CHEMICAL AND SENSORY QUALITY EVALUATION OF NEWLY DEVELOPED TEA CLONES IN KENYA

KILEL EMILY CHERONO

A Thesis submitted to the Graduate School in partial fulfilment for the requirements of the Master of Science Degree in Food Science of Egerton University.

Egerton University

January, 2013

DECLARATION AND RECOMMENDATION

Declaration

This thesis is my original work and has not, wholly or in parts, been presented for an award of a degree, in this or any other university.

Kilel Emily Cherono

KM16/2271/08

Signature …… Date: ……

Recommendation

This proposal is the candidate's original work and has been prepared with our guidance and assistance; it has been submitted with our approval as official university supervisors.

Dr. Abdul K. Faraj, PhD

Department of Dairy and Food Science and Technology,

Egerton University, P.O Box 536 - 20115, EGERTON.

Signature …… Date: ……

Dr. John K. Wanyoko, PhD

Tea Processing and Value Addition Programme (TPVA Programme),

Tea Research Foundation of Kenya, P.O Box 820 -20200, KERICHO.

Signature …… Date: ……

COPY RIGHT

DEDICATION

I dedicate this work to my entire family and friends

ACKNOWLEDGEMENTS

I thank the Almighty God for his mercy and He is still sustaining me to face challenges ahead. I salute my dear husband Mr. Bii for his overall support to me.

I acknowledge the Director, TRFK Prof. F.N. Wachira for availing me an opportunity to conduct the research using the Foundation's facilities.

My supervisors, Dr. J.K. Wanyoko and Dr. A. Faraj supported me unceasingly and may God reward them here on earth and thereafter.

I acknowledge support of all staff in Dairy and Food Science and Technology for their great support to me ever since I joined them in 2006 and Egerton University at large.

Kaisugu Tea Factory management team, Gianchore Tea Factory management team (Rose Maraka, Mr. Mwiti, Mr. Ombae), Toror Tea Factory management team (Mr. Rono and Mr. Rotich) and their supervisors assisted me in tasting black tea and I sincerely thank them. Vincent Mwingirwa of KTDA Headquarters also tasted our green tea samples and I greatly salute him.6

To Dr. S.M. Kamunya of TRFK, who exceptionally supported me during my research, God bless you mighty. The TRFK staff right from senior staff to technical staff overwhelmingly assisted me before, during and after research and I highly acknowledged them.

Dr. E. Cheruiyot and Dr. J. Owuoche were resourceful during data analysis and I highly appreciate. To my fellow Msc. students, Robert Koech, Kelvin Mosete, Dennis Gitau, Janet Too and my Course mates, thank you for your concern. You made my life and work easier, God bless you all.

My role models, Dr. J. Sigei (JKUAT), Mrs. Florence Bet and Mr. Alex Kirui have been uplifting my spirit to this far, they are God sent to me, God bless them.

To all who supported and facilitated this research work in one way or another, thank you and God bless you.

ABSTRACT

A study was conducted on the newly developed purple tea clones in Kenya. Green tea quality analyses on theanine and catechins were done using HPLC, while total polyphenols were done using the Folin- Ciocalteu phenol reagent method. Sensory analysis was also done on green tea samples. Plain black tea quality parameters were determined (total theaflavins and individual theaflavins, brightness and total colour percentages, total thearubigins and fractions and total soluble solids) for all the clones and sensory evaluation was done by experienced tea tasters in three different tea Factories in Kericho County. Completely randomized design with three replications was adopted for this experiment. Data analyses were done using General Linear Model, Correlation procedures of Statistical Analysis System (SAS, version 9.1). Means were separated by Duncan Multiple Range Test at $p \leq 0.05$ significant level. Clone TRFK 91/1 had the highest theanine content of 2.18 % giving it a flavoury taste. All the test clones had higher total polyphenol contents than the Japanese clone Yabukita used as a standard in green tea experiment. Most test clones had lower mean values of total catechins except for clones TRFK 73/7 and TRFK 73/4 which showed similar total catechins like the control Yabukita with 12.15 % and 12.00 %, respectively. On the plain black tea quality parameters, most test clones showed promising results except clones TRFK 73/3, TRFK 73/7 and TRFK 91/1 which showed low mean values of most black tea parameters. Clones K-Purple and TRFK 83/1 showed good quality black tea parameters. On sensory evaluation, clones 73/3, 91/1 and TRFK 91/2 had less strength and briskness but with flavour. Most test clones had thick liquors except TRFK 73/3, TRFK 83/1 and TRFK KS 1 which had lesser thickness. Most of the test clones also had bright liquors though with dull infusions because of the purple colour. It was concluded that most of the test clones can make good green tea while a few like KS 3, KS 2, 91/2, K-Purple and 14/1 can be made into black tea.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

APPENDICES

LIST OF ABBREVIATIONS

BOP	Broken Orange Pekoe
BOPF	Broken Orange Pekoe Fannings
BP I	Broken Pekoe I
C	Catechins
CFT	Clonal Field Trial
CTC	Cut Tear Curl
D I	Dust I
DM	Dry Matter
EC	Epicatechin
ECG	Epicatechin Gallate
ECQ	Epicatechin Quinone
EGC	Epigallocatechin
EGCG	Epigallocatechin Gallate
EGCQ	Epigallocatechin quinone
FBOP	Flowery Broken Orange Pekoe
GDP	Gross Domestic Product
HIV	Human Immunodeficiency Virus
HPLC	High Performance Liquid Chromatography
IBMK	Isobutyl Methylketone
ISO	International Organization for Standardization
JKUAT	Jomo Kenyatta University of Agriculture and Technology
KTDA	Kenya Tea Development Agency
LTP	Laurie Tea Processor
OD	Optical Density

PD	Pekoe Dust
PF 1	Pekoe Fannings 1
PPO	Polyphenol Oxidase
RRF	Relative Response Factor
TBK	Tea Board of Kenya
TC	Total Catechin
TF	Simple Theaflavin
TF – 3 – g	Theaflavin – 3 – monogallate
TF – 3’ - g	Theaflavin – 3’- monogallate
TF dg	Theaflavin – 3 – digallate
TFs	Theaflavins
TPP	Total Polyphenol
TRs	Thearubigins
TRFK	Tea Research Foundation of Kenya
TR S I, TR S II	Thearubigins S I type and S II type, respectively
TSS	Total Soluble Solids
USD	United Status of America Dollars
UV	Ultra violet

CHAPTER ONE

INTRODUCTION

1.1 Background information

Tea (*Camellia sinensis*) (L.) O. Kuntze belongs to Theaceae family and is used to process the most popular beverage worldwide after water (Wheeler and Wheeler, 2004). *Camellia sinensis* consists mainly of two varieties, *Camellia sinensis* variety *sinensis* and *Camellia sinensis* variety *assamica* (Hara *et al*., 1995). Tea trees can attain a height of twenty to thirty metres in nature and its beverage is a major source of dietary flavonoids (Rijken, 2000). Tea plants are grown in a wide range of latitudes in the world, from 45 °N (Russia) to 30 °S (South Africa), and longitudes from 150 °E (New Guinea) to 60 °W (Argentina) (Hara *et al*., 1995). The plant is kept as an evergreen shrub by pruning. Only the apical bud and the first few leaves are plucked for tea processing. In tropical countries, tea leaves are harvested all year around. In temperate countries, harvesting is seasonal. There are many different kinds of products of different quality arising from different cultivation practices, growing conditions and processing methods (Hara *et al*., 1995). Generally, tea can be broadly classified according to the production method as unfermented tea (green tea), semi-fermented tea (Oolong tea), fully fermented tea (black tea) or post-fermented tea (pu-erh tea) (Zhao *et al*.,2006). There are other types of tea produced including white, yellow and reprocessed tea which include flower scented tea, compressed tea, instant tea and herbal teas (Hara *et al*., 1995). White and yellow teas have been regarded as two subclasses of green tea by Harbowy and Balentine (1997). These two types of tea are different from green tea due to differences in variety, processing, geographical and traditional distributions (Lu, 1987).

Tea was first introduced in Kenya from India by a colonial settler G.W. Caine in 1903 and in the 1930's commercial planting began (Watts, 1999). Currently Kenya is a major black tea exporter and has one of the well established tea estates in both the smallholder grower and large scale farms. Planted tea area in Kenya has grown from a mere 21,448 hectares in 1963 to 180,000 hectares currently (Anonymous, 2011). Tea production is split between smallholders and large estates operated by companies such as Unilever Tea, Finlay Tea and Eastern Produce Limited. The large plantations are organized under the Kenya Tea Growers Association and account for about 40 % of the Kenyan tea production. Smallholders tea farmers are organized under the Kenya Tea Development Agency (KTDA) which was set up in 1964. KTDA operates its own tea factories and buys tea from the smallholders who

produce more than 60 % of Kenyan tea. Tea in Kenya is mainly grown in the Rift-valley province in Kericho, Bomet, Sotik, and Nandi districts. In Central province tea is grown in Kiambu, Thika, Maragua, Murang'a, Nyeri, and Kirinyaga districts, while in Eastern province, tea is cultivated in Meru district. Kisii and Nyamira districts of Nyanza province grow tea mainly under KTDA management. In these districts the crop experience favorable weather patterns. The small-scale sectors in these areas have managed to achieve high quality resulting in high auction price as compared to the multinational companies (Anonymous, 2002). Black tea is consumed worldwide, while green and Oolong teas are consumed mainly in Asia and North Africa.

In Kenya, tea is the leading foreign exchange earner, followed by horticulture and employs over three million people directly and indirectly. It has been established that 60 % of Kenya's tea is produced by over 400,000 small scale growers. Earnings from tea have been steadily increasing every year. Kenya earned 62.1 billion shillings (US $ 774 million) from tea exports in 2008 according to the Tea Board of Kenya (Anonymous, 2008). In 2010, tea earnings in Kenya were 97 billion shillings compared to 69 billion shillings earned in 2009 (Anonymous, 2010). Tea production in 2010 was 399 million kg compared to 314 million kg in 2009, accounting for increased earnings. In the year 2011, Kenya earned 109 billion shillings from tea export which was triggered by weak shilling against the US dollars that year (Anonymous, 2011). Summary is shown in Fig. 1.

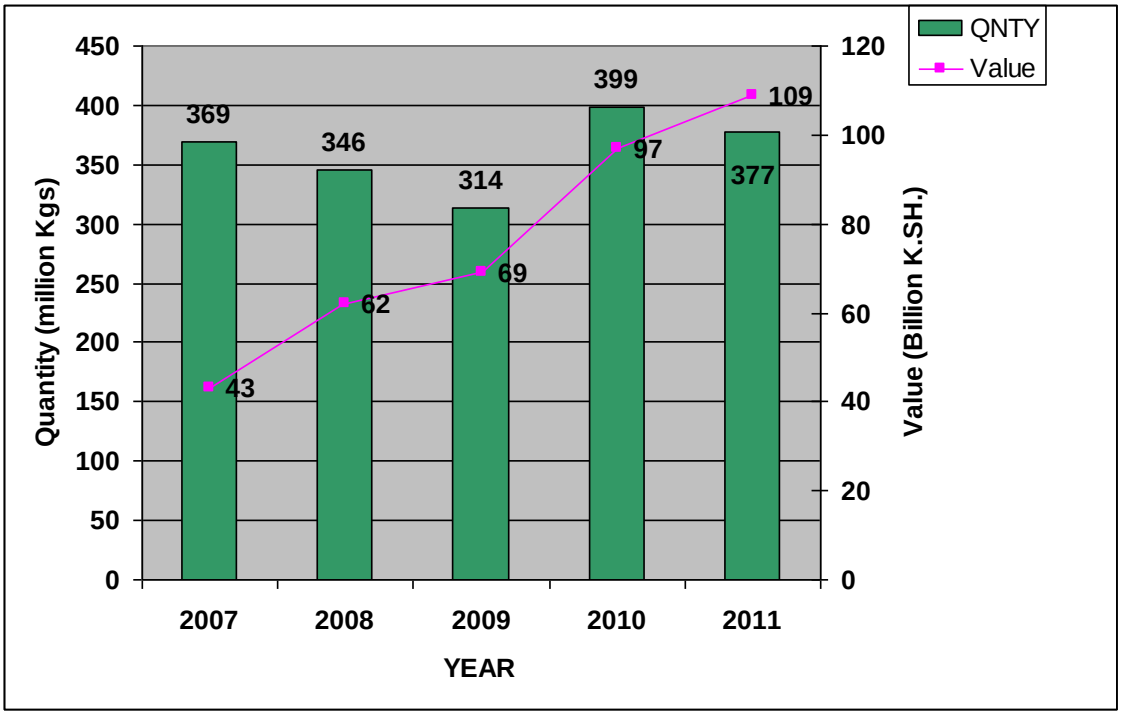


Figure 1: Kenyan tea production and earnings trend (2007-2011) Source: Tea Board of Kenya (2011)

Green tea (*Camellia sinensis* var. *sinensis*) originated in China where it has been used as a beverage and medicine since 2,700 BC. Japan and China are the major green tea producers in the world (Golding *et al.,* 2009). In Kenya, green tea production is just emerging so that currently very few tea factories of Unilever Tea and Finlay Tea produce green tea. Green tea tasters, consequently, are few.

Kenya supplies 22 % of the world's black tea and tea is the major foreign exchange earner for the country (Anonymous, 2011), contributing about 26 % of all foreign exchange earnings and 4 % of the gross domestic product (GDP).

World black tea production has been higher than world demand (Anonymous, 2007), while cost of production has continued to rise (Herath and Weersink, 2007). As a result, only producers of high quality black tea sell at reasonable prices (Anonymous, 2007). Use of superior quality plants (Kamunya, 2003) can improve the profitability of a tea enterprise, provided other agronomic practices are optimized (Owuor *et al.,* 2009). Tea is still treated as an agricultural commodity making it vulnerable to supply and demand pressures. The FAO in 2001, had predicted that Kenya would produce 304, 000 tonnes of black tea by 2010 (FAO, 2001), but in 2011, Kenya produced 377,900 tonnes (Anonymous, 2011). This shows a higher rate of black tea production not only in Kenya but also globally. However, adverse weather condition sometimes affects the production.

As a result of the low profits in tea enterprises, tea farmers, especially in Kenya are yearning for clones which can fetch more money. The tea clones under study are interspecific hybrids between tea and related *Camellia* species which were selected from seed plantation in Kenya, cloned and planted at Kangaita sub-station of the Tea Research Foundation of Kenya (Anonymous, 2006). The newly developed clones might fetch more money because of their inherently high anthocyanin content than the commercial clones in use.

The newly developed clones include: TRFK 73/1, TRFK 73/2, TRFK 73/3, TRFK 73/4, TRFK 73/5, TRFK 73/7, TRFK 91/1, TRFK 91/2, TRFK 83/1, TRFK 14/1, K- Purple, TRFK KS 1, TRFK KS 2 and TRFK KS 3. Most of these clones have purple red leaf pigmentation which is due to anthocyanins. Presence of anthocyanin is a genetical characteristic. The purple red anthocyanin pigment tends to mask the normal green colour of chlorophyll. The clones under study were meant for value addition to the already existing tea clones through blending but now we wish to investigate if their plain black tea quality parameters are comparable to the released standards clones.

The control clones are: AHP S 15/10, TRFK 31/8, TRFK 301/1, TRFK 301/2, TRFK 6/8, TRFK 303/216, TRFK 303/577, EPK TN 14/3, GW Ejulu, ST. 543, and ST. 536 which are released standard clones and most of them are considered the best clones for black tea.

In the tea trade, Kenyan black teas are classified as plain to medium flavoury. The plain black teas are valued for their theaflavins content and the thearubigins. Theaflavins are responsible for the taste, brightness and contribute to the colour of black teas. The thearubigins are responsible for thickness and colour of both the liquors and infusion (Biswas *et al*., 1973). These chemical attributes, including black tea brightness and colour are referred to as the plain tea quality parameters (Biswas *et al.,* 1973*).* Indeed theaflavins have become a critical parameter in estimating the quality of black teas (Owuor and Obanda, 2007).

The current study aims to characterize green tea quality in terms of total polyphenols, total catechins and theanine of the new clones against the Japanese Yabukita variety as a standard and to determine the plain black tea quality parameters of the newly developed tea clones and compare them with the commercially released clones chemically and through sensory evaluation.

1.2 Statement of the problem

Currently Kenya produces mainly black tea from green tea clones which is largely exported making Kenya the leading black tea exporter. Tea industry is currently experiencing problems such as overproduction that is outstripping demand. Though the Mombasa auction prices have lately improved, the cost of production has sharply increased because of escalated fuel prices. There are, however, better prices for branded tea products and therefore Kenyan tea industry needs to brand and diversify its tea products like producing anthocyanin rich teas. Agronomic practices affect the quality of made tea. Little has been done to evaluate the quality of the test clones for comparison with the existing commercial clones grown under the same agronomical practices. The chemical parameters of the test clones need to be ascertained before product development, hence the need for this study.

1.3 Objectives

1.3.1 General objective

The overall objective of the study was to elucidate the quality parameters of the newly developed tea clones in Kenya.

1.3.2 Specific objectives

The specific objectives were;

1. To characterize green tea quality parameters; total polyphenols, total catechins and theanine between the new clones compared to Japanese clone Yabukita.
2. To determine; (a) the total theaflavins and the ratios of the individual theaflavins in the new tea clones and compare with the commercial clones currently in use.

(b) the total thearubigins and their fractions in the new tea clones and compare with the commercial clones currently in use.

(c) the total soluble solids, total colour and brightness percentage in the new tea clones and compare with the commercial clones in use currently.

(d) the residual catechins in black tea made from the test and commercial clones

3. To determine the correlation between sensory evaluation scores with the chemical parameters in both green and black teas.

1.4 Null hypotheses

The hypotheses tested were:

1. There is no difference in the amount of total polyphenol, total catechins and theanine between the new tea clones and Yabukita clone.
2. There is no difference between; (a) The amount of theaflavins and the ratios of the individual theaflavins in the new tea clones and the commercial tea clones.

(b) The amount of thearubigins and their fractions in the new tea clones and the commercial tea clones.

(c) Total soluble solids, total colour and brightness percentage of the test clones and the commercial clones.

(d) Residual catechins in test clones and in commercial clones.

3. There is no correlation between the sensory evaluation scores with the chemical quality parameters in both green and black teas.

1.5 Justification

The tea sub-sector has become a major foreign exchange earner in Kenya outdoing horticulture in 2010 to become the first foreign exchange earner. Kenya is currently the leading black tea exporter in the world. Quantification of the quality parameters of the new tea clones will guide whether they are worth being made into black tea or green tea. Black

teas from these new clones could be marketed as specialty teas because of residual anthocyanin. Quality traits of these clones such as theaflavins content, total thearubigins and total soluble solids have not been quantified because they were developed recently. The clones will be used as parents in the breeding programme at the Tea Research Foundation of Kenya when their chemical and sensory qualities have been ascertained and found to be potentially valuable. Teas from the new clones are expected to contribute to product diversification for improved revenue generation. Green teas from the new clones will also be sold as functional food because of anthocyanin pigment.

1.6 Outcome

The outcome of this study will include;

1. Quantifying the quality parameters of made teas (black and green) from Purple leaf coloured teas.
2. Publication of scientific papers in refereed journals and presentation at a scientific forum.
3. Dissemination of generated data to relevant stakeholders in the tea industry.
4. Writing Master of Science thesis in Food Science.

CHAPTER TWO

LITERATURE REVIEW

2.1 Chemistry of tea

Tea flush (young shoots of tea) consists of the terminal bud and two adjacent leaves. A variety of non-volatile compounds exist in fresh tea flush. These include polyphenols, flavonols and flavonol glycosides, flavones, phenolic acids, amino acids, chlorophyll and other pigments, carbohydrates, organic acids, caffeine and other alkaloids, minerals, vitamins, and enzymes (Hara *et al.*, 1995c). The chemical composition of the tea leaves depends on leaf age, type of clone, soil and climatic conditions, and agronomic practices. The total polyphenols in tea flush ranges from 20 % to 35 %. Flavanols which are mainly catechins are the most important group and occupy 60–80 % of the total amount of polyphenols (Hara *et al.*, 1995c). Four major catechins, namely (-) - epigallocatechin-3-gallate (EGCG), (-)-epigallocatechin (EGC), (-)-epicatechin-3-gallate (ECG), and (-) - epicatechin (EC), constitute around 90 % of the total catechin fraction; and (+) catechin (C) and (+)-gallocatechin (GC) constitute about 6 % of the fraction. Other minor catechins constitute less than 2 % of the total catechins. Being water-soluble and colourless catechins contributes astringency and bitterness in green tea (Scharbert and Hofmann, 2005). Three major flavonol in the fresh leaf are kaempferol, quercetin and myricetin. These substances occur both as free flavonols and as flavonol glycosides. The glycosidic group may be glucose, raminose, galactose, arabinose and orrutinose. These compounds are considered to contribute to bitterness and astringency in green tea (McDowell and Taylor, 1993). Amino acids constitute around 4 % in tea flush. The most abundant amino acid is theanine (5-N-ethylglutamine) which is unique to tea and it is found at a level of 2 % dry weight (50 % of free amino acid fraction) (Juneja *et al.*,1999). Free sugars constitute 3–5 % of the dry weight of tea flush. They consist of glucose, fructose, sucrose, raffinose and stachyose. The monosaccharides and disaccharides contribute to the sweet taste of tea infusion. The polysaccharides present in tea flush can be separated into hemicellulose, cellulose and other extractable polysaccharide fraction. Caffeine is the major purine alkaloid present in tea. The content of caffeine in tea flush is approximately 2–5 % (dry weight basis). Theobromine and theophylline are found in very small quantities. Traces of other alkaloids like xanthine, hypoxanthine and tetramethyluric acid, have also been reported (Graham, 1992). Many volatile compounds, collectively known as the aroma complex, have been detected in tea. The aroma in tea can be

broadly classified into primary or secondary products. The primary products are biosynthesized by the tea plant and are present in the fresh green leaf, whilst the secondary products are produced during tea manufacture (Sanderson and Graham, 1973). Some of the aroma compounds, which have been identified in fresh tea leaves, are mostly alcohols including Z-2-penten-1ol, n-hexanol, Z-3-hexen-1-ol, E-2-hexen-1-ol, linalool plus its oxides, nerol, geraniol, benzylalcohol, 2-phenylethanol, and nerolidol (Saijo and Takeo, 1973). The aroma complex of tea varies with the country of origin. Slight changes in climatic factors can result in noticeable changes in the composition of the aroma complex. Notably, teas grown at higher altitudes tend to have higher concentrations of aroma compounds and superior flavour, as measured by the flavour index (Owuor *et al*., 1990). Growing tea in a shaded environment may change the aroma composition and improves the flavour index. The aroma complex also varies with season and these variations appear to be larger under temperate or sub-tropical climates (Gianturco *et al*., 1974).

2.2 Tea and health

Tea consumption has a long history of over 2,000 years. Originated in China, drinking tea as a habit of daily life has spread all over the world. Currently, tea is one of the most popular beverages globally. Because tea is widely consumed by hundreds of millions of people in a perpetual manner, the possible effects of tea on human health is of particular importance in the field of medical, agricultural, and food research. The general view of tea drinking has experienced a series of changes over the years. Originally in ancient China, tea was taken as a medicine to detoxify or to cure diseases (Balentine *et al.*, (1997). Later on, tea was recognized as a tonic, which is beneficial to human health. In the course of development, tea is widely accepted as a beverage. Despite those changes, tea remains a kind of medicine, at least in part, in traditional Chinese medicine, in which tea is used alone or in most cases used in combination with other herbs to treat a variety of disorders. Modern medical research has found that tea and tea products display a wide spectrum of bioactivity and show therapeutic effectiveness in a number of experimental disease models (Wolfram, 2007; Gomes *et al.,* 1995). The subject of bioactivity and therapeutic potential of tea and tea products has drawn a lot of attention.

A recent study by Karori *et al.*, (2007), found out that Kenyan black tea could attenuate inflammation induced in *Trypanosoma brucei* infected mice. They showed that tea was more efficacious than dexamethasone which is an established anti-inflammatory drug. Some epidemiological studies have associated the consumption of tea with a lower risk of

several types of cancer including those of the stomach, oral cavity, oesophagus and lungs (Hakim and Chow, 2004). Green tea polyphenols especially EGCG have been shown to be an effective chemopreventive agent (Gosslau and Chen, 2004; Hsuu and Chen 2007). Other health benefits, such as enhancing insulin activity (Cabrera *et al.*, 2003), antimicrobial effect (Stapleton, 2004; Almajano *et al.*, 2008), antibacterial activity (Mbata *et al.*, 2008), immuno stimulatory effect (Matsunaga, 2002), anti-inflammatory capacities (Sato and Myata, 2000; Karori *et al.*, 2008), its protective effect against cardiovascular diseases (Sano, 2004; Khan and Mukhtar, 2007) and cerebral ischemic damage (Suzuki, 2004), have been suggested. These beneficial effects have been attributed to the presence of tea compounds such as polyphenols, amino acids, vitamins, carbohydrates, and purine alkaloids (Bolling and Chen, 2009). Researchers have also found that Epigallocatechin gallate (EGCG), a green tea catechin, may have anti-HIV effects when bound to CD4 receptor (Kawai, 2003). Tea has also received a great deal of attention because of its antioxidant properties (Zhang, 2004; Luczaj and Skrzydlewska, 2005; Gramza *et al.*, 2006; Fu and Koo, 2006; Karori *et al.*, 2007; Maurya and Rizvi, 2008).

2.3 Antioxidant properties of tea

Antioxidants play an important role in the prevention of chronic diseases. Tea leaves have high antioxidant activity (Hara *et al.*, 1995d). Tea polyphenols can scavenge free radicals due to possession of a phenolic hydroxyl group attached to the flavan-3-ol structure. Hence tea has been associated with therapeutic action against free radical mediated diseases (Amie *et al.*, 2003*).* Studies have reported that green tea extract has antioxidant, antibacterial, antiviral, anticarcinogenic and antimutagenic functions (Lin *et al.*, 2008). Karori *et al.*, *(*2007), compared the antioxidant activity of Kenyan black tea and popularly consumed vegetable (spinach and onion) and showed that the antioxidant activity of tea was significantly ($P< 0.05$) higher than that of the fresh unprocessed vegetable. This demonstrated the potency of tea as health enhancing beverage.

A study done by Tsong-Ming *et al.*, (2009), where they formulated sponge cake with partial replacement of cake flour with up to 20 % green tea, showed that the cake had bioactive components and pleasant tea flavour as compared to cake prepared with 100 % cake flour. Green tea sponge cake was good in antioxidant properties. They concluded that green tea could be incorporated into cake to have more functional components and more effective antioxidant properties.

In another study done by Ercisli *et al., (*2008), they analyzed fresh tea leaves sampled from Derepazari 7 clone grown in Turkey. They were investigating on the seasonal variation of total phenolic, antioxidant activity, plant nutritional elements and fatty acids in tea leaves of clone Derepazari 7 in three commercial harvest seasons. There were significant differences ($P < 0.05$) among harvest times on antioxidant activity. The second harvest had the highest followed by first and third harvest was last though still high. These could be because of the effect of change of ecological parameters.

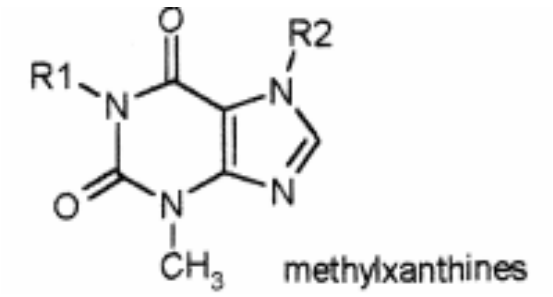
2.4 Chemical constituents of green tea

Green tea has many different components which affect the taste and therefore the quality of the tea. These tea constituents are well characterised and can be useful for the evaluation of tea taste quality (Caffin *et al*., 2004), especially in the absence of trained sensory panels. The constituents of green tea which are of major importance to the taste of green tea are the catechins, caffeine and an amino acid unique to tea called theanine. The catechins are prominent compounds which give green tea its bitter and astringent taste properties (Hara, 2001). Caffeine in green tea is at levels around a third of those in coffee and is also very bitter which adds to green tea's sharpness. Theanine on the other hand, has a sweet and brothy taste which counteracts the astringency and bitterness of the catechins and caffeine. The concentrations and interactions of these different constituents in the tea will determine how intense the tea flavours are. In general, the higher the concentrations of these components within the tea, the more intense the tea flavours will be. However, the balance between these components, especially between the catechins and theanine is very important for overall taste sensation. Green tea with higher theanine fetches more money (Golding *et al*., 2009). There is need therefore to screen the Kenyan clones for theanine as we try to make specific tea products.

Tea contains several alkaloids called methylxanthines as shown in Figure 2 which include caffeine (1, 3, 5- trimethylexanthine) and two dimethylxanthines, theophylline and theobromine (Lin *et al*., 1998).

Tea leaves contain about 2 – 4 % caffeine (% dry weight) and is the major alkaloid present in tea (Graham, 1992). The caffeine content of a typical tea beverage prepared from 2-2.5 g of tea leaves is in the range of 20-70 mg per 170 mL infusion (Caffin *et al*., 2004). However, theobromine and theophyline constitute a very small percentage of tea alkaloids, about 0.1 % (Graham, 1992). In addition to affecting the taste of tea with its sharp bitterness,

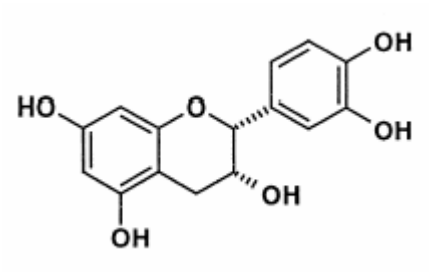
caffeine has mood and cognitive-enhancing properties which adds to its regard as an important constituent of tea contributing to tea quality (Fernandez *et al*., 2002; Caffin *et al*., 2004). Caffeine removes fatigue and sleepy feeling and has diuretic action (Snel and Lorist, 2011)



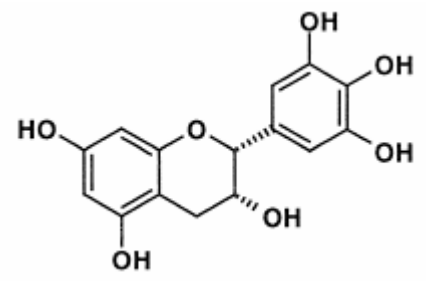
Theobromine: R1=H, R2= CH3 Caffeine: R1=R2=CH3, Theophylline: R1=CH3, R2=H

Figure 2: Chemical structures of methylxanthines

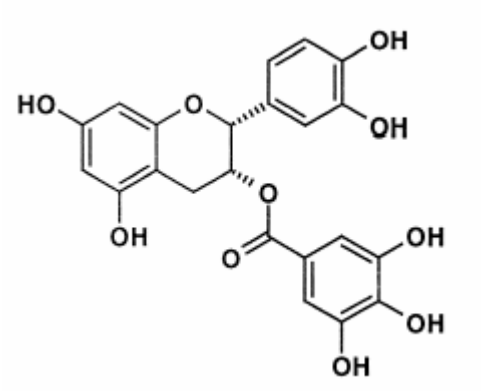
Catechins can make up to 30 % of the dry weight of fresh tea leaf extracts (Graham, 1992; Fernandez *et al*., 2002) and constitute 20-30 % of the dry weight of dried green tea (Wang *et al.,* 2000). The EGCG is considered as the most important component because it is the most abundant catechin in tea leaves and in most green teas (Wang *et al*., 2000; Higdon and Frei, 2003). The gallated catechins (EGCG, ECG) are particularly astringent while the non-gallated catechins (EGC, EC) are far less astringent (Hara, 2001). The ECG can also provide a bitter aftertaste while the slightly sweet aftertaste of some teas can be due to EGC even at concentrations as low as 0.1% (w/v) in aqueous solutions (Hara, 2001). Catechins structures are shown in Figure 3. High concentrations of catechins differentiate green tea from black and oolong teas. In the processing of green tea, the enzyme polyphenol oxidase (PPO) is quickly inactivated after harvest using steam in order to prevent it from oxidising the catechins (Graham, 1992). In contrast, the processing for black tea stimulates the oxidation of 70 % to 90 % of the catechins. In the production of oolong tea, oxidation also occurs but it is stopped before 70 % of the catechins are oxidised.



