour of black tea samples

Table 4.4 shows the variations in the total colour content of the black teas analyzed in the study from the three ecological zones in Kenya. Colour levels fall between 3.38±0.31% for Eberege in Kisii to 5.15±0.28% in Kiru in Muranga.The zonal variation was 4.66±0.41% for Muranga, 4.64±0.28% for Meru and 4.49±0.75% for Kisii zone.The pooled mean colour content for the three zones was 4.60±0.09%.The zonal variations in the colour content were not statistically significant in the agro ecological zones and between the factories but were significant at $p \leq 0.05$ in their interactions thereto.

The data obtained is consistent with the results of other researchers from the TRFK who have obtained similar data for Kenyan black teas, whose ranges are from 4.0 to 5.0 %. Since TRs levels in black tea are thought to contribute to the total colour in black tea, it was expected that the results would mimic those of total TRs, as had been observed by the results depicted in Table 6.

4.2.5 Percent liquor brightness of black tea samples

Table 4.4 shows the variations in the brightness levels of black teas analyzed in this study.

Brightness levels fall between 24.0±1.4% for Tombe in Kisii to 50.0±2.9% for Kiru in Muranga.The zonal variations were 43.8±8.6% for Muranga, 39.2±6.3% for Meru and 30.3±7.3% for Kisii zone.The pooled mean for the three zones was 37.8±6.9%. Zonal variations are evident in the level of brightness of the tea samples from the three zones and these are statistically significant at $p < 0.05$ across the three zones, between the factories and in their interactions thereto. The obtained data is consistent with results of other researchers at the TRFK who have obtained similar data for Kenya black teas whose brightness tend to range between 20% and 50 %. Since TFs levels in black tea contribute significantly to the liquor brightness, it was expected that the results would mimic those of TFs as has been observed by the results depicted.

4.3 Antioxidant activity of black CTC teas from three ecological zones in Kenya

The data on percent antioxidant capacity of the different black CTC teas from the three ecological zones is presented in Table 6. Antioxidant capacities of the black teas assayed were between 71.6±2.8% for Githongo in Meru and 84.2±3.6% for Ngere in Muranga.The zonal averages were 83.0±1.0% for Muranga teas followed by 79.0±3.0% for Kisii teas and 78.3±5.0% for Meru zone.The pooled mean total was 80.1±2.5%. The variations in antioxidant capacities were statistically significant at $p \leq 0.05$ across the agrozones, between the factories and in their interactions thereto.

From these results it appears that Kenyan black teas from the three zones have very high antioxidant capacity and therefore have effective radical scavenging properties. Antioxidant activity was highest in teas from Murang'a followed by Kisii and then Meru. These results are comparable with other research being carried out at the TRFK of Kenya which has found out that antioxidant activities is higher in teas which have high levels of anthocyanins, EGCG, ECG, EC and C implying that the most effective radical scavengers are catechins with a 3, 4 and 5 -trihydroxylated substitution pattern on the B-ring and/ or hydroxyl group at the C-3 position of the catechins structure (Kerio *et al.*, 2012).

4.4 Taster's score and ranking of teas from the three agro-ecological areas

The organoleptic tasting data is presented in Table 4.5 These results reveal significant differences ($p < 0.05$) in the quality of the teas from the three zones with regard to the plain tea quality parameters tested (aroma, briskness, brightness and strength). The total overall score had Muranga teas having a score of 41.4±1.9; followed by Meru at 23.3±6.1 and Kisii teas at 15.6±8.6.These overall score variation was statistically significant at $p \leq 0.05$ across the three agrozones, between the factories and in their interactions thereto. Aroma values were 10.3±0.8 for Muranga, followed by Meru at 5.8±2.0 and Kisii at 3.8±2.4. These variations were statistically significant across the zones, within the factories and in their interactions thereto. Briskness values were 10.6±0.3 for Muranga, followed by Meru at 5.3±1.6 and then Kisii at 4.0±2.6. The variations in briskness levels were statistically significant at $p \leq 0.05$ across the zones, within the factories and in their interactions thereto.Brightness levels of the black teas tasted were 10.1±0.6 for Muranga, followed by Meru at 5.9±1.1 and Kisii at 4.2±2.3. These levels were statistically significant across the agrozones and in their interactions thereto, but were not significant within the factories.Strength which is a very strong indicator of the quality in a cup of tea, was 10.4±0.6 for Muranga tea, followed by Meru tea at 6.3±1.6 and then Kisii at 3.7±1.7.The variations in strength were statistically significant at $p \leq 0.05$ across the zones, and in their interactions thereto but not significant

within the factories.It can therefore be concluded that teas from Murang'a are of significantly superior quality, followed by teas from Meru and Kisii zones respectively according to professional taster evaluations.

