OPTIMIZATION OF BREWING, PROCESSING CONDITIONS AND THEIR EFFECTS ON THE CHEMICAL AND SENSORY QUALITY OF PURPLE-LEAFED KENYAN TEA

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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.

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DEDICATION

I dedicate this work to my family.

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ABSTRACT

The Tea Research Institute (TRI) developed TRFK 306 tea clone which is rich in anthocyanin as a strategy to diversify Kenyan teas. TRFK 6/8 was used as a control in the study being a standard clone used at the TRI. TRFK 306 can be utilized non-processed but there is no well researched, documented data on the best brewing conditions. Consumers add edible acids to its brew to improve the colour and taste but information on the acid effect is scanty. Information on the quality of products manufactured in various ways is equally scanty. Completely randomized block design replicated three times was adopted. Fresh TRFK 306 tea leaves were brewed using time temperature combinations of 70 ^{0}C and 92 ^{0}C timed at 5, 10 and 15 minutes prior to analysing for total anthocyanins and antioxidant activity. Citric acid concentration of 0.1 %, 0.2 %, 0.3 %, 0.4 % and 0.5 % were used to check their effect on total anthocyanins .antioxidant activity and sensory quality. Five- point hedonic scale was used in the sensory evaluation. Non-aerated and aerated orthodox and Crush, Tear and Curl (CTC) teas were processed at different withering times with an interval of 5 hours starting from 0 up to 20 hours. Aeration duration was varied at an interval of 30 minutes, from 30, to 90 minutes. Tea quality parameters were assayed using the Flavognost method, Roberts method, High Performance Liquid Chromatography (HPLC) and UV spectrophotometer. Experienced tea tasters tasted the processed samples subjectively. Data obtained were subjected to general linear model using SAS package version 9.1.3. Means were separated using Least Significant Difference at $p < 0.05$ significance level. Non-processed purple tea leaves brewed for five minutes using boiling water, had anthocyanins level of 92 mg/L and had over 87.7 % antioxidant activity without citric acid added. Citric acid lowered antioxidant activity though it improved the taste and colour. TRFK 306 non-aerated orthodox teas had more total catechins than CTC teas and best withered for 10-15 hours for higher antioxidant activity. Non- aerated TRFK 306 CTC teas had a score of 3.5 in liquor briskness while TRFK 6/8 teas highest scored was 2. Aerated TRFK 306 CTC teas had a highest theaflavins of 14.41 µmol/g compared to TRFK 6/8 CTC teas with highest value of 16.43 µmol/g. Aerated TRFK 306 orthodox teas had a highest liquor flavour score of 4 slightly lower than TRFK 6/8 orthodox teas with 4.4. It was concluded that, TRFK 306 can be utilized non-processed or can be processed into green orthodox or aerated CTC teas at withering hours not exceeding 15 hours and aeration time not exceeding 60 minutes for premium products. TRFK 306 tea leaves are recommended for direct use without much processing.

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LIST OF ABBREVIATIONS AND ACRONYMS

AFA	Agriculture and Food Authority
AFFA	Agriculture, Fisheries and Food Authority
ATB	Africa Tea Brokers
BC	Before Christ
+C	Catechins
CD4	Cluster of Differentiation 4
CFR	Code of Federal Regulations
CTC	Crush Tear Curl
DPPH	2,2-Diphenyl-1-picrylhydrazyl
DM	Dry Matter
DNA	Deoxyribonucleic acid
EC	Epicatechin
ECG	Epicatechin Gallate
EGC	Epigallocatechin
EGCG	Epigallocatechin Gallate
GC	Gallocatechin
GRAS	Generally Recognized As Safe
HIV	Human Immunodeficiency Virus
HPLC	High performance liquid chromatography
IBMK	Isobutyl Methylketone
ISO	International Organization for Standardization
KALRO	Kenya Agricultural and Livestock Research Organization
KTDA	Kenya Tea Development Agency
LTP	Lawrie Tea Processor
OD	Optical density
ODS	Octadecylsilane
OP	Orange Pekoe
RNS	Reactive Nitrogen Species
ROS	Reactive Oxygen Species
SAS	Statistical Analysis Software
TC	Total Colour
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
TPVA	Tea Processing and Value Addition
TRs	Thearubigins
TRFK	Tea Research Foundation of Kenya
TRI	Tea Research Institute
UPASI	United Planters Association of Southern India
UV	Ultra Violet

CHAPTER ONE

INTRODUCTION

1.1 Background information

Tea is a plant whose scientific name is *Camellia sinensis* (L.) O. Kuntze belongs to Theaceae family and its growing shoots are used to process the second most consumed beverage globally, after water (Deb and Pou, 2016). Among some non-alcoholic beverages which include, packaged water, tea, coffee, carbonated soda and juices, packaged water global consumption in 2017 stood at 56.7 % while tea had 35.2 % (Bolton, 2018). According to Bolton, (2018), it is projected that, by 2021, the global consumption of tea and packaged water will in the ratio of 37.7 % to 68.3 %, respectively. These shows, tea will continue to be among widely consumed beverage. Tea has a pleasant taste and a refreshing effect yet relatively cheap compared to other beverages. It is consumed by many people all over the world at any time of the day. The widespread consumption could be because tea has high polyphenol contents which have health benefits (Sharma, 2014). *Camellia sinensis* consists mainly of two varieties of tea namely; *Camellia sinensis* variety *sinensis* and *Camellia sinensis* variety *assamica* (Wium, 2009). Several products can be obtained from the tea plant which can differ on quality due to variations in cultivation practices, processing styles and growing conditions (Ahmed *et al*., 2019). Processed tea can be categorized based on aeration status as non-aerated tea (green tea), semi-aerated (Oolong tea), aerated tea (black tea) and post-aerated tea (Pu-erh tea) (Zhao *et al*., 2006; Hilal, 2017). Other types of tea which can be produced include yellow, white, flavoured tea, instant tea and herbal teas (Hilal, 2017). Other types of teas based on processing methods include white and yellow tea (Ning *et al*., 2017).

Tea has remained among the top foreign exchange earners in Kenya for some years now with revenue rising to 140 billion Kenya shillings in 2018 (AFA, 2018). The production has steadily been increasing (Table 1) over the years and it is projected to increase in future. The trend shows some increase in earnings over a span of six years. Tea prices are driven by supply and demand, and this calls for the tea industry to be cautious and diversify its products especially those meant for exports.

Table 1.Trends in Kenya's tea earnings from 2013 to 2018

Year	kg (million) of tea exported	Ksh. (Billion) foreign exchanged	Source of information
2013	432	114	AFFA (2015)
2014	445	101	AFFA (2016)
2015	443.4	125	AFFA (2016)
2016	480	120	AFA (2017)
2017	415	129	AFA (2018)
2018	474.86	140	AFA(2018)

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 e*t al.,* 2009). In Kenya, green tea production is just emerging such that currently very few tea factories of Unilever Tea and Finlay Tea produce green tea. Green tea tasters, consequently, are few. Ochanda *et al*. (2012) described the green tea production in Kenya being at its infancy stage.

The Tea Research Institute - which replaces the Tea Research Foundation of Kenya in July 2014 and is under the Kenya Agricultural and Livestock Research Organization (KALRO) (Bore, 2015) - has developed an Assamica tea cultivar rich in anthocyanin coded TRFK 306 whose leaves have a purple appearance. The clone is an interspecific hybrid between a brick-red pigmented non-cultivated and cultivated tea cultivars (Kamunya *et al*., 2009b). The clone underwent improvement process for close to 30 years and further works on the content and antioxidant value of their anthocyanins are on going (Kamunya *et al*., 2009a). Special attributes of TRFK 306 include drought, frost, disease and pest resistance; high yield similar to the standard control and commercial clone TRFK 31/8; and wider adaptability (Kamunya *et al*., 2009a; Leonida *et al*., 2013). TRFK 6/8 is an assamica type of tea of local selection known for high quality black tea and is widely adapted to East Africa region (Cherotich *et al*., 2013). The new purple clone which has been released for commercialization can be processed just like the green clones into any of the tea types as is desired. Some steps like withering and aeration are critical steps in tea manufacture. The major physical change which occurs during withering is the moisture loss by the tea leaves which leads to changes in the permeability of the cell membrane. Chemical withering is the process where natural biochemical changes takes place following physical wither (Deb and Jolvis, 2016). Air may be blown to the tea leaves to keep them cool and remove the heat of respiration (Jayasundara,

2008). The chemical changes which take place during chemical withering include the breakdown of proteins into amino acids. Short chemical wither period favour the formation of theaflavins and thus liquor brightness increases (Obanda and Owuor, 1992). Normal wither occurs when air is passed through plucked tea leaves for about 12 to 24 hours, where the tea leaves looses moisture (Sharma and Dutta, 2018). Baruah e*t al.* (2012) in their study found that, reduction of withering time of 12 hours from traditional method to four hours in the modified method induced some favourable biochemical changes for enhancement in brightness and flavour quality in black tea infusion. Chemical wither for longer period produced liquor with better flavour quality and fuller cup characters (Baruah e*t al.,* 2012). Optimal fermentation/aeration duration should be established for every cultivar at different temperatures for production of high quality black teas (Asil e*t al.,* 2012). TRFK 6/8 is an Assam type of tea which was bred by Tea Research Institute, formerly the Tea Research Foundation of Kenya (Leonida *et al*., 2013). It has special attributes which includes high black tea quality and high yield.

Previously these authors studied other types of purple leafed teas, including some green clones (Kilel *et al*., 2012; Kilel, 2013) processed as Crushed Teared and Curled (CTC) teas with uniform withering and aeration time. TRFK 306 was not studied then. They, however, recommended for optimization of the purple-leafed clones manufacturing conditions. The current study aims to determine the quality of liquor made from fresh purple-leafed teas and to manufacture the purple clone through CTC and orthodox rolling at different withering times and aeration prior to quality evaluation. The findings will guide on how best the purple-leafed teas could be brewed and the optimum processing conditions if processed.

1.2 Statement of the problem

TRFK 306 was released in the year 2011 by the Tea Research Institute for commercial utilization in Kenya. Currently most purple tea consumers do brew fresh purple tea leaves then add lemon juice before they drink which help in improving the colour of the brew. There is little documentation on the quality of the product brewed at various time/temperature conditions, especially on the levels of anthocyanins and the antioxidant activity. More over the effect of addition of acids on the quality of the brew is equally scanty. There is a general belief that purple tea can fetch better price, and especially since many countries have not planted the clone. Many stakeholders are emphasising the need to grow, process and market the purple tea clone but its manufacturing conditions have not been well established neither documented. Withering and aeration conditions affect the ultimate

quality of made tea and currently there has been little research on optimization of such processing conditions and the documentation of the quality of such products. As the tea Industry adopts to promote purple tea, it is important to have some research data documented on their optimum manufacturing conditions to ensure quality is preserved hence better price and revenue from this new tea clone.

1.3 Objectives

1.3.1 General objective

The study was aimed at contributing towards improving tea earnings and utilization, through, optimising processing and brewing conditions of purple-leafed Kenyan tea.

1.3.2 Specific objectives

The specific objectives of the research were to:

(i) Determine the effect of brewing regime on the total anthocyanins present in the brew made from freshly plucked, non-processed TRFK 306 tea leaves.

(ii) Investigate the effect of citric acid on the quality of a brew made from fresh, non-processed TRFK 306 teas (total anthocyanins, antioxidant activity and sensory quality).

(iii) Determine the effect of processing conditions on the key chemical parameters of tea products (total polyphenols, total anthocyanins, total soluble solids, catechins, caffeine and gallic acid, total and individual theaflavins, thearubigins, total colour, brightness percentage and total soluble solids) made from TRFK 306 and compare it with tea made from TRFK 6/8.

(iv) Evaluate the effect of processing conditions on the sensory quality of the products made in objective (iii) alongside TRFK 6/8 teas.

(v) Determine the effect of processing conditions on the antioxidant activity of tea products made in objective (iii) alongside TRFK 6/8 teas.

1.4 Hypotheses

The hypotheses tested were:

(i) H_0: Brewing regime has no effect on the total anthocyanins present in the brew made from freshly plucked, non-processed purple tea leaves.

(ii) H_0: Total anthocyanins, antioxidant activity and sensory quality of a brew made from fresh, non-processed purple-leafed teas are not affected by the amount of citric acid.

(iii) H_0: Key quality parameters of teas made from TRFK 306 and TRFK 6/8 are not significantly affected by processing conditions.

(iv) H_0: sensory quality of the products made from TRFK 306 and TRFK 6/8 is not affected by processing conditions.

(v) H_0: Antioxidant activity of products made from TRFK 306 and TRFK 6/8 is not significantly affected by processing conditions.

1.5 Justification

Kenya is a major producer and exporter of black CTC teas in the world. Tea is a major relatively cheap beverage consumed worldwide because of its perceived health benefits. Purple tea clone, TRFK 306 can be grown in areas where the commonly known tea clones are cultivated. This means many people from tea growing zones can have access to this new clone. Though most consumers brew fresh purple tea leaves and add lemon juice before consumption, documentation of the quality of the brewed product at various time/temperature regimes and the effect of added acid is lacking. The information will guide the consumers and other stake holders on the brewing of non-processed fresh purple tea leaves which might open a market of selling the teas just like fresh cut flowers. This diversification of utilization will create a wider market for this new tea variety and hence more revenue. The TRFK 306 clone, however, can be processed like any other tea clone. Its product quality like any other tea product is affected by the processing conditions employed which include withering and aeration duration. Optimizing these two processing conditions (withering and aeration) during purple tea clone processing will guide the potential processors on how best this clone could be processed to get the most acceptable product. Optimization of the processing conditions will help minimise the production cost in terms of time and energy used during processing yet guarantees retention of beneficial phenolic compounds. Moreover, little research if any has been done to check the effect of these processing conditions on the chemical and sensory quality of the final product. Developing various products from the purple tea clone will help to spread the risk in case of unstable tea prices hence cushioning farmers from losses.

1.6 Scope and limitation

The study was carried out at Kenya Agricultural and Livestock and Research Organization –Tea Research Institute, Kericho. The tea leaves were specifically picked from TRFK 306 and TRFK 6/8 tea clones. The environment of tea processing area during samples

preparations was not modified, ambient conditions were used. Orthodox teas were all hand-rolled and CTC teas were processed at the miniature tea factory of TRI using micro tea processors. There were no tea samples from external sources that were tested, all the tea samples were manufactured by the principal investigator. Total monomeric anthocyanins in processed black teas were not investigated in the current study neither were non-processed TRFK 6/8 subjected to any analysis. Non-aerated teas made fromTRFK 6/8 were also not assayed for total monomeric anthocyanins.

1.7 Assumptions

This study was based on the following assumptions:

i) Tea processors do not alter the withering and aeration duration based on tea clones plucked.

ii) The quality parameters assayed are the key aspects in tea quality.

iii) The sensory responses reflect how the products might sell in the market.

iv) That the plucking or use of two leaves and a bud will be representative of all other harvesting methods where more leaves will be included.

1.8 Definition/description of terms

Tea terminologies globally may not necessarily be referring to the same thing always. The definitions/descriptions highlighted here refer to how they were used in the current study.

Withering – As used in tea processing refers to the process of allowing fresh tea leaves to wilt soon after plucking due to moisture loss.

Aeration- Is a term used in tea processing to refer to oxidation of teas soon after leaf disruption (CTC or rolling).

Fixation – Is a term used in green tea processing to refer to a process of inactivating tea enzymes using heat. Other alternate terms include 'kill-green' and enzymes inactivation used differently in tea growing regions.

Sweltering – Is a unique processing technique to yellow teas, where the warm and damp teas leaves from fixation are allowed to be slightly heated in a closed container, which causes the previously green tea leaves to turn yellow.