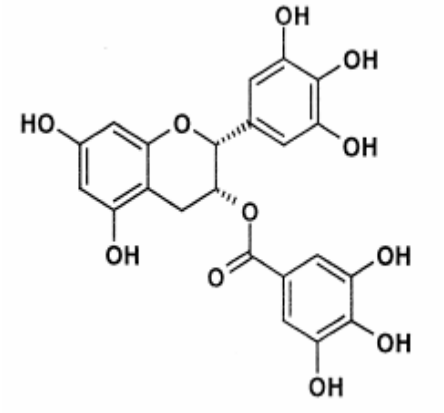
(i) Epicatechin



(ii) Epigallocatechin



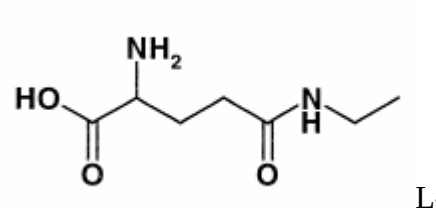
(iii) Epicatechin gallate



(iv) Epigallocatechin gallate

Figure 2: Chemical structures of major catechins

Theanine, γ-glutamylethylamide or 5-*N*-ethyl glutamine shown in Figure 4 is a non-protein amino acid that was first discovered in tea leaves (Sakato, 1949). Theanine is found in 21 species from theaceae family (Ashihara *et al.,* 2010). It is the main free amino acid in teas, representing as much as 50 % of the total amino acids in black tea and 1–2 % of the dry weight of green tea (Hara *et al.,* 1995). Theanine is mainly responsible for the brothy, sweet, and umami taste of tea and hence, plays an important role in green tea quality (Ekborg-Ott *et al*., 1997). It plays an important role in the characteristic flavour, delicate taste of tea and also shows many biological effects such as promoting relaxation, inhibiting caffeine's negative effects, reducing blood pressure, and enhancing anti-tumor activity (Kimura *et al.,* 2007; Sugiyama and Sadzuka, 2003 and Yamada and Terashima, 2009). Moreover, it has been reported to have physiological activities including neuroprotection and anti-obesity (Cho *et al.,* 2008; Egashira *et al*., 2004 and Zheng *et al*., 2005).



L- Theanine

Figure 3: Chemical structure of L-theanine

The amino acid theanine is synthesized in the root of the tea plant with the aid of theanine synthetase and translocated to the developing shoot tips (Ekborg-Ott *et al*., 1997). Theanine is also a precursor for the biosynthesis of the catechins in tea leaves (Kito *et al*., 1968). This conversion is known to be controlled by light because large quantities of theanine accumulate instead of being converted to catechins in shaded tea leaves, and result in lower catechin levels than that in unshaded leaves (Kito *et al*., 1968).

2.5 Anthocyanins in tea

Anthocyanins are members of the flavonoid group of phytochemicals and are predominant in teas, honey, wines, fruits, vegetables, nuts, olive oil, cocoa, and cereals. The major phytochemicals include anthocyanins like cyanidin; the flavonols like quercetin; flavones like apigenin; flavanones like myricetin; flavan-3-ols (catechin, epicatechin, gallocatechin) and, the isoflavones like genistein (Lila, 2004). Phytochemicals in this class are frequently referred to as bioflavonoids due to their multifaceted roles in human health maintenance. Anthocyanins in food are typically ingested as components of complex mixtures of flavonoid components. Daily intake is estimated to be from 0.5g to 1g, but can be several grams per day if an individual is consuming flavonoid supplements like grape seed extract (Skibola and Smith, 2000).

Anthocyanins are phenolic materials, and along with catechins are important components in the medicinal effect of tea. Purplish red tea results from an inherited reaction to unfavourable hot and humid environmental conditions, providing the tea tree with a mechanism for fighting scorching ultraviolet rays. In order to resist damage from this shortwave radiation, tea leaves produce anthocyanin, which can reflect away a portion of the ultra violet light hitting the leaves (Jansen *et al.,* 1998).

Most of the research on anthocyanin is on fruits and recently anthocyanins were extracted from some Kenyan tea cultivars (Kerio *et al.,* 2011). A figure showing a field of anthocyanin rich tea is presented in Figure 5. A review on raspberry, strawberry, blackcurrant

and muscadine grape juice by Tiwari *et al.,* (2009b), showed that, the temperature during juice extraction influences the stability of anthocyanins by affecting the enzymatic activity of PPO, which accelerates the rate of anthocyanin degradation. Inactivation of the enzyme could reduce the loss of anthocyanins. Polyphenol oxidase is a major enzyme found in tea and therefore there is need to study its effect on the residual anthocyanin in black tea.

Figure 4: Field of anthocyanin - rich tea

The presence of various compounds such as ascorbic acid and other polyphenols affects anthocyanin stability. Protective effect was observed between ascorbic acid and retention of anthocyanins in sonicated strawberry juice as shown by Tiwari *et al., (*2009a). A study by Del Pozo-Insfran *et al.,* (2007) on the stability of high hydrostatic processed grape juice fortified with water soluble polyphenolic cofactors from thyme (*Thymus vulgaris* L.), indicated the efficacy of such additions on the stability of anthocyanins in the presence of residual enzymatic activity of polyphenol oxidase. Copigmentation increased anthocyanin retention.

2.6 Importance of anthocyanins

Anthocyanins are plant pigments responsible for the orange, red and blue colours of various fruits and vegetables and are important quality indicators (Wrolstad *et al*., 2005). Anthocyanins are believed to be important to plants as their colour attracts animals, leading

to seed dispersal and pollination. Owing to strong absorption of light, they may also be important in protecting plants from UV-induced damage (Mazza and Miniati ,1993).

Anthocyanins are used as food colorants primarily in the beverage industry. Consumers and food manufacturers desire colorants from natural sources since there is increased public concern about synthetic food dyes. Synthetic dyes commonly used in the food industry have been suspected to cause adverse behavioural and neurological effects (McCann *et al.,* 2007).

The low stability of these pigments, which is influenced by several factors especially the pH of a system (low stability at high pH), limits their incorporation in foods. They are susceptible to light, temperature and different agents like oxygen and enzymes which can cause their degradation (Jackman and Smith, 1996).

Black carrot juice concentrate was added to enhance the colour of strawberry jams prepared from two locally grown cultivars in Turkey, Osmanlı and Kara (Kirca *et al.,* 2007). The natural colorant stabilized the colour of the strawberry jam. It was also found that as the storage temperature increased, the stability of anthocyanins decreased significantly.

Kerio *et al.,* (2011) characterized anthocyanins in Kenyan teas and found out that the major anthocyanin found in these teas is malvidin. The effect of this anthocyanin on tea quality is yet to be ascertained.

2.7 Black tea processing

The general steps of processing black tea are; withering of leaves under certain plucking standards, rolling, aerating, drying and sorting. Each step has a major influence on the made tea quality and many chemical or physical changes occur.

2.7.1 Withering

This is the first step in processing of black tea with the major aim of reducing the moisture content on the fresh leaves. The moisture content of green tea leaves reduces from about 80 % to about 72 % for maceration with the cut-tear-curl method. The loss in moisture makes the leaves amenable to subsequent rolling and aeration. Many physical and biochemical changes take place during the withering process (Hara *et al.,* 1995; Owuor, 1996). Withering can be divided into physical and chemical wither because of physical and chemical changes, respectively. Usually the tea leaves are spread evenly in perforated troughs fitted with a powerful exhaust fan underneath to draw the moisture from the tea leaves and carry the

humid air out of the withering area. Alternatively and especially during humid conditions, hot air is blown from underneath the trough through the tea leaves to remove the moisture. The physical change associated with withering is a loss of moisture from the shoot which leads to changes in cell membrane permeability. The chemical wither involves breakdown of proteins into amino acids and other chemical changes take place. Short chemical wither period favour the formation of theaflavins and thus liquor brightness increases (Obanda and Owuor, 1992). Humid conditions during withering also favour the formation of theaflavins (Obanda *et al.,* 1997). To ensure good quality tea, an even wither is critical (Keegal, 1950). The rate of loss of moisture and temperature of the leaf during withering is related to surface moisture, humidity of the air, altitude, dry-bulb and wet-bulb temperature, air flow, packing density and whether heat is applied during withering (Johnson, 1987, 1990; Hampton, 1992).

2.7.2 Rolling

During rolling of the tea leaves, the leaves are macerated and the cell structures are disrupted, which brings various enzymes into intimate contact with their substrates, the polyphenols. Rolling of tea leaves may be accomplished by orthodox rollers (e. g. rotor vanes), Lawrie Tea Processor (LTP), or crush-tear-curl (CTC) machines. Orthodox rolling is widely used in Sri Lanka, the world's major producer of orthodox teas (Hara *et al*., 1995). India and Kenya are the major producers of CTC teas. In CTC manufacture, after preconditioning, tea leaves are fed between a pair of stainless steel rollers with etched surfaces, one rotating clockwise, the other anti-clockwise at different speeds. Poly phenol oxidase enzymes are activated during rolling and their activities are enhanced with the presence of suitable conditions like temperature, oxygen supply and moisture during rolling process. The chemical and biochemical reactions initiated in the leaves during preconditioning proceed at an accelerated rate during and after the rolling, before the leaves progress to the next stage of aeration (Hara *et al*., 1995).

2.7.3 Aeration

The process which used to be referred to as fermentation is currently referred to as aeration since no microorganisms are used in this kind of enzyme-oxidized black tea (Mo *et al.,* 2008). The ex-CTC dhool is what is aerated immediately after rolling. The high temperature of up to 32^{o}C is progressively reduced by subsequent supply of oxygen where the temperature is reduced to about 22^{O}C and maintained constant by controlling air supply. The principal reaction in aeration is the oxidation of catechins and catechin gallates by

various enzymes especially polyphenol oxidase. Other enzymes like peroxidase are also involved and some non-enzymatic reactions take place to form the unique character of black tea (Hara *et al*., 1995). During the aeration process there is development of colour, strength and quality of tea brews from the production of non-volatile compounds through the enzymatic oxidation of catechins and their gallates to theaflavins and thearubigins (Haslam, 2003). There is also the production of volatile compounds responsible for the characteristic aroma of black tea. The rate of aeration is profoundly influenced by genetic constitution, seasonal and climatic factors, agronomic and management practices, and systems of processing (Cloughley, 1980). High temperatures usually increase the rate of aeration.

2.7.4 Drying

Drying of the aerated dhool is primarily aimed at arresting the aeration through cessation of enzymatic activity and also to reduce moisture to about 3 % dry mass. Changes other than removal of moisture that occur during drying include a significant loss of volatile compounds, an increase in the levels of amino acids, the binding of polyphenols to other tea components, and an increase in carboxylic acids, and Maillard reactions. Firing at an elevated temperature is necessary for the development of the taste, colour, and aroma of black tea (Hara *et al*., 1995).

2.7.5 Sorting and grading

Sorting is done mainly to remove excess fibre in order to have clean teas. Grading follows and it is an important stage for the marketing of tea, ensuring the correct particle size, shape, and cleanliness. The major primary grades in Kenya are Broken pekoe 1 (BP 1), Pekoe Fannings 1 (PF 1), Pekoe Dust (PD) and Dust 1 (D 1). In the tea trade, commonly found grades include broken orange pekoe (BOP), flowery broken orange pekoe (FBOP), and broken orange pekoe fannings (BOPF) (Hara *et al*., 1995).

2.8 Plain black tea quality parameters

Black teas are obtained from aerated green leaf while green teas are from un aerated green tea leaf. Plain black tea quality parameters are; theaflavins, thearubigins, total colour and brightness (Owour and Obanda, 2001). Theaflavins and thearubigins are black tea polyphenols which occur when the tissue is deliberately damaged during cutting (Haslam, 2003). Theaflavins are bright and orange-red while thearubigins are more chemically

heterogeneous and tend to be brownish-red (Brown *et al.,* 1969)). Together, these compounds and flavonol glycosides (McDowell *et al.,* 1991) give black tea liquor most of its taste and colour.

2.8.1 Theaflavins

These are products formed by the enzymatic oxidation and condensation of catechins with dihydroxylated and trihydroxylated B rings. Four major individual theaflavins are commonly formed during black tea processing. These include simple theaflavin (TF), theaflavin -3-monogallate (TF-3-g), theaflavin –3′-monogallate (TF-3′-g) and theaflavin-3, 3′-digallate (TFdg).

The total theaflavins (Wright *et al.,* 2002), or derived theaflavin digallate equivalents (Owuor and Obanda, 1997), have a dominant effect on the quality of black teas. The content of total theaflavins in black tea does not usually exceed 2 % and can be as low as 0.3 % (Balentine *et al*., 1997). Graham (1992) reported that theaflavins ranged 1.5-2.5 % in the dry leaf. An analysis of commercial tea samples from Sri Lanka, Kenya, India and other countries in the German market found that, the total theaflavins range 0.45-1.45 %, with an average of 0.92 % (Steinhaus and Engelhardt, 1989). A study on the commercial black tea from the Kenyan market showed a range of total theaflavins from 1.89-2.27 %, with an average of 2.14 % (Owuor and Obanda, 1995). The formation of a single theaflavin molecule requires a dihydroxy and a trihydroxy flavan-3-ol, as follows:-

Epicatechin (EC) + Epigallocatechin (EGC) = simple theaflavin (TF).

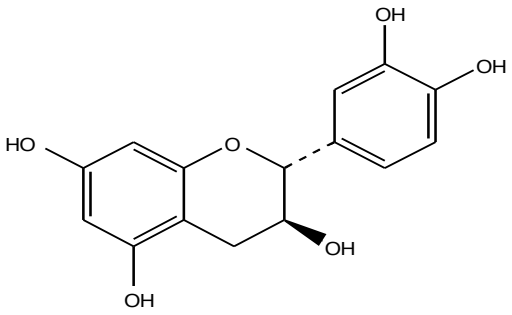
EC + Epigallocatechin gallate (EGCg) = Theaflavin-3-gallate (TF-3-g)

Epicatechin gallate (ECG) + EGC = Theaflavin-3’-gallate (TF-3’- g)

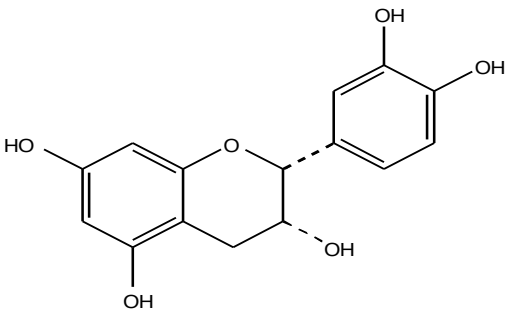
ECG + EGCg = Theaflavin-3, 3’-digallate (TF dg)

Thus the ratio of dihydroxy flavan-3-ol to trihydroxy flavan-3-ol in green leaf may have a major influence on the amount of theaflavins in black tea. The correct balance and amount of dihydroxy flavan-3-ol and trihydroxy flavan-3-ol are therefore necessary to ensure maximum formation of the theaflavins (Wright *et al.,* 2002). The amount of the individual theaflavins formed are largely influenced by the amount of the precursor catechins in green leaf, their redox potential and/or affinity for polyphenol oxidase and activity (Owour and Obanda, 2007).

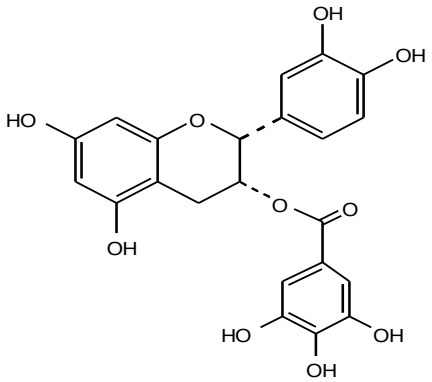
Obanda *et al.*, (2001), confirmed the formation of individual theaflavins according to (Robertson, 1992) as shown in Figure 18.



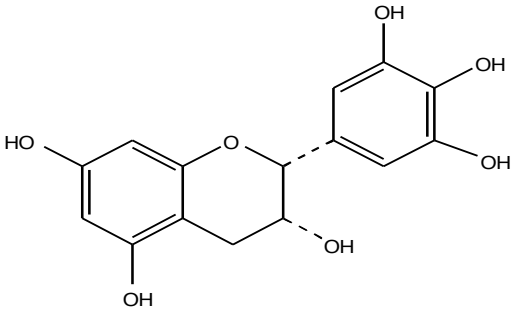
(+) Catechin (C)



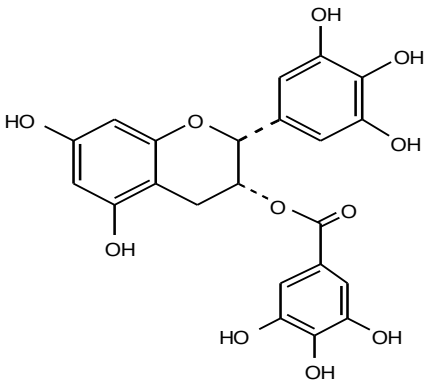
(-) Epicatechin (EC)



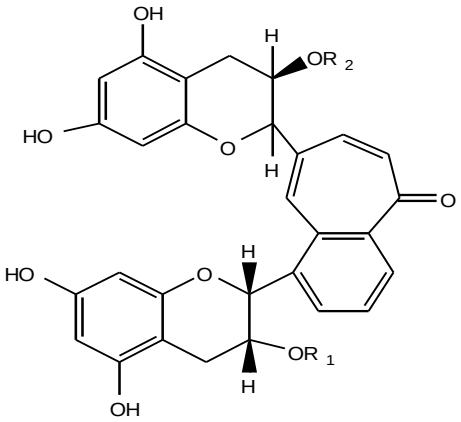
(-) Epicatechin gallate (ECG)



(-) Epigallocatechin (EGC)



(-) Epigallocatechin gallate(EGCG)



Theaflavins

TF: R_1 = R_2 = H ;TF-3-g: R_1=H, R_2= 3,4,5 trihydroxybenzoyl; TF-3'-g: R_1= 3,4,5 trihydroxybenzoyl,R_2= H; TF dg: R1=R2=3,4,5 trihydroxybenzoyl

Figure 6: Catechin structures and general structure of individual theaflavins (Robertson, 1992).

2.8.2 Thearubigins

Thearubigins is a collective name for the brown acidic pigments or the coloured phenolic oxidation products that remains after removing of the yellow neutral pigments from a black tea liquor (Millin, 1987). Their molecular weight and spectral characteristics are known to be very heterogeneous. The thearubigins constitute between 10 and 20 % of the dry weight of black tea (Roberts, 1962) and represent approximately 30-60 % of the solid in liquors after infusion. Although thearubigins have not been fully characterised, it is clear that this complex consists of a group of polymers with various properties. It is therefore likely that the complex will be fully elucidated and characterised in the near future with the further development of modern analytical techniques.

2.8.3 Factors affecting the formation and degradation of theaflavins and thearubigins

(a) Coupled oxidation

The catechins will undergo rapid redox equilibration after they are enzymatically oxidized to their respective *o*-quinones because they are extremely effective electron carriers (Roberts, 1983b). This process will create an imbalance of simple quinones relative to gallocatechin quinones drastically affecting the formation of the theaflavins which ideally require equal concentrations of di- and trihydrolated catechins. These factors are fundamental in directing the majority of the catechins, particularly the gallocatechins into the thearubigin fractions. Theaflavins reduction can further be exacerbated by inherently lower levels of simple catechins relative to gallocatechins in green tea shoots (Robertson, 1983b). As shown in Figure 19 below, any factor which increases the rate of catechin quinone formation above that of redox equilibration will also increase the ratio of simple to gallocatechin quinones and thus enhance theaflavins formation. Oxygen concentration, polyphenol oxidase activity, changes in the concentrations of the individual catechins, pH and temperature, have all been shown to affect these rate constants (Robertson, 1983b).

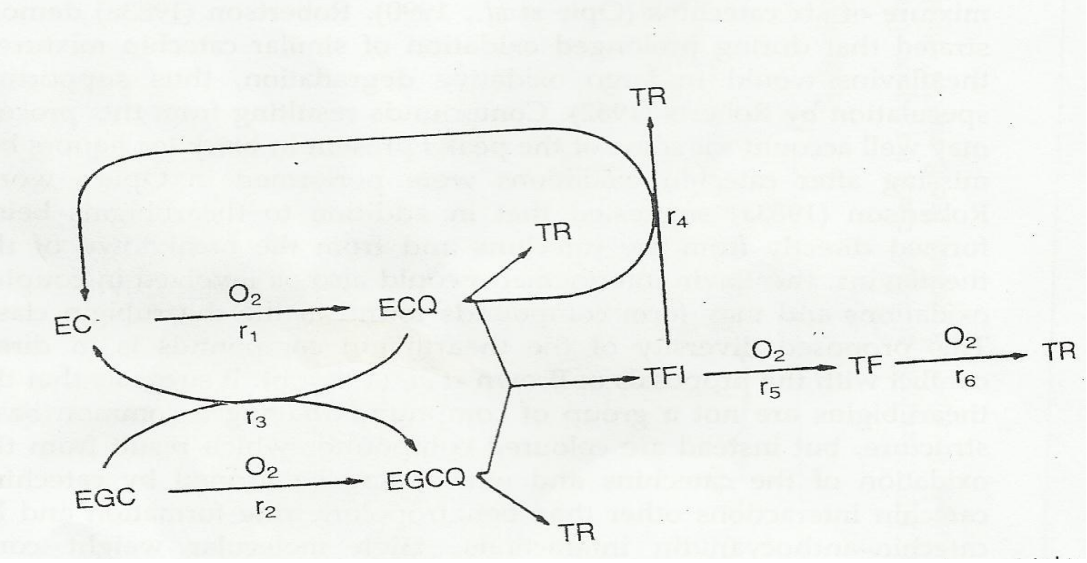


Figure 7: Scheme showing the formation of theaflavin and thearubigin from catechins. (Robertson, 1983b).

Where EC = Epicatechin; EGC = Epigallocatechin; ECQ = Epicatechin quinones; EGCQ = Epigallocatechin quinone; TF I = Theaflavin intermediate; TF = Theaflavin; TR = Thearubigin.

(b) Oxygen

Oxygen is consumed both in catechin quinone, subsequent benzotropolone formation, as well as in the oxidative degradation of the theaflavins. For theaflavins formation to occur oxygen is required to support both quinones and benzotropolone ring formation and therefore maximum synthesis of theaflavins occurs only when excess oxygen is available (Robertson, 1992). Due to polyphenol oxidase preferential demand for oxygen, under limiting oxygen concentration, theaflavin formation can be inhibited at the expense of catechin quinone formation. Competition for oxygen is noticeable during early stages of aeration when the concentration of the catechins is at its highest and enzyme turnover is unimpeded by substrate availability (Robertson, 1992). At this stage, thearubigins formation, mainly from the gallocatechins, will predominate since the simple catechins are unable to react in benzotropolone formation and redox equilibration therefore predominates. If oxygen tension is very low, substrate turnover by polyphenol oxidase will decrease. The rate of redox equilibration with respect to that of catechin oxidation will be more rapid and the steady concentration of simple catechin quinones will drop. In this situation little theaflavins or theaflavins intermediates can be synthesized and the major source of thearubigin compounds will be from the gallocatechins (Robertson, 1992).

(c) Temperature

Significant interactions exist between aeration duration, temperature and all plain black tea quality parameters as reported by Owour and Obanda (2001). Obanda *et al*, (2001), found out that theaflavins formed over time was dependent on temperature. Ngure *et al***.,** (2008) showed that unequal depletion ratios of di- and trihydroxylated catechins led to a decline in total theaflavins and an increase in thearubigin levels. An equitable decline in both groups of catechins corresponded to a subsequent rise in theaflavins content. The decline in the catechins levels was much faster at higher temperature resulting in a shorter aeration time to achieve a peak of the theaflavins content. In the same study by Ngure *et al.,* (2008), it was found out that raising the aeration temperature increases enzymatic oxidation leading to a faster depletion of all catechins. Thearubigins percentage increases with aeration temperature and duration. The rate of formation of chemical quality parameters within aeration temperature and time is clonal dependant (Ngure *et al.,* 2008). At low temperature, redox equilibration plays the lead role with the steady state concentration of simple catechin quinones being dependent on polyphenol oxidase activity. Tea shoots, inherently low in polyphenol oxidase, may therefore produce improved quality black tea at higher temperatures, while those having high enzyme activity will produce higher theaflavins to thearubigin ratios provided enzyme concentration is not so high as to restrict the availability of oxygen for benzotropolone ring formation.

(d) pH

Aeration pH has a major effect on the composition of the pigmented polyphenols with theaflavins and thearubigins formation having specific optimum pH at 5 and 6, respectively (Robertson, 1983a). This effect is due to the availability of simple catechin quinones and is facilitated by virtue of differences in enzyme substrate specificity at these two pH values and possibly redox equilibration reactions.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Samples collection from the field

Fresh tea leaves for the study were plucked from a Clonal Field Trial (CFT), from the Kangaita sub- station field of TRFK planted in 1999 at Kirinyaga, (0º26" south and 37º 15" east elevation 2020 m above mean sea level). The CFT consisted of all the commercial clones and putative test clones planted in three replicated plots. Most commercial clones are the released standard clones while the putative test clones are the ones under study yet to be released to tea farmers.

3.2 Tea manufacture

Young tender shoots of the youngest two leaves and a bud were harvested and processed into green and black tea. Green tea (unaerated) were manufactured by steaming the leaf at 100^{o}C for 1 min, macerated and dried at 120 – 150 ^{o}C in a fluidized bed drier (Tea Craft, UK) for 30 min. Black tea was manufactured by physically withering the leaf for 18 – 22 h to reduce moisture content to between 50 % and 65 % and macerated using a Crush, Tear and Curl (CTC) machine. The crushed leaf was then aerated for 90 min and then dried at the same temperature as for green tea. Both tea products were then milled and stored at 25^{o}C in sealed aluminium packets for further analysis.

3.3 Equipment, apparatus and materials

The following equipment were used; analytical balance capable of weighing to an accuracy of ± 0.001 g, vortex mixer, volumetric flasks, stainless steel electric kettle, 475ml thermos flasks, test tubes, conical flasks, pipettes, measuring cylinders , aluminium dishes, Cecil Digital grating spectrophotometer CE393 series 2 (Cambridge, England), aluminium dishes, mechanical shaker, hot plate, Whatman 541 filter papers, markers, cotton wool, aluminium lined sachets, centrifuge (capable of 2000 Relative Centrifugal Force (typically 3500 r/min), extraction tubes, HPLC , vials, oven at 103 ± 2^{o}C and magnetic stirrer

Figure 8: Modern steaming vessel used in green tea manufacture

3.4 Reagents

The following reagents were used; Isobutyl methyl ketone (IBMK), ethanol, oxalic acid, distilled water, methanol. Flavognost reagent, ethyl acetate, Sodium hydrogen carbonate (1% and 2.5%), anti-bumping granules, 1 % acetic acid, acetonitrile, Folin-Ciocalteu phenol reagent, Gallic acid, Sodium carbonate, L – theanine standard and caffeine standard

3.5 Determination of total polyphenols

Tea sample of a coarse granular structure were milled before analysis. A sample weighing 2 g was put on a pre-weighed aluminium dish and left for 16 hours in an oven at 103^{o}C ± 2^{o}C to dry for the determination of dry matter. For analysis, 0.2g was weighed into an extraction tube. Five millilitres of hot 70 % v/v methanol/distilled water was dispensed into the sample as an extraction mixture and vortexed. Heating of the extraction tube was continued in the water bath maintained at 70^{o}C for 10 minutes with mixing in the vortex mixer after every 5 minutes (the sample is vortexed at zero minute, after 5 minutes and after 10 minutes). The samples were then centrifuged at 3500 revolution per minute (rpm) for 10

minutes. The supernatant was decanted into a graduated tube and the extraction procedure repeated. The extracts were combined and made up to 10ml with cold 70 % methanol/water mixture.

The Folin-Ciocalteu phenol reagent method was used to determine total polyphenols as described by Pourmorad *et al., (*2006).The reagent was used because it contains phosphotungistic acid as oxidants. One millilitre of the sample extract was transferred to a 100ml volumetric flask and topped to the mark with distilled water and mixed. One millilitre of the diluted sample extract was transferred in duplicate into separate tubes. Five millilitre of ten percent (10 % v/v) of dilute Folin-Ciocalteu reagent was pipetted into each tube and mixed. Within 3 - 8 minutes after addition of the Folin-Ciocalteu phenol reagent, 4ml of 7.5 % w/v sodium carbonate solution was added to each tube then a stopper was fitted and mixed well. The mixture was allowed to stand at room temperature for 60 minutes and then optical densities (OD) measured using a CE 393 Cecil digital grating spectrophotometer set at 765nm. A calibration curve was obtained for gallic acid over a concentration range of 10µg/ml to 50µg/ml. The OD readings of the test samples were referenced to the calibration curve to determine the total polyphenols content of the tea samples.

3.6 Determination of catechins

Sample preparation procedure is just like for total polyphenols as described above. A modified HPLC method of (Zuo *et al.*, 2002) was used to assay for the tea catechins. A Shimadzu LC 20 AT HPLC system fitted with a SIL 20A auto sampler and a SPD-20 UV visible detector with a class LC 10 chromatography workstation was used for analysis of the prepared samples. A Gemini 5 Um C6- phenyl, 250mm x 4.6 mm (Phenomenex, Torrance, CA, USA) separation column with a Reodyne precolumn filter disk was used. A gradient elution was carried out using the following solvent system: Mobile phase A (acetonitrile/acetic acid/ double distilled water – 9/2/89 v/v/v), mobile phase B (acetonitrile/acetic acid/ double distilled water – 80/2/18 v/v/v). The mobile phase composition for a binary gradient condition started at 100% solvent A for 10 minutes then over 15 minutes a linear gradient to 60 % mobile phase A ,32 % mobile phase B and held at this composition for 7 minutes. The condition was reset to 100 % mobile phase A and then allowed to equilibrate for 10 minutes before the next injection. The flow rate of mobile phase was 1 ml/min and the temperature in the column was maintained at 35 ± 0.5^{0}C.

The identification of individual catechins was carried out by comparing the retention times and UV – absorbance of unknown peaks with peaks obtained from the mixed known catechin standards under the same conditions. The quantification of catechins was performed at 278nm and was achieved using a caffeine standard with a calibration curve $R^2 = 0.9984$ in conjunction with the consensus individual catechin relative response factor (RRF) values with respect to caffeine calculated on a dry matter basis. Total catechin as percentage by mass on a sample dry matter basis was given on the summation of individual catechins.

% Total catechins = [% ECG + % EC + % ECCG + % C] content

3.7 Determination of theanine in green tea

One gram of finely ground green tea sample was weighed into a 200 ml beaker and 100ml of boiling double distilled water added then allowed to brew for 5 minutes in a magnetic stirrer (500 rpm). The tea brew was allowed to cool down and made to volume with cold double distilled water before putting the extract in vials ready for analysis.

Standard stock solution- 50mg of pure L-theanine was weighed into a 50ml volumetric flask and dissolved with double distilled water by aid of sonication and made to volume with double distilled water. This stock solution was diluted with double distilled water to prepare the standard working solutions in the concentration range of 20-80 mg/L.