Table4.5: Mean sensory quality scores and ranking of tea liquors prepared from black CTC teas as assessed by professional tea tasters (*n* = 2; mean ± SD)

Agro-zone	Factory	Aroma	Briskness	Taster's brightness	Strength	Overall Score	Ranking
Murang'a	Ngere	[##ab]10.0±1.0	[a]10.3±1.2	[a]9.7±1.2	[a]10.3±1.2	**[ab]40.3±0.3**	**3**
	Kenyanya-ini	[a]11.0±0.0	[a]10.3±1.2	[a]9.7±1.5	[a]10.7±0.6	**[ab]41.7±0.6**	**2**
	Gatunguru	[a]11.0±0.0	[a]11.0±0.0	[a]11.0±0.0	[a]11.0±0.0	**[a]44.0±0.0**	**1**
	Kiru	[bc]9.3±1.5	[a]10.7±0.6	[a]10.0±1.0	[ab]9.7±1.2	**[b]39.7±0.6**	**4**
	Mean	**10.3±0.8**	**10.6±0.3**	**10.1±0.6**	**10.4±0.6**	**41.4±1.9**	
Meru	Kinoro	[ef]5.0±1.0	[cd]5.3±1.5	[cd]5.3±1.5	[c]6.0±1.0	**[de]21.7±0.4**	**7**
	Kionyo	[de]5.3±0.6	[de]5.0±1.0	[cd]5.3±0.6	[c]6.0±1.0	**[de]21.7±0.6**	**7**
	Imenti	[f]4.0±8.7	[ef]3.5±0.5	[cd]5.5±1.3	[c]4.8±1.8	**[e]17.8±0.9**	**10**
	Githongo	[c]8.7±1.8	[b]7.3±1.8	[b]7.5±1.0	[b]8.5±1.3	**c32.0±0.7**	**5**
	Mean	**5.8±2.0**	**5.3±1.6**	**5.9±1.1**	**6.3±1.6**	**23.3±6.1**	
Kisii	Nyansiongo	[d]6.3±0.8	[bc]7.0±1.3	[bc]7.0±1.3	[c]4.8±0.6	**[d]25.2±1.0**	**6**
	Nyankoba	[def]5.2±1.3	[de]4.8±0.8	[d]5.0±1.0	[c]5.3±1.5	**[e]20.3±0.2**	**9**
	Tombe	[g]2.7±0.6	[f]3.0±0.0	[e]1.7±1.2	[d]3.0±0.0	**[f]10.3±0.6**	**11**
	Eberege	[h]1.2±0.3	[g]1.0±0.0	[e]3.0±0.0	[d]1.5±0.9	**[f]6.7±0.9**	**12**
	Mean	**3.8±2.4**	**4.0±2.6**	**4.2±2.3**	**3.7±1.7**	**15.6±8.6**	
	Pooled mean	**6.6±3.3**	**6.6±3.4**	**6.7±2.9**	**6.8±3.2**	**26.8±12.6**	
Coefficient of Variation (%)		11.6	15.5	15.3	15.2	**9.2**	
LSD (p = 0.05)	**Agro-zone**	1.4	1.8	1.8	1.8	**4.3**	
	Factory	1.2	1.5	NS	NS	**3.7**	
	Interactions	1.5	1.2	2.1	2.1	**5.0**	

[#] = Standard deviation; [##] = means within a column preceded with the same superscript letter(s) are not statistically significantly different (p > 0.05); NS = not significant; LSD = Least Significant Difference

4.5 Correlation between biochemical constituents and tasters' evaluation of black teas from three agro-ecological areas

Correlation analysis was carried out to determine the relationship between biochemical constituents assayed of the different teas from the three zones and their plain quality tasters' sensory evaluations (Table 4.6 and 4.7).

Aroma depicted a statistically significant correlation with briskness($r=0.977, p\leq0.001$),brightness($r=0.956, p\leq0.001$),strength($r=0.981, p\leq0.001$),EC($r=0.671, p\leq0.01$),+C($r=0.671, p\leq0.01$),EGCG($r=0.793, p\leq0.01$) and EGC($r=0.822, p\leq0.001$).

Briskness was statistically significant with brightness($r=0.960, p\leq0.001$),strength($r=0.957, p\leq0.001$),EC($r=0.690, p\leq0.01$),+C($r=0.690, p\leq0.01$),EGCG($r=0.778, p\leq0.01$),EGC($r=0.790, p\leq0.01$).

Brightness had a statistically significant correlation with strength ($r=0.941$, $p\leq0.001$), EC($r=0.649$, $p\leq0.05$), +C($r=0.649$, $p\leq0.05$), EGCG($r=0.751$, $p\leq0.01$) and EGC($r=0.774$, $p\leq0.01$).

Strength had a positive correlation with EC($r=0.728$, $p\leq0.01$), +C($r=0.728$, $p\leq0.01$), EGCG($r=0.804$, $p\leq0.01$) and EGC($r=0.794$, $p\leq0.01$). The individual catechins also correlated positively amongst themselves for example EC had a positive correlation with ECG($r=0.739$, $p\leq0.05$), EGCG($r=0.898$, $p\leq0.001$) and EGC($r=0.659$, $p\leq0.05$). On the other hand ECG had a positive correlation with +C ($r=0.739$, $p\leq0.01$), and EGCG ($r=0.538$, $p\leq0.001$). It was also noted that +C positively correlated with EGC($r=0.659$, $p\leq0.05$) while EGCG depicted a positive correlation with EGC($r=0.777$, $p\leq0.01$).

There was a statistically significant correlation between the total polyphenols (TP) and TF($r=0.819$, $p\leq0.001$), aroma($r=0.671$, $p\leq0.01$), briskness($r=0.645$, $p\leq0.05$) and strength($r=0.638$, $p\leq0.05$).Total theaflavins (TFs) significantly correlated with total colour ($r = 0.678$, $p\leq0.01$) such that high TFs values tend to give colourly liquors, aroma ($r = 0.700$,$p\leq0.01$) and briskness ($r = 0.706$,$p\leq0.01$) and strength($r=0.647$,$p\leq0.05$). Total Thearubigins (TRs) correlated significantly with total colour (Tcol) ($r = 0.581$, $p\leq0.05$), such that high TRs values will give teas with colour and body.