CTC – Is an abbreviation of 'Crush, Tear and Curl'. It's a method of leaf disruption where a set of 3-4 pair of steel rollers is used to reduce the size of tea leaves. The product of the process is referred to as 'dhool' and made teas from the process are referred to as CTC teas.

Orthodox- Is a method of leaf disruption which involves rolling of the tea leaves. It is an alternative of CTC method of leaf disruption. The products of the rolling process are bigger tea leaf particles than CTC. It uses a rolling table and a hopper containing tea.

Theaflavins – These are products of enzyme initiated de-carboxylation and condensation between a dihydroxy and a trihydroxy flavan-3-ol in the presence of oxygen. They include simple theaflavin, theaflavin-3-monogallate, theaflavin-3'- monogallate and theaflavin-3-3'-digallate. They give briskness, brightness and colour to black tea.

Thearubigins – These are products of condensation and polymerization formed during fermentation of tea. They contribute to the thickness, body and colour of black tea.

Briskness – A tea tasting term used to describe a live taste as opposed to flatness or softness. Black teas with more theaflavins are more brisk.

Thickness- A tea tasting term used to describe liquor viscosity. Thick liquor has a better body as opposed to light liquor. Thick liquor creams well and faster while light liquor doesn't cream well.

Body – Is a term used to describe the strength of the liquor combined with the weight on the tongue. 'Full bodied' is the opposite of 'thin'. Thearubigins contributes to the body of black tea liquor.

Brightness – Is a description of reflective quality of liquor imparted to tea by the presence of theaflavins. The ability of the liquor to reflect light from the surface varies from mirror-like to total lack of reflection. High theaflavins content enhance brightness as opposed to more thearubigins.

Liquor colour – Is a term used to describe tea liquor such that a coloury liquor has a useful depth of colour and strength. Colour is brought about by the ratios of theaflavins to thearubigins.

Infusion- Is a term used to refer to the wet leaf after brewing the tea in readiness for tea tasting. In brewing, we need dry tea leaves and hot water, once these two are separated; we get the liquid part as liquor and the solid part (used wet tea leaves) as an infusion.

CHAPTER TWO

LITERATURE REVIEW

2.1 The tea plant

Linnaeus (1753) in his "Species Plantarum" (1st ed.) nominated *Camellia* cultivated in Japan and the tea plants cultivated in China and Japan as *Camellia japonica* and *Thea sinensis,* respectively. The nomenclature of *Camellia* was for the memory of G. J. Kamel (1661–1706), or Camellus, a Moravian Jesuit traveller in Asia, while Thea from the transliteration of jian's dialect of tea. In 18th century, *Camellia* and *Thea* were widely accepted as two separated genera, but latter were united for the first time by Sweet (1818), who selected the name *Camellia* (L) for the combined genera. Until the fifth decade of the twentieth century, the concept of the genus *Camellia sensulato* was more popularized, and *Thea sinensis* (L) was recombined by O. Kuntze in 1887 as *Camellia sinensis* (L.) O. Kuntze. 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) (Gebrewold, 2018). Tea trees can attain a height of twenty to thirty metres in nature and its beverage is a major source of dietary flavonoids (Hoensch and Oertel, 2015). Tea plant requires temperatures between 10-30 ^{0}C, and an annual rainfall of at least 1200 mm but 2500-3000 mm per year is the optimum. The pH requirement for the growth of tea is in the range of 4.5-5.6, acidic soils, and elevations up to 2000 meters (Hajiboland, 2017). Therefore, the production is geographically selective to a few areas around the world since the growing conditions are highly sensitive .The plant is kept as an evergreen shrub by pruning and only the apical bud and the first few leaves are plucked for tea processing. In tropical countries, tea leaves are harvested all year round though in temperate countries, harvesting is seasonal.

There are several purple-leafed coloured teas which have been identified and researched on in various tea growing countries (Terahara *et al*., 2001; Kamunya *et al*., 2009a; Saito *et al*., 2011; Kerio *et al*., 2012; Kilel *et al*., 2013a; Joshi *et al*., 2015; Lv *et al*., 2015; Lai *et al*., 2016; Shen *et al*., 2018). The purple teas are a bred of *Camellia sinensis* and related tea cultivar (Saito *et al*., 2011; Kamunya *et al*., 2009b). The purple-leafed tea cultivars have been studied for both the chemical composition of tissues and molecular mechanisms of colour formation. A number of studies have characterised anthocyanin contents in purple-leaf tea cultivars (Saito *et al*., 2011; Kerio *et al*., 2012; Joshi *et al*., 2015). The Kenyan purple tea is an interspecific hybrid between a tea plant (*Camellia sinensis*) and a brick-red pigmented non-cultivated (*Camellia irrawadiensis*) which is rich in anthocyanin but not suitable for

processing of palatable tea products (Kamunya *et al*., 2009b). These Kenyan breeders (Kamunya *et al*., 2009a) improved the clone for over 30 years and ensured the clone is resistant to drought, frost, diseases and pests. They also found the clone has high yield comparable to the standard control and commercial clone TRFK 31/8 and can be grown in all tea growing areas. The cultivar can mature in three years just like the green tea clones though highest production is experienced after six years. According to Kamunya *et al*. (2012), TRFK 306 is a clone whose source of seed is TRFK 91/1 and can yield 4,000 kg of made tea per hectare per year. This yield is higher than some released clones whose source of seed is TRFK 6/8. The clone is among the cultivars published in the Regional Variety List of Kenya, Uganda and Tanzania (Association for Strengthening Agricultural Research in Eastern and Central Africa (ASARECA), 2004) and the Kenya National Crop Variety List (Kenya Plant Health Inspectorate Service (KEPHIS), 2004) for wider coverage. Currently there are over 700,000 small scale farmers in purple tea business in Kenya. Purple tea is the most talked of type of tea within the region and globally.

Sunrouge, a Japanese red leaf tea is derived from natural crossing *Camellia taliensis* and *Camellia sinensis* (Nesumi *et al*., 2012). It was bred and released in 2009 as a tea cultivar for use as a functional ingredient rather than a green tea drink. Sunrouge tree is of the medium type and has many branches in comparison to typical cultivars. The survival rate of Sunrouge is lower than 'Yabukita' and other control cultivars in the cutting propagation. Saito *et al*. (2011) studied the anthocyanins content of 'Sunrouge' and the anthocyanins present. They found the new red leaf tea cultivar had the highest content of anthocyanin among the four studied tea cultivars and was eight times higher than that of 'Yabukita'. They researchers also purified and isolated six anthocyanins from 'Sunrouge' by chromatography; which included, delphinidin-3-O- β- D- (6-(E) –*p*- coumaroyl) galactopyranoside, delphinidin-3- O- β- D- (6-(E) –*p*- coumaroyl) glucopyranoside, Cyanidin-3-O- β- D- (6-(E) –P- coumaroyl) galactopyranoside, cyanidin--3- O- β- D- (6-(E) –*p*- coumaroyl) glucopyranoside, delphinidin- (Z)- *p*- coumaroylgalactopyranoside and petunidin- (E) –*p*- coumaroylgalactopyranoside.

Zijuan is the most well-known purple tea cultivar in China. All the bud, leaf and stem of the fresh 'Zijuan' tea shoots as well as its made green tea products appear purple because they are rich in anthocyanins (Lv *et al*., 2015). The anthocyanin content in Zijuan tea cultivar was reported to be almost three times of that found in the other Chinese purple tea cultivars (Yang *et al*., 2009). Zijuan was bred and selected from Yunnan Daye [*Camellia sinensis var, assamica* (Mast.) *Kitamura*], and was classified as a variety for protection (No. 20050031) by

the China State Forestry Administration in 2005 making it of high economic value and likely to be applied in landscaping (Zhang *et al*., 2010). Zijuan tea plant, having purple-coloured stems, leaves and buds is a mutant of the broad- leaf variety of the tea plant (*Camellia sinensis* var, *assamica*) (Jiang *et al.*, 2013). Fermented Zijuan tea owing to its 'large molecular pigments' content were found to have effects on lowering blood lipids in rats consuming a high lipid diet (Wang *et al*., 2012). Lv *et al*. (2015) studied the anthocyanin composition of Zijuan teas and their antioxidant activities. They found eight anthocyanins; pelargonidin-3-5-diglucoside, cyanidin-3-O-galactoside, cyanidin-3-O-glucoside, delphinidin, cyanidin, pelargonidin, peonidin and malvidin. Cuanidin-3-O-galactoside was found to be the most abundant. The researchers found Zijuan green tea has stronger antioxidant activity than Oolong tea and Zijuan black tea.

Lai *et al*. (2016) recently found a tea tree with dark purple young shoots at an altitude of approximately 1500m in Sichuan province, China. They then developed a novel purple-leaved cultivar 'Ziyan' which was found to have a more intense purple colour than Zijuan cultivar. The cultivar was found to have delphinidin, cyanidin and pelargonidin anthocyanins with delphinidin being more predominant than the rest. The researchers also found out that, 'Ziyan' exhibits a preference for synthesing B-ring trihydroxylated catechins, and had lower amounts of total catechins, polyphenols and water extracts than ordinary non- anthocyanins cultivars but with similar levels of caffeine.

Shen *et al*. (2018) recently examined the biochemical variation and mechanism of leaf colour change in Zixin tea cultivar. Zixin is a new purple-leaf cultivar which was selected from a natural purple- leaf mutant in Huangdao, China, via systematic selection. The Zixin tea cultivar has purple tender leaves, stems and green mature leaves. The colour change is brought about by the decreasing anthocyanins and an enhancement of chlorophyll metabolism. The leaf of Zixin has a lustrous surface and is thicker than that of Zijuan cultivar. Indian scientists have analysed the quality of orthodox teas made from anthocyanin-rich tea clones growing in the Kangra valley (Joshi *et al*., 2015).

2.2 History of tea in Kenya

The first tea seedlings (*Camellia sinensis*) were introduced to Kenya from India by G.W.L. Caine in 1903 and planted at Limuru (Watts, 1999). In some literature, it was reported that, tea was introduced into Kenya by the Caine brothers who imported dark-leafed "manipuri" hybrid seed from Assam in 1904 to 1905 to establish a plantation in Limuru, central Kenya (Kamunya *et al*., 2012; Matheson and Bovill, 1950). These progenies since

they had not been selected for high yield and quality, the resultant seedlings populations of mixed genotypes were phenotypically inferior though diverse. These authors continue to highlight that, in 1912, *Camellia sinensis* seed was imported from Sri Lanka to establish a plantation of tea with high yield and quality. There was little interest over the next twelve years except for several small plantations which were established at Limuru in the East of Great Rift Valley, Kericho and Kaimosi in the West of Great Rift Valley (Matheson and Bovill, 1950). Few private farmers established small tea gardens in Limuru and Kericho after the introduction of tea in Kenya.

The commercial cultivation of tea began in 1924 and remained an exclusive activity of the colonialists until 1956 when African farmers were allowed to start growing tea. This was after the colonial government initiated trials assessing if indigenous Kenyans could grow tea in the non-European settled Kenya Highlands in 1950s (Walton, 1969). The trials proved successful and the first factory to process the smallholder leaf was opened at Ragati (Nyeri) in 1957 which was serving about 500 acres of tea (Tiampati, (2015). The Kenyan government in 1960 initiated an Authority (the Special Crop Development Authority) with the mandate of promoting the cultivation of the crop within the smallholder agricultural sub sector (M'Imwere, 1997). Tea breeding work in Kenya commenced in 1961 under the oversight of the Tea Research Institute of East Africa which was renamed Tea Research Foundation of Kenya in 1980 (Wambulwa *et al*., 2016; Mondal, 2014) and currently refered to as Tea Research Institute under Kenya Agricultural and Livestock Research Organization.

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 (Monroy *et al*., 2012). Planted tea area in Kenya has grown from a mere 21,448 hectares in 1963 to over 209, 426 hectares by 2015 (Anonymous, 2015). 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. Small scale 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 following counties situated in the West of Great Rift Valley; Kericho, Bomet, Nakuru, Kisii, Nyamira and Nandi County. Kisii and Nyamira Counties grow tea mainly under KTDA management while most of these other counties have other private tea processors leading to competition for tea leaves. In East of Great Rift Valley, tea is grown in most parts of Kiambu County, Murang'a County, Nyeri County,

Kirinyaga County, Meru County and Embu County mainly under KTDA management. In these Counties the crop experiences favourable weather patterns. The small-scale sectors in these areas have managed to achieve high quality resulting in high auction prices as compared to the multinational companies (Anonymous, 2002).

Tea was introduced into the country in form of seed (Singh, 1979; Anon, 1962) and there was no data on collection and passport descriptors were kept (Wachira, 2002). The early introductions were therefore highly variable forming the initial populations of mixed genotypes. Uniformity and stability in yield and quality of the mixed genotypes could not be maintained; hence this necessitated the search for more uniform high yielding tea cultivars (Kamunya *et al*., 2012). The first phase of the tea improvement was done by mass selection among introduced seedling based on morphological characteristics (Barua, 1963). The initial selections were based on similarity to the Assam varieties, vigour, density of plucking points and large shoot size which later became the open-pollinated seed bearers (Kamunya *et al*., 2012; Green, 1966). The cultivars selected for high yields, were compared mainly to seedling tea, and later cultivar TRFK 6/8 for black tea quality (Kamunya *et al*., 2012). Initially a cultivar was released when it had yields greater than TRFK 6/8 or with quality worse than TRFK 6/8 but better than seedling tea or a cultivar with better quality than TRFK 6/8 and yields greater by 175 % the yield of seedling tea.

The Tea Research Institute has developed over 914 improved clones, out of which 51 clones have been selected for consistent superiority in yield and quality and released for commercial exploitation by both smallholder and large estate growers. Thirteen of these clones yield between 5,000 and 8,000 kg of processed tea per hectare per year. These yield levels are some of the highest in the world and are three times the average yields of unimproved tea varieties. The Tea Research Institute has since reoriented its research agenda to focus more on tea products diversification, value addition and Tea and health. Tea Research Institute has developed a new tea clone ("Purple tea") rich in a pigment called anthocyanin widely used for the manufacturing of fast moving consumer goods such as soaps, shampoo and detergents (TRI, n.d; Bore, 2015).

2.3 Effects of brewing conditions on tea quality

Hot water is normally used to prepare tea liquor for drinking and has been used extensively in brewing tea for research on tea quality (Sharpe *et al*., 2016; Perez-Burillo *et al*., 2017; Xu *et al*., 2017; Liu *et al*., 2018). Other factors affecting the quality of tea liquor include; temperature, time, water/tea ratio and particle size (Liu *et al*., 2018). The quality of

water used in preparation of tea liquor is of paramount importance as it affects the taste quality, catechin concentration and antioxidant activity (Xu *et al*., 2017). These reseachers had used purified water, mineral water, mountain spring water and tap water in their study. They found that, high pH in brewing water influences the stability of catechins in tea liquor though lower pH improved the taste of the tea liquor. High conductivity decreases the extraction yield of catechins and caffeine. Other reseachers had also found pure water which is weakly acidic and low in dissolved ions, achieved the highest catechin content (Zhang *et al*., 2017). The reseachers also found caffeine and theanine were higher in infusions made from spring and tap water, respectively. They also did sensory quality evaluation and pure water was found to be more suitable for brewing white tea with superior colour, aroma and taste. Sharpe *et al*. (2016) had found out that tap water can be used to brew tea without interfering with the antioxidant capacity of the catechins present.