An ISO developed HPLC method was used to essay for the green tea theanine. A Shimadzu LC 20 AT HPLC system fitted with SIL 20A auto sampler and a SPD-20 UV visible detector with a class LC 10 chromatography workstation was used for the analysis. A reverse phase 18 column (phenomenex Aqua 250 x 4.6 mm i.d) was used. Injection volume was 20µL and detection wavelength was 210nm. A gradient elution was carried out using the following solvent system: Mobile phase A (double distilled water), mobile phase B (100 % acetonitrile). Analysis time was 10 minutes with 100 % mobile phase A, and then the next 8 minutes was wash time with 20 % mobile phase A and 80 % mobile phase B. The next 20 minutes was conditioning with 100 % mobile phase A before the next injection. The flow rate of mobile phase was 1ml per minute and the temperature in the column was maintained at 35 ± 0.5^0C. Theanine peak eluted at the sixth minute after injection. To calculate theanine, a theanine calibration graph was drawn using concentration of theanine in the working solutions against theanine peak area and calibration line slope and intercept value (m and b

as in y=mx + b) were obtained. The theanine content, $W_{theanine}$, expressed as a percentage by mass on a sample dry matter basis, is given by the following formula;

$W_{theanine} = [(A_{sample} - b_{intercept}) \times V_{sample} \times d \times 100] / [m_{std} \times M_{sample} \times 10000 \times w_{DM,sample}]$

Where

A_{sample} is the peak area obtained for the sample test solution;

$b_{intercept}$ is the y – intercept;

m_{std} is the slope obtained from the best – fit linear calibration;

M_{sample} is the mass, in grams of the sample test portions;

d is the dilution factor used prior to the injection on to an HLPC;

$w_{DMsample}$ is the dry matter content, expressed as a mass fraction in percent of the test sample determined as; 2 grams of the sample was put into aluminium dishes and heated in an oven at 103 ± 2^0 C for 16 hours to constant weight. The percentage dry matter (DM) in the sample was then calculated when all the moisture has been removed. Dry matter was necessary since all the parameters are expressed on dry weight basis.

3.8 Sensory evaluation of green tea

Five grams of the sample were transferred to infusion cup and boiling water added, covered and left to steep for five minutes. It was then filtered into infusion bowl and the residue (infusion) was collected on the infusion cup lid. Both the liquor and infusion were then analysed accordingly. The liquor was sipped by the taster with a spontaneous breath which brought the liquor in contact with the tongue and other parts of the mouth, sensitive to astringency and flavour. The tasters were allowed to describe the quality then scoring was done using the score sheet shown in Appendix I.

3.9 Testing of plain black tea quality parameters

3.9.1 Dry matter content

Black tea weighing approximately 2 grams was put into aluminium dishes and heated in an oven at 103 ± 2^0 C for 16 hours to constant weight. The percentage dry matter (DM) in the sample was then calculated when all the moisture has been removed. Dry matter was necessary since all the parameters are expressed on dry weight basis.

3.9.2 Total theaflavins content analysis

Flavognost method (Hilton, 1973), was used to determine total theaflavins content. A tea infusion was made by adding 375 ml boiling distilled water into a vacuum flask with 9 g of black tea and shaken for ten minutes in a mechanical shaker. The infusion was filtered through a cotton wool into a flat bottomed flask and allowed to cool to room temperature before pipetting 10 ml of it into a 25 ml volumetric flask. Addition of 10 ml of IBMK was done before shaking for 15 minutes and the mixture transferred to a test tube so as to allow layers to separate. From the upper layer, 2 ml was pipetted into a test tube then 4 ml ethanol and 2 ml flavognost reagent was added and the mixture was shaken in a vortex mixer for 2 minutes before allowing colour development for 15 minutes. Absorbance was read at 625 nm after setting the machine with blank of ethanol/IBMK (1: 1 v/v).

Theaflavin (µmol/g) = A_{625nm} x 47.9 x 100/DM

The factor 47.9 is a conversion factor attributed to the dilution effect in theaflavins analysis

3.9.3 Individual theaflavins ratios determination

High performance liquid chromatography (HPLC) with reverse phase C18 ODS column was used to determine the ratios of individual theaflavins (Mcdowell *et al.,* 1991). Using a 475ml thermos flask, 4g of milled black made tea was weighed and a tea infusion was made with 200ml boiling double distilled water. Shaking for ten minutes was done before filtering through a cotton wool into a 250ml conical flask. Dilution of 1:1 with double distilled water was done then 20 µL was injected onto HPLC. The wavelength was set at 378nm and flow rate was set at 1.5ml/min. Solvent A was 1% acetic acid while acetonitrile was solvent B. A linear gradient from 8 % to 31% solvent B over 60 minutes was used (Bailey *et al.,* 1990). The total amount of theaflavins was allocated to the individual theaflavins, according to the ratios determined by HPLC, assuming the molar absorption coefficients of the four theaflavins are similar at 378nm (Steinhaus and Engelhardt, 1989).
Individual theaflavin in µmoles/g = (its peak area/Total peak area)* TFµmoles/g of that clone determined by Flavognost method.

3.9.4 Total colour measurement

The tea infusion of 1ml (9 g black tea in 375ml boiling distilled water in a thermos flask) was mixed with 9ml of distilled water and topped with methanol in a 25ml volumetric

flask. Its optical density (E) was read at 460nm using the 1ml cell. Distilled water was used as blank.

% Total colour = 6.25 x 4E x DM %

3.9.5 Total thearubigins (TR) and fractions (TR S1 and TR S11) measurement

Roberts and Smith method (1963) was used to determine total thearubigins. Four solutions were made (solution A, B, C and D). First, 50ml of the cooled, well shaken and filtered tea infusion was mixed with 50ml IBMK and gently shaken to avoid formation of an emulsion and the layers were allowed to separate. **Solution A,** a 4ml portion of the IBMK layer was taken and made up to 25ml with methanol in a volumetric flask. **Solution B,** 2ml portion of the aqueous (lower layer) was diluted with 8ml distilled water and made up to 25ml with methanol. **Solution C,** 25ml portion of the IBMK layer was mixed with 25ml of freshly prepared 2.5 % aqueous sodium hydrogen carbonate. The mixture was shaken vigorously and the layers were allowed to separate before discarding the lower layer. A 4ml portion of washed IBMK layer was made up to 25ml with methanol. **Solution D,** 2ml portion of the aqueous layer was diluted with 6ml distilled water and 2ml of a saturated oxalic acid added before topping to 25 ml with methanol in a volumetric flask.

The absorbance A_A,A_B,A_C and A_D of solution A,B,C and D, respectively were read at 380nm and 460nm using a Cecil Digital grating spectrophotometer with distilled water as the blank. The mean absorbance of TR fractions at 380nm is 0.733 (Roberts and Smith, 1963). The value A_A-A_C represents the absorbance due to the IBMK- solution free acid thearubigins S1 type for which $A^{0.2\%}_{460nm} = 0.138$.

At 460nm and following the above partitioning procedures

% TR S1 = (375 x 0.02 x 6.25 [$A_A - A_C$])/(0.138 x 9 x DM/100)

The value A_B represents the absorbance of the IBMK-insoluble thearubigins of S11 type and after acidification with oxalic acid, this change to A_D. These acidified S11 type thearubigins has $A^{0.2\%}_{460nm}$ of 0.233, and are deeply coloured than the S1 type (Roberts and Smith,1963).

Hence at 460nm;

% TR S11 = (375 x 0.02 x 12.5 A_D)/(0.233 x 9 x DM/100).

Total thearubigins were calculated using a method described by Obanda *et al.*, (2001) as;

At 380nm,

% Total TR = (375 x 0.02 x 6.25 [$2A_D + A_A - A_C$]) / (0.733 x 9 x DM/100)

3.9.6 Determination of liquor brightness

The above procedure used in determining total thearubigins was followed where four solutions were made (A, B, C and D). Their corresponding absorbances were read at 460nm. Brightness percentage was calculated as follows;

Brightness % = (100 x A_C)/ (A_A + 2A_B) (Obanda *et al.,* 2001). Where $A_{A,}$ A_B and A_C are the absorbance of solution A, B and C respectively read at 460nm.

3.9.7 Determination of total soluble solids (TSS) in black tea (Beverages method)

Made black tea of 2g was weighed into a 500ml conical flask and a few anti-bumping granules and 200ml of boiling distilled water was added to the flask. The flask was refluxed gently on a hot plate for one hour, swirling occasionally. The infusion was then filtered through a Whatman 541 filter paper carefully to avoid infused leaf going with the liquor to hasten filtration. The hotter the liquor, the faster was the filtration. Ambient temperatures also influence the rate of filtration, the warmer the faster the filtration. Hot distilled water was used to rinse out the liquor until there was no liquor seen in the flask used in refluxing. The filtrate was allowed to cool to room temperature then transferred to a 250ml volumetric flask and made to the volume with cold distilled water. Thorough mixing was done before 50ml was drawn and put in an already weighed beaker (W1) and placed in an oven at $100^oC \pm 2^0C$ and left for 16 hours. The beaker was then transferred to a desiccator to allow it to cool and then weighed to the nearest milligram (W2).

% TSS = (W2-W1) x (250/50/2) x 100 x (100/DM)

DM = Dry matter

3.9.8 Residual catechins in black tea

A modified HPLC method of Zuo *et al.,* (2002) was used to assay for the tea catechins. A Shimadzu LC 20 AT HPLC system fitted with a SIL 20A auto sampler and a SPD-20 UV visible detector with a class LC 10 chromatography workstation was used for analysis of the prepared samples. A Gemini 5 Um C6- phenyl, 250mm x 4.6 mm (Phenomenex, Torrance, CA, USA) separation column with a Reodyne precolumn filter disk was used. A gradient elution was carried out using the following solvent system: Mobile phase A (acetonitrile/acetic acid/ double distilled water – 9/2/89 v/v/v), mobile phase B (acetonitrile/acetic acid/ double distilled water – 80/2/18 v/v/v). The mobile phase composition for a binary gradient condition started at 100% solvent A for 10 minutes then

over 15 minutes a linear gradient to 60 % mobile phase A ,32 % mobile phase B and held at this composition for 7 minutes. The condition was reset to 100 % mobile phase A and then allowed to equilibrate for 10 minutes before the next injection. The flow rate of mobile phase was 1 ml/min and the temperature in the column was maintained at 35 ± 0.5^{0}C.

The identification of individual catechins was carried out by comparing the retention times and UV – absorbance of unknown peaks with peaks obtained from the mixed known catechin standards under the same conditions. The quantification of catechins was performed at 278nm and was achieved using a caffeine standard with a calibration curve $R^2 = 0.9984$ in conjunction with the consensus individual catechin relative response factor (RRF) values with respect to caffeine calculated on a dry matter basis. Total catechin as percentage by mass on a sample dry matter basis was got by summing individual catechins as shown below.

% Total catechins = [% ECG + % EC + % ECCG + % C] content

3.9.9 Sensory evaluation of black ea

Five grams of the sample were transferred to infusion cup and boiling water added, covered and left to steep for five minutes. It was then filtered into infusion bowl and the residue (infusion) was collected on the infusion cup lid. Both the liquor and infusion were then analysed accordingly. The liquor was sipped by the taster with a spontaneous breath which brought the liquor in contact with the tongue and other parts of the mouth, sensitive to astringency and flavour. The tasters were allowed to describe the quality then scoring was done using the score sheet shown in Appendix II.

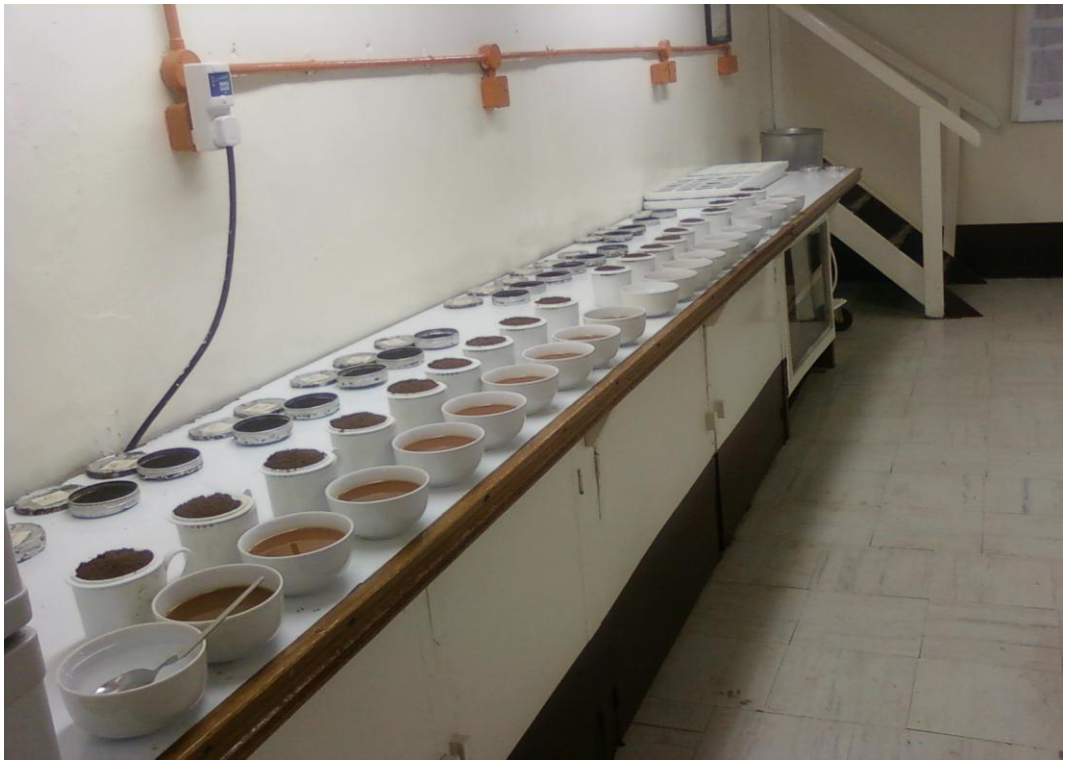


Figure 9: Display of ready- to-taste tea in a tea tasting room

3.10 Experimental design

Leaf for manufacture for each clone was randomly plucked from the three plots. The manufacture was repeated three times in order to have replicated treatments. Completely randomized design with three replications was adopted for this experiment. Data analysis on all chemical parameters was done using General Linear Model, Correlation procedures of Statistical Analysis System (SAS, version 9.1). Data on sensory evaluation were subjected to analysis of variance using MSTAT version 2.10. Means were separated using Duncan multiple range test.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.0 Introduction

The results are presented graphically in a descending order for ease of comprehension whenever necessary and the test clones are shaded purple (light) while the commercial clones are shaded black (dark) in all the results. Yabukita variety from Japan was used as a control in green tea experiment, while TRFK 6/8 was used in black tea experiment because of their respective good green and black tea quality.

4.1.1 Total polyphenols

Total polyphenols (TPP) results are presented in Figure 7 and Appendix VII. There were significant differences ($p \leq 0.05$) observed in TPP. All the test clones had a mean value above 20 % total polyphenols. Among the commercial clones, GW Ejulu and TRFK 6/8 showed relatively higher mean values of TPP % with 27.27 % and 26.54 % respectively. Most commercial clones which are known to produce good black tea like TRFK 301/1, TRFK 303/577, and TRFK 303/216 also showed relatively high mean values of TPP. Yabukita and Hanlu tea varieties which are green varieties used in Japanese and Chinese, respectively showed the lowest TPP. Clones K-Purple and TRFK 91/1 showed relatively higher mean values of TPP among the test clones with 25.86 % and 23.87 % TPP, respectively. These values are higher than some commercial clones mean values.

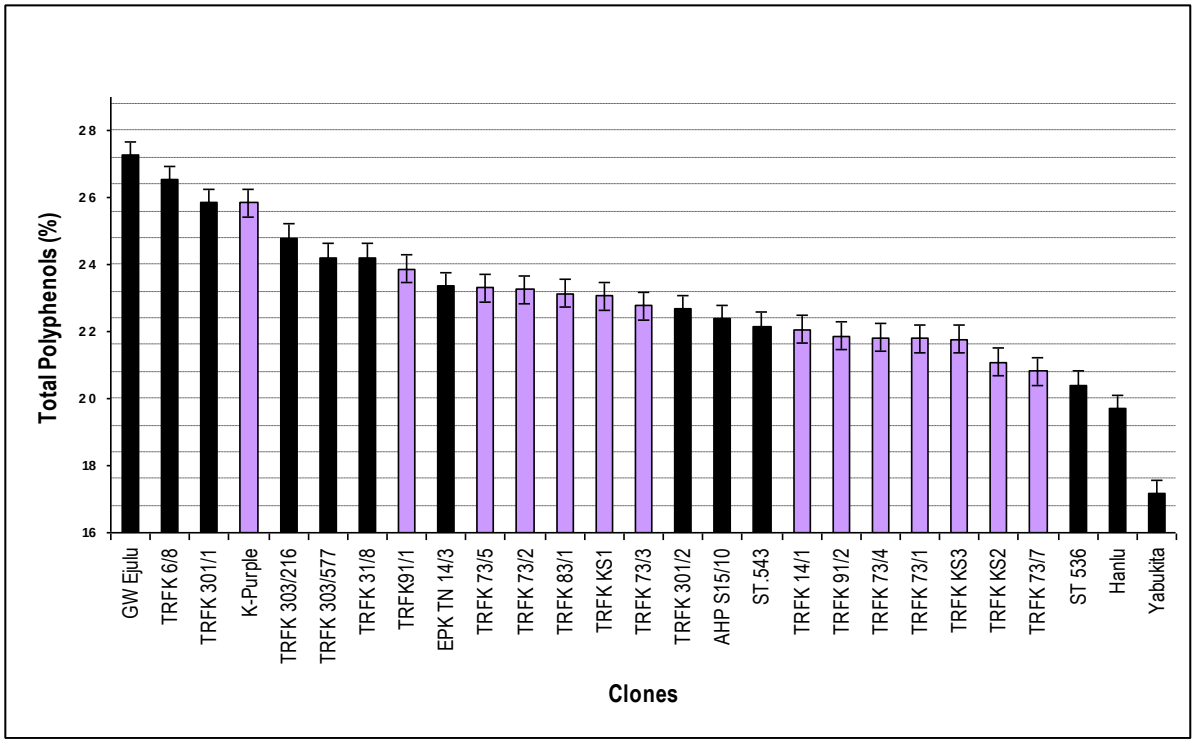


Figure 10: Total polyphenols content in the studied clones/varieties

4.1.2 Total catechins

The results are presented in Figure 8 and Appendix VIII. There were significant differences ($p \leq 0.05$) noted in total catechins. Some test clones like; TRFK 73/4, TRFK 73/7, TRFK 73/1, TRFK 83/1, TRFK 73/2, TRFK KS 2 and TRFK KS 3 showed higher mean values among the test clones than Yabukita clone. The rest of the test clones showed lower mean values than the standard clone. Most test clones showed mean values above 8.5 % total catechins except clone TRFK 73/3 which had relatively low total catechins. Clone TRFK 301/2 showed the highest mean value of total catechins with 13.79 % among the commercial clones. Hanlu had relatively high mean value of total catechins than Yabukita. Clone TRFK 6/8 and TRFK 303/577 had low total catechins though with high TPP.

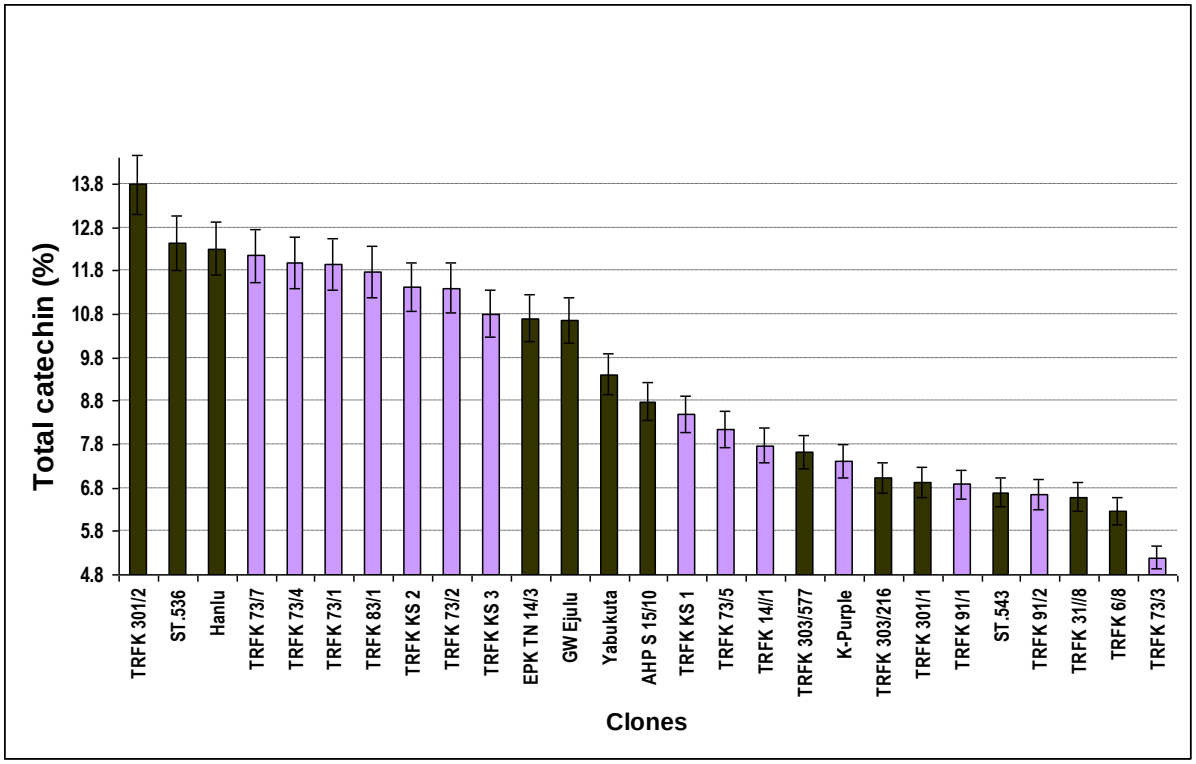


Figure 11: Clonal variations in total catechins in the studied clones/varieties

4.1.3 Non gallated catechins

Free catechins include Epigallocatechins (EGC) and Epicatechins (EC) and the results are presented in Figures 9, 10, 11 and in Appendix VIII. There were significant differences ($P< 0.05$) observed in EGC among the studied clones. Some test clones like TRFK 83/1, TRFK 73/2, TRFK 73/7 and TRFK 73/1 had higher mean values of EGC than the standard clone Yabukita. GW Ejulu had the lowest EGC among the commercial clones. Clones TRFK 91/2, TRFK 91/1 and K-Purple showed the lowest mean values of EGC with 1.3 %, 0.46 % and 0.38 %, respectively. Significant difference ($P< 0.05$) was also noted in EC mean values. Most test clones had higher EC than the standard clone Yabukita except clones TRFK 83/1, TRFK 73/5, K-Purple, TRFK KS 1, TRFK 73/3, TRFK 91/2 and TRFK 91/1. Clones TRFK 73/7 and TRFK 73/1 had relatively high mean values of EC among the test clones while TRFK 91/2 and TRFK 91/1 had low mean values. Among the commercial clones, TRFK 301/2 and ST.536 showed relatively high mean values of EC while TRFK 31/8 showed the lowest mean values.

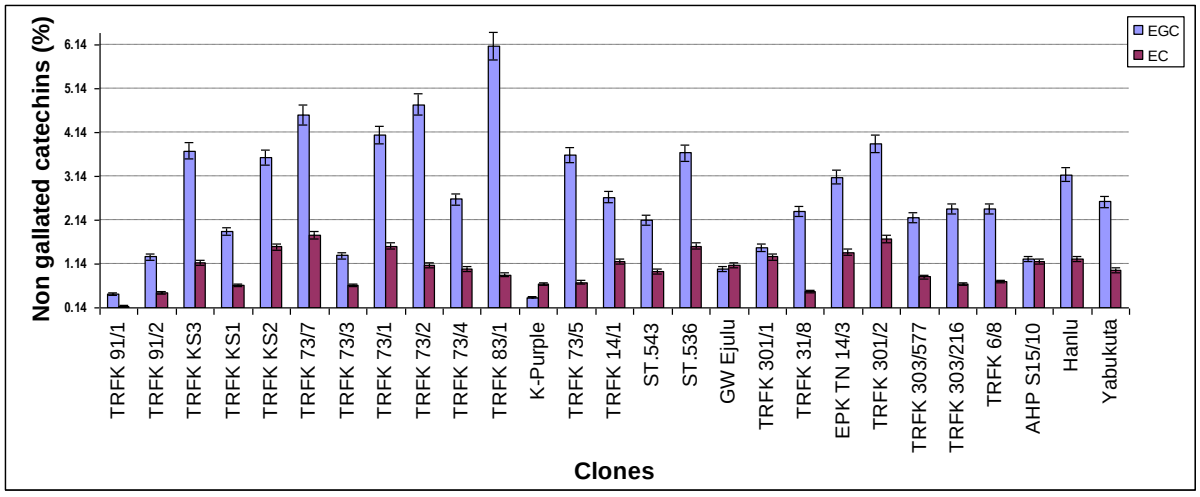


Figure 12: Non gallated catechins in the studied clones / varieties

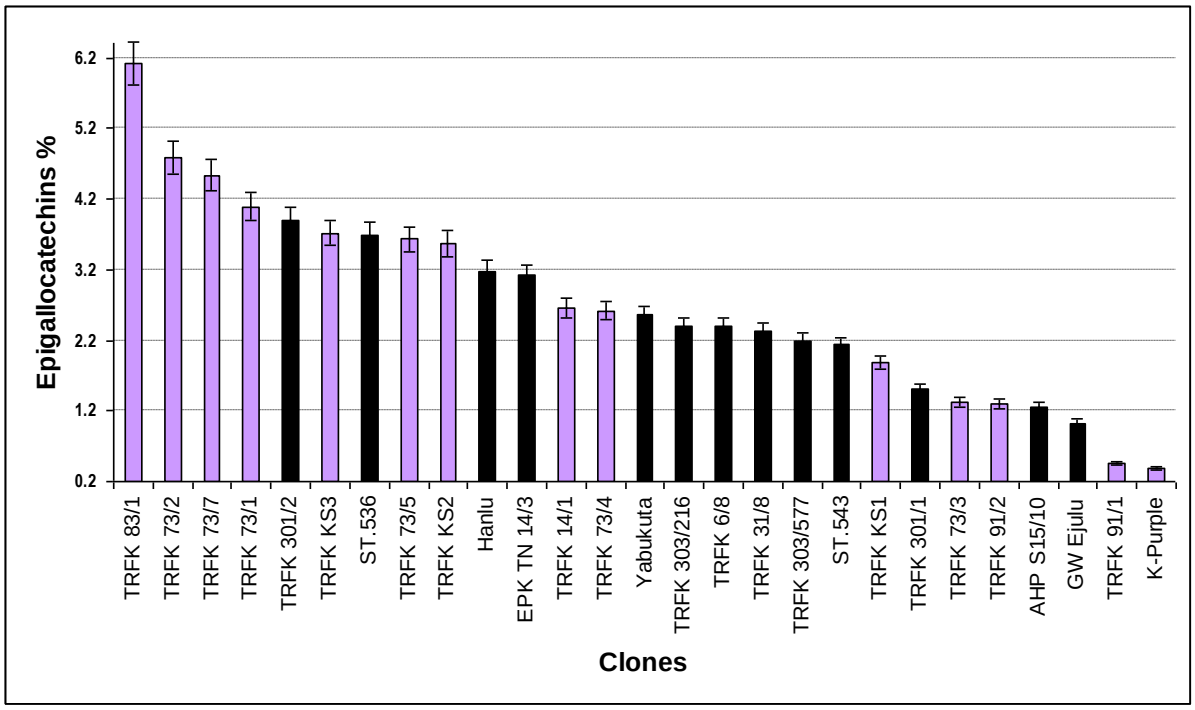


Figure 13: Epigallocatechins in the studied clones/varieties

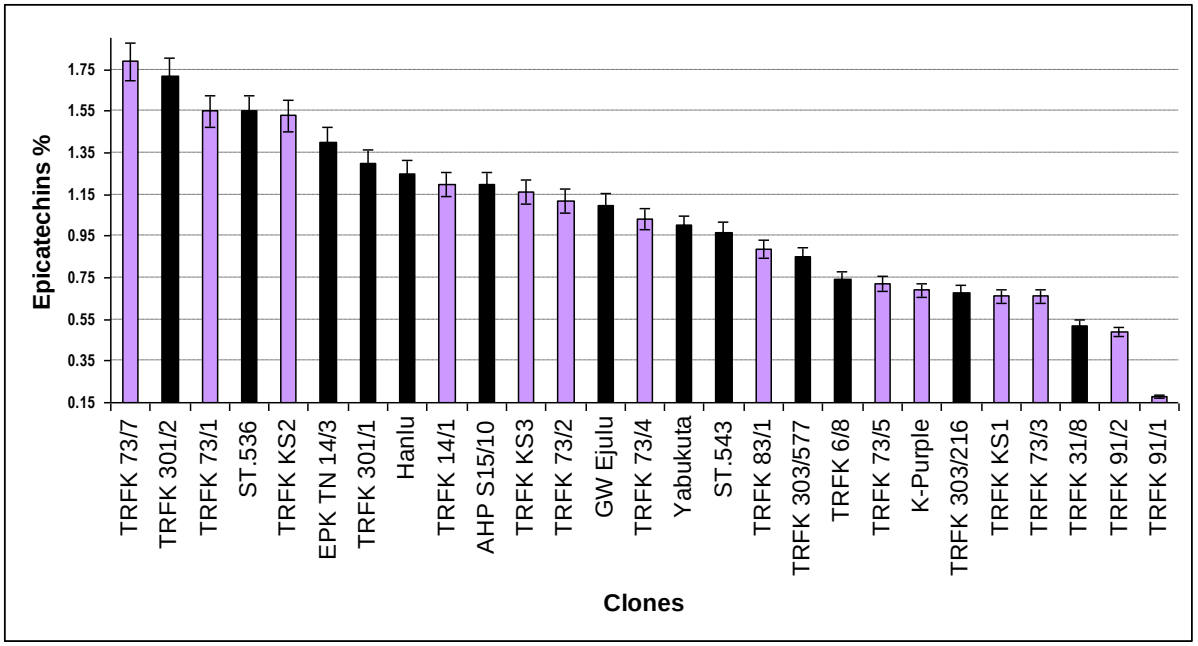


Figure 14: Epicatechins in the studied clones/varieties

4.1.4 Gallated catechins

These include Epigallocatechin gallates (EGCG) and Epicatechin gallates (ECG). The results are presented in Figures 12, 13 and 14 below and in Appendix VIII. Significant differences ($P< 0.05$) were noted in both EGCG and ECG. EGCG was the most dominant in all the clones except in K-Purple which had ECG dominating. Clone TRFK 73/4 had the highest mean value of EGCG with 6.25 % and did not differed significantly ($P< 0.05$) with TRFK 301/2 which had 5.97 %. Clone TRFK 91/1 also had relatively high EGCG but low free catechins. Some test clones like TRFK KS 3, TRFK 73/1, TRFK 91/2, TRFK 83/1, K-Purple, TRFK 14/1, TRFK 73/5 and TRFK 73/3 showed lower EGCG percent than the Yabukita clone. There were significant differences ($P< 0.05$) noted in ECG where we had clone GW Ejulu having the highest ECG content and did not differed significantly ($P< 0.05$) with K-Purple. Clones TRFK 73/7, TRFK 73/4, TRFK KS 2 and TRFK KS 3 had higher ECG content than Yabukita clone. Clone TRFK 14/1 had the lowest ECG content among the test clones while TRFK 6/8 had the lowest mean value among the commercial clones.