TFs and unoxidised catechins are thought to have considerable human health benefit (Cheng *et al.,* 1986, 1991; Sano *et al.,* 1991).There was a positive correlation between briskness and brightness ($r = 0.960$), $p\leq0.001$, and strength ($r = 0.957$,$p\leq0.001$). This tends to show it is briskness that accounts for the major variations in brightness and strength of black tea. The result also showed a positive correlation ($r = 0.581$, $p\leq0.05$) for total colour of black tea liquor with % total thearubigins (TRs) content. There was also a positive correlation between total strength and aroma with total TP, total TF and TR levels.

Table 4.6: Correlation coefficient matrix analysis between aroma briskness, brightness, strength, gallic acid, caffeine and the individual catechins (+C, EC, EGC, EGCG and ECG)

Aroma	Briskness	Brightness	Strength	GA	EC	ECG	+C	Caff	EGCG	EGC	GC	
1.000	0.977***	0.956***	0.981***	0.424	0.671**	0.375	0.671**	0.228	0.793**	0.822***	0.349	**Aroma**
	1.000	0.960***	0.957***	0.425	0.690**	0.388	0.690**	0.230	0.778**	0.790**	0.377	**Briskness**
		1.000	0.941***	0.354	0.649*	0.326	0.649*	0.007	0.751**	0.774**	0.404	**Brightness**
			1.000	0.464	0.728**	0.413	0.728**	0.164	0.804**	0.794**	0.373	**Strength**
				1.000	0.797**	0.560*	0.797**	0.296	0.699**	0.666*	0.498	**GA**
					1.000	0.739*	1.000	0.179	0.898***	0.659*	0.672*	**EC**
						1.000	0.739**	0.126	0.538***	0.492	0.674*	**ECG**
							1.000	0.179	0.898	0.659*	0.672*	**+C**
								1.000	0.259***	0.231	-0.009	**Caff**
									1.000	0.777**	0.666*	**EGCG**
										1.000	0.614*	**EGC**
											1.000	**GC**

**Correlation is significant at the 0.05 (95 %) level*

*** Correlation is significant at the 0.01 (99 %) level*

**** Correlation is significant at the 0.001 (99.9 %) level*

Table 4.7: Correlation coefficient matrix analysis between antioxidant capacity, total polyphenols, total theaflavins, total thearubigins, total colour, aroma, briskness, brightness and strength

AC	TP	TF	TR	T Col	Aroma	Briskness	Brightness	Strength	
1.000	0.118	-0.137	-0.225	-0.145	0.177	0.248	0.236	0.269	**AC**
	1.000	0.819***	0.387	0.299	0.671**	0.645*	0.513	0.638*	**TP**
		1.000	0.514	0.678**	0.700**	0.706**	0.592	0.647*	**TF**
			1.000	0.581*	0.328	0.217	0.176	0.349	**TR**
				1.000	0.363	0.408	0.249	0.355	**T Col**
					1.000	0.977***	0.956***	0.981***	**Aroma**
						1.000	0.960***	0.957***	**Briskness**
							1.000	0.941***	**Brightness**
								1.000	**Strength**

**Correlation is significant at the 0.05 (95 %) level*

*** Correlation is significant at the 0.01 (99 %) level*

**** Correlation is significant at the 0.001 (99.9 %) level*

4.6 Inorganic nutrient content of black CTC tea

Recent findings (Kwach *et al.,* 2012) revealed that tea leaf nutrients vary with clones and locations. The results presented herein (Table 4.8) for the inorganic nutrients studied (K, P, Ca, Mn, Fe, Zn and Cu) agree with this observation since variations with factory of production as well as the ecological area were evident. For each inorganic nutrient, the concentration ranges observed varied, demonstrating variations in the factors influencing their distribution in the different ecological areas as well as the factories of origin (Omwoyo *et al.,* 2013) and their uptake and accumulation by the tea plant. For instance, the Fe content ranged between 136 ± 8 and 320 ± 5 µg/g whereas the Ca content ranged from 0.16 ± 0.01% to 0.62 ± 0.03%. The differences in the levels of each inorganic nutrient in black tea from the 12 factories were statistically significant ($p < 0.05$) as can be seen in Table 4.8. This observation is in corroboration with (Kumar *et al.,* 2005) and (Moseti *et al.,* 2013; Omwoyo *et al.,* 2013), whose recent findings have demonstrated variations in the micronutrient content of black tea from different locations in India and different factories in Kenya respectively. Further, comparisons of the mean inorganic nutrient contents in black tea from the three tea growing ecological areas reveal statistically significant differences ($p < 0.05$) in all the inorganic nutrients studied except for Zn and P as indicated in Table 10. Consequently, the highlighted differences result in significant ($p < 0.05$) interaction effects between the ecological areas and factories for all the inorganic nutrients studied (Table 4.8). Based on the current data, the inorganic nutrients studied can be arranged in descending order as K > P > Ca > Mn > Fe > Zn > Cu. K and Cu were the most and least abundant inorganic nutrients in the black teas with concentrations obtained being in the ranges 1.6 ± 0.05 % to 2.1 ± 0.01 % and 10 ± 3 % to 16 ± 1 µg/g respectively (Table 4.8). Moreover, these data demonstrates the different extents to which different micronutrients are accumulated in the tea shoots of the plant. K, P, Ca and Mn were present in percent levels whereas Fe, Zn and Cu were present in the parts per million levels (Figure 4.8) on a dry weight basis.