Time/temperature combination also affects the quality of the tea liquor obtained, though other factors also influences. Saklar *et al*. (2015) in their studies found that brewing Turkish tea at 85 ^{0}C for 3 minutes is optimal for EGCG extraction. A study on white tea by Perez-Burillo *et al*. (2017) found out that brewing at 98 ^{0}C for 7 minutes was the best condition to obtain a high content of polyphenols, antioxidant activity and pleasant sensory properties. Liu *et al*. (2018) in their study found optimal brewing conditions for overall acceptability of green tea infusion was 82^{0}C for 5-7 minutes. Key independent factors in optimal brewing conditions of Oolong tea by subjective rating are; amount of tea, water temperature used and brewing time allowed (Lin and Chen, 2015). In their study, they concluded that, 2.25 g of tea, with water temperature of 98^{0}C and brewing time of 63 seconds were the optimal brewing combination of Oolong tea when 120 mL porcelain teapot is used. A study was carried out on green tea using cold brewing and hot brewing (Lin *et al*., 2013). Cold brewing, resulted in a lighter, less coloured and higher sensory-rated tea infusions with less astringency and bitter taste. Hot brewed infusions, however, resulted in higher contents of EGCG and EGC infusions leading to lower sensory results. Another study was carried out to determine the influence of commonly used steeping time, temperatures and leaf size on the antioxidant activity and sensory attributes of tea (Castiglioni *et al*., 2015). They found that maximum extraction effeiency occurs with cold water for 120 minutes and hot water at 90 ^{0}C for 7 minutes.

Tea phytochemicals extraction increases with a decreasing water/tea ratio (Liu *et al*., 2018; Shi *et al*., 2005). Quality of the liquor is also affected by the particle size (Liu *et al*.,

2018; Castiglioni *et al*., 2015; Vuong *et al*., 2011b). The current study used water: tea ratio of 20:1 at 70 ^{0}C and 92 ^{0}C, the tea used were freshly plucked non-processed purple tea leaves.

2.4 Citric acid

2.4.1 The chemistry and production of citric acid

Citric acid ($C_6H_8O_7$) is a weak organic tricarboxylic acid found in citrus fruits. Citrus fruits (lemons, oranges, tomatoes, beets, among others) are those fruits which contain sufficient amount of citric acid and they are classified as acid fruits (Kanse *et al*., 2017). Pure citric acid is colourless, readily soluble in water with a molecular weight of 210.14 mg/mol (Angumeenal and Venkappayya, 2013). It is biodegradable, eco-friendly, economical, safe and versatile chemical sequestering, buffering, wetting, ckeaning and dispersing and it is especially used in food, beverage and pharmaceutical industries (Vandenberghe *et al*., 2017). The acid is mainly used in the preparation of medicinal citrates, confectionary, soft drinks and effervescent salts (Angumeeral and Venkappayya, 2013). These researchers in their review paper presented the conversion of low cost substrates to citric acid by fermentation. They proposed use of plant biomass as raw material for fermentation with utilization of Aspergillus and Candida microorganisms. Many researchers previously had also studied the production of citric acid through fermentation processes (Cevrimli *et al*., 2010; Dhillon *et al*., 2011; Hanapi *et al*., 2011). Moreover, Kirimura *et al*. (2011) showed that citric acid is exclusively produced by fermentation with the filamentous fungus, *Aspergillus niger*. The industrial production is performed by carbohydrates or agro-industrial residues as substrates by three different types of processes: submerged, surface and solid fermentations. Citric acid yielding can be enhanced using mutant strains of *Aspergillus niger* on agro waste such as cassava peels, coupled with a suitable combination of optimal operational parameters (Adeoye *et al*., 2015). The production process of citric acid by *Aspergillus niger* can be monitored using a hybrid electronic tongue based on potentiometric/voltammetric sensors and appropriate chemometric techniques (Olesiuk *et al*., 2014). The designed hybrid electronic tongue was able to evaluate the progress and correctness of the ferementation process better than classical methods. Recently, solid state fermentation using agro-based waste materials (apple pomace, peanut shell and a mixture of both apple pomace and peanaut shell nut with 50:50 ratio) was used in production of citric acid (Ali *et al*., 2016). Due to new biotechnological production of citric acid especially in China, its global supply has risen from 0.5 to over 2 million tonnes becoming the single largest chemical obtained via biomass fermentation and the most widely employed organic acid (Ciriminna *et al*., 2017).

2.4.2 Application of citric acid

Citric acid is used primarily in the food industry because of it has a pleasant acidic taste and has high solubilty in water (Vandenberghe *et al*., 2017). Citric acid is accepted worldwide as generally recognized as safe and is approved the joint FAO/WHO Expert Committee on Food Additives (Soccol *et al*., 2006). The acid is also affirmed as GRAS by Food and Drug Administration (FDA, 21CFR84.1033, 2018; Show *et al*., 2015). Citric acid is used in food industry to enhance the activity of antioxidant preservatives and as a flavouring agent since its sharp, acid taste help mask the unpleasant tastes (Ciriminna *et al*., 2017). These researchers also stated that, the acid is used in beverages as an acidulant and a pH stabilizer. It is used in dairy products as; an emulsifier especially in ice creams and processed cheeses; as an antioxidant and acidifying agent in cheese products (Show *et al*., 2015; Grewal and Kalra, 1995). There are research on new uses and technological applications of citric acid as shown by (Apelblat, 2014).

2.5 The 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 including anthocyanins, carbohydrates, organic acids, caffeine and other alkaloids, minerals, vitamins, and enzymes (Monobe *et al*., 2008; Wei *et al*., 2010; Chaturvedula and Prakash, 2011; Xiong *et al*., 2012). The chemical composition of the tea leaves depends on leaf age, type of clone, soil and climatic conditions, and agronomic practices (Turkmen *et al*., (2009). The total polyphenols in tea flush ranges from 20 % to 35 %. Kenyan teas however have higher total polyphenols (Kilel *et al.,* 2013a) than some China and Japan clones. It was discovered that most Kenyan clones have a total polyphenol ranging from 21 % to 27 % in green made tea compared to China and Japan clones with 19.7 % and 17.2 %, respectively. Flavanols which are mainly catechins are the most important group and occupy 60–80 % of the total amount of polyphenols (Hara *et al.*, 1995b). Four major catechins, namely (-) - epigallocatechin-3-gallate (EGCG), (-)-epigallocatechin (EGC), (-)-epicatechin-3-gallate (ECG), and (-) - epicatechin (EC) (Reygaert, 2017; Reygaert, 2018), constitute around 90 % of the total catechin fraction. Catechins constituting about 6 % of the fraction includes; (+) catechin (C), (+)-gallocatechin (GC) and (-) catechin gallate (CG) (Namita *et al*., 2012). 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; Kim *et al*., 2016).

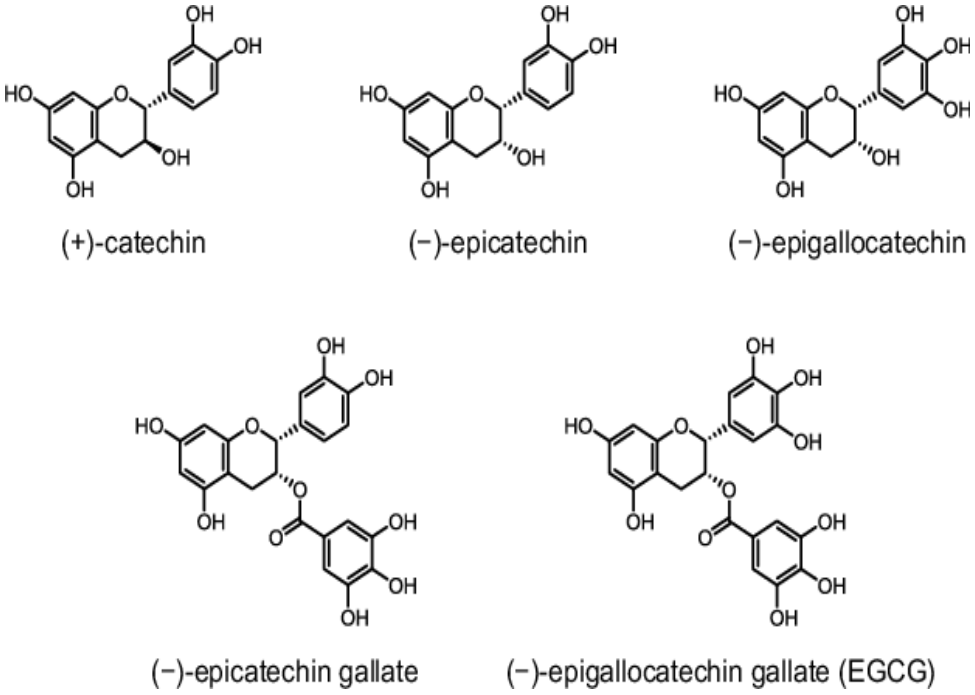


Figure 1: **Chemical structures of (+) catechin and major green tea catechins (**Suzuki *et al*., 2016).

Three major flavonol in the fresh leaf are kaempferol, quercetin and myricetin (Jeganathan *et al*., 2016). 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). Kenyan purple clones also have theanine (Kilel *et al.,* 2013a), with clone TRFK 91/1 having relatively higher amount. Other amino acids present in tea leaves are; glutamic acid, tryptophan, glysine, serine, aspartic acid, tyrosine, valine, leucine, threonine, arginine and lysine (Jayakeerthana, 2016).

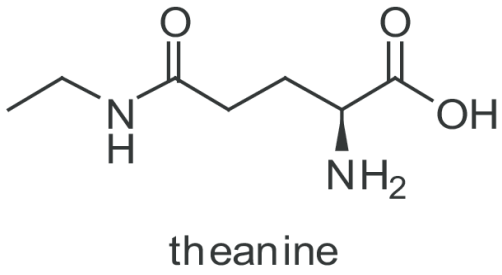


Figure 2: Chemical structure of theanine (Vuong *et al*., 2011a)

Free sugars constitute 3–5 % of the dry weight of tea flush. They consist of glucose, fructose, sucrose, raffinose and stachyose (Du *et al*., 2016). 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. Methylxanthines are a group of phytochemicals derived from the purine base xanthine and obtained from plant secondary metabolism (Monteiro *et al*., 2016; Sanchez, 2017). Methylxanthines of relevance are caffeine, theobromine and theophylline (Montero *et al*., 2016). 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).

Figure 3: Chemical structures of purine alkaloids (Monteiro *et al*., 2016)

Many volatile compounds, collectively known as the aroma complex, have been detected in tea (Jayakeerthana, 2016). Volatile compounds are important components in tea aroma, a key attribute of sensory quality (Zheng *et al*., 2016; Yang *et al*., 2013; Zeng, *et al*., 2018). The flavour of tea can be broadly classified into aroma contributed by the volatile compounds; and taste which is contributed by the non-volatile compounds (Ho *et al*., 2015). Volatile compounds including linalool, 2, 3-methyl butanal, 2-heptanone, and 3-5-octadien-2-one drive consumer liking of green tea products (Kim *et al*., 2016). 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. Teas grown at higher altitudes tend to have higher concentrations of aroma compounds and superior flavour, as measured by the flavour index (Pripdeevech *et al*., 2017). Growing tea in a shaded environment may change the aroma composition and improves the flavour index. The aroma complex varies with season, soil, climate, pre- and postharvest treatments (Qin *et al*., 2013; Zeng *et al*., 2018).

Tea like any other plant has various pigments mainly chlorophyll and carotenoids (Yashin *et al*., 2015). Chlorophyll is an important pigment in tea and is found in photosynthesizing tissues. They constitute a key element of photosynthesis, needed for light absorption (Hortensteiner and Krautler, 2011). Chlorophyll gives green tea its final greenish colour. During aeration and heating processes, chlorophyll is degraded into their derivatives such as pheophorbides, pheophytins and pyropheophytin, which gives rise to the dark colour of black tea (Suzuki and Shioi, 2003; Cartaxana *et al*., 2003). Carotenoids in fresh tea leave include β-carotene and lutein (Wijaya *et al*., 2010). Carotenoids can be converted into cis-isomers especially by heating (Zuzuki and Shioi, 1998). Recently purple teas have been discovered which have different pigmentation and they are rich in anthocyanins (Terahara *et al*., 2001; Kamunya *et al*., 2009a; Saito *et al*., 2011; Jiang *et al*., 2013; Lai *et al*., 2016). Anthocyanins act as pigments in a variety of fruits, vegetables, and other plants where their colour intensity, hue and stability are of importance (Welch *et al*., 2008). These properties are highly influenced by structure, pH, temperature, light, oxygen, and a number of other factors. Structurally anthocyanins undergo transformations with changes in the pH, which has dramatic effect on colour. Anthocyanin structure (Figure 4) has 7 positions labelled R. R basically means that it can be occupied by almost any organic group like methoxyl group, sugar, and the number of R that are occupied by specific substitutions would determine the colour of the anthocyanin (Kong *et al*., 2003). The most common anthocyanidin found in edible plants include pelargonidin, peonidin, cyanidin, malvidin, petunidin and delphinidin (Iwashina, 2000). These anthocyanins are grouped according to their substitution as follows; Peonidinin and cyanidin have substituted at 3’ and 4’ positions, Petunidin, malvidin and delphinidin are trisubstituted at 3’, 4’ and 5’ positions, pelargonidin is monosubstituted (Kong *et al*., 2003).

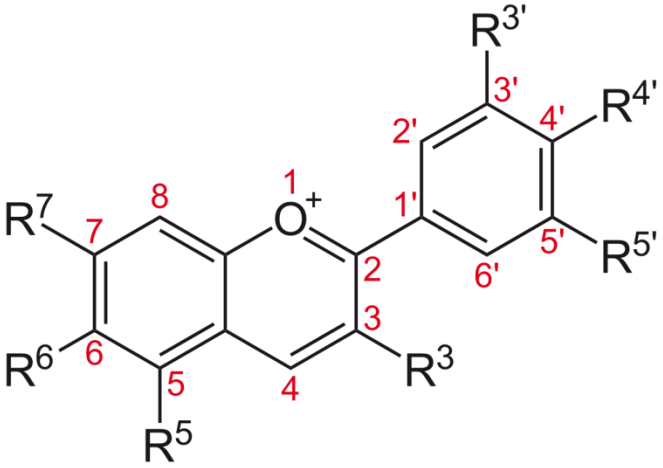
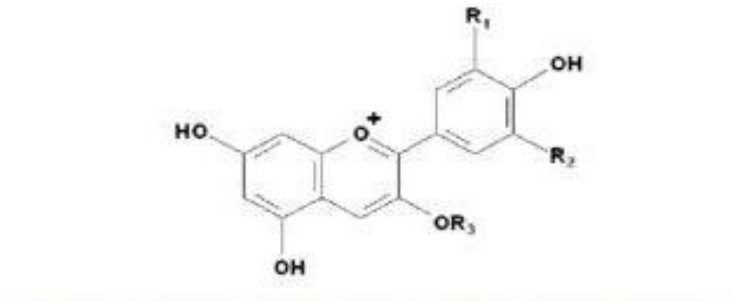


Figure 4: Structure of anthocyanin (Wahyuningsih *et al*., 2017)



Name	**R_1**	**R_2**	**R_3**
Pelargonidin	H	H	H
Cyanidin	OH	H	H
Delphinidin	OH	OH	H
Peonidin	OCH_3	H	H
Malvidin	OCH_3	OCH_3	H
Pelargonidin-3-*O*-glucoside	H	H	Glu
Cyanidin-3-*O*-glucoside	OH	H	Glu
Delphinidin-3-*O*-glucoside	OH	OH	Glu
Peonidin-3-*O*-glucoside	OCH_3	H	Glu
Malvidin-3-*O*-glucoside	OCH_3	OCH_3	Glu

Figure 5: **Structures of the monomeric anthocyanins,**

occuring naturally in *Vitis vinifera* wines and their corresponding anthocyanidins (Jackson, 2008).