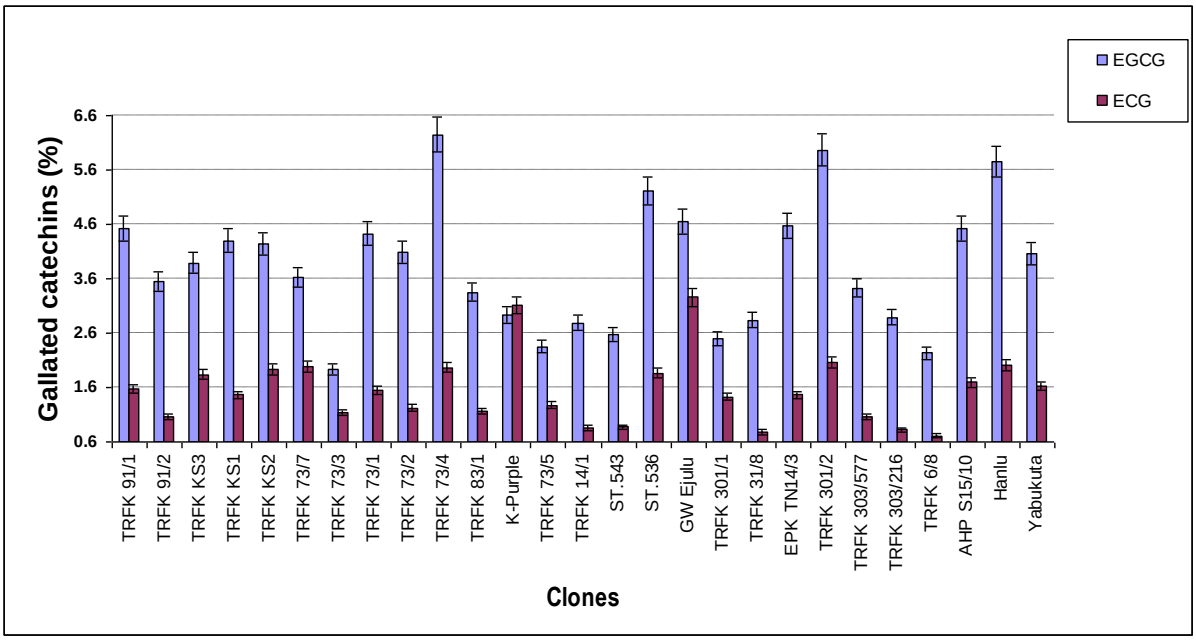


Figure 15: Gallated catechins in the studied clones/varieties

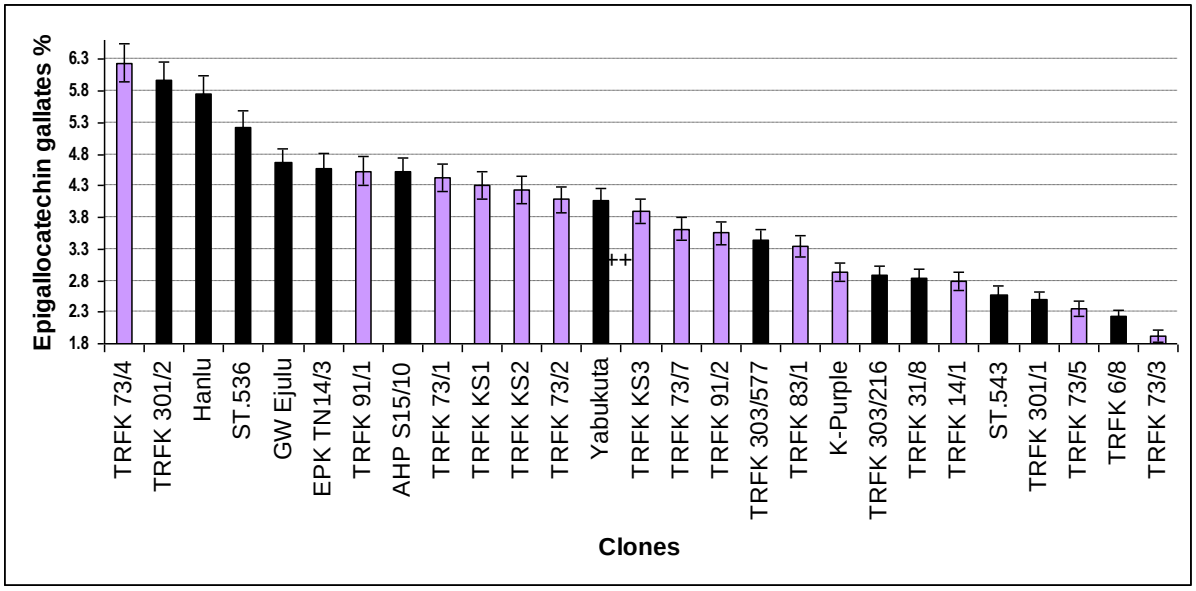


Figure 16: Epigallocatechin gallates in the studied clones/varieties

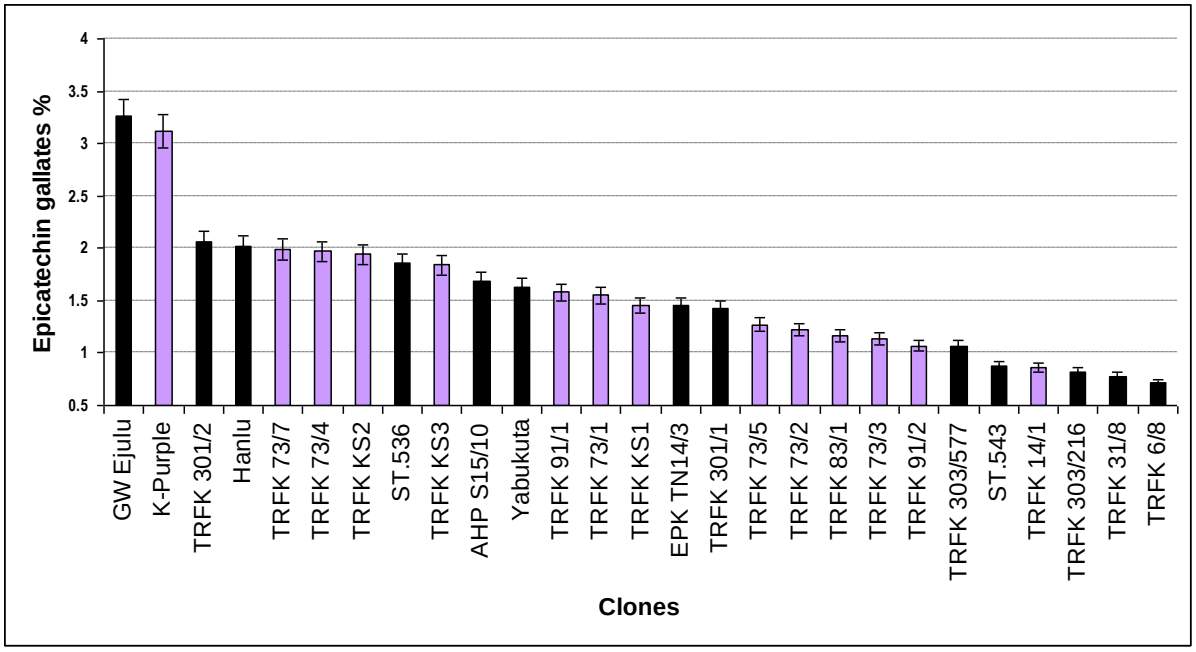


Figure 17: Epicatechin gallates in the studied clones/varieties

4.1.5 Gallic acid

The results are presented in Figure 15 below and in Appendix VIII. There were significant differences (P< 0.05) observed in gallic acid where we had TRFK 91/1 showing highest mean value of 1.09 %. Most test clones had higher mean value of gallic acid than the standard clone Yabukita except clones TRFK 73/4, TRFK 73/3 and TRFK 73/7.

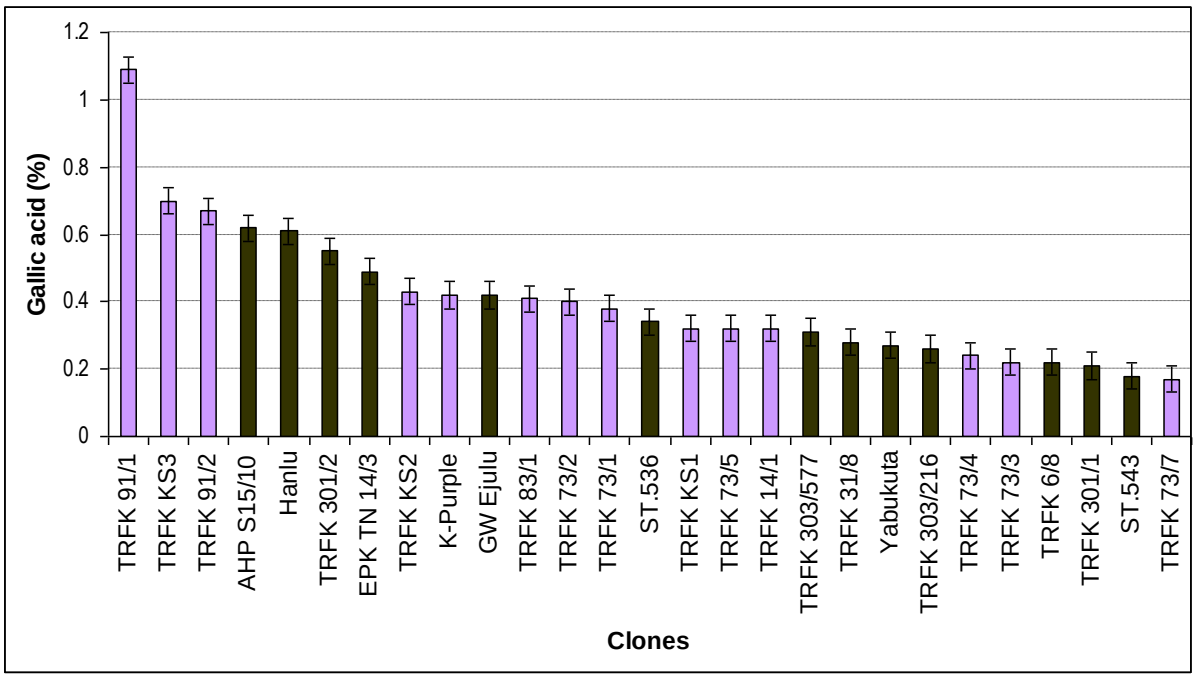


Figure 18: Gallic acid in the studied clones/varieties

4.1.6 Caffeine

Caffeine content results are presented in Figure 16 and in Appendix VIII. Significant difference (P< 0.05) was noted in caffeine content in the studied clones with clone TRFK KS 3 having the highest (2.33 %) mean value among the test clones. AHP S 15/10, EPK TN 14/3, TRFK 301/2, Hanlu and GW Ejulu had relatively higher caffeine content among the commercial clones. Most test clones had lower caffeine than Yabukita clone except clones TRFK KS 3, TRFK KS 2 and TRFK 83/1. Clone TRFK 73/5 had the lowest caffeine content with 1.16 % among the test clones but higher than TRFK 6/8 which had the lowest on the studied clones.

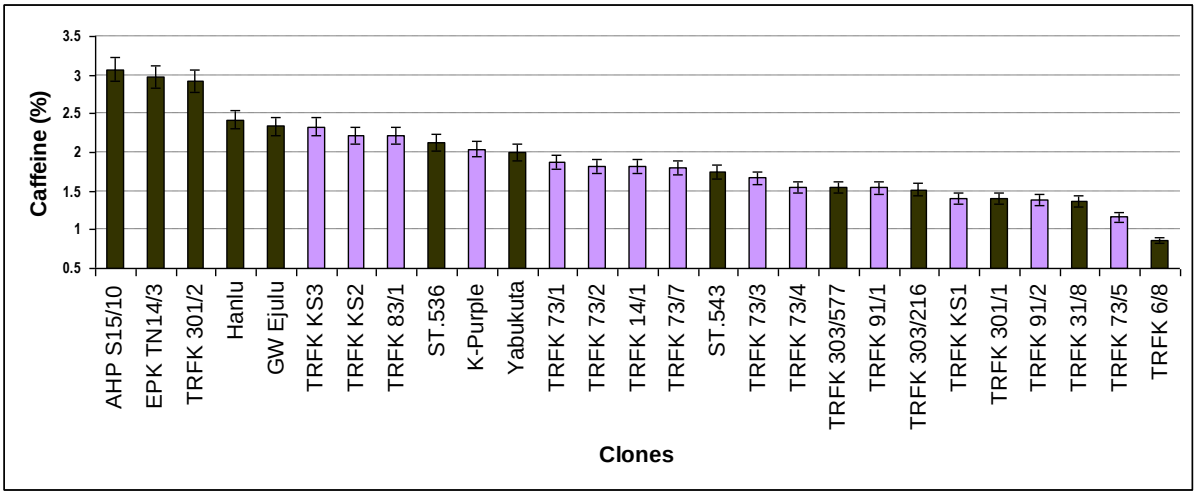


Figure 19: Caffeine content in the studied clones/varieties

4.1.7 Theanine

The results are presented in Figure 17 and Appendix VII. The results showed that most of the clones had theanine percentage above one percent on dry weight basis except clone TRFK 6/8. It can also be observed that most clones meant for black tea like TRFK 303/577 and TRFK 31/8 showed lower theanine percentage with clone TRFK 303/577 being not significantly different (P< 0.05) from TRFK 6/8. As presented in Figure 17 below, there were significant differences (P< 0.05) observed in theanine levels. Clone TRFK 91/1 had the highest level theanine with 2.18 % followed by TRFK KS 3, TRFK 73/3 among the test clones. Clone TRFK 301/1 had relatively higher percentage of theanine among the commercial clones and it also showed good results as a black tea. Clones TRFK 83/1 and K-Purple showed relatively low levels of theanine with 1.1 % each.

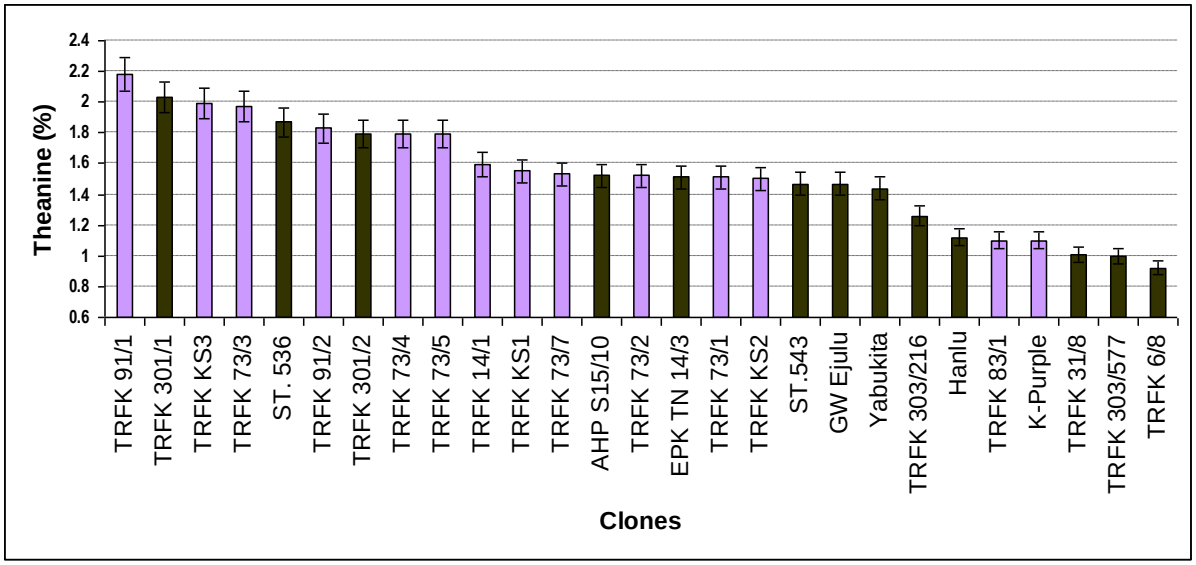


Figure 20: Theanine content in the studied clones/varieties

4.1.8 Green tea sensory evaluation

The results are presented in Table 1. Significant difference (P< 0.05) was observed in all the tea tasting parameters accept in liquor body. The samples were coded 1-27 such that a taster could not know which clone is which but we had our reference. Green tea taster could distinguish TRFK KS 1 as a purple tea. From the lab results, clone TRFK KS I had average mean values of catechins, TPP and even theanine but its taste was distinctly of purple tea. Clones TRFK 91/1, ST.536, TRFK 73/3 and TRFK 73/4 were said to have some flavour. The above first three clones were also noted to have flavour in black tea. Their flavoury characteristic could be attributed to relatively high levels of theanine as seen from the results. Most liquors both of test and commercial clones were thick, strong and brisk. The liquors were, however, not as green as is desired in most clones except for clones TRFK KS 2, TRFK 73/3, TRFK 73/1, TRFK 73/2, TRFK 83/1 and TRFK 14/1 which showed good green liquor. Clone TRFK 91/1, TRFK K S I, TRFK 73/7, TRFK 73/4, K-Purple and TRFK 73/5 had greenish liquors. Clones TRFK 91/2 and TRFK K S 3 had bright amber liquors. Most clones had thick liquors except clones TRFK 31/8, TRFK 303/216 and TRFK 73/4 which had fairly thick liquors. Clones TRFK 73/2 and TRFK 83/1 had very strong liquors while clones TRFK 91/1, TRFK 91/2, TRFK K S1, TRFK 73/3 and TRFK 14/1 had strong liquors. Clone TRFK 73/1 scored last in liquor strength as all commercial clones produce strong liquors. Most liquors were brisk, some were astringent like GW Ejulu, TRFK 301/1, TRFK 6/8, TRFK 91/2, TRFK KS 2, TRFK 73/3 and TRFK 73/5. Yabukita clone scored last in briskness with 3.33, others with fairly brisk liquors were, TRFK 303/216, ST. 536, TRFK 73/7 and TRFK

83/1. Most commercial clones had fairly bright liquors except clones TRFK 303/216, ST.536 which had bright infusions. Among the test clones, TRFK K S 1, TRFK K S 2, TRFK 73/7, TRFK 73/2, TRFK 83/1, K-Purple and TRFK 14/1 had bright infusions. Clone TRFK 73/4 had very bright infusions with a score of one.

Table 1: Sensory evaluation of green tea

	Liqour Colour	Liqour Body	Liquor strength	Liqour Briskness	Infusion Colour	**Average**
Commercial clones						
GW Ejulu	4.33^{ab}	2.00^{a}	2.00^{bcd}	1.00^{e}	3.17^{abc}	**2.50**
TRFK 301/1	4.33^{ab}	2.00^{a}	1.67^{cd}	1.33^{de}	3.67^{a}	**2.60**
TRFK 31/8	3.33^{bcde}	3.00^{a}	2.00^{bcd}	1.5^{de}	3.33^{ab}	**2.63**
EPK TN 14/3	5.00^{a}	2.00^{a}	2.33^{bcd}	2.00^{cd}	3.33^{ab}	**2.93**
TRFK 301/2	3.00^{bcde}	2.00^{a}	2.00^{bcd}	2.00^{cd}	3.67^{a}	**2.53**
TRFK 303/577	3.00^{bcde}	2.00^{a}	2.00^{bcd}	1.67^{cde}	3.00^{abc}	**2.33**
TRFK 303/216	3.67^{abcd}	3.00^{a}	1.67^{cd}	3.00^{ab}	2.33^{bc}	**2.73**
TRFK 6/8	4.33^{ab}	2.00^{a}	2.00^{bcd}	1.00^{e}	3.33^{ab}	**2.53**
AHP S 15/10	5.00^{a}	2.00^{a}	2.00^{bcd}	2.00^{cd}	3.00^{abc}	**2.80**
ST.543*	4.00^{abc}	2.00^{a}	2.00^{bcd}	2.00^{cd}	2.33^{bc}	**2.47**
ST.536*	2.00^{e}	2.00^{a}	2.50^{bcd}	3.00^{ab}	2.33^{bc}	**2.37**
Hanlu*	4.00^{abc}	2.00^{a}	2.50^{bcd}	2.00^{cd}	3.00^{abc}	**2.70**
Yabukita	3.00^{bcde}	2.67^{a}	2.00^{bcd}	3.33^{a}	3.33^{ab}	**2.87**
Test clones						
TRFK 91/1*	3.50^{abcde}	2.50^{a}	2.00^{bcd}	2.33^{bc}	3.67^{a}	**2.80**
TRFK 91/2	4.00^{abc}	2.00^{a}	2.00^{bcd}	1.67^{cde}	3.67^{a}	**2.67**
TRFK KS 3	4.00^{abc}	2.00^{a}	3.00^{b}	2.00^{cd}	3.67^{a}	**2.93**
TRFK KS 1	3.67^{abcd}	2.00^{a}	2.00^{bcd}	2.00^{cd}	2.00^{cd}	**2.33**
TRFK KS 2	2.00^{e}	2.00^{a}	3.00^{b}	1.00^{e}	2.00^{cd}	**2.00**
TRFK 73/7	3.50^{abcde}	2.00^{a}	3.00^{b}	3.00^{ab}	2.00^{cd}	**2.70**
TRFK 73/3*	2.00^{e}	2.67^{a}	2.00^{bcd}	1.50^{de}	3.00^{abc}	**2.23**
TRFK 73/1	2.00^{e}	2.00^{a}	5.00^{a}	2.00^{cd}	3.00^{abc}	**2.80**
TRFK 73/2	2.00^{e}	2.00^{a}	1.50^{d}	2.00^{cd}	2.33^{bc}	**1.97**
TRFK 73/4*	3.67^{abcd}	3.00^{a}	3.00^{b}	2.00^{cd}	1.00^{d}	**2.53**
TRFK 83/1*	2.33^{de}	2.00^{a}	1.50^{d}	3.00^{ab}	2.00^{cd}	**2.17**
K-purple	3.67^{abcd}	2.00^{a}	3.00^{b}	2.33^{bc}	2.33^{bc}	**2.67**

TRFK 73/5	3.67^{abcd}	2.00^{a}	3.00^{b}	1.67^{cde}	2.33^{bc}	**2.53**
TRFK 14/1	2.67^{cde}	2.50^{a}	2.00^{bcd}	2.00^{cd}	3.00^{abc}	**2.43**
CV %	**29.27**	**43.78**	**27.02**	**22.8**	**26.61**	

* Flavoury

Note : The scores ranged from 1 – 5, with 1 being the best and 5 is the poorest score.

4.1.9 Correlation analysis

Quality parameters including theanine, total polyphenols and total catechins were correlated with tasters score on liquor colour, liquor strength, liquor briskness and infusion colour. The results are presented in Table 2. There was no correlation between total catechins and liquor characteristics. A weak correlation however was observed between theanine, TPP and liquor colour. Correlation exists between infusion and theanine with correlation coefficient (r) of 0.524. A strong correlation was observed between TPP and theanine with an r-value of 0.95. Total polyphenol was also correlated with individual catechins and coefficients are presented in Table 3. Strong correlation exists between the total polyphenols and the individual catechins.

Table 2: Correlation coefficients (r) between tea quality parameters and tasters score. N = 27

	L/Colour	L/Body	L/Strength	Briskness	Infusion	Theanine	TPP	T/Catechin
L/Colour	1							
L/Body	- 0.121	1						
L/Strength	- 0.181	-0.155	1					
Briskness	- 0.203	0.197	-0.018	1				
Infusion	0.277	-0.118	-0.185	-0.262	1			
Theanine	0.477	-0.030	-0.331	-0.302	0.524*	1		
TPP	0.427	-0.044	-0.286	-0.262	0.450	0.950***	1	
T/Catechin	- 0.086	-0.238	0.353	-0.204	0.212	-0.004	0.080	1

* Correlation significant at the $p \leq 0.05$ level

*** Correlation significant at the $p \leq 0.001$ level

L/Colour = Liqour colour

L/Body = Liqour bod

L/ strength = Liqour strength

TPP = Total polyphenols

T/catechin = Total catechins

Table 3: Correlation coefficient matrix analyses between TPP and individual catechins. N=27

	EGCG	*EGC*	*ECG*	*EC*	*TPP*
EGCG	1				
EGC	0.983***	1			
ECG	0.958***	0.962***	1		
EC	0.983***	0.974***	0.941***	1	
TPP	0.961***	0.959***	0.922***	0.978***	1

*** Correlation significant at the $p \leq 0.001$ level

EGCG Epigallocatechin EGC Epigallocatechin

ECG Epicatechin gallate EC Epicatechin

TPP Total polyphenols

4.2.1 Total theaflavins

The results are presented in Figure 21 and in Appendix XI. Clone TRFK 6/8 was used as a standard clone in this set of experiment because of its proven high quality. The clones under study differed significantly ($p \leq 0.05$) in total theaflavins determined using Flavognost method. Clone TRFK 6/8 showed the highest mean value of 25.07µmol/g while TRFK 91/1 had the lowest mean value of the same with 9.54 µmol/g. Clones TRFK 303/577, EPK TN 14/3, TRFK 301/1 and TRFK 303/216 were also among the commercial clones with relatively high levels of TF. Most test clones had mean values above 16 µmol/g but clones TRFK 91/1, TRFK 73/3 had lower than 16 µmol/g values of theaflavins. TRFK KS 3 had the highest theaflavins (23.47µmol/g) among the test clones.

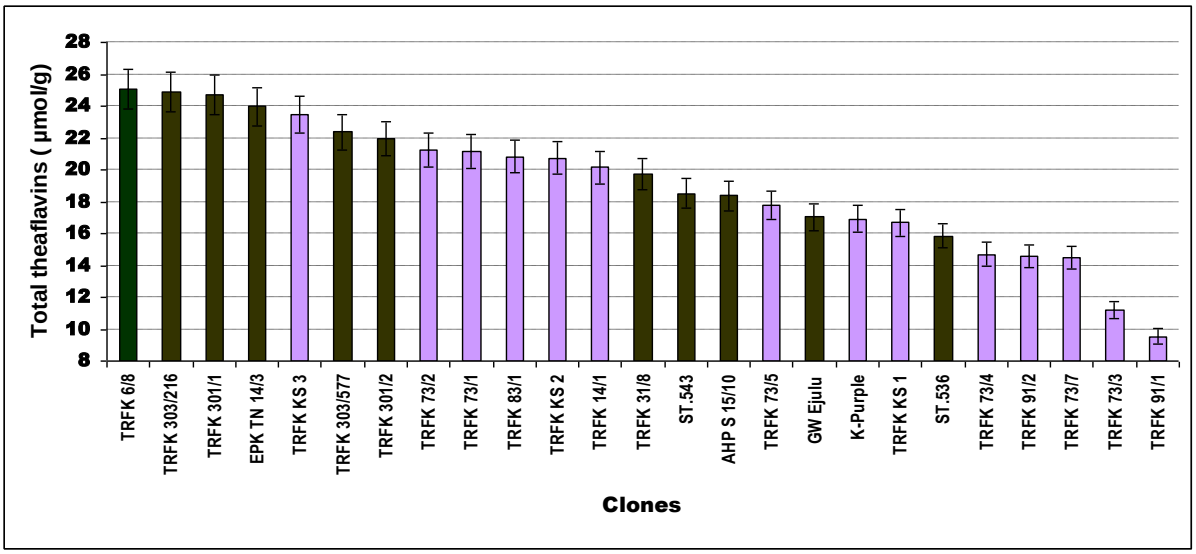


Figure 21: Total theaflavins by Flavognost method in the studied clones/varieties

4.2.2 Total colour

Results are presented in Figure 22 and in Appendix XI. There was a significant difference ($p \leq 0.05$) observed in total colour in the studied clones. Most commercial clones had better colour than the test clones. Clones TRFK 301/1 and TRFK 303/216 had high mean values of total colour among the commercial clones while clones ST.536 and ST.543 showed the lowest total colour. Most test clones had colour values below 5 % total colour with clone TRFK KS 3 showing the highest mean value with 5.72 % total colour.

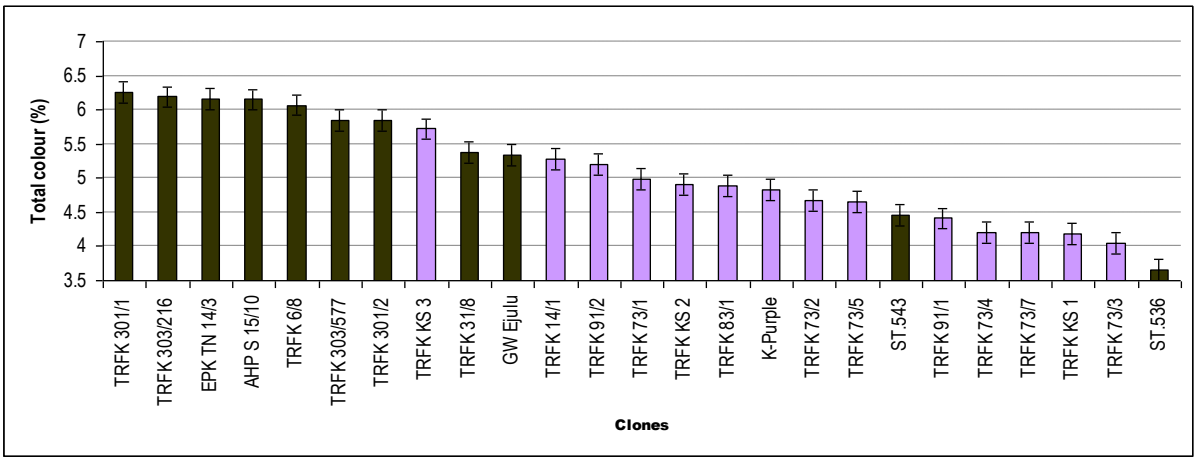


Figure 22: Total colour in the studied clones/varieties

4.2.3 Brightness

The results for brightness are presented in Figure 23 and Appendix XI. There was a significant difference ($p \leq 0.05$) observed in brightness percentage measured by spectrophotometer. Clone EPK TN 14/3 had the highest mean value of 34.38 % while clone AHP S 15/10 showed the lowest mean value of brightness among the commercial clones. Among the test clones, clones TRFK 91/1 and TRFK 91/2 showed low levels of brightness with 10.79 % and 17.20 %, respectively as TRFK 73/2 showed the highest mean value of 33.35 % which never differed significantly ($p \leq 0.05$) with TRFK 6/8. TRFK K S 3 and TRFK K S 2 were also among the test clones with relatively higher brightness.

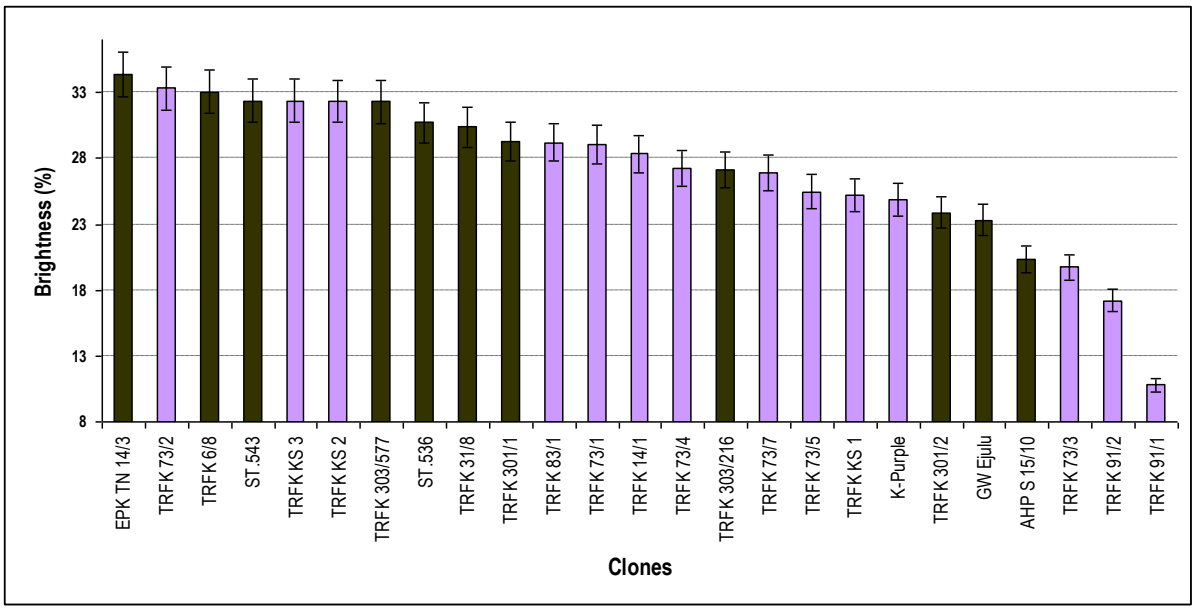


Figure 23: Clonal variation in brightness in the studied clones/varieties

4.2.4 Individual theaflavins

The quantities of the different individual theaflavins are presented in Figure 24 – 27. Black teas from the clones studied differed significantly ($P< 0.05$) in the levels of simple theaflavins (TF), theaflavin-3-monogallate (TF3MG), theaflavin-3'-monogallate (TF3'MG), and theaflavin-3-3'-digallate as shown in respective figures below. Clone TRFK 303/216 and clone TRFK 6/8 had the highest mean of simple theaflavins and never differed significantly ($p < 0.05$) (Appendix IX and Figure 24) among the commercial clones. TRFK K S 3 showed the highest simple theaflavins among the test clones. Clones TRFK 73/3, TRFK 91/1 had the lowest mean of simple theaflavins among the test clones. Significant difference ($p < 0.05$) was also observed in TF-3-MG (Figure 25) with clone TRFK 301/1 having the highest mean. Clones TRFK K S 3 and TRFK 73/1 had the highest mean values among the test clones while clones TRFK 73/3 and TRFK 91/1 showed the lowest mean value of TF-3-MG among the test clones. Clones TRFK 83/1, TRFK K S 2 and TRFK 73/2 never differed significantly ($p < 0.05$) with TRFK 6/8. Clones EPK TN 14/3, TRFK 6/8, TRFK 303/216 and TRFK 303/577 showed relatively higher mean values of TF-3'-MG (Figure 26) among the studied clones. TRFK 91/1 and TRFK 73/3 showed the lowest mean values of TF – 3'- g with 1.68µmol/g and 1.59µmol/g, respectively. Significant difference was also noted ($p < 0.05$) in TF dg with clone GW Ejulu showing the highest mean as clone ST. 536 and clone TRFK 73/7 showed the lowest means (Figure 27). Clone TRFK K- Purple (test clone) had the highest mean value of TF dg with 5.37 µmol/g. Clones TRFK K S 1, TRFK 73/5, TRFK 73/1 and TRFK 83/1 never differed significantly ($p < 0.05$) with TRFK 6/8 (Appendix IX). Most clones had

simple theaflavins dominating among the four theaflavins. Clone Ejulu had theaflavin digallate dominating whereas clones AHP S 15/10, TRFK 301/1, TRFK 301/2, TRFK 73/5, TRFK 91/2, TRFK 91/1, TRFK KS 1 and K-Purple had theaflavins-3-monogallate dominating. Clone TRFK 91/1 had the lowest values of all the theaflavins with theaflavin digallate dominating and simple theaflavins was the least.