The Fe content obtained for the sample from the fourth factory from the Kisii ecological area, Eberege (320 ± 5 µg/g) was significantly high compared to the obtained mean Fe contents of 202 and 180 µg/g obtained for black tea from the Kisii ecological area and all the 12 tea processing factories under study respectively. In the same factory, a similar observation was made for Ca, where the level obtained (0.62 ± 0.03, Table 10) was significantly high compared than the mean Ca contents for the ecological area (0.32 µg/g) and the grand mean for all the teas from all regions (0.25 µg/g). High levels of Fe in tea leaves have been associated with mature leaf (Wanyoko & Njuguuna, 1983), implying that the high Fe content in black tea from Eberege factory may be attributed to coarse plucking standards and long plucking intervals among other factors hence the need for strict adherence to Good Agricultural Practices (GAP). Further, (Moseti *et al.*, 2013) observed the introduction of additional Fe along the CTC black tea processing chain. This is possibly due to wear and tear of the CTC segments during the rolling stage in tea manufacture [Personal Observation]. This observation further underlines the need for strict adherence to Good Manufacturing Practices (GMP) in the tea industry.

Table 4.8: K, P, Ca, Mn, Fe, Zn and Cu in black tea from Murang'a, Meru and Kisii agro-ecological areas

Agro-zone	Factory	K (%)	P (%)	Mn (%)	Ca (%)	Fe (µg/g)	Zn (µg/g)	Cu (µg/g)
Murang'a	Ngere	*a2.1±0.01	c0.30±0.01	b0.07±0.009	g0.16±0.01	b235±5	b33±1	bc11±3
	Kenyenya-ini	bc2.0±0.00	abc0.32±0.02	ab0.09±0.003	cd0.23±0.01	c193±2	bc32±4	bc12±5
	Gatunguru	cd1.9±0.05	ab0.35±0.05	ab0.10±0.001	cdef0.22±0.02	f148±9	c28±2	c10±3
	Kiru	de1.9±0.05	a0.36±0.02	ab0.10±0.002	cde0.23±0.04	g136±8	ab35±2	abc14±1
	Mean	**2.0±0.03**	**0.33±0.03**	**0.9±0.04**	**0.21±0.03**	**178±6**	**32±2**	**12±2**
Meru	Kinoro	c2.0±0.05	abc0.33±0.02	ab0.11±0.004	efg0.18±0.03	de162±5	ab35±0	abc13±2
	Kionyo	c2.0±0.05	abc0.32±0.01	ab0.11±0.004	fg0.17±0.03	d166±9	b33±3	abc13±2
	Imenti	e1.8±0.05	bc0.30±0.01	ab0.11±0.005	b0.33±0.02	d170±5	bc31±3	ab15±2
	Githongo	e1.8±0.03	abc0.33±0.01	ab0.10±0.001	fg0.17±0.01	ef151±4	ab35±1	ab14±1
	Mean	**1.9±4**	**0.32±0.01**	**0.11±0.03**	**0.21±0.02**	**162±6**	**34±2**	**14±2**
Kisii	Nyansiongo	ab2.0±0.05	a0.36±0.02	ab0.12±0.003	cdefg0.21±0.03	fg141±9	a39±7	ab14±2
	Nyankoba	a2.1±0.00	a0.37±0.04	a0.13±0.001	defg0.19±0.02	f149±8	b33±2	ab15±1
	Tombe	cd1.9±0.05	abc0.34±0.01	a0.13±0.004	c0.25±0.04	c197±9	b34±4	a16±1
	Eberege	f1.6±0.05	c0.30±0.01	ab0.12±0.003	a0.62±0.03	a320±5	c27±1	ab14±1
	Mean	**1.9±0.04**	**0.34±0.02**	**0.13±0.03**	**0.31±0.03**	**201±8**	**33±3**	**15±1**
Coefficient of Variation (%)		2.2	7.0	3.6	9.7	3.7	9.2	17.5
LSD (p=0.05)	**Agrozone**	0.032	**NS	0.023	0.023	4.85	NS	1.68
	Factories	0.024	0.018	0.018	0.018	3.59	1.62	1.24
	Interactions	0.021	0.015	0.015	0.015	3.13	1.42	1.08

* Means within a column preceded with the same letter are not statistically significantly different at $p \leq 0.05$; ** No significant difference

CHAPTER FIVE

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

This study presents a comparison of the biochemical parameters of black tea from multiple clones processed under identical conditions and grown under different ecological zones by small scale farmers in Kenya. The results of the study noted differences in biochemical composition of fresh leaf and black CTC tea in different ecological zones in Kenya (Tables 3.1 to 4.8). There are several differences in the growing conditions, agronomic practices and altitudes in Kisii, Murang'a and Meru ecological zones that could lead to variations in the biochemical profiles in the samples and therefore cause quality differences. Agronomic practices have been found to affect the quality of black tea from *Camellia sinensis* (L) O. Kuntze and one such practice that has been reported is the plucking standard. Coarse plucking reduces black tea quality (Owuor *et al.*, 1987, 1990; Owuor, 1989, 1990) due to decline in catechin levels (Forrest & Bendall, 1960) and changes in polyphenol oxidase isoenzyme composition and activity (Takeo and Baker, 1973; Obanda &Owour,1992) leading to a general decline in total TFs levels (Owuor *et al.*,1987; Owuor, 1990). Coarse plucking also leads to an increase in unsaturated fatty acids in the leaf (Owuor *et al.*, 1990) leading to production of less aromatic black teas (Owuor *et al.*, 1987). The fact that there is a significant difference in the quality of the plucked leaf count in the three zones would therefore explain the reported biochemical variations in terms of catechin, caffeine, polyphenols, TFs, TRs, color,aroma and brightness of the teas analyzed in this study.