Anthocyanin come from the words ‘‘Antho’’ which means flower and ‘‘cyanin’’ meaning blue. Anthocyanins are considered as functional ingredients because of their health-promoting properties (Montensen, 2006). The basic colours of blue, purple, red and orange have a direct relation with the number of hydroxyl groups and indirect relation with the number of methoxyl groups (He *et al*., 2010).

2.6 Common types of tea based on processing method

A summary of processing steps in most commercial types of teas is illustrated (Figure 6). The steps highlighted common steps in a general view. Otherwise in most cases, processors can have their own way of processing to get a particular type of tea product.

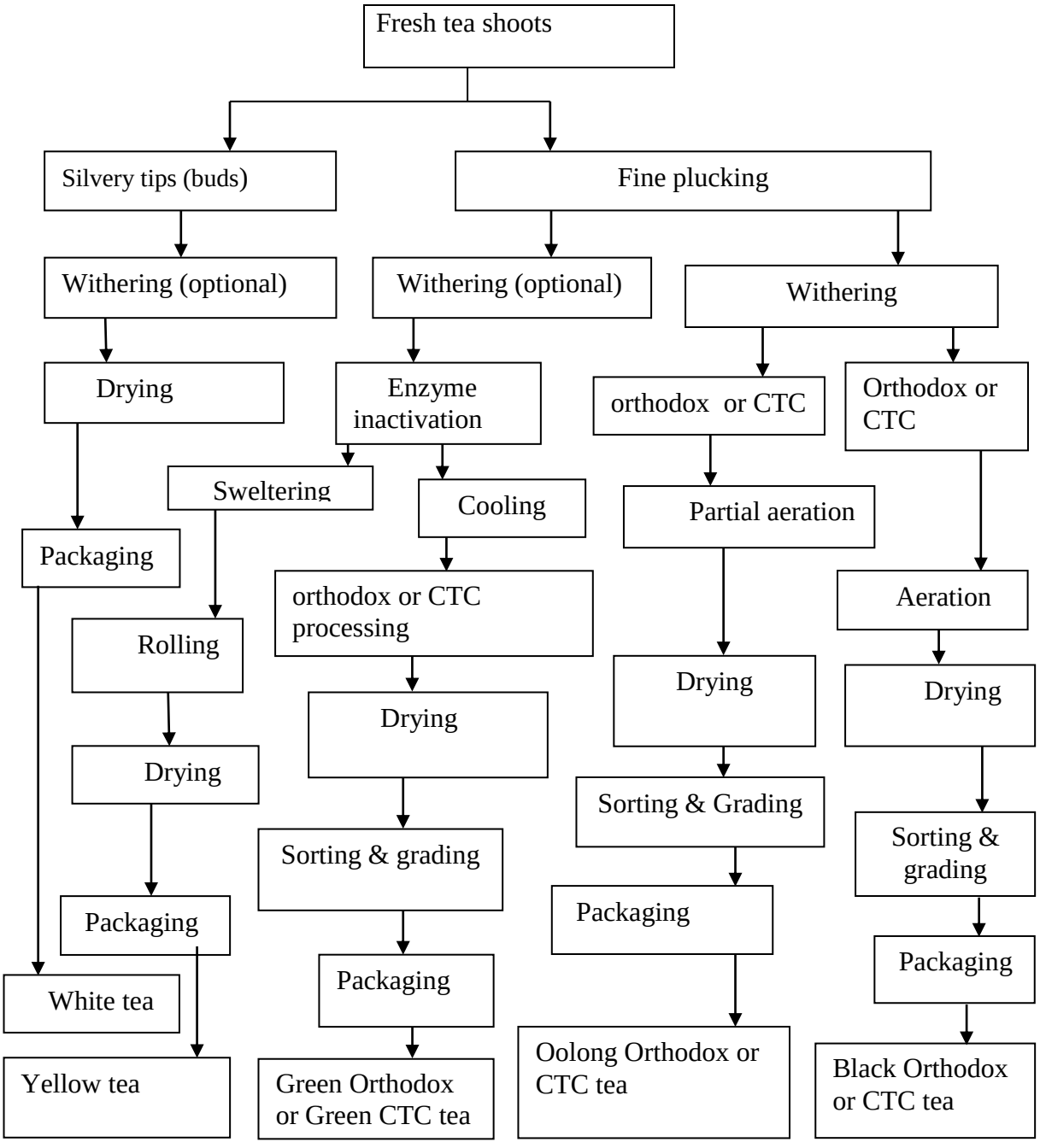


Figure 6*:* Flow chart for the manufacture of common types of tea.

The different types of teas available in the market- black tea, green tea, Oolong tea, yellow tea and white tea- arise from their different processing techniques (Tran, 2013). Kenya produces all the above teas but in different percentages. Black tea is the most

commonly produced especially CTC black teas and the most consumed beverage after water globally (Deb and Pou, 2016). Black teas can be grouped into many types based on cultivar, region of origin, harvesting seasons, processing methods among other factors. These varying factors produce different flavour attributes (Duan *et al*., (2016). It can be produced through CTC or orthodox method of manufacture (Kosinska and Andlauer, 2014). It is a fully fermented/aerated tea (Li *et al*., 2013) and this process of aeration distinguishes black tea from the rest of the teas. CTC teas are smaller in size compared to orthodox as shown in Plate 1 below. CTC black teas are graded into primary and secondary grades for marketing purposes. In Kenya, CTC teas are graded in decreasing size as BP 1(Broken Pekoe 1, PF1 (Pekoe Fannings 1), PD (Pekoe Dust) and D1 (Dust 1). The PF1 grade is the most traded within though blending has recently taken centre stage in tea trade.

Aerated orthodox teas

Aerated CTC teas

Plate 1: Aerated orthodox and CTC teas

Green tea is produced in a similar manner just like black tea but the leaves must be enzyme inactivated and it never undergoes aeration. Green tea in China can be grouped in four categories based on the different fixation and drying method used, namely basket-fired green tea, roasted green tea, steamed green tea and sundried green tea (Lv *et al*., 2015). Green tea can be orthodox or CTC green tea depending on whether the leaf disruption method was by rolling or maceration by CTC rollers, respectively.

Oolong tea processing steps are similar to black tea processing except that its aeration duration is shorter (Schillinger *et al*., 2010). It is commonly referred to as a semi-aerated tea and has more residual catechins though some are partially oxidixed and transformed during handling and withering process (Meng *et al*., 2018). They can also be processed as orthodox or CTC teas. Though shaking was not shown as one of the steps (Figure 6) some processors

especially in Taiwan, shake the teas during Oolong tea processing as was studied by Lin *et al*. (2016). These researchers carried out a study to check the effect of shaking process on correlation between catechins and volatiles in Oolong tea. The reseachers found out that, shaking affects the chemical transformation of the compounds in Oolong tea. The study revealed that the concentrations of the main catechins (EGCG, ECG, EGC and EC) were fluctuating with shaking and that shaking helped in imparting the characteristic features to Oolong tea. They however, recommended for proper adjustment of the shaking time because it influences the changes in aroma.

Yellow teas production steps are similar to green tea except that immediately after enzyme inactivation, the teas are allowed to yellow in a process referred to as sweltering. During sweltering, the teas are held up in a basket where the heat obtained from enzyme inactivation process is maintained for some hours. This will allow the teas to loose the green colour and turn to a yellow colour. The resulting tea leaves produce a beverage that has a distinctive yellowish-green hue due to transformations of the leaf chlorophyll. Yellowing is done at temperature close to human body temp for 6–8 hours. The amino acids and polyphenols in the processed tea leaves then undergo chemical changes to give the tea its distinct briskness and mellow taste**.** Yellow tea also known as 'huangcha' in Chinese is a lightly fermented tea unique to China (Xu *et al*., 2018). They are mainly produced in Sichuan, Anhui, Hunan, Hubei, Guanglong, Zheijiang and Guizhou provinces of China.

White tea is the least processed teas and the dearest in the market. It can be made from silvery tips to get a product usually refered to as 'silver Needle' or it can be from 1or 2 leaves and a bud. The processing style and naming of the final product differ from region to region. In China, there are four major types of white tea (White Tea Guide, n.d.). These are; silver Needle (Baihae Yinzhen), white peony (Bai Mudan), tribute eyebrow (Gong Mei) and Longetivity eyebrow (Shou Mei). The plucked tips or tea leaves are allowed to wither either naturally using natural environmental conditions or artificially where appropriate heaters are used to remove moisture.

2.7 Processing of aerated (black) CTC teas

Black tea is the most prevalent type of tea in the world accounting for roughly 75 % of the world's tea trade (Duan *et al*., 2016). Tea processing aim to initiate and regulate the biochemical reaction to produce desired quality attributes which are acceptable to different consumers (Borah *et al*., 2012). Normally two young leaves and a bud are plucked, withered before leaf disruption then aerated, dried, sorted and graded accordingly. Each of these

processing steps has a major influence on the made tea quality and many chemical or physical changes occurs.

2.7.1 Plucking

The term plucking refers to the harvesting/picking of the youngest tea leaves for purposes of further processing. It is considered labour intensive and a costly operation in tea processing (Wijeratne, 2012). Usually two leaves and a bud are hand plucked in small fields while at multinationals fields more tea leaves and a bud can be hand plucked. Highly skilled and experienced pluckers are recommended in order to get high quality shoots and minimize tea leave damage (Nandagopalan *et al*., 2014). Machine plucking can also be employed, where one or two people can operate. Use of mechanical tea harvesters replacing hand plucking is currently trending as technological changes happens (Kitur and Rop, 2016). Mechanization of plucking operation is good to increase workers' productivity and to reduce cost of production (Nandagopalan *et al*., 2014). Plucking standards can be fine or coarse plucking (Akbar *et al*., 2014) mainly dictated by the tenderness of the tea leaves plucked. The choice of plucking style is key when high quality teas are required (Nandagopalan *et al*., 2014). Fine plucking involves picking 2 or 3 youngest tea leaves and a bud while for the coarse plucking more tea leaves with or without a bud are plucked. Fine plucking is usually achieved by hand plucking and if handled carefully can make high quality teas. Coarse plucking will produce teas with a lot of fiber and flaky in nature with poor cup qualities (Sarkar *et al*., 2016).

2.7.2 Withering

Withering is a process which starts immediately the fresh tea leaves are plucked. It involves spreading the tea leaves over perforated troughs fitted with powerful exhaust fan underneath to draw the moisture from the tea leaves. The fan blows in air necessary for moisture removal (Singh *et al*., 2014). During humid conditions, hot air is blown from underneath the trough through the tea leaves to remove the moisture. It is the first step in processing of black tea with the major aim of reducing the moisture content on the fresh tea leaves (Baruah *et al*., 2012). The moisture content of green tea leaves could reduce from about over 80 % to 60 % or below according to processors or researchers (Omiadze *et al*., 2014; Jabeen *et al*., 2015). The loss in moisture makes the tea leaves amenable to subsequent rolling and aeration thus preventing the shoots from damage during maceration or rolling (Jolvis Pou, 2016). Many physical and biochemical changes take place during the withering process (Owuor, 1996; Deb and Pou, 2016). Withering can be divided into physical and

chemical wither because of physical and chemical changes, respectively. The physical change associated with withering is a loss of moisture from the shoot which leads to changes in cell membrane permeability. Physically the tea leaf transforms from a sturdy crisp leaf to limp and pliable during withering (Franks *et al*., 2019). The chemical wither involves breakdown of proteins into amino acids and other chemical changes take place (Deb and Pou, 2016). 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 and must be monitored carefully for perfect wither (Botheju *et al.,* 2011; Baruah *et al*., 2012; Dep and Jolvis Pou, 2016). 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 (Hampton, 1992).

A study Baruah *et al.* (2012) on impact of moisture loss and temperature on biochemical changes during withering concluded that; catechin degradation was less at low temperature as compared to that of higher temperature for the clones studied in India. High leaf temperature during withering decreased theaflavins, brightness and flavour index for both the clones studied. According to Baruah *et al*. (2012), reduction of withering time from twelve hours to four hours in a modified method induced some favourable biochemical changes which enhance brightness and flavour quality in black tea infusion. Chemical wither for a long period produced liquor with better flavour quality and fuller cup characters according to the above researchers.

2.7.3 Leaf disruption - CTC maceration

During leaf disruption, the tea leaves are macerated and the cell structures are disrupted which brings various enzymes into intimate contact with their substrates, the polyphenols (Sarkar *et al*., 2016). Tea leaf disruption involves use of a rotor vane and CTC rollers. India and Kenya are the major producers of CTC teas. In CTC manufacture, after preconditioning, the tea leaves are fed between a pair of stainless steel rollers with etched surfaces, one rotating clockwise, the other anti-clockwise at different speeds. Polyphenol 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.,* 1995c).

2.7.4 Aeration

Soon after CTC, the product is referred to as a dhool. It is bright green in colour with high temperature of about 32 ^{0}C. Aeration is a term currently being used instead of fermentation since no microorganisms are used in this kind of enzyme-oxidized black tea (Mo *et al.,* 2008). The dhool is what is aerated immediately after leaf disruption/maceration. The high temperature of up to 32 ^{0}C is progressively reduced by subsequent supply of oxygen where the temperature is reduced to about 22 ^{0}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 (Sanyal, 2011). 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 which is time dependent (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. The following factors affect the formation and degradation of theafalvins and thearubigins (Wilson and Clifford, (1992); coupled oxidation, presence of oxygen, aeration temperature, pH and green leaf catechin composition.

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 (Robertson, 1983b). This process will create an imbalance of simple quinones relative to gallocatechinquinones drastically affecting the formation of the theaflavins which ideally require equal concentrations of di- and trihydrolatedcatechins. These factors are fundamental in directing the majority of the catechins, particularly the gallocatechins into the thearubigin fractions. Theaflavins reduction can further be worsened by inherently lower levels of simple catechins relative to gallocatechins in green tea shoots (Robertson, 1983b). As shown (Figure 7), any factor which increases the rate of catechinquinone formation above that of redox

equilibration will also increase the ratio of simple to gallocatechinquinones 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).

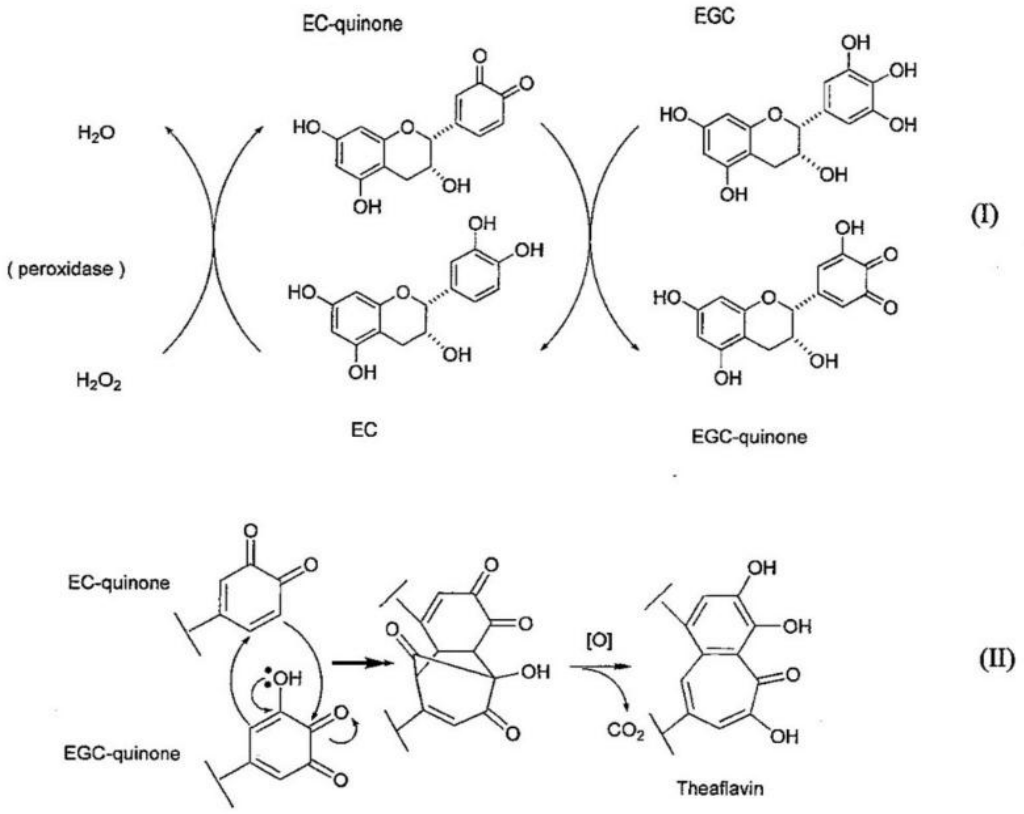


Figure 7: Synthesis of theaflavins from catechins.