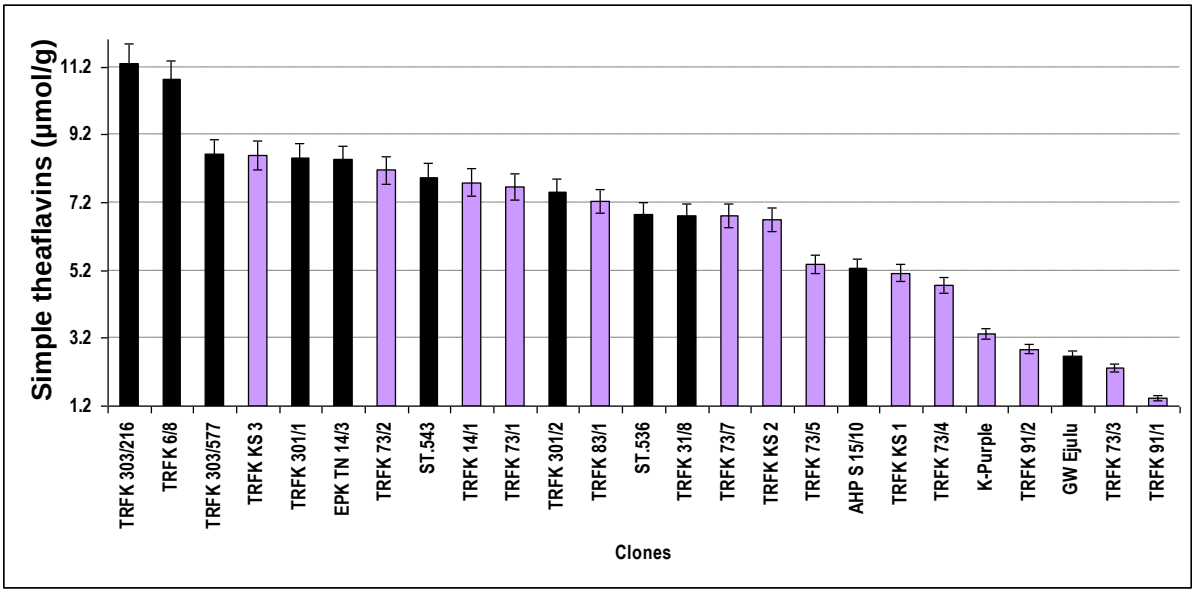


Figure 24: Simple theaflavins content in the studied clones/varieties

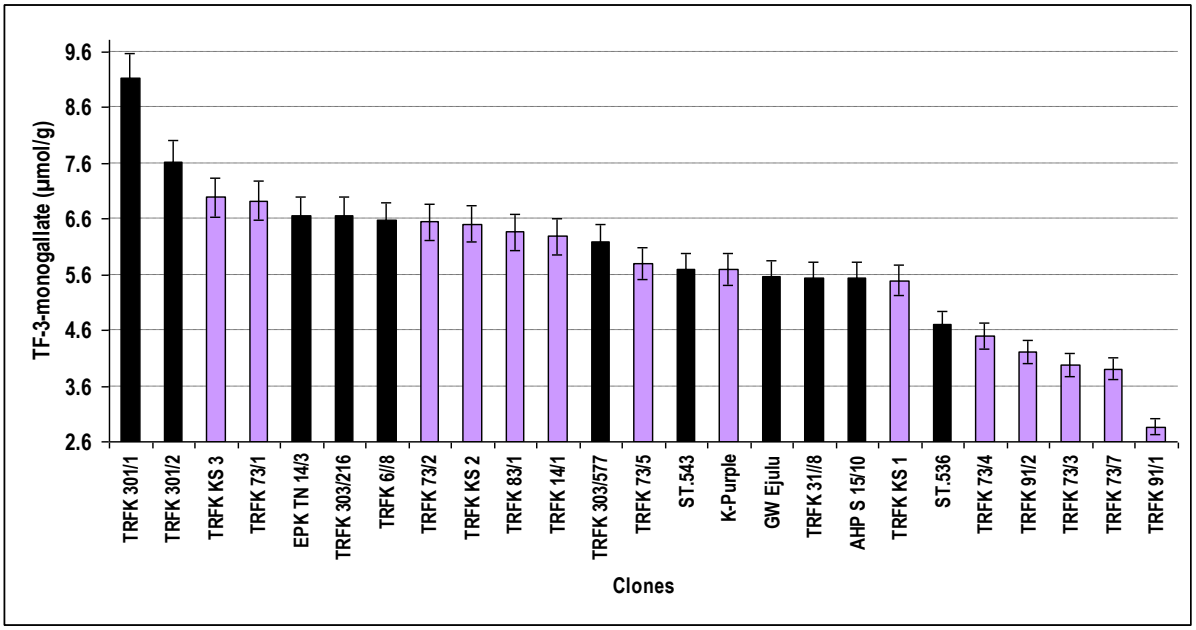


Figure 25: TF – 3 – monogallate content in black tea studied

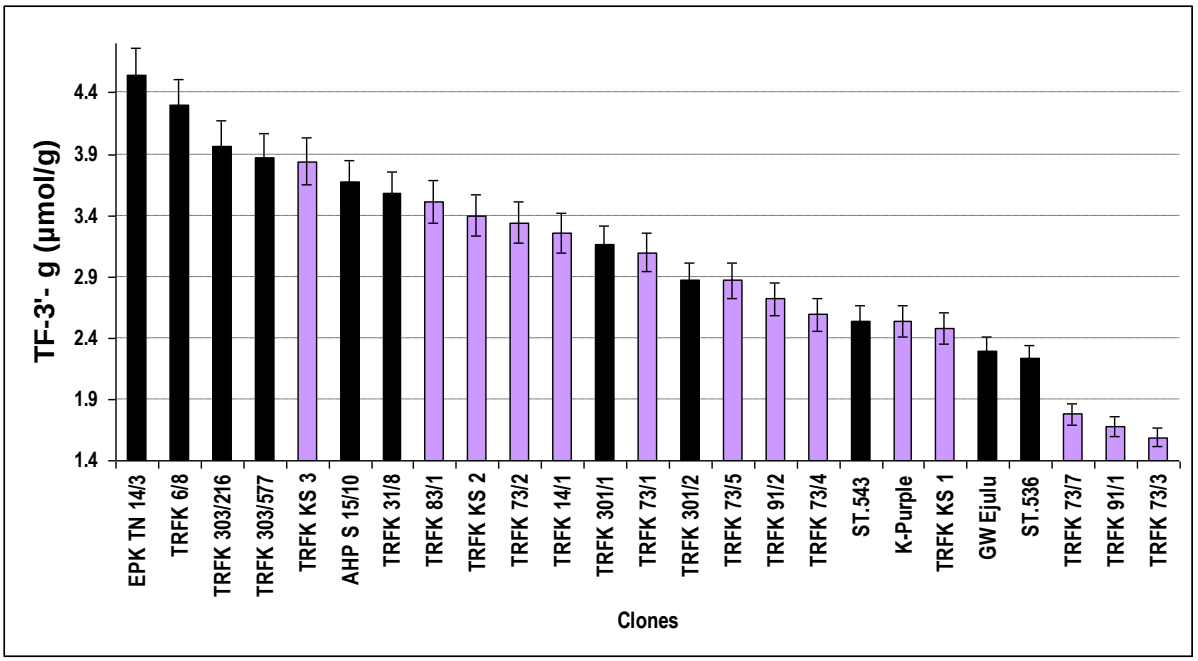


Figure 26: TF – 3'- monogallate content in black tea studied

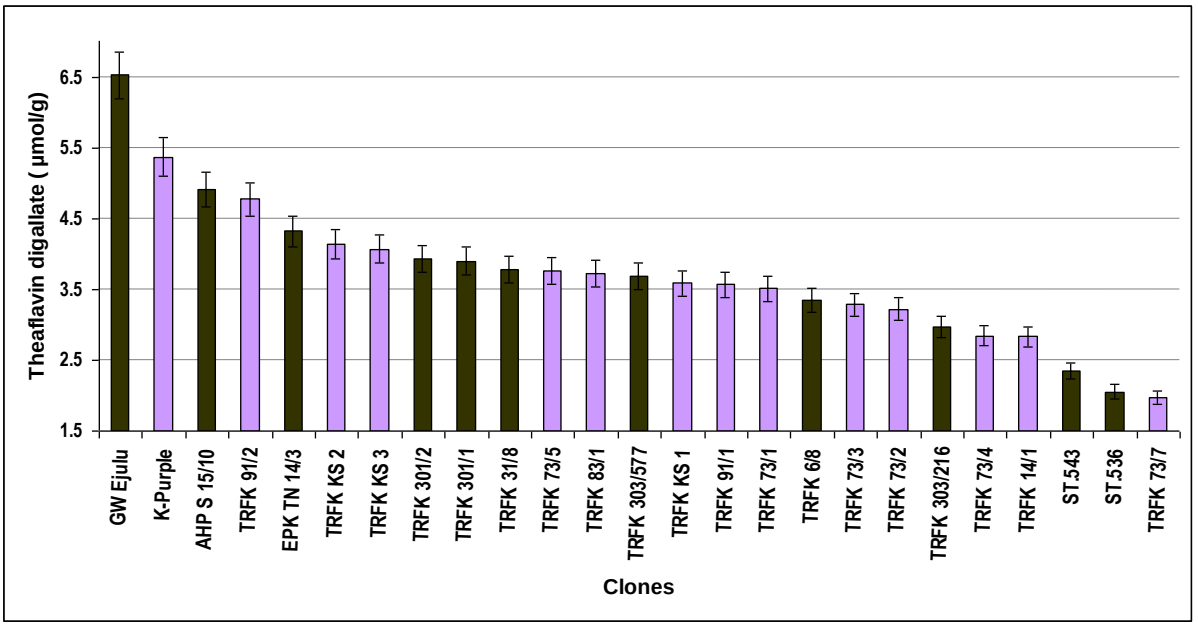


Figure 27: Theaflavin digallate content in the studied clones/varieties

4.2.5 Total thearubigins, thearubigins S I and thearubigins S II

The results are presented in Figures 28, 29, 30 and in Appendix X. There were no significant differences ($p \leq 0.05$) observed in total thearubigins as shown by Duncan multiple range test. There were variations observed in the studied clones in thearubigins S I (Figure 29). Clone TRFK 6/8 and ST. 543 showed relatively higher values of thearubigin S I among the commercial clones with 4.87 % and 4.82 %, respectively. Clone TRFK 73/1 and clone

TRFK KS 3 showed higher values among the test clones with 5.18 % and 6.30 % TR S I, respectively while clone TRFK 91/1 showed the lowest mean value of thearubigin S I with 1.69 %. Clones TRFK 6/8 and TRFK 301/2 were among the commercial clones with relatively high thearubigins S II with 12.40 % and 11.57 %, respectively (Figure 30). Clones ST.536 and ST. 543 showed the lowest mean values of thearubigins S II as 5.57 % and 7.49 %, respectively. Clone TRFK 91/2 showed the highest thearubigin S II among the test clones, followed by TRFK 91/1 with 12.28 % and 10.73 %, respectively. As shown in Figure 28, TR S II dominates TR S I across all the clones.

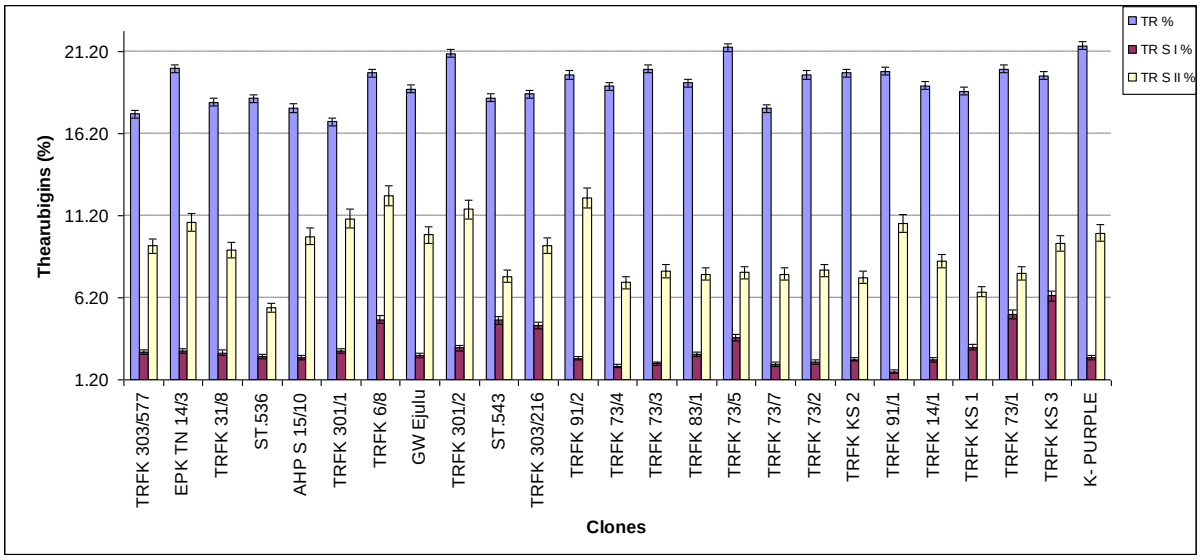


Figure 28: Total thearubigins and TR S I and TR S II fractions in the studied clones/varieties

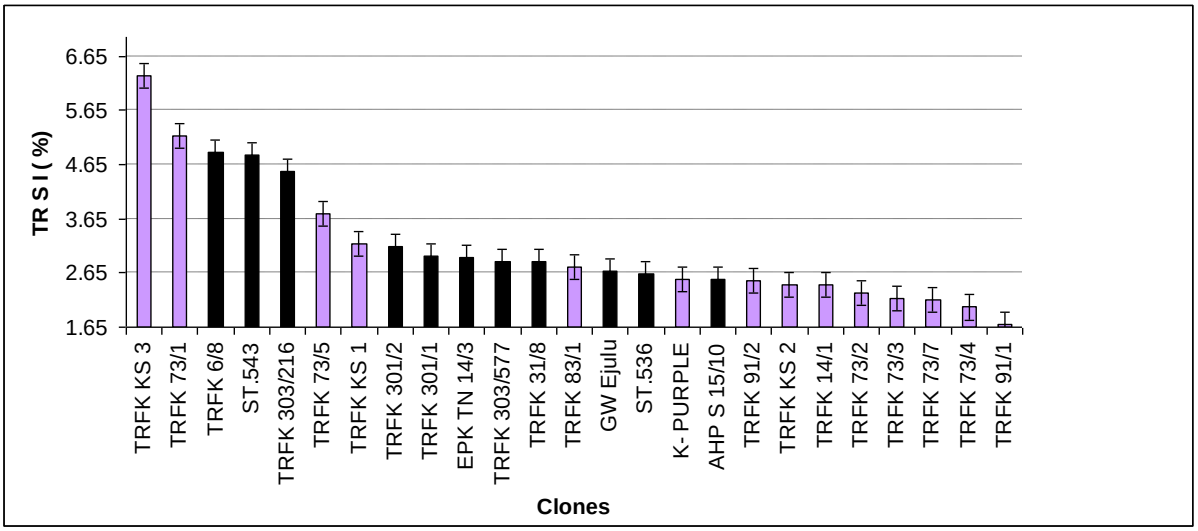


Figure 29: TR S I content in black tea studied

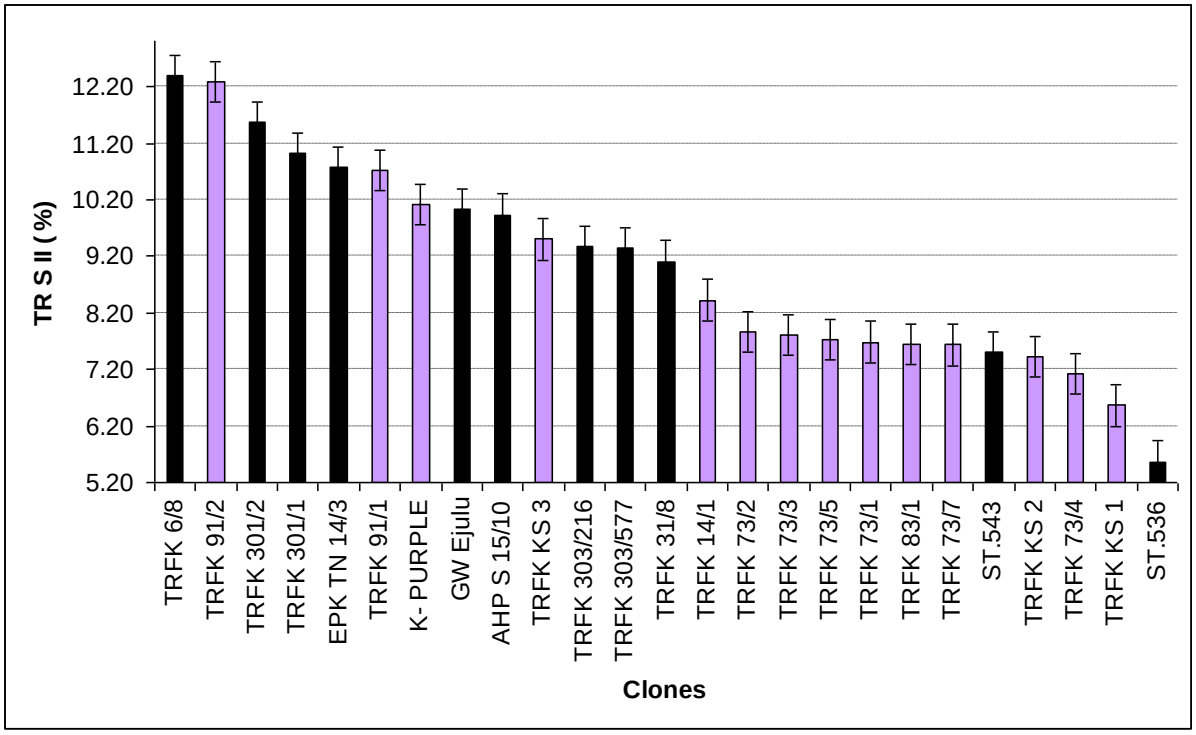


Figure 30: TR S II content in black tea studied

4.2.6 Total soluble solids (TSS)

Both the test and commercial clones had high total soluble solids as presented in Figure 31. Clones TRFK 91/2, TRFK K S 3, TRFK 83/1, TRFK 14/1, EPK TN 14/3, ST.536, AHP S 15/10, TRFK 301/1, and ST. 543 never differed significantly ($p < 0.05$) with TRFK 6/8 (Table 11). GW Ejulu and TRFK 303/216 had relatively high TSS content of all the clones. TRFK K S 1, TRFK 91/1 and TRFK K S 2 were the test clones with relatively high mean values with, 39.83, 39.33 and 37.96 %, respectively. None of the commercial clones had less than 33 % TSS. Most test clones had above 31 % TSS except clone TRFK 73/2 which had 28.48 % TSS.

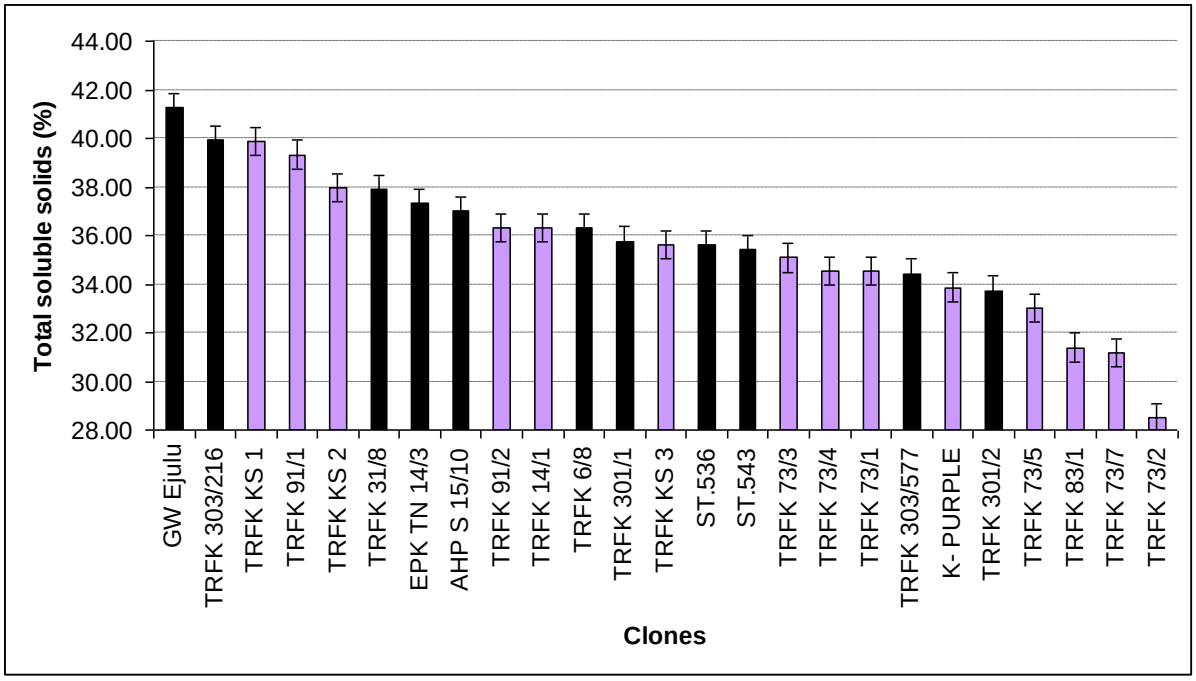


Figure 31: Total soluble solids content in the studied clones/varieties

4.2.7 Residual catechins in black tea

The major catechins presented here are four, namely; Epicatechins (EC), Epigallocatechins (EGC), Epicatechin gallate (ECG) and Epigallocatechin gallate (EGCG).

EC results are presented in Figure 32 and Appendix XII. Significant difference ($p < 0.05$) was observed in EC where TRFK KS I had the highest mean value. TRFK 6/8 followed with 0.78 % and never differed significantly ($p < 0.05$) with TRFK 303/216 which had 0.76 %. Clones TRFK 73/3 and TRFK 83/1 had relatively lower mean values among the test clones.

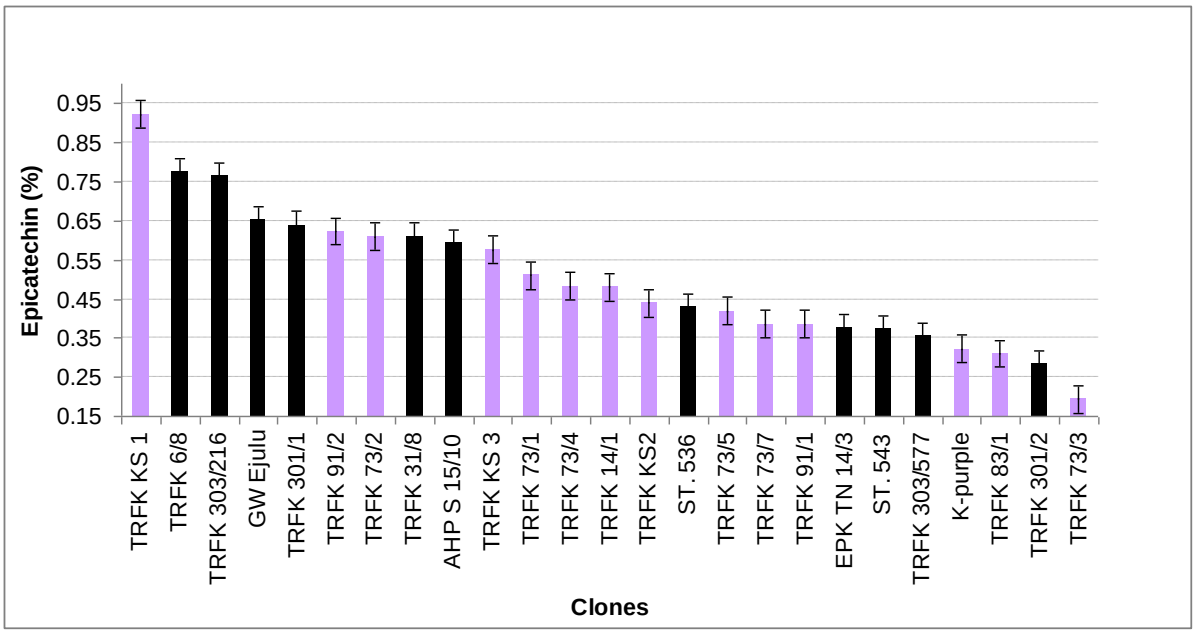


Figure 32: Epicatechin content in the studied clones/varieties

EGC results are presented in Figure 33 and Appendix XII. There was significant difference ($p < 0.05$) observed in EGC and TRFK 6/8 had the highest mean value while K-Purple had the lowest

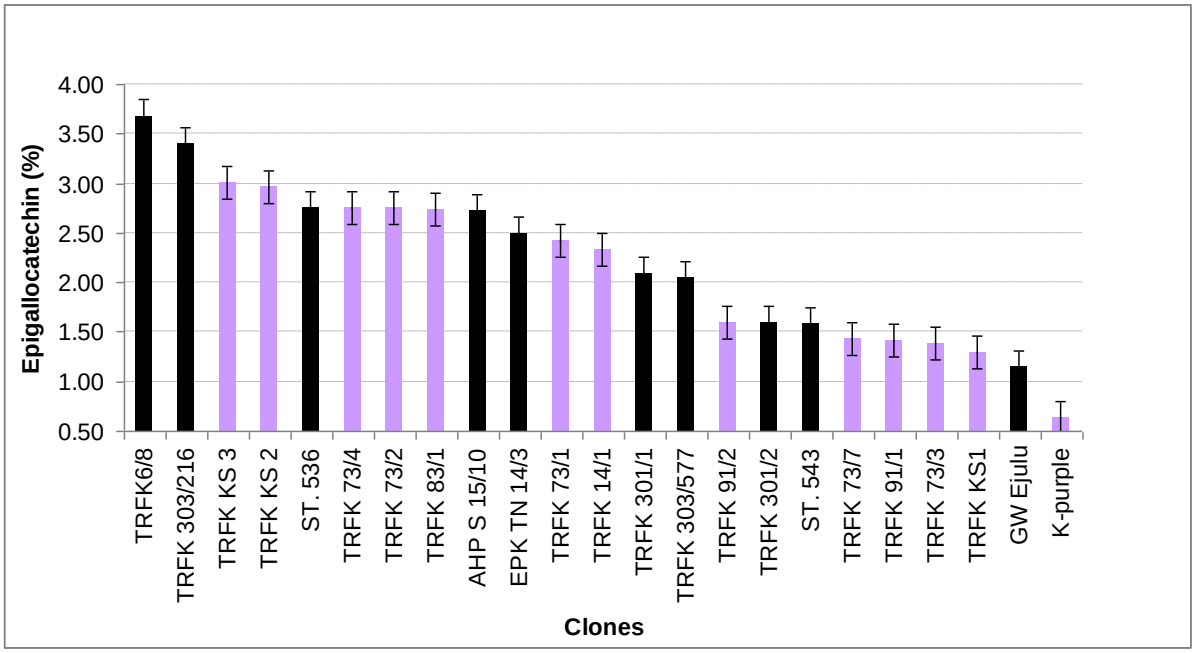


Figure 33: Epigallocatechin content in the studied clones/varieties

ECG results are presented in Figure 34 and Appendix XII. GW Ejulu had the highest mean value of 1.95 % while ST.543 had the lowest mean value of 0.60 %. K-Purple had the highest mean value among the test clones with a mean of 1.80 %.

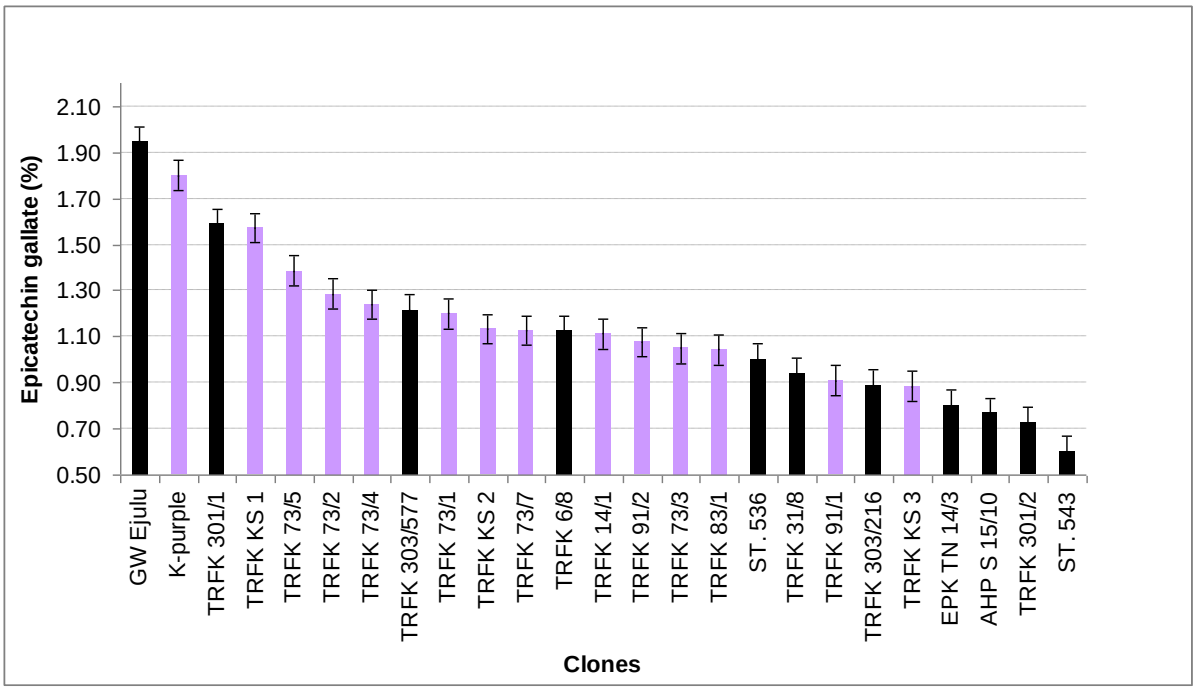


Figure 34: Epicatechin gallate content in the studied clones/varieties

EGCG results are presented in Figure 35 and Appendix XII. There was a significant difference ($p < 0.05$) observed in EGCG where TRFK 73/7 had the highest mean value of 1.53 %. Clones TRFK 91/1 and TRFK 301/2 had the lowest mean values of EGCG with 0.36 %.