Factors which affect the growth rate of the tea plant normally lead to variations in biochemical composition and quality of black tea (Robertson, 1983; Owour, 1993). It was noted that in the three ecological zones, Kisii found in the West of the Rift Valley is lower in altitude than the other zones and the area is largely mountainous covered by the

Kisii highlands which have altitudes of between 1500 and 2200 m above sea level. Ambient temperature falls over a small region averaging between 20 and 25 ^{0}C. The rainfall precipitates by conventional mode as a result of the influence of Lake Victoria, the largest water reservoir in Africa. The rainfall is well distributed throughout the year giving rise to evenly distributed tea production with only relatively minor peaks between the months of April and May and October and November. With relatively lower altitudes, warmer temperatures and evenly distributed rainfall, the growth rate of tea in Kisii is therefore faster unlike that of Murang'a and Meru which are in the East of Rift Valley. These latter regions have altitudes between 2000 to 3000 m a.m.s.l. They are on the slopes of the snowcapped Mt Kenya, one of the highest Mountains in Africa that is on the Equator and which is surrounded by the cool Aberdare Highlands and Nyambene Hills. Temperature in these zones at times fall to 4 ^{0}C during the very cold spell between July and September referred to as the "Kathano" where there is suppressed growth of the shoots in these areas. The suppressed ambient temperatures lead to slower growth rates of tea shoots and this is expected to affect the chemical composition of the teas. Rainfall precipitates by relief. However, Murang'a is on the windward side of the mountain while Meru is on the leeward side of the mountain. This location of the mountain has created the two areas to be in different ecological zones. Therefore the variations noted in biochemical composition of teas grown and processed in these zones would be attributed to difference in environmental conditions prevalent in the three ecological zones. Earlier, Obaga *et al.*, (1988) observed that there was a decrease in growth rates of some cultivars of tea with rise in attitude, even within a radius of only 10 km, where variations in environmental conditions could be expected to be minimal. Under these conditions there was slower growth at higher altitude leading to yield decline. The processed black teas however showed an increase in quality with rise in altitude (Owour *et al.*, 1990). One factor that can cause quality differences for black teas produced in the three zones is the variation in altitude of the three zones. The findings of this study are similar to those of Owuor *et al.*, (2006), who found out that production of black tea from the same vegetatively propagated cultivars in Kenya and Malawi show variations in both chemical

composition and quality. The variations in black tea quality were due to differences in environmental conditions leading to different shoot growth rates and biochemical compositions. Similarly Fernandez *et al.*, (2001) found out that teas from different geographical origins of China, Japan, Kenya, Sri Lanka and India have significant differences in their chemical compositions which they attributed to climate and agricultural practices including soil, water and fertilizers.

Generally Kenyan black tea is classified as plain to medium flavoury in the tea markets. Such teas are sold for their TFs, TRs, brightness, briskness, strength and total colour. The effect of growing teas in different ecological zones on plain black tea quality parameters is presented in Tables 6, 7 and 10. High theaflavins levels in black teas have tended to lead to higher tea prices (Ellis & Cloughley 1981; Davies 1983) and therefore theaflavin levels are useful plain black tea quality parameters that would be used for price or/and sensory evaluation (Cloughley, 1980; 1981; Hilton & Palmer, 1975). More recently a relationship between TFs and sensory evaluation for Kenyan teas has been improved by normalizing the contribution of the individual TFs to the taste of tea (Owour & Obanda, 1995). Indeed by normalizing this contribution of the individual TFs, a significant relationship was demonstrated for both Kenya and Malawi black teas (Owour *et al.*, 2006). It was demonstrated that the actual theaflavin factor (theaflavin digallate equivalent) vary with growing weather conditions with the epigallogatechins gallate (EGCG) dominating under fast growing conditions (Robertson, 1983) while the total catechins could vary as reflected in the TRs levels. In the current study, the levels of TFs, TRs and even antioxidant capacities of teas were quite significantly high among Meru and Murang'a teas and hence explaining why the market prefers teas from these zones and hence the yearly price differentials realized in them.

The concept of black tea quality is broadly divided into the sensations of sight (colour), taste and aroma. The non-volatile components are responsible for taste, with some of these components also being responsible for the colour. However, the volatile components comprises aroma. Although many aroma compounds are primary tea plant

metabolites, many Volatile Flavour Compounds (VFC) in tea are secondary metabolites derived from carotenes, amino acids, unsaturated fatty acids plus other lipids and terpene glycosides during tea processing (Robinson & Owour, 1992).The composition and concentrations of the VFC plays a vital role in the valuation and or pricing of black tea (Owour, 1992; Owour *et al.*, 1988; Wicremasinghe *et al.*, 1973; Yamanish *et al.*, 1968a, 1968b). Generally teas from the Kisii zone were less aromatic than those from Murang'a and Meru. This is probably due to coarse plucking, lower altitude and higher temperatures in the Kisii ecological zone which result into faster growth rates of teas in this zone. Factors which increase growth rate e.g. decrease in altitude (Mahanta *et al.*, 1988; Owour *et al.*, 1990), high rates of nitrogen fertilizers (Owour *et al.*, 1987c), and pruning (Owour & Lagat, 1988) reduce black tea aroma. The lower aroma in Kisii teas noted by tasters and in the biochemical analysis of plain tea quality parameters explains the noted observations in the tea trade that Kisii teas are generally plainer than Murang'a and Meru teas.