(Tanaka *et al*., 2002)

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

Presence of oxygen

Oxygen is consumed both in catechinquinone, 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 catechinquinone 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 unhindered 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 catechinquinones 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).

Aeration temperature

Significant interactions exist between aeration duration, temperature and all plain black tea quality parameters as reported by Owuor and Obanda (2001). Obanda *et al.* (2001), found out that theaflavins formed over time was dependent on temperature. Ngure *et al.* (2009) showed that unequal depletion ratios of di- and trihydroxylatedcatechins 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.* (2009), 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.,* 2009). At low temperature, redox equilibration plays the lead role with the steady state concentration of simple catechinquinones 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.

Aeration 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.

Green leaf catechin composition

The initial concentration of the catechins- epigallocatechins, epigallocatechin gallate, epicatechin and epicatechin gallate – is extremely important. The availability of simple catechin quinones for theaflavin formation during fermentation is highly dependent on this composition. High concentrations of the catechin gallates have been shown to inhibit polyphenol oxidase activity and this will affect the theaflavins and thearubigins composition of the black tea.

A recent research by Jiang *et al*. (2018) studied the effect of variety, season and region on theaflavins content. They found out that high ratio of polyphenol oxidase to peroxidase activity and high content of certain individual catechins led to high yield of theaflavins production. Theaflavins 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 %. 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, (Sang *et al*., 2003) as shown;

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

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

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

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

Takino *et al*. (1964) demonstrated the formation of theaflavins in the laboratory as shown below.

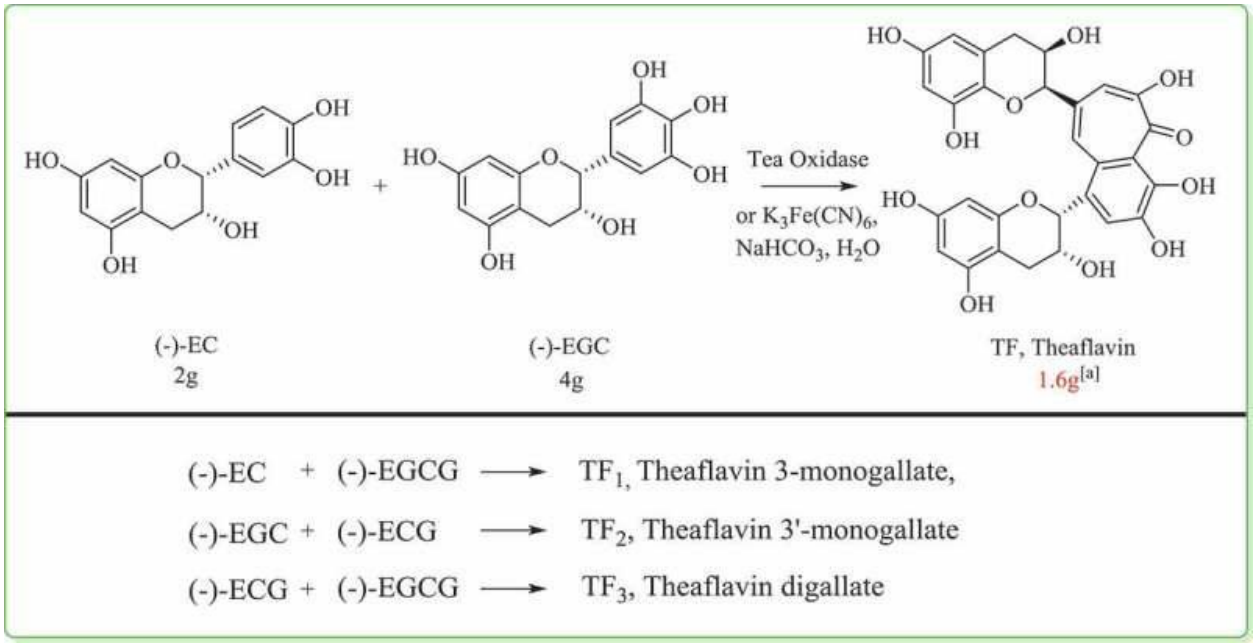


Figure 8: Generation of the four dominant theaflavins

[a] *Reaction condition: 2 g of GC and 4 g of EC were dissolved in 600 mL of water, then 30 mL of an oxidizing reagent which was prepared by combining equal volume each of aqueous potassium ferricyanide (3.08 g in 10 mL) and aqueous sodium hydrogen carbonate (0.78 g in 10ml) was added to the solution of catechins, dropwise under ice- cooling.*

The ratio of dihydroxy flavan-3-ol to trihydroxy flavan-3-ol in green leaf may therefore 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 (Owuor and Obanda, 2007). Theaflavins are natural compounds characterized with benzopolone skeleton (Figure 9; He, 2017) and the pigments have a benzotropolone chromophore (Kusano *et al*., 2015).These products of fermentation were originally found by Professor E.A.H Roberts (Roberts *et al*., 1957). Recent studies have since found out more

products obtained from enzymatic oxidation of theaflavins (Kusano *et al*., 2015). The researchers found out oxidation of theaflavins with peroxidase yield a new product named theacoumarin A together with known pigment theanaphthoquinone, the structures of which are depicted (Figure 10).

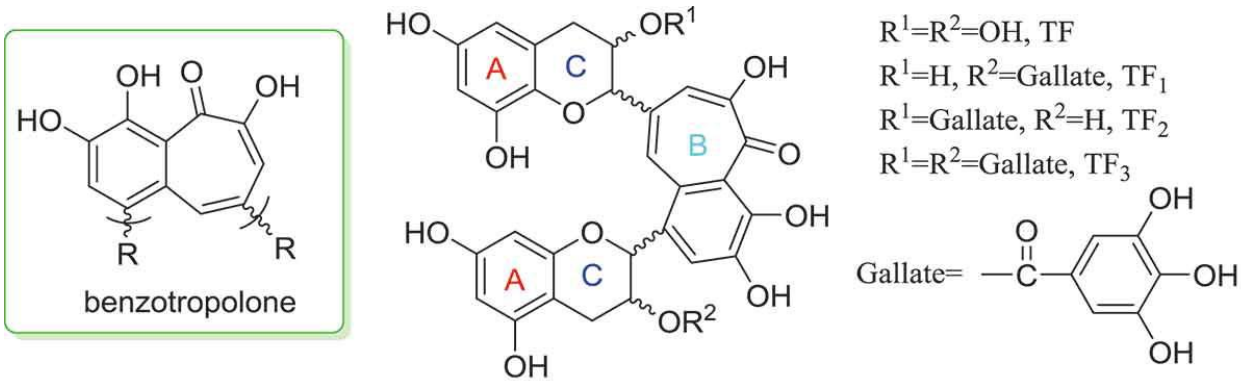


Figure 9: Chemical structure of theaflavins (He, 2017)

TF= Simple theaflavin; TF1= Theaflavin- 3-monogallate; TF2= Theaflavin-3'-monogallate
TF3= Theaflavin-3-3'- digallate

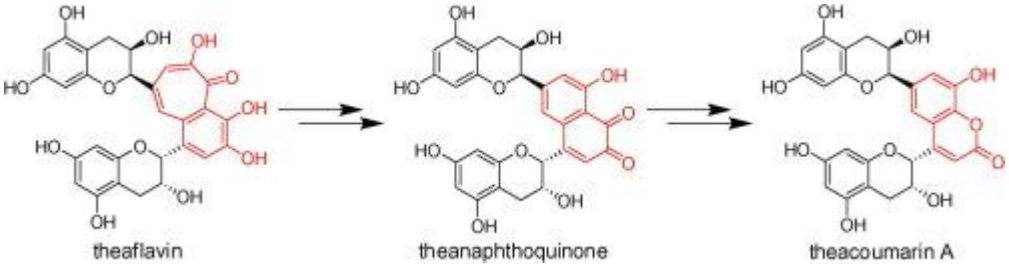


Figure 10: Products of theaflavin oxidation (Kusano *et al*., 2015)

Thearubigins is a collective name for the brown acidic pigments or the coloured phenolic oxidation products which remained after removing the yellow neutral pigments from a black tea liquor .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 (Izawa *et al*., 2010) and represent approximately 30-60 % of the solid in liquors after infusion. Thearubigins have not been fully characterised and its chemical nature is still a mystery (Teshome *et al*., 2013). It is however clear that its structure is complex consisting polymers as depicted (Figure 11).

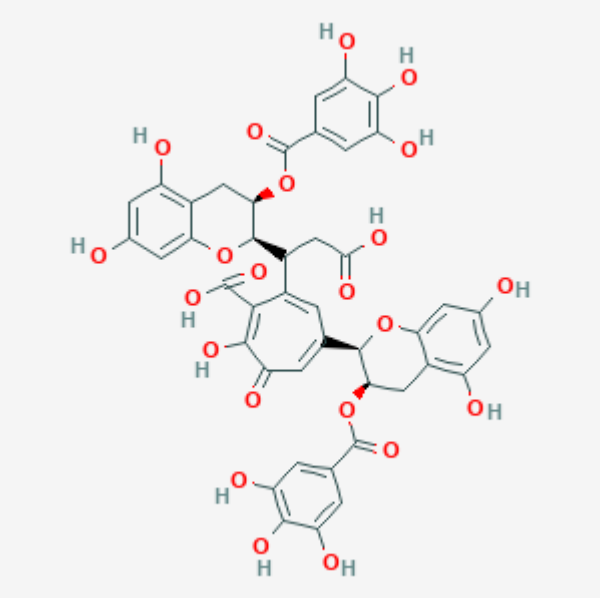


Figure 11: Structure of thearubigins

Source: (Athanasios, 2019).

A recent research by Yassin *et al*. (2015b) studied the mechanism of thearubigins formation from green tea favan-3-ols, based on electrochemical oxidation of epigallocatechin gallate. They found out that oxidation mainly takes place on the B- ring and galloyl group, where the oxidized components undergo oxidative coupling for the formation of theaflavins, theasinensins and polyhydroxylated flavan-3-ols as precursors for thearubigins formation.

2.7.5 Drying

Drying is a simultaneous heat and mass transfer process, where heat is supplied to wet tea by heated air and the evaporated moisture is carried away by the air (Akhtaruzzaman *et al*., 2013). Drying of the aerated dhool (ex- CTC teas) 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, however, it has been observed that as temperature increased with duration the biochemical composition and quality of black tea were decreased (Teshome *et al*., 2013). These researchers recommended that, for production of good quality black tea, a treatment combination of 100 ^{0}C with 25 minutes was a recommended optimum treatment combination to be used, assuming other factors are at optimum. Drying can be done using

fluid bed driers and temperatures and timing should be controlled. Fluid bed driers have high rate of heat and mass transfer while maintaining good thermal contact between the tea particles and drying medium. Case-hardening is rare in fluidized systems and particle to particle attraction is minimized because each particle is surrounded by its own fluid. Improvements and advacements in drying have been explored to evercome challenges associated with tea drying especially high cost of energy (Rumaisa *et al*., 2018). Many studies have been done on tea drying using various methods including microwave (Hatibaruah *et al*., 2012) and super heated steam (Rumaisa *et al*., 2018). Superheated dried tea leaves were found to exhibit better colour attribute and had higher phenolic content. Rumaisa *et al*. (2018) in their study concluded that super heated steam seemed an attractive and highly viable option in the application of tea leaves drying.

2.7.6 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). Secondary grades includes; Fannings, Broken Mixed Fannings (BMF) and Dust. Packaging should be done properly to maintain quality and avoid any moisture loss and absorption (Sarkar *et al*., 2016).

2.8 Processing of non-aerated teas (green)

Green tea is processed the same way as black tea except that the two products differ on two major steps, aeration and enzyme inactivation. The first step is also plucking and fine plucking is recommended since coarse plucking reduces price and quality (Lee *et al*., 2014). Plucked tea leaves meant for green tea manufacture can also be sufficiently withered since withering process has positive effect on the final quality of green tea (Yin *et al*., 2008). Enzyme inactivation is carried out before leaf disruption because enzymatic reaction should be slowed. Enzyme inactivation can be achieved by quick application of high temperature either with steam, typical of Japanese- style or by dry heating (pan-firing) the traditional Chinese method (Kosinska and Andlauer, 2014). Green tea manufacture has mainly the following three steps: enzyme inactivation, rolling or maceration and drying. Steaming has the following objectives; to inactivate the inherent polyphenol oxidase enzyme which would otherwise enzymatically oxidize the polyphenols (catechins); to get the proper colour; to give

off grassy smell in order to release the aroma; to evaporate part of the water in the fresh leaves to soften the leaves to make rolling easier.

Rolling is done to the panned or steamed tea leaves by passing through rollers or macerated using CTC rollers with an objective of reducing the leaf size. Drying of macerated or rolled tea leaves may be done using a fluid bed drier or chain conveyor belt driers.

2.9 Processing of aerated orthodox teas

Orthodox is a type of tea processing alongside CTC method. Historically the term 'Orthodox' refers to a method of producing tea in India (Poudel, 2010). Manufacture of aerated orthodox tea involves the following steps; plucking, withering, rolling/twisting, aeration, drying, sorting, grading and packaging (Kumar *et al*., 2018). During plucking, young tea leaves of 4-6 days old are hand-plucked because the younger the tea leaves, the better the quality (Sarkar *et al*., 2016). Tea leaves meant for Orthodox teas can be hand rolled by skilled personnel or by machines/rollers.

2.9.1 Withering

Normally withering is carried out by spreading the leaves thinly on banks of trays or open troughs fitted with perforated trays under forced air circulation (Fard *et al*., 2015). There are two types of withering; open withering and closed withering (Kothari, 2017). According to Kothari, (2017), the open type of withering is open by virtue of the tea leaves are placed on troughs or trays and are the left in an open place. In open withering system, the tea leaves are spread on the troughs at a desired thickness and air is blown upwards from the bottom of the perforated bed. Closed withering on the other hand, have the troughs enclosed with a cover to ensure less leaf damage. Unnecessary leaf damage influences subsequent step of aeration negatively (Sanyal, 2011). During rainy season, the tea leaves have higher water content and thus requires controlled heat treatment to remove the surface moisture (Kothari, 2017). There is no definite withering duration but 14-18 hours is considered optimum, and the duration is greatly determined by the quality of plucked tea leaves (Deb and Pou, 2016).