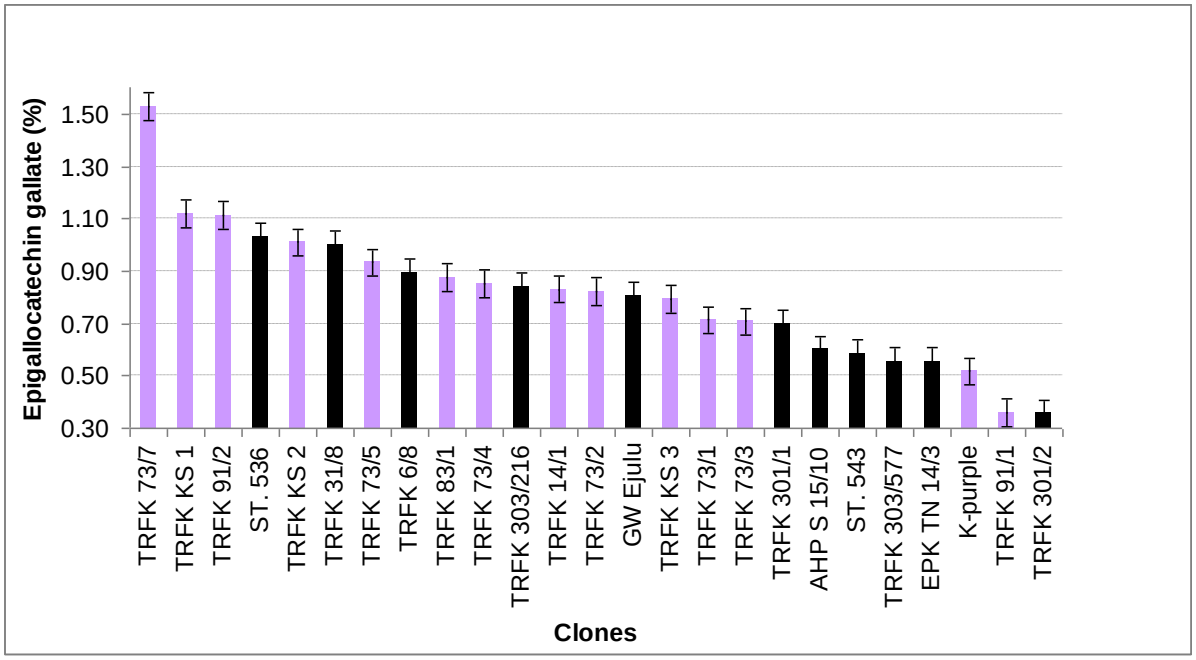


Figure 35: Epigallocatechin gallate content in the studied clones/varieties

4.2.8 Caffeine content

Results are presented in Figure 36 and Appendix XII. Significant difference ($p < 0.05$) was observed in caffeine content where TRFK 301/1 had the highest mean value of 3.96 % while TRFK 91/1 had the lowest with 1.96 %. Most test clones had lower caffeine content than the commercial clones.

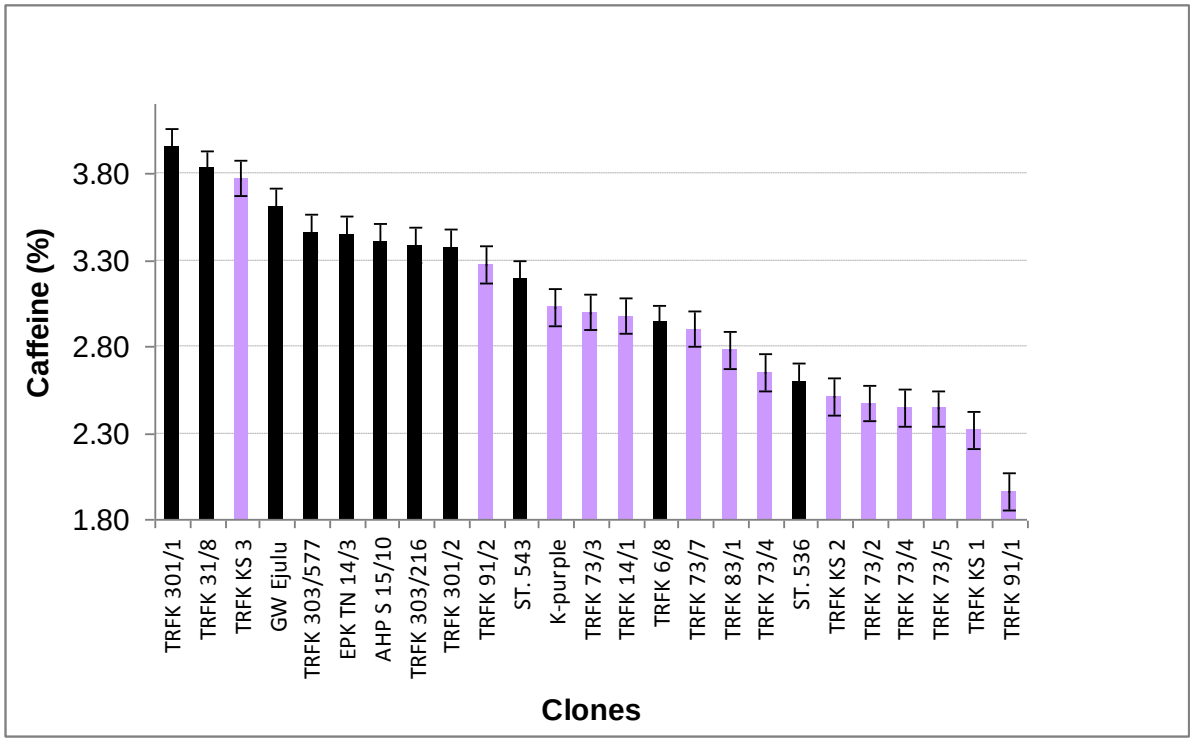


Figure 36: Caffeine content in black tea studied

4.2.9 Sensory evaluation

The taster's score on black tea is presented in Table 4 where mean scores are shown. The scores are from 1-5. The lowest score represent the best quality and the highest score, represent lowest quality as shown in Appendix II. There was a significant difference ($P< 0.05$) observed in all the sensory parameters. In this study, it was found out that, most clones showed bright liquors. Some were brighter like clone EPK TN 14/3, TRFK 301/1, TRFK 301/2, TRFK 91/2 and clone TRFK 73/3. Clones TRFK 91/2 and clone TRFK73/3 are coloured clones and their infusion colour was also bright as oppose to other coloured clones which were fairly bright at best otherwise dull. Clones TRFK KS1 and TRFK KS 3 were

fairly bright in their liquors but dull and fairly bright infusions, respectively. On liquor body (thickness), most clones were thick with a few test clones being fairly thick (TRFK 73/3, TRFK 83/1 and TRFK KS1). Clones TRFK 73/2, TRFK 73/4, TRFK 73/7, TRFK KS1 and K-Purple had liquors which were tending soft. Clone TRFK 73/3 and clone TRFK 14/1 among the test clones had strong liqours. Most test clones had brisk liquors - scores between 2 and 3 (Table 4) – but clone K-Purple was the most brisk with a score of 1.72. TRFK 73/7 scored last in briskness. Infusion colour varied, with most test clones showing dull infusions. The tea leaves colour intensity differ such that more purplish tea leaves appear duller in the infusion. Some test clones e.g TRFK 14/1 and TRFK 91/2 however, had bright infusions though the former is green and the latter is a purple clone.

Table 4: Sensory evaluation of black tea

	Liqour colour	Liqour body	Liqour strength	Liqour briskness	Infusion colour	**Average score**
Commercial clones						
TRFK 303/577	3.11^{abc}	3.00^{ab}	3.22^{abcd}	2.78^{abcd}	1.61^{j}	**2.74**
EPK TN 14/3	2.92^{bc}	3.25^{ab}	3.53^{abc}	2.39^{abcde}	3.39^{abcde}	**3.10**
TRFK 31/8	3.00^{bc}	3.50^{ab}	3.33^{abcd}	2.78^{abcd}	2.28^{fghij}	**2.98**
ST.536*	3.11^{abc}	3.92^{a}	3.67^{abc}	2.78^{abcd}	2.28^{fghij}	**3.42**
AHP S 15/10	3.00^{bc}	3.08^{ab}	3.00^{abcde}	2.72^{abcde}	2.28^{fghij}	**2.92**
TRFK 301/1	2.83^{bc}	3.08^{ab}	3.56^{abc}	2.89^{abc}	2.28^{fghij}	**2.88**
TRFK 6/8	3.11^{abc}	3.08^{ab}	2.73^{cde}	2.39^{abcde}	2.28^{fghij}	**2.69**
GW Ejulu	$3.11a^{bc}$	3.00^{ab}	3.72^{ab}	2.50^{abcde}	2.28^{fghij}	**2.87**
TRFK 301/2	2.92^{bc}	3.00^{ab}	3.83^{ab}	2.22^{cde}	2.28^{fghij}	**2.89**
ST.543	3.00^{bc}	3.33^{ab}	3.78^{ab}	3.08^{ab}	2.28^{fghij}	**3.20**
TRFK 303/216	3.22^{abc}	3.00^{ab}	3.78^{ab}	2.89^{abc}	2.28^{fghij}	**3.01**
Test clones						
TRFK 91/2*	2.77^{c}	3.08^{ab}	3.00^{abcde}	2.44^{abcde}	1.97^{ij}	**2.65**
TRFK 73/4	3.08^{abc}	3.08^{ab}	3.92^{a}	2.33^{bcde}	4.08^{a}	**3.30**
TRFK 73/3	2.92^{bc}	3.69^{ab}	2.11^{e}	2.83^{abcd}	2.92^{cdefg}	**2.89**
TRFK 83/1	3.11^{abc}	3.67^{ab}	2.89^{bcde}	2.06^{de}	3.36^{abcde}	**3.02**
TRFK 73/5	3.11^{abc}	3.00^{ab}	2.89^{bcde}	2.28^{cde}	3.44^{abcde}	**2.94**
TRFK 73/7	3.11^{abc}	3.00^{ab}	3.78^{ab}	3.17^{a}	4.00^{a}	**3.41**
TRFK 73/2	3.00^{bc}	2.92^{ab}	3.50^{abc}	2.97^{abc}	2.86^{defgh}	**3.05**

TRFK KS 2	3.00^{bc}	3.14^{ab}	3.36^{abcd}	2.78^{abcd}	3.06^{bcdef}	**3.07**
TRFK 91/1	3.03^{abc}	3.00^{ab}	3.45^{abc}	2.72^{abcd}	3.39^{abcde}	**3.12**
TRFK 14/1	3.08^{abc}	3.00^{ab}	2.44^{de}	2.44^{abcde}	2.61^{efghi}	**2.71**
TRFK KS 1	3.78^{ab}	3.44^{ab}	3.61^{abc}	2.50^{abcde}	4.00^{a}	**3.47**
TRFK 73/1	3.08^{abc}	2.78^{b}	3.50^{abc}	2.78^{abcd}	3.86^{ab}	**3.20**
TRFK KS 3	3.99^{a}	3.08^{ab}	3.39^{abc}	2.61^{abcd}	2.67^{efghi}	**3.15**
K-Purple	3.00^{bc}	3.00^{ab}	3.58^{abc}	1.72^{e}	3.75^{abc}	**3.01**
CV %	**18.69**	**20.93**	**17.48**	**18.4**	**18.48**	

*Flavoury. Means followed by the same letter along the column are not significantly different at $p< 0.05$. n= 75.

4.2.10 Correlation coefficient between the quality parameters and sensory evaluation

Results on correlation are shown in Table 5 below. There was a significant correlation between total thearubigins and tasters score on the liquor body and strength with r - value of 0.587 and 0.5568, respectively. The low r - value when thearubigin was correlated with liqour briskness shows there was no correlation. There was, however, a weaker correlation with an r – value of 0.4021 between the total theaflavins and total colour scored by the tasters, compared to stronger correlation between TF and tasters score on briskness of the liquor with an r - value of 0.6137. The correlation between total colour and liquor strength, briskness was very strong with r - values of 0.9737 and 0.8330, respectively. Correlation between brightness percentage and liquor body and briskness was also very strong with r - values of 0.9093 and 0.9960, respectively. The correlation between total soluble solids and liquor body was weak with an r - value of 0.3997.

Table 5**:** Matrix for tea quality parameters and tasters score. N = 75

	Liqour colour	Liqour body	Liqour strength	Liqour briskness	TR	Total colour	Brightness	TSS	TF
Liqour colour	1								
Liqour body	0.3	1							
Liqour strength	0.03	0.76***	1						
Liqour briskness	0.75***	0.01	0.07	1					
TR	0.13	0.59*	0.56*	0.04	1				
Total colour	0.39	0.02	0.97***	0.83***	0.68**	1			
Brightness	0.4	0.91***	0.23	0.99***	0.59*	0.81***	1		
TSS	0.1	0.4	0.05	0.16	0.08	0.35	0.57*	1	
TF	0.4	0.12	0.03	0.61**	0.3	0.54*	0.65**	0.62**	1

* Correlation significant at the $p \leq 0.05$ level

** Correlation significant at the $p \leq 0.01$ level
*** Correlation significant at the $p \leq 0.001$ level
TR = Total Thearubigins
TSS = Total soluble solids; TF = Total theaflavins

4.3 Discussion

Total polyphenols is a general term referring to catechins, catechin gallates, phenolic acids and flavonoid glycosides. The level of total polyphenols of fresh tea shoots is important to tea quality, and may be a reliable parameter for identifying and propagating potential high quality clonal tea plants (Obanda *et al.*, 1997). At The Research Foundation of Kenya, clone 6/8 is used as a reference clone in total polyphenol such that teas can be categorized as follows;

High quality teas have 24.80 - 27.07 % TPP; Medium high quality have 22.47- 24.40 % TPP; Medium quality have 19.57 – 22.33 % TPP; Low quality teas have 17.53 – 19.17 % TPP. From the results of this study, we can group the test clones as;

High quality only K- Purple ; Medium high quality, include, TRFK 91/1, TRFK 91/2, TRFK KS1, TRFK 73/3, TRFK KS3, TRFK 83/1, TRFK 73/5 and TRFK 73/2; Medium quality, include, TRFK 14/1, TRFK 73/4, TRFK 73/1, TRFK 73/7 and TRFK KS2. There were no low quality teas as far as TPP is concerned. Most test clones had a mean above 21 % on TPP except clone TRFK 73/7 which had 20.82 %, though better than the control clone Yabukita which had 17.16 %. The differences in polyphenol content among the clones could be due to differences in their botanical origin. Yabukita clone is a Japanese clone while K-purple which had high total polyphenol is of Kenyan origin. The high polyphenol content in Kenyan tea is expected since the tea breeding programme in Kenya has indirectly and consistently selected tea germplasm for high total phenol content to produce good teas with high levels of theaflavins and thearubigins. A study by Wachira and Kamunya, (2005), confirmed the superiority of Kenyan tea germplasm in total polyphenol content and that is why Kenyan teas are used for blending in other countries. Tea germplasm from Japan and China that is traditionally used for green tea manufacture has low total polyphenols and consequently low in astringency and bitterness. Usually green teas originating from India or Sri Lanka have higher polyphenol content than those from China (Harbowy and Balentine, 1997). The 73 series clones (TRFK 73/1, TRFK 73/2, TRFK 73/4, and TRFK 73/5) are from China seedlings while TRFK 91/1 and TRFK 91/2 are a selection from *C. irrawadiensis* seedlings from Tocklai, India (Anonymous, 1998). The variation in TPP could also be genetic since

clones TRFK 303/577 and TRFK 303/216 are progenies of TRFK 6/8 which they all had high TPP content.

The major catechins in green tea leaf consist of (-) epicatechin , (-) epigallocatechin, (-) epigallocatechin gallate and epicatechin gallate. The low mean values of total catechins in clones TRFK 91/1, TRFK 73/3, TRFK 91/2 could be attributed to high mean values of theanine content and this trend was vice versa for clone TRFK 83/1. The low mean values of total catechins in K-Purple could be because of low precursor theanine as shown by the results. This is supported by work done by Kito *et al.*, 1968. It is expected that those clones with high TPP should be having high total catechins but from the results, though clones TRFK 6/8 and TRFK 31/8 had high TPP, they showed low total catechins. This shows that other phenolic compounds could be contributing to TPP. Individual catechins were also investigated and this was necessary since it can help predict expected theaflavins in black tea manufacture.

Caffeine and gallic acid (GA; 3, 4, 5-trihydroxybenzoic acid), are important natural polyphenolic compounds in tea. Phenolic acids are aromatic secondary plant metabolites, widely spread through the plant kingdom (Anonymous, 2009). The basic feature of phenolic acids is the presence of one or more hydroxylated benzene rings. High gallic acid content can be found in gallnuts, grapes, sumac, witch hazel, tea leaves, hops, and oak bark. Caffeine content decreases with increasing leaf maturity of tea. Gallic acid is commonly used in the pharmaceutical industry (Fiuza *et al.*, 2004). It is also used as a standard for determining the phenol content of various analytes by the Folin - Ciocalteau assay. The high levels of gallated catechins shown by clone TRFK 91/1 is because of high gallic acid. AHP S 15/10 had high gallation probably because of low free catechins and high galloyl catechins. Caffeine is responsible for the briskness of tea liquors and these can explain the astringency noted in clones; GW Ejulu, TRFK K S 2, TRFK 73/3. Caffeine in tea prevents tumorigenesis (Lin and Liang, (2000). These results can guide breeders for selection of low caffeine tea or otherwise. This study found TRFK 6/8 and TRFK 73/3 having low caffeine and this is important when low caffeine tea is required.

Most of the test clones had above 1.5 % theanine except clones K- Purple and TRFK 83/1 which both had 1.10 %. Subsequently the tea taster's comments on clone K-purple reflected the results, in the sense that, though it was bright and thick, it had fair strength and briskness. Taster's comments on clone TRFK 83/1 were, however, better, meaning the theanine content in this clone were sufficient enough to make good tea liqour. The two clones (K-Purple and TRFK 83/1) were also found to be flavoury by the taster and this can be

attributed to theanine content because it's sweet and brothy (Hara *et al.,* 1995c). The taster's comment on the rest of the test clones were good except a few which fermented slightly may be because of the long time of processing considering the many samples handled. Theanine is a precursor for the biosynthesis of the catechins in tea leaves (Kito *et al.,* 1968). This conversion is known to be controlled by light because large quantities of theanine accumulate instead of being converted to catechins in shaded tea leaves and result in lower catechin levels than that in unshaded leaves (Kito *et al.,* 1968). In this study some clones showed this trend of high theanine, low total catechins like clone TRFK 301/1. Some clones however, showed low theanine and low catechin levels like clone TRFK 6/8 but high total polyphenols. The astringency showed by some clones could be attributed to high total polyphenol and Yabukita clone which had the lowest TPP had the lowest score in briskness.

Most clones had good liquor body because most of them had high TPP though Yabukita showed low liquor body may be because of low TPP. The flavoury taste in clones, ST.536, TRFK 91/1, TRFK 73/3 and TRFK 73/4 can be attributed to high levels of theanine. The green tea liquors in this study were generally of good briskness because of the displayed high quality parameters. Infusion colour of all clones studied were good, displaying high quality teas even in clones generally meant for black tea. The low strength liquor of K-Purple could be due to the low theanine content. GW Ejulu was astringent and this could be because of high levels of ECG since gallated catechins are particularly astringent (Hara, 2001). From the average scores, it was observed that, most test clones, in fact the first best five, were the test clones including TRFK 73/2, TRFK KS 2, TRFK 83/1, TRFK 73/3 and TRFK K S 1. TRFK KS 3 had the poorest average among the test clones but indeed had good black tea quality parameters. TRFK 303/577 had good sensory scores both in green and black tea. TRFK 73/1 scored poorly in sensory evaluation both as green and black tea.

There was no correlation found between some variables, however, the strong correlation between TPP and individual catechins is expected since catechins constitutes 60-80 % of the total amount of polyphenols (Hara *et al.,* 1995c).

For the purpose of discussion in black tea experiment, clone TRFK 6/8 will be used as a reference standard. Clone TRFK 6/8 is used at the Tea Research Foundation of Kenya as quality standard clone in most trials.

The standard clone TRFK 6/8 had the highest mean value of TF by Flavognost method supporting its known superiority in quality. TRFK KS3 had the highest mean value

among the test clones with 23.47µmoles/g. This might have contributed to the good liquor colour and briskness noted by the tea tasters. Other test clones were of average theaflavins mean values as shown in Figure 21 except clone TRFK 73/3 and TRFK 91/1 which showed relatively low mean values. The tasters noted the low strength in TRFK 91/1 though it was rated as flavoury. Theaflavins affects the total colour of the tea infusion, as observed from the results, those clones showing high values of theaflavins showed high total colour (Figure 22).

Brightness percentage measured by spectrophotometer, showed clone TRFK 73/2 having higher mean value than the standard TRFK 6/8 with 33.35 % and 33.07 %, respectively though not significantly different ($p < 0.05$) (Figure23). This can be attributed to high mean values of theaflavins. This is because theaflavins contribute to brightness of the liquor (Hilton and Ellis, 1972). Most of the test clones had mean values above 20 % brightness though clones TRFK 73/3, TRFK 91/2 and TRFK 91/1 had lower mean values of brightness. These clones also had less strength and briskness but had flavour. This could be because of the relatively low values of theaflavins, both simple and total theaflavins (Figures 24 and 21). The low percentage brightness in clones TRFK 73/3, TRFK 91/2 and TRFK 91/1 could also be attributed to the relatively higher mean values of TR S II. This is because TR S II influences liquor brightness negatively (Obanda *et al.,* 2001). The dull infusion colour shown by most test clones could be because of the purplish colour of the leaves. The tea leaves colour intensity differ such that more purplish tea leaves appear duller in the infusion. Some test clones e.g TRFK 14/1 and TRFK 91/2 however, had bright infusions though the former is green and the later is a purple clone. Clone TRFK 14/1 infusion brightness could be because of high TF by Flavognost method.

There was no significant difference ($p< 0.05$) noted between the standard and the test clones in total thearubigins as reported in Appendix XI. This could be because same duration and time of fermentation was used uniformly during black tea manufacture. This trend of non-significance also may indicate that total thearubigins might not be used in clonal selection in quality experiments. There was, however, significant difference ($p< 0.05$) noted in TR S 1 and TR S 11. TR S 11 has been found to have more influence in liquor brightness (Obanda *et al.,* 2001) than TR S 1 may be because of its relatively higher amount. Clones TRFK 91/2, TRFK 91/1 and K-purple had mean values above 10 % of TR S II. These high values could be because of the high total polyphenols which these clones had as shown in green tea experiment.

Total soluble solids results are impressive since nearly all clones had above 33 % except TRFK 73/2 which had 28.48 % though it had high TPP and total theaflavins. This shows this clone can still make good black tea.

As reported in literature, the formation of a single theaflavins molecule requires a dihydroxy and a trihydroxy flavan-3-ol (Obanda *et al.*, 2001). The formation is as follows; Epicatechin (EC) + Epigallocatechin (EGC) = simple theaflavin (TF).

EC + Epigallocatechin gallate (EGCg) = Theaflavin-3-gallate (TF-3-g)

Epicatechin gallate (ECG) + EGC = Theaflavin-3'-gallate (TF-3'-g)

ECG + EGCg = Theaflavin-3, 3'-digallate (TF dg).

According to Owuor and Obanda, (2007), the amount of the individual theaflavins formed are largely influenced by the amounts of the precursor catechins in green leaf, their redox potential and / or affinity for polyphenol oxidase and activity. Clones TRFK 303/216 and TRFK 6/8 had high residual EC and EGC (Figures 32 and 33) and consequently relatively high simple theaflavins (Figure 24). Clones TRFK 91/1, TRFK 73/3, K-Purple and GW Ejulu on the other hand had relatively low EC, EGC and consequently low simple theaflavins mean values. The relatively high mean value of TF- 3-g showed by clone TRFK 303/1 could be attributed to the relatively high EC content (Appendix IX). Clones TRFK 73/3 and TRFK 91/1 on the other hand showed low TF-3-g mean values may be because of relatively low EC and EGCG values. It's however worth noting here that, TRFK 301/2 had low EC and EGCG (Appendix VIII) but high TF-3-g (Figure 23). Clones TRFK 6/8, TRFK 303/216 and TRFK K S3 had relatively high TF-3'-g and this may be attributed to the relatively high EGC though TRFK had low ECG. The relatively low TF-3'-g mean values by clones TRFK 91/1, TRFK 73/3 and TRFK 73/7 could be attributed to the low values of EGC. GW Ejulu had high ECG, low EGC and consequently low TF-3'-g meaning that EGC is a limiting factor in theaflavin formation. Clones GW Ejulu and K-Purple had relatively higher TF dg may be because of the relatively high ECG mean values. The two clones never had high EGCG meaning that, it is ECG which is limiting the formation of TF dg. The low TF dg mean value showed by clone ST. 543 can be attributed to the low ECG and EGCG (Appendix VIII).

Residual catechins results reveals that despite the oxidation which takes place during aeration, some catechins remains unoxidised though when compared to green tea is relatively

low. Special interest is on EGCG which is the most abundant and bioactive tea catechin (Cabrera *et al.*, 2006). It has potential health benefits including cancer chemoprevention (Hsuuw and Chen, 2007), improving cardiovascular health (Hirai *et al.*, 2007), and antioxidant properties (Fu and Koo, 2006). There is however evidence that EGCG is a pro-oxidant in higher concentrations (Bandele and Sheroff, 2008; Lambert *et al*., 2010, Rohde *et al.*, 2011) and this warrant clear clinically proven recommended tolerant concentrations.

Caffeine acts as a diuretic, cardiac muscle stimulant, central nervous system stimulant, smooth muscle relaxant, gastric acid secretion stimulant, elevates plasma free fatty acids and glucose (Harbowy and Balentine, 1997). Caffeine levels vary from 5.30 % for 1 bud 1 leaf; to 4.20 % for 1 bud 2 leaves; to 3.80 % for 1 bud 3 leaves; and to 3.20 % for 1 bud 4 leaves (Dev Choudhury *et al*., 1991). Thus, the caffeine content decreases as the leaf ages or matures. Accordingly, teas made from pruned shoots have higher caffeine contents because of more young tender shoots. During withering and the entire processing of black tea, caffeine content increases marginally. This explains why caffeine is higher in black tea than in green tea (Appendix XII and Appendix VIII). Caffeine of an infused brew is responsible for the briskness of the tea liquor; this is due to its association with theaflavins. Results of this study found out the clonal variations in caffeine levels. The levels ranged from 3.95 – 1.96 %, though the samples were the same in quality (2 leaves and a bud), meaning caffeine levels vary from clone to clone. These results can guide on clonal selection for low or high caffeine tea products.

During the sensory evaluation of black tea, the tasters' judgement is usually based on the colour, strength, briskness, flavour and overall quality of the tea (Hilton and Ellis, 1972). Theaflavins contribute to all of these characteristics but some other components such as thearubigins, caffeine and volatile flavour compounds also have some effects (Owuor, 1982). Theaflavin digallate can better describe the quality of Kenyan black teas than the use of total theaflavins (Owour and Obanda, 1995). Among the four common theaflavins, (TF, TF-3-g, TF-3'-g and TF dg) theaflavin digallate (TF dg), influences tea liquor characteristic even at very low concentration because it possesses the strongest astringency (Owour and Obanda, 1995). The tasters noted low liquor briskness in clone TRFK 73/7 and this can be attributed to low TF dg. Clone TRFK 73/4 had tea liquor tending to soft and this can also be attributed to the low TF dg mean values displayed by this clone. From the results, it can be seen, TF dg was a dominating theaflavin in GW Ejulu clone. These results agree with an earlier study

done by Owour *et al., (*2006), where they found most commercial Kenyan cultivars have simple theaflavin dominating including TRFK 6/8 but GW Ejulu had TF dg dominating. The good briskness of K – Purple and GW Ejulu can be attributed to the high TF dg they displayed. K-Purple had high TFdg because it had low EGC hence allowing the reaction between EGCG and ECG to form high TF dg. K- purple's high briskness is also necessitated by high residual ECG because retention of higher epicatechin gallate (ECG) and epigallocatechin gallate (EGCg) levels produce more brisk tea liquors (Obanda *et al.,*2001). From the average scores, TRFK 91/2 and TRFK 14/1 had the best score value of 2.65 and 2.71, respectively with the former scoring best in infusion colour and the latt78iujer in liquor strength and briskness. TRFK 91/2 had relatively high amount of theaflavin digallate, TR S II and even caffeine giving good liquor briskness. TRFK 14/1 had high total theaflavins and consequently total colour and brightness though it had low theaflavins digallate. These results showed that many factors including the above determine the liquor strength and briskness alongside theaflavins digallate. TRFK K S 1 and TRFK 73/7 averaged poorest with 3.47 and 3.41 scores, respectively but particularly poor in infusion colour. TRFK K S I however, had good liquor briskness. It is also worth noting here that, TRFK K S I was among the best in green tea.

CHAPTER FIVE

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

(1) Most test clones had more theanine content than the control clone Yabukita except clone TRFK 83/1 and K- Purple. All the test clones had higher total polyphenols than the Japanese clone (Yabukita) showing superiority of Kenyan green tea. Except for clones TRFK 73/7 and TRFK 73/4 which had similar total catechins as Yabukita, the rest of the test clones had lower mean values of total catechins than the control (Yabukita).

(2) (a) Most commercial clones had more total theaflavins than the test clones, however, clone TRFK KS 3 had more total theaflavins than some commercial clones. The control clone, TRFK 6/8, however had more total theaflavins than all the test clones. Among individual theaflavins, theaflavin digallate is the most important in liquor quality because it influences astringency even at very low concentration. Some test clones like K- Purple, TRFK KS 3, TRFK KS 2 and TRFK 91/2 had more theaflavins digallate than clone TRFK 6/8. Some test clones however had lower theaflavins digallate like clone TRFK 73/7, TRFK 14/1 and TRFK 73/4 than the control clone, TRFK 6/8.

(b) There is no significant difference ($p > 0.05$) among the test clones and commercial clones on total thearubigins. Clones TRFK K S 3 and TRFK 73/1 had higher TR S I fraction than the standard clone TRFK 6/8. The rest of the test clones had lower mean values of TR S I than

the reference clone. Clone TRFK 91/2 had higher TR S II percentage than the rest of the test clones and even higher than the standard clone.

(c) Clones TRFK 91/1, TRFK K S I, TRFK KS II and TRFK 83/1 had relatively higher total soluble solids than TRFK 6/8, while the rest of the test clones had lower or equal percent of total soluble solids. Clone TRFK 73/2 had similar brightness as clone TRFK 6/8 but the rest had lower brightness than the control. All the test clones were lower in total colour than clone TRFK 6/8. Some however, can still make good black tea as seen from the sensory evaluation.

(3) Total colour and brightness correlate strongly with liquor briskness and strength meaning, the higher the above parameters, the stronger and brisker the liquor. Total soluble solids also affect the strength of the liquor such that the more the soluble solids, the higher the tea liquor strength.

5.2 Recommendations

- Low levels of catechins and theanine in K-Purple should be investigated. Low catechins and high total polyphenols in TRFK 91/1 warrant an investigation.
- Other polyphenols apart from catechins should be studied since it might not be true that the higher the catechins the higher the total polyphenols.
- When theanine is the target parameter, the teas should be shaded or harvested at night since in presence of sunlight, theanine is converted to catechins
- Since this work was done using samples from Kangaita only , I recommend that these test clones be planted in other areas and their quality assessed.
- Optimal manufacturing conditions of each clone especially those that can be made black should be confirmed since all the clones were manufactured using same standard time of 90 minutes and uniform controlled aeration.
- Study on the variation of theanine levels with seasons should be done in these clones.
- The effect of anthocyanin presence in relation to black tea quality paratemers (theaflavins and Thearubigins) should be studied in details.
- Recommended clones for green tea; TRFK 91/1, TRFK 91/2, TRFK 73/2, TRFK 73/3, TRFK 73/4, TRFK 73/5 , TRFK 83/1, TRFK K S 1,TRFK K S 2, TRFK K S 3
- Since the taster could repeatedly identify TRFK K S 1 as a purple tea, it can be used as a reference standard for green purple tea.

- Recommended clones for black tea based on these findings; TRFK K S 3, TRFK 91/2, K- Purple, TRFK K S 2, TRFK 14/1

REFERENCES

Almajano, P.M., Rosa, C., Jimenez, L., and Gordon, H. (2008). Antioxidant and antimicrobial activities of tea infusions. *Food Chem*., **108**: 55-63.