5.1.1 Total catechins

In general the total catechins contents and individual catechins contents were found to differ significantly across the ecological zones with EGCG, EGC, ECG, and GC recording higher levels, with +C being less abundant. The finding of this study corroborated that of Karori *et al.*, 2007 who researched on the catechins contents of teas in Kenya. Black teas are obtained by a post-harvest auto oxidation which is catalyzed by the enzyme polyphenols oxidase (PPO). The enzymatic oxidation of catechins located in the cell vacuole of the tea leaf is as a result of polymerization of flavan-3-ol monomers to form TFs and TRs which are compounds that have a significant influence in the quality of black tea. The catechin content of teas is therefore a very important quality indicator which may be used to gauge the quality potential of a tea cultivar as has been demonstrated in this study.

Thus it can be concluded that catechins are adequate descriptors to differentiate different tea samples from different ecological zones in Kenya. The catechin, polyphenol and caffeine profiles of green leaf can be used to train a classifier accurately discerning the geographic origin of the teas (Eetu Makela., 2012). Nevertheless further research using a higher number of samples covering aspects such as the technology used in the manufacturing and seasonal variation would be necessary to confirm the connection between the contents of catechins with ecological area of origin.

5.1.2 Total polyphenols

The levels of polyphenols in the different types of tea samples across the three zones were determined in this study. The results of the study reported superior levels of polyphenols amongst the black teas across the ecological zones that were statistically significant ($p < 0.05$). The rolling and cutting of tea shoots in CTC manufacture causes a release of the enzyme polyphenol oxidase which interacts with phenolic compounds, simple catechins, and gallocatechins (GC) to produce TFs and TRs (Mahanta & Hemanta, 1992). During black tea manufacture, the gallocatechins are first oxidized and dimerized to TFs and TRs because of their high oxidation potential and high concentrations in leaves. At the same time several factors have been known to influence the polyphenols content. These include genotype, geographical origin, soil composition, harvesting time, post-harvest treatment and physical structure of the leaves (Lin *et al.*, 2003). The results obtained in this study would therefore vary with seasons and it could be important to carry out a study to establish the seasonal variations of the total polyphenols. However owing to the fact that tea contains several polyphenols, it is likely that even such a proposed study may reveal some derivatives which were more stable than the others.

5.1.3 Theaflavins (TFs) and thearubigins (TRs)

Catechins are the major component of green tea leaves. In black tea, they are oxidized and dimerized during fermentation to the yellow orange pigments, TFs are polymerized to the red pigments called TRs. Black teas used in its study had high level of TFs and TRs across the ecological zones. These results colloborated those of Obanda *et al.,* (2004) and Li *et al.,* (2005) who reported that theaflavins are oxidized further to form thearubigins that are heterogeneous in nature and contribute significantly towards taste, colour and body of teas. Wilson and Clifford (1992) explained the factors affecting the formation and degradation of TFs and TRs in black tea and observed that maximum synthesis of TFs occurs when oxygen is in excess to support benzotropolone ring formation. This may suggest that TFs are not the only source of TRs. However under a limiting oxygen concentration, polyphenol oxidase which has higher affinity for the substrate has a preferential demand for oxygen and TF formation is suppressed at the expense of catechin quinone formation.

5.1.4 Antioxidant activity

In this study there was a high radical scavenging activity in the DPPH radical by the black teas across the zones, though there was more inhibition in Murang'a at 82.6 %, followed by Kisii at 79.14 % and the Meru teas, at 77.01 %. The antioxidant activity is mostly attributed to the presence of high levels of bioactive catechins and polyphenols that have the ability to donate hydrogen ions to stabilize the free radicals. The high antioxidant effect of polyphenols is due to the presence of the phenolic hydroxyl groups in their structures that make them protect free radical scavengers (Amie *et al.,* 2003). The hydroxylation confers a higher degree of stability in the Catechins phenoxyl radical by participating in electron delocalization that is as an important feature of the anti-radical potential. This explains why radical scavenging is high in the gallocatechins including EGCG and EGC that are potent antioxidants (Zhu *et al.*, 2001; Amie *et al.*, 2003; Rao *et al.,* 2006). These results are similar to those of Karori *et al.,* (2007) who

reported the antioxidant effects of black tea. The antioxidant capacity in black tea could also be augmented by TFs in the teas that have been shown to have higher antioxidant activity than EGCG, which is the strongest antioxidant among all catechins and a precursor of TFs (Leung *et al.,* 2001). TFs have more hydroxyl groups which are considered to be necessarily for exerting radical scavenging activity than do catechins, since TFs are dimers of catechins.

The antioxidant activity of TRs in black tea can be explained by the presence of three hydroxyl groups which are more or less esterified by gallic acid in the TRs structure. However this is a highly speculative hypothesis since to date no definite data on TRs structure (Li *et al.,* 2005) has been documented. Therefore the high percentage inhibition exhibited by black teas in this study shows the conversion of the Catechins to TFs and TRs does not affect the radical scavenging activity of the highly dimerized products. These findings are in agreement with those of Leung *et al,* 2001.