2.9.2 Rolling

Rolling is done when satisfactory wither has been achieved, which twists the leaf, breaks it up and expresses the juices enabling the substrates and enzymes to come in contact (Abhiram *et al*., 2018). Accordingly, oils are released with rolling which imparts a unique aroma to tea. The machines used vary in size and design but their principles are alike, they compress and turn the leaf over. This step facilitates mixing up of polyphenols and

polyphenol oxidase enzymes thereby starting aeration (Kumar *et al*., 2013). Rolling process should be controlled to ensure; optimal aeration of each set of dhool thus ensuring even aeration (Abhiram *et al*., 2018). One of the facrors to control is pressure application in machine rolling; where for an epicyclical pressure roller, pressure is applied laterally by the central fitting, and in battern-pressure cap technique, it is applied from the top by lowering the pressure cap (Kumar *et al*., 2013).

2.9.3 Aeration

Though actual aeration starts at rolling it is continued in the subsequent stage of aeration (Sarkar *et al*., 2016). The sifted leaves are spread out in thin layers on tables or perforated aluminium trays or into aluminium drums, in order to continue the oxidative process. According to Sarkar *et al*., (2016) modern Continous Fermenting Units are widely used during aeration**,** where the leaf changes colour and turns into a dark coppery tone. Typical aroma develops at this stage. The ideal conditions for aeration are dhool temperature less than 30 ^{0}C, moisture content of 55 %, pH 4.5 to 5.0 and relative humidity above 90 %. When the aeration is judged to be sufficient (colour and nose assessment) the aerated dhool is transferred to the drier.

2.9.4 Drying

Endless chain pressure driers are also commonly used in orthodox tea manufacturing otherwise other types of driers include; hot-feed drier, fluid-bed drier and ding dong drying machine (Sarkar *et al*., 2016). Ding dong drying machine is more complicated to used and is usually suitable for small scale operation. After firing, the tea is spread out to cool and then temporarily stored to wait sorting. Modern innovations on the drier are the hot-feed drier, where hot air is supplied separately to the feeder to arrest aeration immediately as the dhool is fed, and the fluid-bed drier where the leaf moves from one end of the chamber to the other over a perforated plate in a liquid fashion.

2.9. 6 Sorting and grading

There are several sorting machines used in sorting orthodox teas (Sarkar *et al*., 2016). According to Sarkar *et al*., (2016), sorting machines includes; Arnott sotter, Myddleton sorter, Vibro fibre extractor and magnetic sorter. Grading is carried out on mechanically oscillated sieves, similar to those used in the green leaf stage and fitted with meshes of appropriate size. Finished grades are stored in air-tight bins until a sufficient quantity has been accumulated to fill a consignment.

Orthodox teas are categorized differently (UPASI, n.d; Sarkar *et al*., 2016). It includes the following; Flowery Pekoe (FP), Whole leaf, broken, fannings and dust. Whole leaf has further been categorized into seven grades, including; Fine Tippy Golden Flowery Orange Pekoe (FTGFOP), Tippy Golden Flowery Orange Pekoe (TGFOP 1), Golden Flowery Orange Pekoe (GFOP), Flowery Orange Pekoe (FOP), Orange Pekoe (OP), Broken Orange Pekoe (BOP), Golden Flowery Broken Orange Pekoe (GFBOP). Brokens have been categorized into five grades which includes; Broken Pekoe Souchong (BPS), Golden Broken Orange Pekoe (GBOP), Flowery Broken Orange Pekoe (FBOP), Broken Orange Pekoe (BOP) and Golden Orange Fannings (GOF). Fannings have six grades according to this source, namely; Flowery Orange Fannings (FOF), Broken Orange Pekoe Fannings (BOPF), Orange Pekoe Dust (OPD), Orange Churamani Dust (OCD), Broken Orange Pekoe Dust (BOPD) and Broken Orange Pekoe Fine Dust. Dust grades include; Fine Dust (FD), Dust – A (D-A), Special Dust (Spl. Dust) and Golden Dust (GD) (UPASI, n.d).

2.10 Medicinal properties of tea

Tea has been considered a medicine since ancient times because of its polyphenols. Green tea (*Camellia Sinensis*) is a famous herb, and its extracts have been used in traditional Chinese medicinal system (Saeed *et al*., 2017). Green tea catechins are polyphenols which are believed to provide health benefits (Botten *et al*., 2015). Green tea consumption is associated with the prevention of many different types of cancers such as mouth, lung, gastrointestinal and mammary glands (Jayakeerthana, 2016). Green tea polyphenols especially EGCG have been shown to be an effective chemopreventive agent (Gosslau and Chen, 2004; Hsuuw and Chen, 2007). Researchers have also found that Epigallocatechingallate (EGCG), a green tea catechin, may have anti-HIV effects when bound to CD4 receptor (Kawai, 2003; Steinmann *et al*., 2013). Research on the effects of tea on human health continues to be fuelled by the growing need to provide naturally healthy diets which include plant-derived polyphenols. 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. 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 (Gomes *et al.,* 1995; Wolfram, 2007). The subject of bioactivity and therapeutic potential of tea and tea products has drawn a lot of attention.

A 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. Black tea has been linked to lower the risk of cardiovascular diseases including coronary heart disease, blood pressure and platelet aggregation (Greyling *et al.,* 2014). The fermented tea has also been shown to prevent oxidative damage, dental caries, cancers, gut microflora, mood and mental performance (Fatima and Rizvi, 2011; Bhattacharjee, 2015).

A study by Rashid *et al.* (2014) found out compelling evidence that consumption of Kenyan purple tea resulted in reinforcement of brain antioxidant capacity in mice. 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). Other health benefits, such as enhancing insulin activity (Cabrera *et al.*, 2003), antimicrobial effect (Stapleton, 2004; Almajano *et al.,* 2008; Koech *et al.,*2013), 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).

Oolong tea is known to have a significant role in reducing obesity, cardiovascular diseases, controlling diabetes, protecting bones and teeth (Ng *et al*., 2017). It has also been shown to exhibit stronger anti-mutagenic activity as compared to green and black teas (Su *et al*., 2007).

2.11 Antioxidant properties of tea

Antioxidants play an important role in the prevention of chronic diseases. Oxidation is an essential biological process for energy production reactions in living organisms which generate reactive oxygen species (ROS). Many living cells can generate ROS such as mitochondria, lysosome, peroxide, cytosol, plasma membrane and endoplasmic recticulum (Meo *et al*., 2016). In a lifespan, a living organism is continuously exposed to ROS and small amounts of ROS can act as signal transduction molecules, however, excessive *in vivo* production of reactive oxygen species leads to oxidative damage in lipids, proteins and DNA (Fernado and Soysa, 2015; Meng *et al*., 2017). Tea has received a great deal of attention because of its antioxidant properties (Hara *et al.,* 1995c; Zhang, 2004; Luczaj and Skrzydlewska, 2005; Gramza *et al.,* 2006; Fu and Koo, 2006; Karori *et al.,* 2007; Maurya and Rizvi, 2009; Sharma and Borua, 2017). The biological properties of tea include effects on

central nervous system and antioxidant effects attributed to the presence of methylxanthines such as caffeine and phenolic compounds, especially catechins (Shagana and Geetha, 2017). Tea polyphenols can prevent the formation of ROS and are strong metal chelators (Kanwar *et al.,* 2012). 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 non-processed vegetable. This demonstrated the potency of tea as health enhancing beverage. Oolong tea has the potential to scavenge superoxide radicals and therefore possesses anti oxidative properties (Su *et al*., 2007; Ng *et al*., 2017). Green tea polyphenols can inhibit cognitive impairement induced by chronic cerebral hypoperfusion via modulating oxidative stress (Xu *et al.,* 2010). These researchers also found out that, administration of green tea polyphenols with a dose of 400 mg/kg per day to model tars were found to scavenge oxygen free radicals, enhance antioxidant potential, decrease lipid peroxide production and reduce oxidative DNA damage. Green tea polyphenols can be direct antioxidants by scavenging reactive oxygen species or chelating transition metals as has been demonstrated *in vitro* (Forester and Lambert, 2011); otherwise they may act directly by up-regulating phase II antioxidant enzymes. These researchers in their review paper indicated that green tea polyphenols can also be potent pro-oxidants, *in vitro* and *in vivo*, leading to the formation of hydrogen peroxide, the hydroxyl radical and superoxide anion.

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. Anthocyanins have been found to have the following health benefits; it can reduce blood pressure, they can improve visual acuity, prevent tumor development, reduce cancer cell proliferation, can prevent diabetes, reduce the risk of cardiovascular diseases, anti-inflamatory, antibacterial and modulate cognitive and motor function (Guha, 2015; Yousuf *et al*., 2016).

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.

2.12 Sensory evaluation of tea

Sensory evaluation is a scientific discipline that analyses and measures human responses to the composition and nature of foods and drink. Sensory evaluation is a key method used to assess the flavour quality of foods because it measures what consumers really perceive; however it is a subjective method (Yang and Boyle, 2016).

Tea as a global product is usually subjected to rigorous sensory analysis. Each tea has a distinctive taste which is affected by cultivation and production techniques (Harney, 2008). The flavour of tea can be divided into two categories, taste (non-volatile compounds) and aroma (volatile compounds) (Ho *et al*., 2015). These researchers also found out that, these aroma molecules are generated from carotenoids, lipids, glucosides precursors and from maillard reactions. The made tea is usually assessed in three areas namely; the leaf - which can be black, green, yellow, or any other- manufactured in various ways as is desired; the liquor; and the infusion. The method described by ISO 1303:1980, is the most widely adopted method when preparing tea liquor for sensory evaluation. In this method ‘‘The method consists in extracting of soluble substances in dried tea leaf, containing in a porcelain or earthenware bowl, examination of the organoleptic properties of the infused leaf, and of the liquor with or without milk or both’’. The standard (ISO 3103), dictates that, a pot with a lid as shown (Plate 2) which fits loosely .The pot should hold a maximum of 300 mL (±8 mL) and must weigh 200 g (±10 g). If a small pot is used, it should hold a maximum of 150 mL (±4 mL) and must weigh 118 g (±10 g).

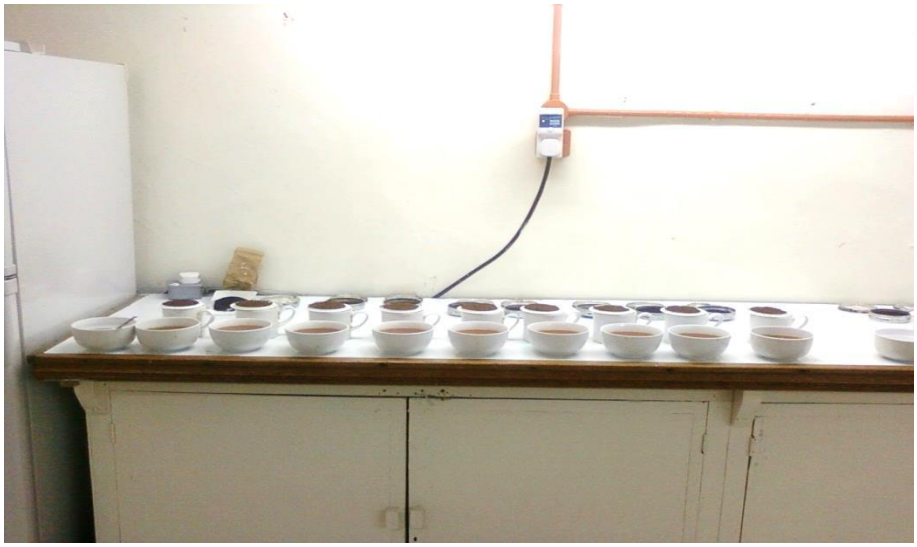


Plate 2: Set tea ready for sensory evaluation

Experienced and trained panellists usually taste the provided tea samples and describe accordingly or they rank as is necessary. The terms used to describe tea quality depends on; the type of tea, geographical regions, tea brokers who sells the tea and buyers of tea. Tea sensory evaluation commonly referred to as 'tea tasting' may be done for purposes of; setting selling prices at the tea auction, research, product development and improvement, and for ascertaining quality assurance during normal production processes. Tea tasting can also be done by tea factories producing similar products as a joint venture. Sensory evaluation exercise can be executed differently but key parameters to note are the amount of tea infused in a specified volume of water and the time used in brewing. Han *et al*. (2017) in their study used 0.01g of processed tea infused in 150 mL of distilled boiling water for 5 minutes. The sensory evaluation was carried out by five trained panellists. The panellist were instructed to evaluate the sensory responses regarding taste, aroma and overall flavour quality by giving a score within 100 and also noting down the flavour cahracteristics of the samples. Grading system has also been used in China basing on a maximum score of 100 of which 10 % was awarded for the dry tea appearance, 30 % for the aroma, 10 % for the liquor colour, 30 % for the taste and 20 % for the infused leaf (Liang *et al*., 2008).

2.12.1 Black tea sensory evaluation

During black tea quality evaluation, the leaf appearance, the liquor characteristics and infusion colour and appearance are key aspects which are assessed. There are universally accepted terminologies to describe all the three aspects (ATB, 1995; Imperial Tea Garden, n.d).

Tea Terms Describing Dry Leaf

Black: A black appearance is desirable, preferably with "bloom". This term is used with Orthodox or Rotorvane manufacture.

Blackish: This is a satisfactory appearance for CTC and LTP (Lawrie Tea Processor) manufacture teas and denotes careful sorting.

Bloom: It is a sign of good manufacture and sorting (where the reduction of leaf has mainly taken place before firing). A "sheen" appearance which has not been removed by over handling or over sorting.

Bold: Particles of leaf, which are too large for the particular grade.

Brown: A brown appearance, with CTC and LTP manufacture, normally reflects too hard treatment of the leaf.

Choppy: Orthodox (or Rotorvane) manufactured leaf, which is cut by a "breaker".

Clean: Leaf which is free from fiber, dust, and any extraneous matter.

Curly: The leaf appearance of whole leaf grade Orthodox teas such as OP as opposed to wiry.

Even: Size is true to grade and of consistent, even size.

Flakey or flaky: Flat, open, and often light in texture.

Grey: Caused by too much abrasion during sorting.

Grainy: Describes well-made CTC or LTP primary grades, particularly Pekoe Dust, and Dust 1 grades.

Leafy: Orthodox manufacture leaf tending to be on the large or long side.

Light: A tea light in weight and of poor density. Sometimes referred to as flakey.

Make: A well made tea and must be true to the particular grade.

Stalk and fibre: Should be minimal in primary or top grades, but generally acceptable in the lower grades.

Tip: A sign of fine plucking and apparent in the top grades of Orthodox manufacture.

Uneven and mixed: "Inconsistent" pieces of leaf indicating poor sorting and untrue to the particular grade of tea.

Well twisted: Applies to orthodox manufacture. Often referred to as "well made" or "rolled" and used to describe whole leaf grades.

Wiry: The appearance of a well twisted, thin leaf orthodox tea.

Powdery: Fine light dust.

Tea Terms Describing Liquors

Aroma: Smell or scent denoting "inherent character".

Bakey: An over fired liquor.

Body: liquor having both fullness and strength, as opposed to being thin.

Bright: A lively appearance that usually indicates bright liquor as well.

Brisk: The most "live" characteristic resulting from good manufacture.

Coppery: Bright tea which indicates a well manufactured tea.

Coloury: Indicates useful depth of colour and strength.

Cream: A precipitate obtained after cooling.

Dull: lack brightness and usually denotes a poor quality tea. Can be due to faulty manufacture, firing, or higher moisture content.

Dark: A dark or dull color, which indicates a poor quality leaf.

Flavour: A most desirable extension of "character" caused by slow growth at high elevations and rarity.

Dry: Indicates slight over-firing.

Earthly: Normally caused by damp storage. Ataste which can at times be "climatically inherent" in leaf from certain origins.

Flat: Not fresh (usually due to age).

Full: A good combination of strength and colour.

Fruity: Can be due to over-fermentation and/or bacterial infection before firing. An over ripe taste.