Amie, D., Amie, DD., Beslo, D. and Trinajstie, N. (2003). Structure-radical scavenging activity relationships of flavonoids. *Croat.Chem. Acta*.***76:***55-61.

Anonymous. (1998). TRFK Annual Report for 1998. Tea Research Foundation of Kenya. pp 32-33.

Anonymous, (2002). The Tea Growers Hand Book. 5th Edn. The Tea Research Foundation of Kenya Printing Services, pp: 31.

Anonymous (2006). Annual technical report. Tea Research Foundation of Kenya. (TBK), 48-49.

Anonymous (2007). Annual bulletin of statistics. International Tea Committee (London).

Anonymous, (2008). *Tea Board of Kenya Annual report and accounts*. Tea Board of Kenya

Anonymous. (2009). Food and Agricultural Organization of the United Nations, (FAO)

Anonymous, (2010). Kenyan tea industry performance in 2010. The Tea Board of Kenya.

Anonymous (2011). Kenya Tea Industry Performance in 2011. The Tea Board of Kenya.

Anonymous, (2011). *Tea Board of Kenya Statistics*.Nairobi: Tea Board of Kenya.

Ashihara, H., Deng, W.W. and Ogita, S. (2010). Distribution and biosynthesis of theanine in Theaceae plants. *Plant Phy. And Biochem.* **48 (1):** 70-72.

Bailey, R.G., McDowell, I. and Nursten, H.E. (1990). Use of an HPLC Photodiode-Array detector in a study of the nature of black tea liquor. *J. of Sci. and Food Agric.* **52:** 509–525.

Balentine, D.A., Wiseman, S.A. and Bouwens, L.C.M. (1997). The chemistry of tea flavonoids. *Crit. Rev. Food Sci. Nutr*. **37(8):** 693-704.

Bandele, O.J. and Osheroff, N. (2008). (-)-epigallocatechin gallate, a major constituent of green tea, poisons type II topoisomerases. *Chem. Res. Toxicol*., **21**: 936-943.

Biswas, A. K.,Sarkar, A. R., and Biswas, A. K. (1973). Biological and chemical factors affecting the valuations of North East India planters. III. Statistical evaluation of the biochemical constituents and their effects on colour, brightness, and strength of black teas. *J. Sci. and Food Agric*. **24**: 1457–1477.

Bolling, B.W. and Chen, C.Y. (2009). Tea and health: Preventive and therapeutic usefulness in the elderly. *Current Opinion in Clinical Nutrition and Metabolic Care.* **12**: 42–48.

Brown, A.G., Eyton, W.B., Hohnes, A. and Ollis, W.D. (1969). The identification of thearubigins as polymeric proanthocyanidins. *Phytochem.*, **8:** 2333–2340.

Cabrera, C., Gimenez, R., and López, M.C. (2003). Determination of tea components with antioxidant activity. *J. Agric. Food Chem*., **51 (15):** 4427-4435.

Cabrera, C., Artacho, R. and Gimenez, R. (2006). Beneficial effects of green tea - A review. *J. Am.Coll. Nutr*., **25**: 79-99.

Caffin, N., D'Arcy, B., Yao, L. and Rintoul, G. (2004). *Developing an index of quality for Australian tea*. Rural Industries Research and Development Corporation Publication No. 04/033. ISBN 0 642 58743 4.

Cho, H.S., Kim, S., Lee, S.Y., Park, J.A., Kim, S.J. and Chun, H.S. (2008). Protective effect of the green tea component, l-theanine on environmental toxins-induced neuronal cell death. *Neuro Toxicol.* **29**: 656–662.

Cloughley, J.B. (1980a). The effect of fermentation temperature on the quality parameters and price evaluation of Central African black teas. *J. Sci. Food Agric*. **31**: 911-919.

Del Pozo-Insfran, D., Del Follo-Martinez, A., Talcott, S.T. and Brenes, C.H. (2007). Stability of copigmented anthocyanins and ascorbic acid in muscadine grape juice processed by high hydrostatic pressure. *J. Food Sci.* **72 (4):** 247–253.

Dev Choudhury, M.N., Rahman, F. and Barbora, B.C. (1991). Caffeine content in teas of North East India - a review. Two and A Bud. **38(1/2**): 3-8.

Egashira, N., Hayakawa, K., Mishima, K., Kimura, H., Iwasaki, K. and Fujiwara, M. (2004). Neuroprotective effect of γ-glutamylethylamide (theanine) on cerebral infarction in mice. *Neuroscie. Letters.* **36**: 58–61.

Ekborg-Ott, K. H., Taylor, A., and Armstrong, D. W. (1997). Varietal differences in the total and enantiomeric composition of theanine in tea. *J. of Agric. and Food Chem.* **45:**353-363.

Ercisli, S., Orhan, E., Ozdemir, O., Sengul, M. and Gungor, N. (2008). Seasonal variation of total phenolic, antioxidant activity, plant nutritional elements, and fatty acids in tea leaves (*Camellia sinensis* var. *sinensis* clone Derepazari 7) grown in Turkey. *Pharmac. Biol*. **46 (10):** 683-687.

Fernandez, P. L., Pablos, F., Martin, M. J. and Gonzalez, A. G. (2002). Study of catechin and xanthine tea profiles as geographical tracers. *J. of Agric. and Food Chem.* **50:** 1833-1839.

Fiuza, S.M., Gomes, C., Teixeira, L.J.,Giraoda cruz M.T., Cordeiro M.N., Milhazes N., Borges F. and Maques M.P. (2004). Phenolic acid derivatives with potential anticancer properties – a structure – activity relationship study. Part 1. Methyl,prophyl and octyl esters of caffeic and gallic acids. *Bioorg. Med. Chem*. **12(13):** 3581- 3589.

Food and Agricultural Organization of the United (FAO) corporate document repository, (2001). Committee on commodity problems. Fourteenth session of the Intergovernmental Group on Tea, New Delhi,India, 10-11 Oct. 2001.

Fu, Y. and Koo, M.W. (2006). EGCG protects HT-22 cells against glutamate - induced oxidative stress. *Neur. Res*., **10**: 23-30.

Graham, H.N. (1992). Green tea composition, consumption, and polyphenol chemistry. *Prev. Med*. **21**: 334-350.

Gianturco, M.A., Biggers, R.E., Riddling, B.H. (1974). Seasonal variations in the composition of the volatile constituents of black tea. A numerical approach of the correlation between composition and quality of tea aroma. *J. Agric. Food Chem.* **22**: 758–764.

Golding, J. Roach, P. and Parks, S. (2009). Production of high quality export green tea through intergrated management. *Rural Industries Research & Development corporation publication* No. 09/124 pp. 1 and 43.

Gomes, A., Veda Siromoni,J.R.,Das,M.,Sharma,R.M. and Ganguly, D.K. (1995). Anti-hyperglycemic effect of black tea (*Camellia Sinensis)* in rat. *J.Ethnopharmacol.* **45(3)**:223-226.

Gosslau, A. and Chen, K.Y. (2004). Nutraceuticals, apoptosis, and disease prevention. *Nutr.* **20 (1):** 95-101.

Graham, H.N. (1992). Green tea composition, consumption, and polyphenol chemistry. *Prev. med.* **21**(3): 334-350.

Gramza, A., Khokhar, S., Yoko, S., Swiglo, A.G., Hes, M., and Korczak, J. (2006). Antioxidant activity of tea extracts in lipids and correlation with polyphenol content. *Eur. J. Lipid Sci. Tech.*,**108**: 351-362.

Hakim, I.A. and Chow, S.H. (2004). Green tea, polyphenol and cancer prevention in: "Proceedings International Conference on Ocha (Tea) Culture and Science". Nov 4-6, 2004. Shizuoka. Japan

Hampton, M. G. (1992). Production of black tea. In *Tea Cultivation to Consumption*, ed. K. C. Willson & M. N. Clifford. Chapman and Hall, London, pp. 459-511.

Hara, Y., Luo, S.J., Wickremasinghe, R.L. and Yamanishi, T. (1995). Special issue on tea. *Food Rev. Int.* **11(3):** 371-545.

Hara, Y., Luo, S.J., Wickremasinghe, R. L. and Yamanishi, T. (1995c). Chemical composition of tea. *Foods Rev. Int.* **11**: 435-456.

Hara, Y., Luo, S.J., Wickremasinghe, R. L. and Yamanishi, T. (1995d). Biochemistry and processing black tea. *Foods Rev. Int.* **11**: 457-471.

Hara, Y. (2001). Green tea: *Health benefits and applications*. Marcel Dekker, Inc., New York, USA.

Harbowy, M.E. and Balentine, D.A. (1997). Tea chemistry. *Crit. Rev. Plant Sci*. **16(5):** 415–480.

Haslam, E. (2003). Review on the chemistry of tea leaf fermentation. *Phytochem.* **64 (1):** 61-73.

Herath, D. and Weersink, A. (2007). Peasants and plantations in the Sri Lanka tea sector: Causes of change in their relative viability. *Aust. J. of Agric. Res. Econ.* **51:**73–89.

Higdon, J. V. and Frei, B. (2003). Tea catechins and polyphenols: Health effects, metabolism, and antioxidant functions. *Critical Reviews in Food Sci. and Nutr*. **43:** 89-143.

Hilton, P.J. and Ellis, R.T. (1972). Estimation of the market value of Central African tea by theaflavins analysis. *J. Sci. Food Agric*. **23**: 227-232.

Hilton, P. J. (1973). Tea. In:. *Ency.of Indust. Chem. Analysis.* Snell, F.D. and Ettre, L.C. **18:** 455-516. John Wiley, New York.

Hirai, M., Hotta, Y., Ishikawa, N., Wakida, Y., Fukuzawa, Y., Isobe, F., Nakano, A., Chiba, T. and Kawamura, N. (2007). Protective effects of EGCG or GCG, a green tea catechin epimer against postischemic myocardial dysfunction in guinea-pig hearts. *Life Sci*., **80**: 1020-1032.

Hsuuw, Y. and Chen, W. (2007). Epigallocatechin gallate dose-dependently induces apoptosis or necrosis in Human MCF-7 cells. *Ann. N.Y. Acad. Sci*., **1095**: 428-440.

Jackman, R. L. and Smith, J. L. (1996) In: *Natural Food Colorants*. Hendry G. A. F. and Houghton J.D. (Ed.), London, Glasgow. PP 244-309.

Jansen, M. A. K., Gaba, V. and Greenberg, B.M. (1998). Higher plants and UV-B radiation balancing damage, repair and acclimation. *Trends in Plant Sci.* **3**:131-135.l

Johnson, A. L. (1987). Some comments on withering. Tea Research Foundation (Central Africa), P.O. Box 51, Mulanje, Malawi.

Johnson, A. L. (1990). Manufacture - Section 14, Tea Planters Handbook. Tea Research Foundation (Central Africa), P.O. Box 51, Mulanje, Malawi

Juneja, L. R., Chu, D.C., Okubo, T., Nagato, Y., and Yokogoshi, H. (1999). L-theanine--a unique amino acid of green tea and its relaxation effect in humans. *Trends in Food Sci. and Technol.* **10**: 199-204.

Kamunya, S. M. (2003). Tea improvement: Strides, constraints and opportunities. *Tea*. **24**: 38– 47.

Karori, S.M.,Wachira, F. N., Wanyoko, J. K. and Ngure, R. M. (2007). Antioxidant capacity of different types of tea products. *Afri. J. Biotechnol.* **6 (19):** 2287-2296.

Karori, S.M., Ngure, R.M., Wachira, F.N., Wanyoko, J.K. and Mwangi, J.N. (2008). Different types of tea products attenuate inflammation induced in *Trypanosoma brucei* infected mice. *Parasit. Int.*, **57**: 325-333.

Kawai, K. (2003). Epigallocatechin gallate, the main component of tea polyphenol, binds to CD4 and interferes with gp120 binding. *J. Allergy Clin Immunol*. **112 (5):** 951-957.

Keegal, E. L. (1950). Quick withering at high temperature. *TRIP Ceylon Bulletin*, **32:**32-33.

Kerio, L.C., Wachira, F.N., Wanyoko, J.K. and. Rotich, M.K. (2011). Characterization of anthocyanins in Kenyan teas: Extraction and identification. *Food Chem.***131 (1):** 31-38.

Khan, N. and Mukhtar, H. (2007). Tea polyphenols for health promotion. *Life Sci.* **81**: 519–533.

Kimura, K., Ozeki, M., Juneja, L.R., and Ohira, H. (2007). L-theanine reduces psychological and physiological stress responses. *Bio. Psych.* **74**: 39–45.

Kirca, A., Ozkan, M. and Cemeroglu, B. (2007). Storage stability of strawberry jam colour enhanced with black carrot juice concentrate. *J. Food Proc. and Pres.***31 (5):** 531-545.

Kito, M., Kokura, H., Izaki, J., and Sasaoka, K. (1968). Theanine, a precursor of the phloroglucinol nucleus of catechins in tea plants. *Phytochem.* **7:** 599-603.

Lambert, J.D., Kennet, M.J., Sang, S., Reuht, K.R., Ju, J, and Yang, C.S. (2010).

Hepatotoxicity of high oral dose (-)-epigallocatechin-3-gallate in mice. *Food Chem.Toxicol*., **48**: 409-416.

Lila, M.A. (2004). Anthocyanins and human health: An in vitro investigative approach. *J. Biomed. Biotechnol.***5:**306-313.

Lin, J.K., Lin, C.L., Liang, Y.C., Lin-Shiau, S.Y., and Juan, I.M. (1998). Survey of catechins, gallic Acid, and methylxanthines in green, oolong, pu-erh, and black teas. *J. of Agric. and Food Chem.* **46**:3635-3642.

Lin, J.K. and Liang, Y.C. (2000). Cancer chemoprevention by tea poluphenols. *Proc Natl. Sci. Counc. Repub. China B.* **24(1):**1-13.

Lin, S. D., Lin, E. H. and Mau, J.L. (2008). Effects of different brewing methods on antioxidant properties of steaming green tea. IWT- *Food sci. and Technol.* **41:**1616-1623.

Lu, S. H. (1987). Tea Evaluation and Inspection. 2nd edition. *Agric. Press*. Beijing

Luczaj, W. and Skrzydlewska, E. (2005). Antioxidative properties of black tea- A review. *Prev. Med*. **40:** 910-918.

Matsunaga, K. (2002). Epigallocatechin gallate, a potential immunomodulatory agent of tea components, diminishes cigarette smoke condensate-induced suppression of anti-*Legionella pneumophila* activity and cytokine responses of alveolar macrophages. *Clin. Diagn. Lab. Immunol*. **9 (4):** 864-71.

Maurya, P.K. and Rizvi, S.I. (2008). Protective role of tea catechins on erythrocytes subjected to oxidative stress during human aging. *Nat. Prod. Res*., pp. 1-8.

Mazza, G. and Miniati, E. (1993). Grapes. In *Anthocyanins in Fruits, Vegetables and Grains*, ed. G. Mazza, E. Miniati, pp. 149–99. Boca Raton, FL: CRC Press.

Mbata, T. I., Debiao, L. U. and Saikia, A. (2008). Antibacterial activity of the crude extract of Chinese green tea (Camellia sinensis) on *Listeria Monocytogenes. Afr. J. of Biotech*. **7 (10):** 1571-1573.

McCann, D. Barrett, A. Cooper, A. Crumpler, D. and Dalen, L. (2007). Food additives and hyperactive behavior in 3-year-old and 8/9-year-old children in the community*:* a randomised, double- blinded, placebo controlled trial. *Lancet.* **370**:1560–67.

McDowell, I., Feakes, J. and Gay, C. (1991). Phenolic composition of black tea liquors as a means of predicting price and country of origin. *J. Sci. Food Agric.* **55:** 627–641.

McDowell, I. and Taylor, S. (1993). Tea: types, production and trade. In R.Macrae, R.K. Robinson and M.J.Sadler, (eds). *Encyclo. of Food Sci., Food Technol. and Nutr.*, Academic Press, London. **7:** 4521–4533.

Millin, D.J. (1987). Factors affecting the quality of tea. In. *Quality Cont. in the Food Indust.* S.M. Herschdoerfer (Ed). **4**: 127-160. Academic Press. London.

Mo, H., Zhu, Y. and Chen, Z. (2008). Microbial fermented tea- a potential source of natural food preservatives. *Trends in Food Sci. and Technol.* **19:** 124-130.

Ngure, F. M., Wanyoko, J. K., Mahungu, S. M. and Shitandi, A.A. (2008). Catechin depletion patterns in relation to theaflavins and thearubigin formation. *Food Chem.***115 (1):** 8-14.

Obanda, M. and Owuor, P.O. (1992). The effect of chemical wither duration and dryer type on quality of black tea manufactured in a commercial factory. *Tea* **13(1):** 50-61.

Obanda, M., Owuor, P.O. and Bore, J.K. (1997). Effects of moisture loss and temperature of leaf during withering on black tea quality parameters. *Tea*. **18(1)**: 45-50.

Obanda, M. Owuor, P.O. and Taylor, S.J. (1997). Flavanol composition and caffeine content of green leaf as quality potential indicators of Kenyan black teas. *J. Sci. Food Agric*. **74 (2)**: 209-215.

Obanda, M., Owour, P.O. and Mangoka, R. (2001). Changes in the chemical and sensory quality parameters of black tea due to variations of fermentation time and temperature. *Food chem*., **75**: 395-404.

Owuor, P.O. 1982. Can theaflavins content alone be adequate parameter in black tea quality estimation? A review. *Tea*. **3**: 36-40.

Owour, P.O and Obanda, M. (1995). Clonal variations in the individual theaflavins levels and their impact on astringency and sensory evaluations. *Food chem.* **54:** 273-277.

Owuor, P.O. (1996). Development of reliable quality parameters for black tea and their application to quality improvement in Kenya. *Tea*. **17(2)**: 82-90.

Owuor, P.O and Obanda, M. (1997). The effects of some agronomic and processing practices and clones on the relative composition of the theaflavins in black tea. *Food Sci.Technol. Int. Tokyo* **3 (4):** 344–347.

Owuor, P. O. and Obanda, M. (2001). Comparative responses in plain black tea quality parameters of different tea clones to fermentation temperature and duration. *Food Chem.* **72:** 319-327.

Owuor, P.O**,** Obanda, M., Nyirenda, H. E., Mphangwe, N. I K., Wright, L. P. and Apostolides Z. (2006). A relationship between some chemical parameters and sensory evaluations for plain black tea (*Camellia Sinensis*) produced in Kenya and comparison with similar teas from Malawi and S.Africa. *Food Chem.* **97 (4):** 644- 653.

Owuor, P. O., and Obanda, M. (2007). The use of green tea (*Camellia sinensis*) leaf flavan-3-ol composition in predicting plain black tea quality potential. *Food Chem*. **100**:873–884.

Owuor, P. O., Kamau, D. M., and Jondiko, E. O. (2009). Response of clonal tea to location of production and plucking intervals. *Food Chem*. **115:** 290–296.

Owuor, P.O., Obaga, S.O., Othieno, C.O. (1990). Effects of altitude on the chemical composition of back tea. *J. Sci. Food Agric*. **50:** 9–17.

Pourmorad, F., Husseinimehr, S.J. and Shahabimajd, N. (2006). Antioxidant, phenol and flavanoid contents of some selected Iranian medicinal plants. *Afric. J. of Biotech.* **5**:1142-1145.

Rijken, P.J. (2000). Antioxidant and other properties of green and black tea. in: E.Cadenas and L.Packer . *Handbook of Antioxidants. 2nd ed*. **19:** 371-399. Marcel Dekker, New York.

Roberts, E.A.H. (1962). Economic importance of flavonoid substances: tea fermentation. In: *The Chemistry of Flavonoid Compounds*. T.A. Geissman (Ed). pp 409-512. Pergamon press, Oxford.

Roberts, E.A.H. and Smith, R.F. (1963). Phenolic substances of manufactured tea.11. Spectrophotometric evaluation of tea liquors. *J. Sci. Food Agri.* **14:** 689-700.

Robertson, A. (1983a). Effects of physical and chemical conditions on the *in vitro* oxidation of tea leaf catechins. *Phytochem.* **22 (4):** 889-896.

Robertson, A. (1983b). Effect of catechin concentration on the formation of black tea polyphenols during *in vitro* oxidation. *Phytochem.* **22 (4):** 897-903.

Robertson, A. (1992). The chemistry and biochemistry of black tea production, the non volatiles. In: *Tea: Cultivation to consumption.* pp 555-601*;* K.C. Wilson and M.N. Clifford, Ed., Chapman and Hall: London, U.K.

Rohde, J., Jacobsen, C. and Kromann-Andersen, H. (2011). Toxic hepatitis triggered by green tea. *Ugeskr Laeger*, **173**: 205-206.

Saijo, R., and Takeo, T. (1973). Volatile and non-volatile forms of aroma compounds in tea leaves and their changes due to injury. *Agric. Biol. Chem*. **37**: 1367–1373.

Sakato, Y. (1949). The chemical constituents of tea III. A new amide theanine. *J. of Agric. and Food Chem.* **23**: 262–267.

Sanderson, G.W. and Graham, H.N. (1973). On the formation of black tea aroma. *J. Agric. Food Chem.* **21**: 576–585.

Sano, J. (2004). Effects of green tea intake on the development of coronary artery disease. *Circ J.* **68:** 665-70.

Sato, T. and Myata, G.(2000). The nutraceutical benefit, Part I: Green tea. *Nutr.* **16:** 315-317.

Sharbert, S. and Hofmann, T. (2005). Molecular definition of black tea taste by means of quantitative studies, taste reconstitution and omission experiments. *J. of Agric. and Food Chem*. **53**:5337-5384.

Skibola, C. and Smith, M. (2000)**.** Potential health impacts of excessive flavonoid intake. *Free Radic. Biol. Med*. **29 (3-4**):375–383.

Snel, J. and Lorist, M.M. (2011). Effects of caffeine on sleep and cognition. *Prog. Brain Res*.**190**:105-117.

Stapleton, P.D. (2004). Modulation of β-lactam resistance in *Staphylococcus aureus* by catechins and gallates. *I. J. Antimic. Ag*. **23:** 462-7.

Steinhaus, B. and Engelhardt, U.H. (1989). Comparison of the flavognost and HPLC analysis of theaflavins- Preliminary results. *Z. Lebensm. Unters Forsch.* **188:** 509–511.

Sugiyama, T. and Sadzuka, Y. (2003). Theanine and glutamate transporter inhibitors enhance the antitumor efficacy of chemotherapeutic agents. *Biochimica et Biophysica Acta.* **1653:** 47–59.

Suzuki, M. (2004). Protective effects of green tea catechins on cerebral ischemic damage. *Med. Sci. Monit*. **10 (6):** 166-74.

Tiwari, B.K., O'Donnell, C.P., Patras, A., Brunton, N.P. and Cullen, P.J. (2009a). Effect of ozone processing on anthocyanins and ascorbic acid degradation of strawberry juice. *Food Chem.* **113 (4):** 1119–1126.

Tiwari, B.K., O'Donnell, C.P. and Cullen, P.J. (2009b). Effect of non thermal processing technologies on the anthocyanin content of fruit juices. *Trends in Food Sci. & Technol*. **20 (3-4):**137-145.

Tsong- Ming, L., Lee, C.C. Mau, J. L. and Lin, S. D. (2009). Quality and antioxidant property of green tea sponge cake. *Food Chem.*, **119 (3):**1090 – 1095.

Wachira, F.N. and Kamunya, S. (2005). Kenyan teas are rich in antioxidant.*Tea.***26:**81-89.

Wang, H., Provan, G. J. and Helliwell, K. (2000). Tea flavonoids: their functions, utilisation and analysis. *Trends in Food Sci. and Technol.* **11:** 152-160.

Watts, R., (1999). Improving farmer's income. Africa Farming and Food Processing, **9:** 26-28.

Wheeler, D.S., and Wheeler, W.J., (2004). The medicinal chemistry of tea. *Drug Dev. Res*. **61:**45-65.

Wolfram, S. (2007). Effects of green tea and EGCG on cardiovascular and metabolic health. *J.Am.Coll.Nutr.***26 (4)**:3735-3885.

Wrolstad, R.E., Durst, R.W. and Lee, J. (2005). Tracking color and pigment changes in anthocyanin products. *Trends Food Sci. Technol*. **16**: 423-428.

Wright, L.P., Mphangwe, N.I.K., Nyirenda, H.E. and Apostolides, Z. (2002). Analysis of the theaflavin composition in black tea (*Camellia sinensis)* for predicting the quality of tea produced in Central and Southern Africa. *J. Sci. Food Agric.* **82**:517–525.

Yamada, T. and Terashima, T. (2009). Theanine, γ-glutamylethylamide, a unique amino acid in tea leaves, modulates neurotransmitter concentrations in the brain striatum interstitium in conscious rats. *Amino Acids.* **36:** 21–27.

Zhang, M.H. (2004). Determination of total antioxidant capacity in green tea by near-infrared spectroscopy and multivariate calibration. *Talant.* **62**: 25-35.

Zhao, J.W., Chen, Q.S. and Huang, X.Y. (2006). Qualitative identification of tea categories by near infrared spectroscopy and support vector machine, *J. of Pharmac. and Biomed. Anal.* **41:** 1198–1204.

Zheng, G., Bamba, K., Okubo, T., Juneja, L.R., Oguni, I. and Sayama, I.K. (2005). Effect of theanine, γ-glutamylethylamide, on bodyweight and fat accumulation in mice, *Animal Sci. J.* **76:** 153–157.

Zuo,Y., Chen, H. and Deng, Y. (2002). Simultaneous determination of catechins, caffeine and gallic acid in green tea, Oolong, black and Puerh teas using HPLC with a photodiode array detector. *Talanta* **57:** 307-361.

APPENDICES

Appendix I: Score sheet for green tea

Liqour colour	1. Green 2. Fairly green 3. Greenish 4. Bright amber 5. Amber
Liqour body	1. Very thick 2. Thick 3. Fairly thick 4. light 5. Very light
Liqour briskness	1. Astringent 2. Brisk 3. Fairly brisk 4. Not brisk 5. Stewy/fermented
Liqour strength	1. Very strong 2. Strong

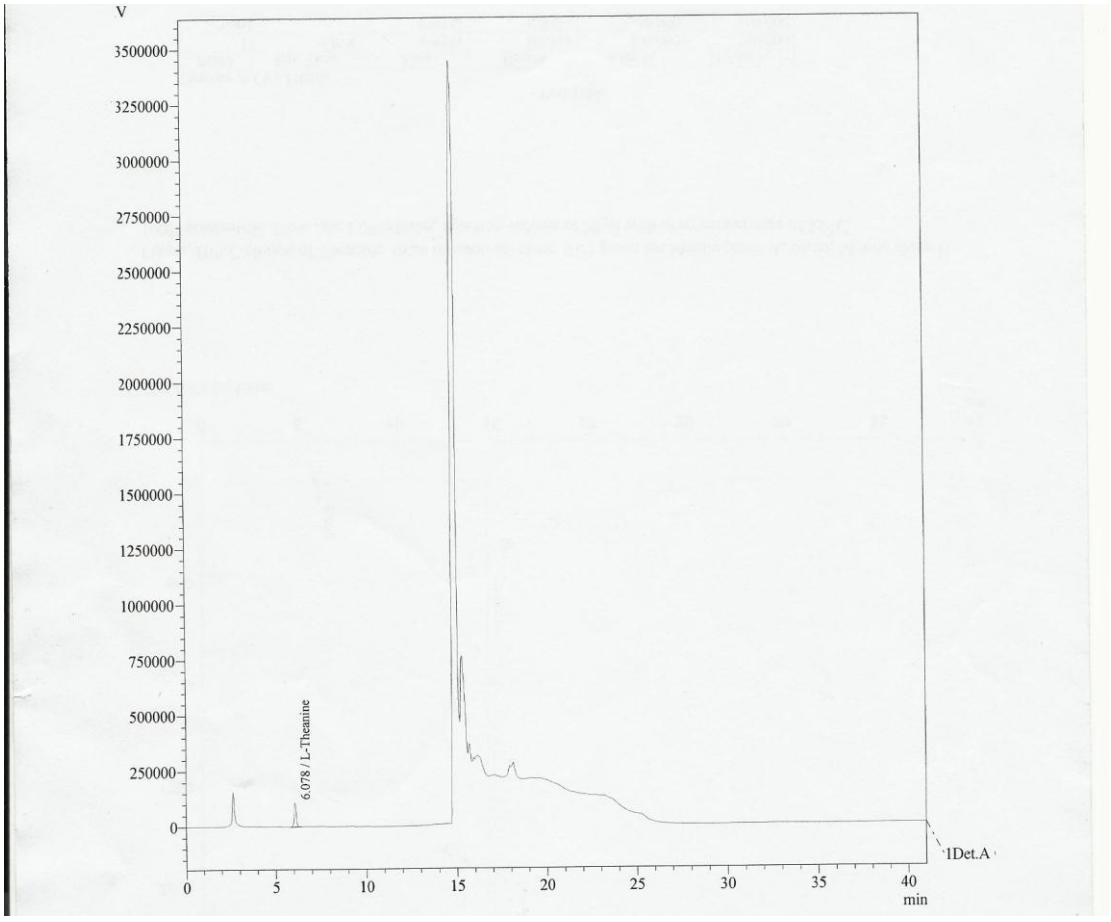
	3. Fair strength 4. Soft 5. Smooth
Infusion colour	1. Very bright 2. Bright 3. Fairly bright 4. Dull 5. Greenish

Appendix II: Score sheet for black tea

Liqour colour	1. Coppery 2. Very bright 3. Bright 4. Fairly bright 5. Dull
Liqour body	1. Creamy 2. Very thick 3. Thick 4. Fairly thick 5. Light
Liqour briskness	1. Very brisk 2. Brisk 3. Fairly brisk 4. Not brisk 5. Coarse
Liqour strength	1. Pungent 2. Strong 3. Fair strength 4. Soft

	5. Harsh
Infusion colour	1. Very bright 2. Bright 3. Fairly bright 4. Dull 5. Greenish

Appendix III : HPLC elution of theanine in an infusion of clone TRFK 91/1 green tea

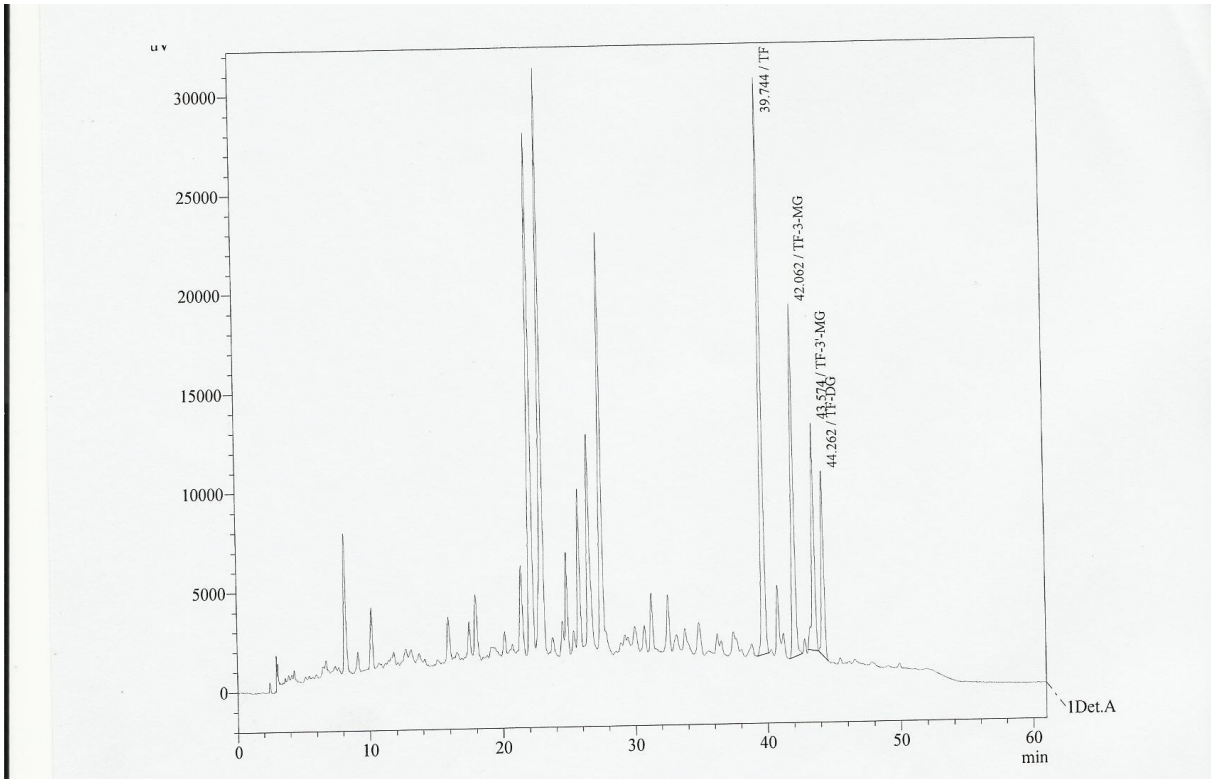


Key

X- Retention time,min

Y- Intensity, µV

Appendix IV: HPLC elution of theaflavin fractions in an infusion of clone TRFK 6/8 black tea



Key

X- Retention time,min

Y- Intensity, µV

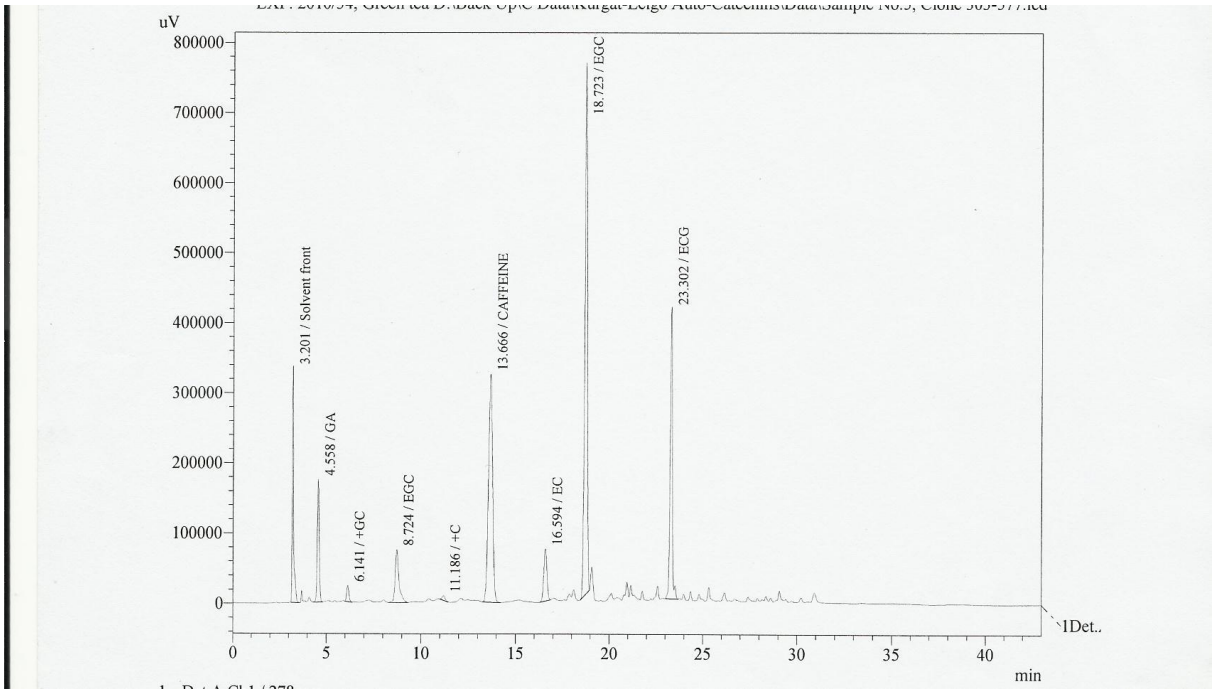
TF Simple theaflavins

TF-3-MG Theaflavin -3-monogallate

TF- 3’-MG Theaflavin- 3’- monogallate

TF-DG Theaflavin digallate

Appendix V: HPLC elution of individual catechins in an infusion of clone 303/577 green tea.