The high radical scavenging activities exhibited by EGCG and EGC is due to the Catechins 3'3' and 5' trihydroxylated substitution patterns in the B-ring and /or a hydroxyl group of the C-3 position of the Catechins. This is an important feature on them as they confer a high degree of stability of the catechins phenoxyl radical that participates in electron delocalization (Rao *et al.,* 2006). Kerio *et al.,* (2012) have reported higher antioxidant activity of the purple coloured variety tea products due to the high levels of anthocyanins in this type of teas. The antioxidant activity of anthocyanins can be related to several parameters like the hydroxyl groups in the molecules, the catechol moiety in the B-ring, the oxonium ion structure in the C-ring, the hydroxylation and the methylation patterns and to the acylation by phenolic components (Sang *et al.,* 2002). Many studies have also demonstrated that both catechins and TFs have strong free radical scavenging activity both in vitro and in vivo.

5.1.5 Inorganic nutrient content

Triplicate analyses of all the analytes in all the samples showed that the coefficients of variation (CV) of the results were within ± 10 % except for Cu whose results gave a CV of 17.5 % as indicated in Table 4.8. Reagent blank analyses indicated no contamination or interferences from the reagents used and the calibration curves obtained for all the elements demonstrated adequate linearity ($r^2 > 0.999$). Being a fundamental element of method validation, the detection limit for each analyte was also estimated. Limit of detection (LOD) is the smallest mass of analyte that can be distinguished from the statistical fluctuations in a blank under specified test conditions (Armbruster & Prry, 2008). LODs of the current method calculated as the concentration corresponding to a signal three (3) times of the blank analyses were 0.015, 0.031, 0.060, 0.025, 0.074, 0.013 and 0.043 for K, P, Ca, Mn, Fe, Zn and Cu respectively. The average percentage recovery for the two tea reference samples (A and B) for K, P, Ca, Mn, Fe, Zn and Cu were 98 ± 2.3%, 98 ± 3.4%, 97 ± 2.8%, 96 ± 1.9%, 95 ± 2.6%, 98 ± 2.3%, and 92 ± 5.7% respectively demonstrating good accuracy of the data obtained.

5.2. Recommendations

The data obtained from this study revealed that different clones/cultivars of tea grown in different ecological zones have different levels of catechins, polyphenols and inorganic micronutrients which give them their characteristic biochemical profiles. The different teas from the different regions have varying levels of bioactive compounds that make them produce different concentrations of TFs and TRs during processing. This generates unique quality characteristics in teas emanating from individual factories and therefore the differing prices realized at the auctions. Arising from these findings, the following recommendations are made.

i) This knowledge should be fully exploited to engage the individual factory companies to engage in value addition and diversification of tea products.

Cottage industries set up should pack tea and brand them stating the different catechin, polyphenol content and inorganic micronutrients in their compositions.In this way, there will be increase of revenue in the factories.

ii) This study has demonstrated the ability of the tea plant to accumulate micronutrients though in varying proportions. Tea consumers stand to gain by being furnished with the data on inorganic nutrients necessary for various biological processes.

iii) For purposes of blending, the study recommends the sourcing of teas from Murang'a and Meru zones due to their high phenolic and catechin content, polyphenol, TFs, TRs, briskness, strength, colour and aroma.

iv) To improve productivity and increase their earnings, farmers in Kisii ecological zone are encouraged to increase their plucking rounds from the current two rounds per month to three or four rounds per month as is being done in Meru and Murang'a so as to reduce the caffeine content in their teas and to improve their TFs levels content and productivity from their farms.

v) A review of the current Kenyan black tea quality specifications needs to be carried out in order to include some parameters that had been left out in earlier standards such as polyphenol content, caffeine,s antioxidant capacity, level of amino acids, lipids, etc so as to certify the true status of the Kenyan tea that enters the world value chain.

vi) It's recommended that the Tea Directorate under Agricultural, Fisheries and Food Authority (AFFA) takes the initiative to get the Kenyan standard KS 65: 2009 revised.Because of variation in tea biochemical components, teas should be classified and marketed based on plain quality tea parameters such as briskness, strength, colour, and aroma

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APPENDICES

Appendix I: KTDA factories grouped into regions

REGION	FACTORY
Region 1	Kambaa, Kagwe, Theta, Mataara, Gachege, Kiru*, Njunu, Ngere*, Makomboki, Ikumbi, Nduti, Gacharage.
Region 2	Gitambo, Kanyenya-ini*, Gathunguri*, Kiru, Chinga, Iriani, Gitugi, Gathuthi, Ragati
Region 3	Ndima, Mununga, Kangaita, Kimunye, Thumaita, Mungania, Rukuriri, Katangariri
Region 4	Kinoro*, Imenti*, Githongo*, Michimikuru, Kiegoi, Kionyo*, Weru, Igembe
Region 5	Litein, Tegat, Kapkatet, Momul, Toror, Rianyamwamu, Kapkoros, Mogogosiek, Kapset, Tirgarga Rorok, Olenguruone.
Region 6	Nyansiongo*, Kebirigo, Kiamokama, Ogembo, Itumbe, Eberege*, Nyankoba*, Nyamache, Tombe*.
Region 7	Chebut, Mudete, Kapsara, Kaptumo.