Gone off: A flat or old tea. Often denotes high moisture content.

Green or raw: Caused by under fermentation and sometimes under withering, or characteristic of leaf from immature tea bushes (liquors are often raw or light). Can also be caused by poor rolling with Orthodox teas.

Harsh: A taste generally related to under withered leaf and very rough.

High-fired: Over-fired but not bakey or burnt.

Light: Lacking strength and any depth of colour.

Plain: A liquor which is "clean" but lacking in the desirable characteristics.

Pungent: Astringent with a good combination of briskness, brightness, and strength. Often reserved for the best quality Assam and Ceylon teas.

Quality: Refers to "cup quality" and denotes a combination of the most desirable liquoring properties.

Soft: The opposite of briskness and lacking any "live" characteristics. Caused by inefficient fermentation and/or firing.

Strength: Substance in the liqour. It is desirable.

Stewed: A soft liquor with an undesirable taste caused by faulty firing at low temperatures and often insufficient air flow.

Taints: Characteristics or tastes which are "foreign" to tea. Such as petrol, garlic, among others. Often due to being stored next to other commodities with strong characteristics of their own.

Thin: Bland light liquor which lacks any desirable characteristics.

Weedy: A grass or hay taste related to under withering.

Tea Terms Describing Infused Leaf

Bakey: An over-fired tea in which too much moisture has been driven off.

Bright: Denotes a lively fresh tea indicative of bright liquors.

Burnt: Extreme over-firing.

Mixed or Uneven:Tea leaves of varying colour and grades.

Dull: Not clear and lacking any brightness. Usually denotes a poor tea. Can be due to faulty manufactur, firing, or high moisture content.

Green: An immature "raw" character. Often due to under fermentation and sometimes under withering.

Fard *et al*. (2015a) studied the impact of withering process on sensory properties of black tea. They found out that, the impact of the air flow rate on dry tea appearance, liquor colour, taste, aroma, infused leaves and final product quality was significant at the 1 % level. They also discovered that, the interaction between air flow rate and withering time was also significant on dry tea appearance, liquor colour, infused leaves and final product quality at 1 % and aroma at 5 % probability levels, respectively. Another study on the effect of chemical components of black tea was carried out (Qu *et al*., 2019). They found out that, Halogen-lamp- microwave dried teas got best sensory quality, whereas, microwave dried tea had higher polyphenols and theaflavins but lower amino acids. The researchers also found out that, microwave and halogen-lamp- microwave dried teas got new aroma compounds and are more suitable for black tea drying.

2.12.2 Green tea sensory evaluation

Green tea processing can vary from processor to processor depending on the market being targeted. The liquor characteristics and leaf appearance are important parameters in green tea sensory evaluation as was discovered in the current study (Appendix I and Appendix V). Lee and Chambers, (2007) previously developed a green tea Lexicon evaluating over 100 green tea samples using descriptive analysis methods. They categorized

attributes as; green, brown, fruity/floral, mouthfeel, basic tastes, among other attributes as were found in the studied samples. Green tea sensory quality can be predicted precisely through process parameters (Zhu *et al*., 2017). These researchers concluded that, leaf temperature, moisture content measured during production could effectively predict the sensory quality of green tea with parameters as image information of green tea being able to effectively evaluate the sensory quality. Lee *et al*. (2008) studied the development of sample preparation, presentation procedure and sensory descriptive analysis of green tea. They found optimum infusing time-temperature combinations of 3 min at 60 ^{0}C or 1min at 80 ^{0}C. They discovered the intensity of fermented-like flavour increased, but cut grass and floral flavour decreased with the lower-graded tea leaf. Samples infused at 60 ^{0}C for 3 minutes were sweeter but less bitter than samples prepared at 80 ^{0}C for 1 minute. Since brewing styles affect the flavour and basic taste (Lee and Chambers, 2007), it is always prudent to state the brewing method used as sensory results are presented. Multiple brewing times has also been found to affect aroma and flavour of green teas (Lee *et al*., 2013). These researchers found out that, green tea may be brewed up to four times; the first two brews providing stronger flavour, bitterness and astringency whereas the third and fourth brews provided milder flavour, bitterness and astringency. Green tea flavour are affected by the aromatic volatile composition of green tea and many researchers have studied them extensively (Kumazawa and Masuda, 2002; Hattori *et al*., 2005; Ho *et al*., 2015). The country of origin has a strong influence on the flavour of green tea because, similar processing methods are widely used within each particular processing country and flavour is dependent, in part, on processing (Lee *et al*., 2013). These researchers compared the flavour of green teas around the world and found out that, roasted-processed teas were mostly responsible for brown-related flavours and steamed- processed teas were responsible for green- related flavours. Previously, Lee and Chambers (2009) had suggested that brown, brown-related attributes (ashy/soothy, burnt/scorched), bitterness and astringency become stronger; and green, green-related attributes (green beans, spinach) become weaker as the brewing time and water temeparture increased. They studied 3 different green teas from Korea using 3 different temperatures (50, 70 and 95 ^{0}C) for 1, 2, 5 and 20 minutes. Green tea polyphenols are also responsible for distinctive aroma, colour and taste (Senanayake, 2013). Green tea evaluation is also done for various reasons like in black tea. There is need to develop a comprehensive study on green tea sensory in Kenya and come up with universally accepted terms like the ones we have for black tea.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study site

Freshly plucked two leaves and a bud of the studied clones were obtained from the fields of Timbili Estate of the Tea Research Institute (TRI), Kericho (Latitude 0 ^{o}22'South, longitude 35 ^{o}21' East, with an elevation of 2180 m above mean sea level). The samples were manufactured accordingly (as is appropriate because of the varying manufacturing conditions) at the mini tea factory at TRI. Most of the chemical analyses were conducted at the biochemistry laboratory of TRI, Kericho and the tea tasting for sensory evaluation were carried out in seven tea factories and at the Kenya Tea Development Agency (KTDA) headquarters. Completely randomized design with three replications was adopted in this study.

Plate 3: A field of purple tea within Timbilil Estate where the samples were collected.

3.2 Experimental design

For objective one and two, laboratory experiment was laid out in a Completely Randomized Design (CRD) in three replications and two time/temperature combinations were used in checking the effect of brewing on anthocyanins levels.

The statistical linear model was; $Y_{ij} = \mu + \alpha_i + \epsilon_{ij}$ …….. (1)

where;

Y_{ij}= Overal observation,

μ= Overal mean,

α_i = Effect due to i^{th} time/temperature combination,

ϵ_{ij} = Random error.

For objectives 3 and 4 where teas were processed and subjected to chemical analyses, tea leaves materials for the study were sourced from a field within the Timbilil estate of TRI. Clone TRFK 306 was used for all the experiments with clone TRFK 6/8 as a control. For each experiment, sufficient amount of fresh tea leaves were plucked. Each treatment was replicated three times. Withering and aeration duration were varied. The samples for the experiment were plucked and/or analysed/manufactured the same day such that the design was a randomized complete block design (RCBD).

The statistical model was; $y_{ijk} = \mu + C_i + R_{ij} + P_i + CP_{ij} + A_i + AP_{ij} + AC_{ij} + APC_{ij} + W_i + WP_{ij} + WC_{ij} + WPC_{ij} + WA_{ij} + WAP_{ij} + WAC_{ij} + WAPC_{ij}$ …….(2)

Where;

y_{ij} is the observation of the i^{th} treatment and j^{th} replication (quantity of the quality parameter),

μ is the overall mean (constant),

C_i is the effect of the i^{th} treatment on the clones,

R_{ij} is the replication of i^{th} treatment and j^{th} replication,

P_i is the effect of the i^{th} treatment on the processing,

CP_{ij} is the effect on the interaction between clones and processing,

A_i is the effect of the i^{th} treatment on aeration time,

AP_{ij} is the effect on the interaction between aeration time and processing,

AC_{ij} is the effect on the interaction between aeration time and clones,

APC_{ij} is the effect on the interaction between aeration time, processing and clone,

W_i is the effect of the i^{th} treatment on withering time,

WP_{ij} is the effect on the interaction between withering time and processing,

WC_{ij} is the effect on the interaction between withering time and clone,

WPC_{ij} is the effect on the interaction between withering time, processing and clone,

WA_{ij} is the effect on the interaction between withering time and aeration time,

WAP_{ij} is the effect on the interaction between withering time, aeration time and processing,

WAC_{ij} is the effect on the interaction between withering time, aeration time and clone,

$WACP_{ij}$ is the effect on the interaction between withering time, aeration time, clone and processing.

For sensory evaluation of fresh TRFK 306 teas where a 5-point hedonic scale was used, forty panellists participated. For processed tea products, 42 trained and experienced tea tasters were used to describe the quality of the various tea products. The experimental design was Randomized Complete Block Design (RCBD). The following statistical linear model was used;

$Y_{ij} = \mu + \alpha_i + \beta_j +$ random error …… (3)

Where; Y_{ij} = Overal observation,

μ = Overal mean,

α_i = Effect due to i^{th} time/temperature combination or processing treatment,

β_j = Effect due to j^{th} replicate (panellist).

3.3 Assay of fresh TRFK 306 tea leaves

This section describes; brewing, analyses of total anthocyanins, antioxidant activity, effect of citric acid on liquor quality and sensory evaluation.

3.3.1 Brewing of non-processed TRFK 306 tea leaves

Freshly plucked youngest two leaves and a bud of TRFK 306 tea weighing 50 g were put into a 1.5 L thermos flask and mixed with 1000 mL of drinking water having varying temperature (brewing) from 70 ± 1 ^{0}C, 90 ± 1 ^{0}C and boiling point (92 ± 1 ^{0}C). The ratio of tea leaves to boiling water used was 1:20 similar to what Bhuyan and Saikia (2005) used. For each temperature, there were three samples timed at 5 minutes, 10 minutes and 15 minutes brewing time, each in separate thermos flask. To ensure less temperature variation, two thermos flasks were used per experiment such that the hot water was kept in another thermos and brewing was done in another thermos flask. Preliminary study was done using the above time temperature combinations then later on based on the results, 70 ^{0}C was ignored for antioxidant activity assay since a significant total soluble solids could not be obtained which could have correlated to the required volume from the sample to be analysed. As for 90 ^{0}C, the results were similar to those for 92 ^{0}C and were also ignored in both (antioxidant and anthocyanin) analyses. For total monomeric anthocyanins, 70 ^{0}C and 92 ^{0}C (boiling point) were adopted. Total monomeric anthocyanins, antioxidant activity and the effect of citric acid, pH and sensory evaluation were then determined.

3.3.2 Analysis of total monomeric anthocyanins

The brew from fresh non-processed purple tea leaves brewed at 70 ^{0}C and boiling point (92 ^{0}C) were filtered through a membrane filter (0.45 µM) into a flask connected to a vacuum pump. The filtrate was then passed through a reverse phase (RP) C_{18} solid phase extraction (SUPELCO, SPE) (Sigma–Aldrich, USA) catridges previously activated with 10 % MeOH/HCl followed by 0.01 % HCL v/v in distilled water to purify the anthocyanins. The anthocyanins were adsorbed onto the column while sugars, acids and other water soluble compounds were washed out using 0.01 % HCL in distilled water. Anthocyanins were recovered using acidified MeOH (10 % formic acid v/v) and collected in small tubes. Then extracted pure anthocyanins were then transferred into graduated test tubes and topped to 20 mL with acidified MeOH (10 % formic acid v/v). The purified extracts were kept at -10 ^{o}C whenever quantification was not immediate. Quantification of anthocyanin was done using pH differential method (Giusti and Wrolstad, 2001). For each sample, sixteen well labelled test tubes were used, because the analyses were carried out in three replicates using the pH differential method (Giusti and Wrolstad, 2001) and two buffer systems:

(i) 0.025 M potassium chloride (KCl) buffer at pH 1.0

(ii) 0.4 M sodium acetate ($NaC_2H_3O_2$) buffer at pH 4.5.

Pipeting was done where, 1.8 mL of 1pH of potassium chloride buffer was put into eight test tubes and to the other eight test tubes, and 1.8 mL of 4.5 pH of sodium acetate was put. A small amount of the sample, 0.2 mL were then put in all the test tubes and kept in a dark place for 15 minutes before reading the absorbance at a wavelength of 520 nm and at 700 nm using UV-1800 spectrophotometer (Shimadzu, Japan). The difference in absorbance of the sample was determined as follows.

A (A 520 nm - A 700 nm) at pH 1.0 – (A 520 nm – A 700 nm) at pH 4.5

The total monomeric anthocyanin pigment concentration in the original sample expressed as mg/L, was calculated using the following formula

Monomeric anthocyanins pigment = (A *MW * DF *1000) /ἐ * 1..... (4)

Where; A = absorbance (absorbance difference in the two pH ranges);

ἐ = cyanidin-3-glucoside molar absorbance (26,900);

MW = molecular weight of anthocyanin (449.2);

DF = dilution factor.

3.3.3 Analysis of antioxidant activity

A modified method of Brand-Williams *et al*. (1995) was used to assay for antioxidant activity. The method uses 2,2-phenyl-1-picrylhydrazyl radical whose inhibition is by the sample is equivalent to the antioxidant activity of that sample.

Liquors from fresh non-processed purple tea leaves were prepared by brewing at 92 ^{0}C, boiling point for 5, 10 and 15 minutes and 100 mL of each set was put into 100 mL conical flasks. Pipette of 10 mL was used to draw 10 mL of the brew into already weighed ashing tubes which were then dried overnight in an oven at 103 ± 2 ^{0}C. This was to evaporate all the water and remain with solids. The remaining brew in the 100 mL conical flask was kept in a fridge having a temperature of -10 ^{0}C awaiting further analysis. The dried ashing tubes were then weighed after cooling. The difference in weight which is in grams was converted to millligrams and it is equivalent to the total soluble solids present. The obtained weight implies C_1 in the formula (equation 5) which was used to calculate how much volume of the sample was to be removed from the original brew for antioxidant activity analysis.

$C_1V_1 = C_2V_2$ …… …… (5)

Where; C_1 represents obtained weight (mg)

V_1 represents an unknown volume to draw from the sample (mL).

C_2 represents an assumed constant of 0.5 mg

V_2 represents a constant with 25 mL because a 25 mL volumetric flask was used

The obtained V_1 volume was picked respectively and put into a 25 mL volumetric flask then topped to the mark with 50 % methanol. Using a micro pipette, 100 uL of the sample was drawn and put in a 10 mL test tube. From freshly prepared DPPH, 4 mL was pipetted into test tube with the sample in a dark room and held for 15 minutes before reading the absorbance at 517 nm using a CE 393 digital grating spectrophotometer. The blank used was distilled water with added 4 mL of DPPH. All determinations were performed in three replicates. The (%) inhibition of DPPH radical was calculated from the absorbance data according to (Olugbami *et al*., 2015).

% Inhibition against DPPH = $[(A_B - A_A)/A_B] *100$..... (6)

Where A_B is the absorbance of the blank sample (100 µL double distilled water and 4 mL DPPH) and

A_A is the absorbance of the tested sample after 15 minutes.

3.3.4 Effect of citric acid on total monomeric anthocyanins

This was done after getting the results of total monomeric anthocyanins using various time/temperature combinations. A 5 minute brewing time using boiling water was used for the experiment because of relatively higher total anthocyanins. Once the brew was ready, food grade citric acid of 0.1, 0.2, 0.3, 0.4 and 0.5 % were added to the brew and determination of total monomeric anthocyanins following the described protocol in section 3.3.2.