Key

X- Retention time,min

Y- Intensity, µV

GA – Gallic acid

GC – Gallocatechin

EGC – Epigallocatechin

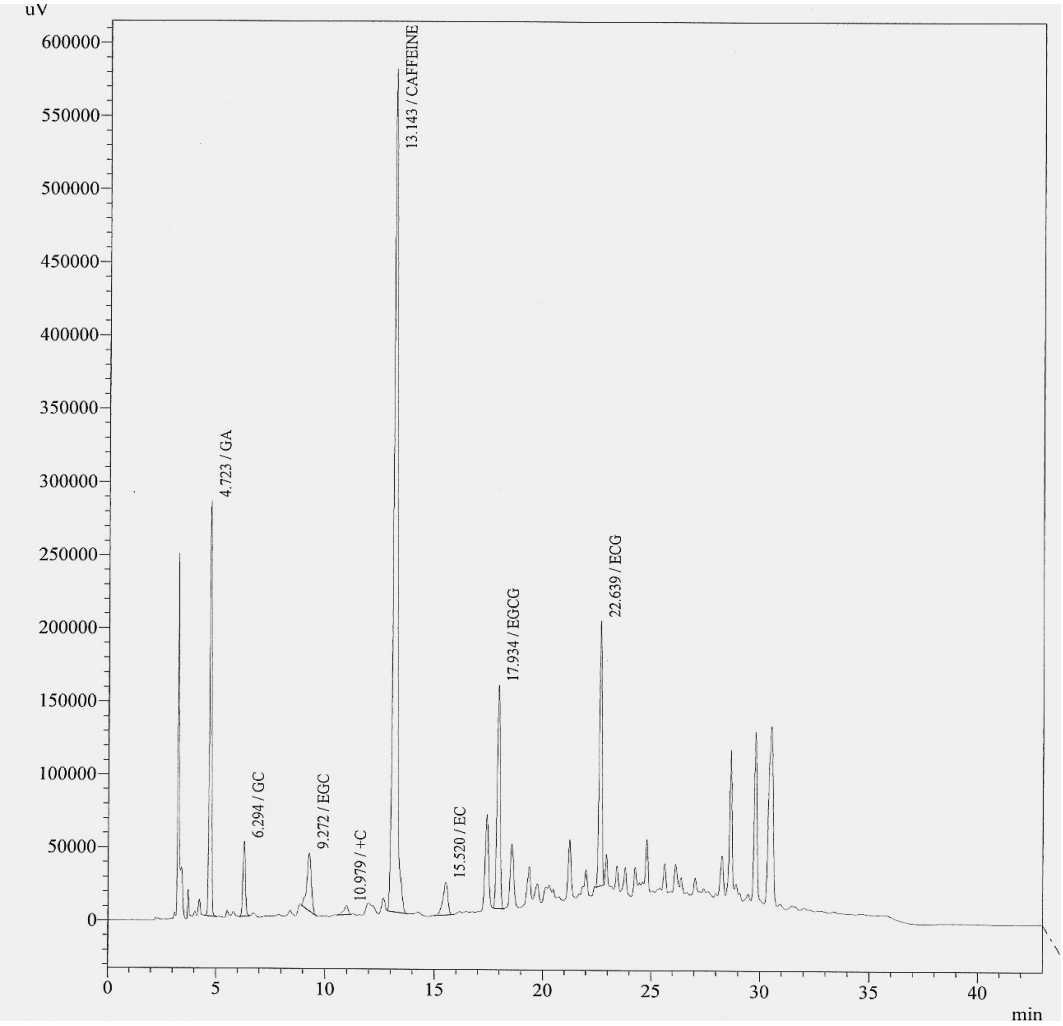
+C – Catechin

EC – Epicatechin

EGCG – Epigallocatechin gallate

ECG – Epicatechin gallate

Appendix VI: HPLC elution of individual catechins in an infusion of clone TRFK 6/8 black tea



Key

X- Retention time,min

Y- Intensity, µV

GA – Gallic acid EC – Epicatechin

EGCG – Epigallocatechin gallate

ECG – Epicatechin gallate

GC – Gallocatechin

EGC – Epigallocatechin

+C – Catechin

Appendix VII: Theanine and total polyphenol (TPP) percentage in studied clones

	Theanine	**TPP**
Test clones		
TRFK91/1	2.18^{a}	23.87^{bcdef}
TRFK 91/2	1.83^{abcd}	21.88^{bcdef}
TRFK KS3	1.99^{ab}	21.79^{defg}
TRFK KS1	1.55^{cdef}	23.07^{bcdefg}
TRFK KS2	1.50^{defg}	21.09^{efg}
TRFK 73/7	1.53^{def}	20.82^{efg}
TRFK 73/3	1.97^{abc}	22.79^{cdefg}
TRFK 73/1	1.51^{defg}	21.81^{defg}
TRFK 73/2	1.52^{defg}	23.28^{bcdef}
TRFK 73/4	1.79^{abcd}	21.83^{defg}
TRFK 83/1	1.10^{gh}	23.15^{bcdefg}
K-Purple	1.10^{gh}	25.86^{abc}
TRFK 73/5	1.79^{abcd}	23.32^{bcdef}
TRFK 14/1	1.59^{bcde}	22.07^{defg}
Commercial clones		
ST.543	1.47^{defg}	22.18^{defg}
ST. 536	1.87^{abcd}	20.41^{fg}
GW Ejulu	1.47^{defg}	27.27^{a}
TRFK 301/1	2.03^{a}	25.87^{abc}
TRFK 31/8	1.01^{h}	24.21^{abcde}
EPK TN 14/3	1.51^{defg}	23.36^{bcdef}
TRFK 301/2	1.79^{abcd}	22.69^{cdefg}
TRFK 303/577	1.0^{h}	24.23^{abcde}
TRFK 303/216	1.26^{efgh}	24.80^{abcd}

TRFK 6/8	0.92^{h}	26.54^{ab}
AHP S15/10	1.52^{defg}	22.38^{cdefg}
Hanlu	1.12^{fgh}	19.70^{gh}
Yabukita	1.44^{defg}	17.16^{h}
CV	**14.34**	**7.62**

Means followed by the same letter along the same column are not significantly different at p< 0.05. n = 81.

Appendix VIII: Clonal variation in gallic acid, caffeine and catechins (%)

	Gallic acid	Caffeine	EGC	EGCG	C	EC	ECG	TC
Test clones								
TRFK 91/1	1.09^{a}	1.54^{efgh}	0.46^{l}	4.53^{cd}	0.14^{hijk}	0.18^{n}	1.58^{def}	6.88^{ijk}
TRFK 91/2	0.67^{bc}	1.38^{gh}	1.30^{k}	3.55^{efgh}	0.24^{cdef}	0.49^{m}	1.07^{ijk}	6.65^{jk}
TRFK KS3	0.70^{b}	2.33^{bc}	3.72^{cd}	3.89^{def}	0.22^{defgh}	1.16^{efgh}	1.84^{bcd}	10.82^{cd}
TRFK KS1	0.32^{ijkjm}	1.40^{fgh}	1.88^{ij}	4.30^{de}	0.21^{defghi}	0.66^{klm}	1.46^{efg}	8.50^{efg}
TRFK KS2	0.43^{fgh}	2.22^{bcd}	3.57^{def}	4.24^{de}	0.16^{efghijk}	1.53^{c}	1.94^{bc}	11.43^{bcd}
TRFK 73/7	0.17^{o}	1.80^{defg}	4.54^{b}	3.62^{efg}	0.21^{defghi}	1.79^{a}	1.99^{bc}	12.15^{b}
TRFK 73/3	0.22^{mno}	1.67^{hi}	1.33^{k}	1.93^{k}	0.14^{ghijk}	0.66^{klm}	1.14^{hij}	5.20^{l}
TRFK 73/1	0.38^{ghijk}	1.87^{cdef}	4.09^{c}	4.43^{cd}	0.34^{b}	1.55^{bc}	1.55^{def}	11.95^{b}
TRFK 73/2	0.40^{fghij}	1.82^{defg}	4.78^{b}	4.08^{def}	0.23^{defg}	1.12^{efgh}	1.22^{ghi}	11.41^{bcd}
TRFK 73/4	0.24^{lmno}	1.55^{efgh}	2.62^{gh}	6.25^{a}	0.14^{hijk}	1.03^{ghi}	1.97^{bc}	12.00^{b}
TRFK 83/1	0.41^{fghij}	2.21^{bcd}	6.12^{a}	3.35^{fghi}	0.27^{bcd}	0.89^{ij}	1.16^{ghij}	11.78^{bc}
K-Purple	0.42^{fghi}	2.04^{bcd}	0.38^{l}	2.93^{ghij}	0.32^{bc}	0.69^{kl}	3.12^{a}	7.42^{hij}
TRFK 73/5	0.32^{ijklm}	1.16^{hi}	3.63^{cde}	2.35^{jk}	0.18^{efghij}	0.72^{jk}	1.27^{fghi}	8.15^{fgh}
TRFK 14//1	0.32^{ijklm}	1.82^{defg}	2.66^{g}	2.79^{hij}	0.28^{bcd}	1.20^{efg}	0.86^{jkl}	7.78^{fghi}
Commercial clones								
ST.543	0.18^{o}	1.75^{defg}	2.14^{hi}	2.58^{ijk}	0.15^{fghijk}	0.97^{hi}	0.87^{jkl}	6.70^{ijk}
ST.536	0.34^{hijkl}	2.12^{bcd}	3.68^{cd}	5.22^{bc}	0.15^{fghijk}	1.55^{bc}	1.86^{bcd}	12.45^{b}
GW Ejulu	0.42^{fghi}	2.34^{bc}	1.03^{k}	4.66^{cd}	0.62^{a}	1.10^{fgh}	3.26^{a}	10.66^{d}
TRFK 301/1	0.21^{no}	1.40^{fgh}	1.51^{jk}	2.49^{jk}	0.22^{defgh}	1.30^{de}	1.43^{efgh}	6.94^{ijk}
TRFK 31//8	0.28^{klmn}	1.37gh	2.33^{ghi}	2.84^{ghij}	0.12^{jk}	0.52^{lm}	0.78^{kl}	6.59^{jk}
EPK TN 14/3	0.49^{ef}	2.98^{a}	3.12^{f}	4.58^{cd}	0.15^{ghijk}	1.40^{cd}	1.46^{efg}	10.71^{d}
TRFK 301/2	0.55^{de}	2.92^{a}	3.89^{cd}	5.97^{a}	0.16^{efghijk}	1.72^{ab}	2.06^{b}	13.79^{a}
TRFK 303/577	0.31^{jklm}	1.55^{efgh}	2.20^{ghi}	3.43^{fgh}	0.08^{k}	0.85^{ijk}	1.07^{ijk}	7.62^{ghij}
TRFK 303/216	0.26^{lmno}	1.52^{efgh}	2.39^{gh}	2.89^{ghij}	0.24^{cde}	0.68^{kl}	0.82^{kl}	7.02^{ijk}
TRFK 6/8	0.22^{mno}	0.86^{i}	2.39^{gh}	2.23^{jk}	0.22^{defgh}	0.74^{jk}	0.71^{l}	6.28^{k}

AHP S 15/10	0.62^{bcd}	3.07^{a}	1.25^{k}	4.52^{cd}	0.13^{ijk}	1.20^{efg}	1.69^{cde}	8.79^{ef}
Hanlu	0.61^{cd}	2.42^{b}	3.18^{ef}	5.75^{ab}	0.13^{ijk}	1.25^{def}	2.02^{b}	12.32^{b}
Yabukuta	0.27^{lmno}	2.00^{bcde}	2.56^{gh}	4.06^{def}	0.16^{efghijk}	1.00^{hi}	1.63^{de}	9.41^{e}
CV	**13.33**	**13.06**	**9.82**	**21.02**	**9.72**	**11.09**	**10.79**	**6.06**

Means followed by the same letter along the same column are not significantly different at p< 0.05. n = 81.

Appendix IX: Clonal variations in individual theaflavins (µmol/g)

Test clones	**TF**	**TF-3-g**	**TF-3'-g**	**TF dg**
TRFK 91/1	1.43^{h}	2.86^{h}	1.68^{kl}	3.57^{fgh}
TRFK 91/2	2.88^{gh}	4.21^{g}	2.72^{efghi}	4.77^{bcd}
TRFK 73/1	7.66^{b}	6.92^{bc}	3.10^{cdefghi}	3.51^{efgh}
TRFK 73/2	8.16^{b}	6.54^{bcd}	3.34^{cdefg}	3.22^{fghi}
TRFK 73/3	2.33^{h}	3.98^{gh}	1.59 ^{l}	3.28^{fghi}
TRFK 73/4	4.76^{efg}	4.50^{fg}	2.59^{fghij}	2.84^{hij}
TRFK 73/5	5.38^{cde}	5.80^{cde}	2.87^{defghi}	3.76^{efgh}
TRFK 73/7	6.81^{bcde}	3.90^{gh}	1.78^{jkl}	1.97^{j}
TRFK KS 1	5.12^{def}	5.49^{def}	2.48^{ghijk}	3.59^{efgh}
TRFK KS 2	6.70^{bcde}	6.50^{bcd}	3.40^{cdef}	4.14^{cdef}
TRFK KS 3	8.58^{b}	6.98^{bc}	3.84^{abc}	4.07^{cdef}
TRFK 83/1	7.24^{bc}	6.36^{bcd}	3.51^{bcde}	3.73^{efgh}
TRFK 14/1	7.80^{b}	6.28^{cd}	3.26^{cdefg}	2.83^{hij}
K-Purple	3.33^{fgh}	5.69^{cdef}	2.54^{fghij}	5.37^{b}
Commercial clones				

TRFK 303/577	8.62^{b}	6.19^{cd}	3.87^{abc}	3.68^{efgh}
EPK TN 14/3	8.46^{b}	6.66^{bcd}	4.54^{a}	4.32^{cde}
TRFK 31/8	6.83^{bcde}	5.54^{def}	3.58^{bcde}	3.77^{efgh}
ST.536	6.87^{bcd}	4.70^{efg}	2.23^{ijkl}	2.05^{j}
AHP S 15/10	5.27^{cdef}	5.54^{def}	3.67^{bcd}	4.90^{bc}
TRFK 301/1	8.50^{b}	9.12^{a}	3.16^{cdefg}	3.90^{defg}
TRFK 6/8	10.85^{a}	6.57^{bcd}	4.30^{ab}	3.34^{efgh}
GW Ejulu	2.68^{h}	5.56^{def}	2.29^{hijkl}	6.52^{a}
TRFK 301/2	7.52^{b}	7.62^{b}	2.87^{defghi}	3.93^{defg}
ST.543	7.95^{b}	5.70^{cdef}	2.54^{fghij}	2.35^{ij}
TRFK 303/216	11.32^{a}	6.65^{bcd}	3.97^{abc}	2.97^{ghi}
CV	**17.06**	**11.61**	**14.95**	**13.68**

Means followed by the same letter along the same column are not significantly different at p< 0.05. n = 75.

Appendix X: Total thearubigins and their fractions in studied clones (%)

Test clones	**TR**	**TR SI**	**TR SII**
TRFK 91/1	20^{a}	1.69^{c}	10.73^{abcd}
TRFK 91/2	19.77^{a}	2.51^{bc}	12.28^{a}
TRFK 73/1	20.11^{a}	5.18^{ab}	7.68^{cdefg}
TRFK 73/3	20.12^{a}	2.18^{bc}	7.80^{cdefg}
TRFK 73/4	19.07^{a}	2.02^{bc}	7.12^{efg}
TRFK 73/5	21.44^{a}	3.75^{abc}	7.72^{cdefg}
TRFK 73/7	17.71^{a}	2.14^{bc}	7.63^{cdefg}
TRFK KS 1	18.76^{a}	3.18^{bc}	6.56^{fg}
TRFK KS 2	19.88^{a}	2.44^{bc}	7.42^{defg}
TRFK KS3	19.71^{a}	6.30^{a}	9.50^{abcdef}
TRFK 83/1	19.29^{a}	2.76^{bc}	7.64^{cdefg}
TRFK 14/1	19.10^{a}	2.43^{bc}	8.43^{bcdefg}
K-Purple	21.54^{a}	2.54^{bc}	10.11^{abcde}
TRFK 73/2	19.77^{a}	2.29^{bc}	7.86^{cdefg}
Commercial clones			

TRFK 303/577	17.39^{a}	2.86^{bc}	9.34^{abcdef}
EPK TN 14/3	20.14^{a}	2.95^{bc}	10.78^{abcd}
TRFK 31/8	18.10^{a}	2.85^{bc}	9.11^{abcdef}
ST. 536	18.32^{a}	2.63^{bc}	5.57^{g}
AHP S 15/10	17.74^{a}	2.54^{bc}	9.94^{abcdef}
TRFK 301/1	16.91^{a}	2.95^{bc}	11.02^{abc}
TRFK 6/8	19.88^{a}	4.87^{abc}	12.40^{a}
GW EJULU	18.91^{a}	2.68^{bc}	10.03^{abcde}
TRFK 301/2	21.07^{a}	3.14^{bc}	11.57^{ab}
ST.543	18.36^{a}	4.82^{abc}	7.49^{defg}
TRFK 303/216	18.61^{a}	4.52^{abc}	9.37^{abcdef}
CV %	**12.88**	**50.71**	**19.12**

Means followed by the same letter along the same column are not significantly different at p< 0.05. n = 75.

Appendix XI: Total soluble solids (TSS), total colour, brightness, and theaflavins (TF)

Test clones	**TSS (%)**	**Brightness (%)**	**Total colour (%)**	**TF µmoles/g**
TRFK 91/1	39.33^{abc}	10.79^{g}	4.41^{ijkl}	9.54^{j}
TRFK 91/2	36.33^{abcde}	17.20^{fg}	5.20^{defghi}	14.59^{hi}
TRFK 73/1	34.54^{bcde}	29.09^{abcd}	4.99^{efghij}	21.19^{abcde}
TRFK 73/2	28.48^{f}	33.35^{ab}	4.68^{ghijk}	21.26^{abcde}
TRFK 73/3	35.08^{bcde}	19.74^{ef}	4.04^{kl}	11.19^{ij}
TRFK 73/4	34.55^{bcde}	27.29^{abcde}	4.21^{jkl}	14.69^{hi}
TRFK 73/5	33.02^{def}	25.49^{abcdef}	4.66^{ghijk}	17.82^{defgh}
TRFK 73/7	31.18^{ef}	26.89^{abcde}	4.20^{jkl}	14.48^{hi}
TRFK KS 1	39.86^{abc}	25.24^{abcdef}	4.19^{jkl}	16.68^{fgh}
TRFK KS 2	37.96^{abcd}	32.33^{abc}	4.91^{fghijk}	20.73^{abcdef}
TRFK KS 3	35.63^{abcde}	32.38^{abc}	5.72^{abcdef}	23.47^{ab}
TRFK 83/1	37.03^{abcde}	29.23^{abcd}	4.89^{fghijk}	20.84^{abcdef}
TRFK 14/1	36.32^{abcde}	28.35^{abcde}	5.28^{cdefghi}	20.16^{bcdefg}
K-Purple	33.87^{bcdef}	24.93^{bcdef}	4.83^{fghijk}	16.93^{efgh}
Commercial clones				
TRFK 303/577	34.44^{bcde}	32.30^{abc}	5.84^{abcde}	22.37^{abc}
EPK TN 14/3	37.34^{abcde}	34.38^{a}	6.16^{abc}	23.99^{ab}
TRFK 31/8	37.90^{abcd}	30.42^{abc}	5.38^{abcdefg}	19.72^{bcdefg}
ST.536	35.61^{abcde}	30.74^{abc}	3.66 ^{l}	15.86^{gh}

AHP S 15/10	37.03^{abcde}	20.35^{def}	6.15^{abc}	18.38^{cdefgh}
TRFK 301/1	35.77^{abcde}	29.31^{abcd}	6.26^{a}	24.69^{a}
TRFK 6/8	36.29^{abcde}	33.07^{ab}	6.07^{abcd}	25.07^{a}
GW Ejulu	41.28^{a}	23.34^{cdef}	5.33^{bcdefgh}	17.05^{efgh}
TRFK 301/2	33.73^{cdef}	23.90^{cdef}	5.84^{abcde}	21.95^{abcd}
ST.543	35.43^{abcde}	32.39^{abc}	4.46^{hijkl}	18.53^{cdefgh}
TRFK 303/216	39.95^{ab}	27.13^{abcde}	6.19^{ab}	24.92^{a}
CV %	**8.65**	**16.81**	**9.16**	**12.05**

Means followed by the same letter along the same column are not significantly different at $p< 0.05$. n = 75

Appendix XII: Residual catechins in black tea

Clones	Gallic acid	Caffeine	EGC	EGCG	C	EC	ECG	TC
TRFK 91/1	0.49^{ab}	1.96^{o}	1.42^{fgh}	0.36^{f}	0.13^{d}	0.39^{fghij}	0.91^{defgh}	3.21^{gh}
TRFK 91/2	0.53^{a}	3.27^{defgh}	1.593^{defgh}	1.11^{ab}	0.16^{cd}	0.62^{bcde}	1.08^{defgh}	4.56^{cdefgh}
TRFK KS3	0.29^{cdef}	3.77^{abc}	3.01^{abc}	0.79^{bcdef}	0.27^{bcd}	0.58^{bcdefg}	0.88^{efgh}	5.53^{bcd}
TRFK KS1	0.36^{bcd}	2.32^{mn}	1.29^{gh}	1.12^{ab}	0.28^{bc}	0.92^{a}	1.57^{abc}	5.18^{bcd}
TRFK KS2	0.30^{cdef}	2.51^{klmn}	2.96^{abc}	1.01^{bcd}	0.22^{bcd}	0.44^{cdefghi}	1.13^{cdefg}	5.77^{abc}
TRFK 73/7	0.20^{f}	2.90^{hijkl}	1.43^{efgh}	1.53^{a}	0.14^{cd}	0.39^{fghij}	1.13^{cdefg}	4.61^{cdefg}
TRFK 73/3	0.26^{cdef}	3.00^{fghij}	1.39^{fgh}	0.71^{bcdef}	0.17^{cd}	0.19^{j}	1.05^{defgh}	3.51^{efgh}
TRFK 73/1	0.26^{cdef}	2.65^{jklmn}	2.42^{bcde}	0.71^{bcdef}	0.17^{cd}	0.51^{cdefgh}	1.20^{cdefg}	4.98^{bcd}
TRFK 73/2	0.28^{cdef}	2.47^{lmn}	2.75^{abc}	0.82^{bcde}	0.16^{cd}	0.61^{bcde}	1.29^{cde}	5.64^{bcd}
TRFK 73/4	0.29^{cdef}	2.45^{mn}	2.75^{abc}	0.85^{bcde}	0.17^{cd}	0.48^{cdefghi}	1.24^{cdef}	5.50^{bcd}
TRFK 83/1	0.30^{cdef}	2.78^{ijklm}	2.74^{abc}	0.88^{bcde}	0.23^{bcd}	0.31^{hij}	1.04^{defgh}	5.20^{bcd}
K-Purple	0.21^{def}	3.03^{efghij}	0.63^{h}	0.52^{ef}	0.15^{cd}	0.32^{hij}	1.80^{ab}	3.42^{fgh}
TRFK 73/5	0.25^{cdef}	2.44^{mn}	2.33^{cdef}	0.93^{bcde}	0.15^{cd}	0.42^{efghi}	1.39^{bcd}	5.23^{bcd}
TRFK 14//1	0.26^{cdef}	2.98^{fghij}	2.33^{cdef}	0.83^{bcde}	0.16^{cd}	0.48^{cdefghi}	1.11^{cdefg}	4.91^{bcde}
Commercial clones								
ST.543	0.20^{ef}	3.19^{defghi}	1.58^{defgh}	0.59^{def}	0.27^{bcd}	0.37^{ghij}	0.60^{h}	3.34^{gh}
ST.536	0.25^{cdef}	2.60^{jklmn}	2.75^{abc}	1.03^{bc}	0.15^{cd}	0.43^{defghi}	1.00^{defgh}	5.37^{bcd}
GW Ejulu	0.36^{bcde}	3.61^{abcd}	1.15^{gh}	0.80^{bcde}	0.24^{bcd}	0.65^{bc}	1.95^{a}	4.78^{cdef}
TRFK 301/1	0.23^{cdef}	3.96^{a}	2.09^{cdefg}	0.70^{bcdef}	0.35^{b}	0.64^{bcd}	1.59^{abc}	5.36^{bcd}
TRFK 31/8	0.37^{bc}	3.83^{ab}	2.38^{cdef}	1.00^{bcd}	0.23^{bcd}	0.61^{bcde}	0.94^{defgh}	5.18^{bcd}

EPK TN 14/3	0.26^{cdef}	3.45^{bcde}	2.50^{bcd}	0.56^{ef}	0.15^{cd}	0.38^{ghij}	0.80^{efgh}	4.39^{cdefgh}
TRFK 301/2	0.20^{ef}	3.37^{cdefg}	1.59^{defgh}	0.36^{f}	0.20^{cd}	0.28^{ij}	0.73^{gh}	3.16^{h}
TRFK 303/577	0.22^{cdef}	3.46^{bcde}	2.05^{cdefg}	0.56^{ef}	0.17^{cd}	0.35^{hij}	1.22^{cdef}	4.34^{defgh}
TRFK 303/216	0.29^{cdef}	3.39^{bcdef}	3.4^{ab}	0.84^{bcde}	0.35^{b}	0.76^{ab}	0.89^{efgh}	6.24^{ab}
TRFK 6/8	0.28^{cdef}	2.94^{ghijk}	3.68^{a}	0.89^{bcde}	0.58^{a}	0.78^{ab}	1.13^{cdefg}	7.06^{a}
AHP S 15/10	0.33^{cdef}	3.40^{bcdef}	2.72^{abc}	0.60^{cdef}	0.22^{bcd}	0.59^{bcdef}	0.77^{fgh}	4.9^{bcde}
CV %	**31.98**	**9.06**	**27.66**	**33.42**	**39.6**	**26.35**	**26.15**	**17.71**

Appendix XIII: The GLM Procedure

Dependent Variable: TR

Source	DF	Sum of Squares	mean Square	F Value	Pr > F
Model	26	129.896	4.996	0.81	0.7125
Error	48	295.431	6.155		
Corrected Total	74	425.328			

R-Square	Coeff Var	Root MSE	TR Mean
0.305	12.876	2.481	19.268

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Rep	2	24.499	12.249	1.99	0.148
Clone	24	105.398	4.392	0.71	0.813

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Rep	2	24.499	12.249	1.99	0.148
Clone	24	105.398	1qa 4.392	0.71	0.813

Appendix XIV: The GLM Procedure

Dependent Variable: Theanine

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	28	9.930	0.355	7.35	<.0001
Error	51	2.461	0.048		
Corrected Total	79	12.391			

R-Square	Coeff Var	Root MSE	Thea Mean
0.801	14.336	0.219	1.532

Source	DF	Type I SS	Mean Square	F Value	Pr > F
rep	2	0.809	0.405	8.38	0.0007
clone	26	9.121	0.351	7.27	<.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
rep	2	0.794	0.397	8.23	0.0008
clone	26	9.121	0.351	7.27	<.0001

Appendix XV**:** An ANOVA Table on residual catechins on black tea
Dependent variable: EGC

K Value	Source	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Prob
1	Replication	2	5.202	2.601	7.0380	0.0021
2	Factor A	24	41.807	1.742	4.7139	0.0000
-3	Error	48	17.738	0.370		
	Total	74	64.746			

Coefficient of Variation: 27.66%

s_ for means group 1: 0.1216 Number of Observations: 25
y

s_ for means group 2: 0.3510 Number of Observations: 3
y

Appendix XVI: Submitted Papers

1. Kilel, E.C., Wanyoko, J.K.*, Faraj, A. K., Wachira F. N. and Mwingirwa, V. **Green tea from purple leaf coloured tea clones in Kenya – their quality characteristics**. *Food Chemistry.* Submitted on 28/9/2012, expected to be published Jan. 2013.

2. Kilel, E.C., Wanyoko, J.K.*,Faraj, A.K. and Wachira, F.N. **Plain black tea quality parameters of purple leaf coloured tea clones in Kenya.** *Food Research International.* Submitted on 30/8/2012, expected to be published Jan. 2013