*indicates the factory selected from each region (source KTDA, 2013)

Appendix II: A sample Analysis of Variance (ANOVA) output of the test results (total polyphenols) from the MSTAT statistical analysis package

Data file: MT MOSE

Title: Mose_TP

Function: FACTOR

Experiment Model Number 8: Two Factor Randomized Complete Block Design

Data case no. 1 to 36.

Factorial ANOVA for the factors:

Replication (Replicates) with values from 1 to 3

Factor A (Agro-zone) with values from 1 to 3

Factor B (Factory) with values from 1 to 4

Variable 4: Total Polyphenols (TP)

Grand Mean = 16.794 Grand Sum = 604.600 Total Count = 36

T A B L E O F M E A N S

1	2	3	4	Total
1	*	*	16.742	200.900
2	*	*	16.850	202.200
3	*	*	16.792	201.500
*	1	*	17.833	214.000
*	2	*	16.458	197.500
*	3	*	16.092	193.100
*	*	1	17.456	157.100
*	*	2	17.044	153.400
*	*	3	17.244	155.200
*	*	4	15.433	138.900
*	1	1	18.733	56.200
*	1	2	18.500	55.500

*	1	3	18.600	55.800
*	1	4	15.500	46.500
*	2	1	16.433	49.300
*	2	2	17.033	51.100
*	2	3	15.467	46.400
*	2	4	16.900	50.700
*	3	1	17.200	51.600
*	3	2	15.600	46.800
*	3	3	17.667	53.000
*	3	4	13.900	41.700

A N A L Y S I S O F V A R I A N C E T A B L E

K		Degrees of	Sum of	Mean	F	
Value	Source	Freedom	Squares	Square	Value	Prob
1	Replication	2	0.071	0.035	0.3875	

2	Factor A	2	20.234	10.117	111.1320	0.0000
4	Factor B	3	22.992	7.664	84.1879	0.0000
6	AB	6	29.659	4.943	54.3002	0.0000
-7	Error	22	2.003	0.091		
	Total	35	74.959			

Coefficient of Variation: 1.80%

s_ for means group 1: 0.0871 Number of Observations: 12
y

s_ for means group 2: 0.0871 Number of Observations: 12
y

s_ for means group 4: 0.1006 Number of Observations: 9
y

s_ for means group 6: 0.1742 Number of Observations: 3
y

Appendix III: A sample of the Least Significant Diffrerence (LSD) test results (total polyphenols) output from the MSTAT statistical analysis package

Data File: MT MOSE

Title: Mose_TP

Case Range: 117 - 128

Variable 4: Total Polyphenols

Function: RANGE

Error Mean Square = 0.09100

Error Degrees of Freedom = 22

No. of observations to calculate a mean = 3

Least Significant Difference Test

LSD value = 0.5108 at alpha = 0.050

Original Order Ranked Order

Mean	1 =	18.73 A	Mean	1 =	18.73 A
Mean	2 =	18.50 A	Mean	3 =	18.60 A
Mean	3 =	18.60 A	Mean	2 =	18.50 A
Mean	4 =	15.50 E	Mean	11 =	17.67 B
Mean	5 =	16.43 D	Mean	9 =	17.20 BC
Mean	6 =	17.03 C	Mean	6 =	17.03 C
Mean	7 =	15.47 E	Mean	8 =	16.90 CD
Mean	8 =	16.90 CD	Mean	5 =	16.43 D
Mean	9 =	17.20 BC	Mean	10 =	15.60 E
Mean	10 =	15.60 E	Mean	4 =	15.50 E
Mean	11 =	17.67 B	Mean	7 =	15.47 E
Mean	12 =	13.90 F	Mean	12 =	13.90 F

Appendix IV: An abstract of a paper published on the inorganic nutrient content of black CTC teas

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Scientific Research

Selected Inorganic Nutrients in Black Tea from Three Tea Growing Agro-Ecological Areas in Kenya

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ABSTRACT

The tea plant absorbs dissolved nutrients from soils for its normal growth and development, though to different extents. Nutrients play vital roles in various metabolic processes, their deficiency or excess being deleterious to living organisms. A study was carried out to quantitatively assess the inorganic nutrient content (K, P, Ca, Mn, Fe, Zn and Cu) of twelve black tea samples sourced from Murang'a, Meru and Kisii tea growing agro-ecological areas in Kenya. K and P were quantified using a flame photometer and a UV-Vis spectrophotometer respectively whereas Ca, Mn, Fe, Zn and Cu were quantified using an Atomic Absorption Spectrophotometer (AAS). The general accumulation pattern of the inorganic nutrients in the tea samples was established to be; K (1.6% ± 0.05% - 2.1% ± 0.01%) > P (0.30% ± 0.01% - 0.37% ± 0.04%) > Ca (0.16% ± 0.01% - 0.62% ± 0.03%) > Mn (0.07% ± 0.009% - 0.13% ± 0.004%) > Fe (136 ± 8 - 320 ± 5 µg/g) > Zn (27 ± 1 - 39 ± 7 µg/g) > Cu (10 ± 3 - 16 ± 1 µg/g). Statistically significant differences ($p < 0.05$) were observed in the inorganic nutrient contents of the black tea from the different tea factories as well as agro-ecological areas. These data demonstrate the tea plant's ability to accumulate the studied nutrients, further underlining tea consumption as a potential dietary source of the nutritionally essential inorganic nutrients.

KEYWORDS

Black Tea; Inorganic Nutrients; Spectrophotometry; Kenya