3.3.5 Effect of citric acid on antioxidant activity

When the results of antioxidant activity had been obtained, a brew timed at ten minutes was used to check the effect citric acid concentration. Ten minutes was used since it had marginally higher percentage though not statistically significant ($p< 0.05$) from the other two timings (5 and 15 minutes). Once the brew was ready, food grade citric acid of 0.1, 0.2, 0.3, 0.4 and 0.5 % were added to 100 mL of the brew. Analysis of antioxidant activity were done following the described procedure in 3.3.3 to check the effect of the citric acid since most users of fresh purple tea leaves add lemon to improve the colour of the liquor. Citric acid is a dominant organic acid in lemon fruits hence the choice of the acid, moreover, citric acid is also safe for human consumption (Li *et al*., 2012).

3.2.6 Determination of pH

A pH meter (OAKLON pH 700- pH/Mv/^{0}C/^{0}F meter) was used to measure the pH of each sample at room temperature and values were recorded accordingly. Determination of pH was necessary since addition of citric acid was expected to increase the acidity of the brew.

3.3.7 Sensory evaluation of fresh purple tea liquor

Sensory evaluation was done after getting the results of anthocyanin and antioxidant activity. Five minutes and ten minutes timing with boiling water were used. The brews were prepared with food grade citric acid of different quantities (0, 0.1, 0.2, 0.3, 0.4 and 0.5 %). Five and ten minutes were used in brewing since, they showed relatively higher total anthocyanins and there was no significance difference ($p< 0.05$), in antioxidant activity, between the duration used. Freshly plucked youngest two leaves and a bud of purple - coloured teas weighing 50 g were put into a 1.5 L thermos flask and steeped with 1000 mL of boiling drinking water and timed for five and ten minutes as is appropriate. 100 mL of each brew were dispensed into clear tasting cups where citric acid of different percentages were put and coded accordingly.

Plate 4: Samples of freshly brewed purple tea leaves ready for sensory evaluation.
From left to right are A= plain liquor without citric acid, B,C,D, E and F are with citric acid at levels ranging from 0.1 to 0.5 % , respectively.

The samples were coded before subjecting them to sensory evaluation using a 5-point hedonic scale (Graham *et al*., 2017), (Appendix VII) and forty panellists participated in the study**.** The panellists were first explained what is expected of them and cautioned not to loudly express their feeling about any of the samples. Panellists were accorded sufficient time to taste the twelve samples and score them accordingly.

Colour and taste were the sensory attributes which were evaluated using a 5-point hedonic rating scale, with 1 meaning 'dislike extremely' and 5 meaning 'like extremely' (Appendix VIII). The coded samples were placed on the table with drinking water placed aside for mouth rinsing. Consistent tasting room conditions (lighting, enough space, less distraction, unnecessary movement) were maintained as much as possible.

3.4 Processing of non-aerated (green) teas

TRFK 306 and TRFK 6/8 were processed through orthodox and CTC types of manufacture. These two methods are currently the most common types of tea leaf disruption in the Kenyan tea industry.

3.4.1 Processing of non-aerated (green) CTC teas

Plucked tea leaves from TRFK 306 and TRFK 6/8 were divided into five equal portions of about 2 kg ± 5 g each. The five portions were subjected to withering as follows;

none withered (0), five hours withering, ten hours withering, fifteen hours withering and twenty hours withering time. The objective was to investigate whether withered tea leaves from these two clones can produce better quality products or otherwise. Comparison between the two clones was also necessary since TRFK 306 is a new clone while TRFK 6/8 is a reference standard clone used at Tea Research Institute, Kenya. The average moisture content for the tea samples withered at various timings were; 80 ± 1 %, 76 ± 1 %, 72 ± 1 %, 65 ± 1 % and 56 ± 1 %, respectively. The withering time limit was guided by previous studies (Owuor and Orchard, 1989) who found out that beyond twenty hours withering, the quality of tea deteriorates. Some other researchers had experimented on four hours wither time (Baruah *et al*., 2012). The non-withered portion was immediately steamed in a steamer (Black and Decker: model no. HS 3000. 220-240V. 50-60HZ.900W. capacity 1 L) for one minute before spreading in withering troughs to cool and drain excess water then crushed in the CTC rollers three times, dried in a fluid bed drier (Tea Craft, UK) set at 120 ^{0}C for thirty minutes before being sieved and packaged in aluminium lined sachets to await further analysis. The other portions were withered for the desired hours before steaming, cooling, crushing, drying, sieving, packaging as described above. The teas were not graded before analyses neither were they sorted. The chemical analysis which were subjected to these teas include; total polyphenols, total and individual catechins, caffeine, gallic acid, antioxidant activity and total soluble solids. Teas from TRFK 306 were analysed for total monomeric anthocyanins and sensory evaluation was also performed on these teas.

3.4.2 Processing of non-aerated (green) orthodox teas

About 10 kg ± 1g of freshly plucked tea leaves from TRFK 306 and TRFK 6/8 each were divided into five equal portions of about two kilograms. The five portions obtained represented non-withered, five hours wither, ten hours wither, fifteen hours wither and twenty hours wither. The non-withered tea leaves were immediately steamed for one minute, spread in withering troughs to cool and drain excess water, then hand rolled for twenty five minutes by trained personnel. The rolled tea leaves were then dried at 120 ^{0}C in a fluidized bed drier (Tea Craft, UK) for 30 minutes before packaging in aluminium lined sachets awaiting further analysis. The withered tea leaves were timed appropriately then steamed, drained and cooled, rolled, dried and packaged as in non-withered tea leaves**.** The teas were not graded before analyses neither were they sorted. These teas were analysed for the following; total polyphenols, total and individual catechins, caffeine, gallic acid, antioxidant activity and total

soluble solids. Teas from TRFK 306 were analysed for total monomeric anthocyanins. Sensory evaluation was also performed on these teas.

3.5 Processing of aerated (black) CTC teas

Freshly plucked tea leaves from TRFK 306 and TRFK 6/8 were withered at varying interval of five hours, from five hours to twenty hours. The withered leaf was put through the mini CTC machine and macerated three times to expose the enzymes for aeration and to get the required dhool. The dhool was divided into three equal parts and aerated separately. Some were aerated for 30 minutes, 60 minutes and 90 minutes at controlled temperatures (22 ^{0}C for both wet and dry bulb temperatures). Drying was done initially at 120 ^{0}C to arrest the enzymatic reactions. When the aerated leaves had turned black, the temperature was lowered to 100 ^{0}C to avoid burning the tea and the drying time took 30 minutes at most. The ungraded made teas were then packed in aluminium lined sachets ready for the analyses and sensory evaluation. These teas were analysed for; total and individual theaflavins, thearubigins, total colour, brightness, antioxidant activity, total soluble solids and sensory evaluation.

3.6 Processing of aerated (black) orthodox teas

Freshly plucked tea leaves of TRFK 306 and TRFK 6/8 were withered at varying duration with an interval of five hours from five hours to twenty hours. Each portion weighed about 2 Kg ± 1 g. The withered tea leaves were then hand-rolled as described in green tea processing above. The rolled tea leaves were then aerated as is desired, dried then packaged as is described in 3.4, black CTC processing. These aerated orthodox teas were analysed for; total and individual theaflavins, thearubigins, total colour, brightness, antioxidant activity, total soluble solids and sensory evaluation.

3.7 Chemical analyses of non-aerated teas

These include; total polyphenols, antioxidant activity, individual catechins, caffeine, gallic acid, total soluble solids and total monomeric anthocyanins for TRFK 306 teas.

3.7.1 Assay of total polyphenols

Extraction of individual catechins and polyphenols was done according to the procedure described by Karori *et al*. (2007). Tea sample of a coarse granular structure was 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 ± 2 ^{0}C to dry for the determination of dry matter. For total polyphenol analysis, 0.2 g was weighed into an extraction tube. Five millilitres of hot 70 %

v/v methanol/distilled water was then dispensed into the sample as an extraction mixture and vortexed using a vortex mixer (Model VM-1000). Heating of the extraction tube was done in the water bath maintained at 70 ^{0}C for 10 minutes with mixing in the vortex mixer after every 5 minutes (the sample was vortexed immediately, after 5 minutes and after 10 minutes). The samples were then centrifuged at 3500 revolution per minute (rpm) for 10 minutes (HSCEN-204). The supernatant was decanted into a graduated tube and the extraction procedure repeated. The extracts were then combined and made up to 10 mL 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 is used because it contains phosphotungistic acid as oxidants. One millilitre of the sample extract was then transferred to a 100 mL volumetric flask and topped to the mark with distilled water and mixed. Then 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 (Na_2CO_3) solution were added to each tube then a stopper were 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 a wavelength of 765 nm. A calibration curve was obtained for gallic acid over a concentration range of 10 µg/mL to 50 µg/mL. The OD readings of the test samples were referenced to the calibration curve to determine the total polyphenols content in the tea samples.

3.7.2 Assay of antioxidant activity

The stable 2,2-diphenyl-2-picrylhydrazyl radical (DPPH) was used for the determination of free radical scavenging of the tea extracts using a modified method of Brand-Williams *et al*. (1995). A sample of 5 g was infused in 100 mL of boiling double-distilled water followed by stirring with a magnetic stirrer for ten minutes at room temperature. The extracts were strained through a nylon mesh (120 µm) followed by a filter paper (Whatman No. 2 with a pore size of 8 µm). Ten millilitres of the extract was pipetted into ashing tubes of known weight and dried in oven at 103 ^{0}C overnight. The difference in weight equals to the total soluble solids present. The procedure described in section 3.3. 3 were then followed to the end where calculation of inhibition percentage against DPPH was done.

3.7.3 Determination of individual catechins, caffeine and gallic acid

Sample preparation procedure used in this section was like the one used for total polyphenols as described in section 3.7.1. A modified HPLC method of Zuo *et al*. (2002) was used to assay for the tea catechins including gallated and non-gallated catechins, caffeine and gallic acid. 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, 250 mm x 4.6 mm (Phenomenex, Torrance, CA, USA) separation column with a Reodyne precolumn filter disk was used. A gradient elution was then 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 $35 \pm 0.5\ ^{0}C$.

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 278 nm and achieved using a caffeine standard with a calibration curve $R^2 = 0.9984$ in conjunction with the consensus individual catechins relative response factor (RRF) values with respect to caffeine calculated on a dry matter basis. Total catechins as percentage by mass on a sample dry matter basis was given on the summation of individual catechins. Sample of chromatographs of individual catechins of both clones from both processing methods are shown in Appendices XI to XIV.

% Total catechins = [% ECG + % EC + % ECCG + % C] content ….....…….. (7)

3.7.4 Determination of total soluble solids

A sample of 5 g was infused in 100 mL of boiling double-distilled water followed by stirring with a magnetic stirrer for ten minutes at room temperature. The extracts were strained through a nylon mesh (120 µm) followed by a filter paper (Whatman No. 2, pore size of 8 µm). Ten millilitres of the extract was pipetted into ashing tubes of known weight and

dried in an oven at 103 ± 2 ^{0}C overnight. The difference in weight equals to the total soluble solids present expressed as mg/mL.

3.7.5 Assay of total monomeric anthocyanins in non-aerated TRFK 306 teas

The anthocyanins were first extracted and purified before determining the amount using UV- spectrophotometer.

Extraction of anthocyanins

A 5 g weight of milled sample were put into 125 mL conical flask and rubbed with aluminium foil after inserting a magnetic stirrer. A 50 mL methanol/formic acid (99/1 v/v) were added into the flask and stirred at 900 rpm for 4 hours at room temperature before filtration. A cotton wool was initially used in the filtration before concentrating in a vacuum rotary evaporator (Buchi Rotavapour R-300, Switzerland) under reduced pressure at 35 ^{0}C. The extract was dissolved in 10 mL distilled water and filtered under vacuum using 0.45 µm membrane filters into sintered glasses. The resultant extract was passed through reversed phase (RP) C_{18} solid phase extraction (SUPELCO, SPE) (Sigma Aldrich, USA) cartridges. The cartridges had been activated with 10 % MEOH/HCL. Anthocyanins were adsorbed into the column while other compounds including sugar, acids and other water soluble compounds were washed out using 0.01 % HCL in distilled water. Acidified methanol (10 % formic acid) was used to recover the adsorbed anthocyanins. The extracted anthocyanins in graduated test tubes rubbed with aluminium foils, were kept at a temperature of -10 ^{0}C awaiting further analysis.

Determination of total monomeric anthocyanins

The described protocol in section 3.3.2 (Giusti and Wrolstad, 2001) was followed in determination of total monomeric anthocyanins of non-aerated teas from TRFK 306.

3.8 Sensory evaluation of non-aerated (green) teas

Sensory evaluation was also done so as to get a feedback from the tasters which would guide on how the perceived consumers would feel about the products. Five grams of the coded sample was transferred into a 250 mL infusion cup and boiling water added, covered and steeped 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 for both orthodox and CTC teas. The liquor was sipped by the experienced tasters with a spontaneous breath which brings 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 (Appendix I and Appendix V) for CTC and orthodox teas, respectively.

3.9 Assay of aerated (black) teas quality parameters

These include; total theaflavins, thearubigins, total colour, brightness, individual theaflavins, antioxidant activity, total soluble solids and sensory evaluation.

3.9.1 Determination of dry matter of black tea

Black tea (2 ± 0.05 g) was weighed into an already weighed aluminium disc and heated in an oven at a temperature of 103 ± 2 ^{0}C for at least 16 hours to constant weight. The percentage dry matter was then calculated when all the moisture has been removed. Dry matter determination was necessary since most of the results were expressed on dry weight basis.

3.9.2 Determination of theaflavin content in aerated (black) tea

Flavognost method (Hilton, 1973) was used to determine total theaflavins content. A tea infusion was prepared by adding 375 mL of boiling distilled water into a tared vacuum flask with 9 g black tea then agitated in a mechanical shaker for 10 minutes. It was then filtered through a cotton wool into a flat bottomed flask. Tea liquor of 10 mL was pipetted into a test tube and 10 mL double distilled iso- butyl methyl ketone 4- methyl-penta-2-one (IBMK) added then shaken for 15 minutes and the test tube was left to stand to allow two layers to separate. From the upper layer, 2 mL was pipetted into a test tube and 4 mL ethanol and 2 mL of diphenylboric acid 2-amino- ethyl ester were added and shaken for exactly 2 minutes. The colour was allowed to develop by letting the test tube stand for exactly 15 minutes and then absorbance (A) read quickly at 625 nm. The machine was first set with blank Ethanol/IBMK (1:1 v/v) before reading the samples.

Theaflavin (µmol/g) = A_{625} * 47.9 * 100/DM..... (8)

Where DM = Dry matter

3.9.3 Assay of thearubigins in aerated (black) tea

The method of Roberts and Smith (1963) was used to determine total thearubigins. A tea infusion was prepared by adding 375 mL of boiling distilled water into a tared vacuum flask with 9 g black tea, corked then agitated in a mechanical shaker for 10 minutes. It was then filtered through a cotton wool into a flat bottomed flask and allowed to cool to room temperature. Pipetting into a separating funnel under a fume-cupboard of 6 mL of the cooled infusion was done before adding 6 mL of 1 % (w/v) aqueous solution of anhydrous disodium

hydrogen orthophosphate. The mixture was vigorously shaken for one minute after adding 10 mL of ethyl acetate which does extraction then settling was allowed to take place. Two layers formed after settling and the lower layer was drained off carefully. Then 5 mL of ethyl acetate was added to the ethyl acetate extract (upper layer) containing theaflavin fraction in the separating funnel before drawing 10 mL of the extraction into a 25 mL volumetric flask. Methanol was used to top up to the mark to obtain sample E1 whose optical density was measured using 10 mm cell at 380 nm and 460 nm as is required. From the cooled tea infusion prepared above, 1 mL was mixed with 9 mL of distilled water and made up to 25 mL in a volumetric flask with methanol where E2 was obtained whose optical density was measured as E1 above. Still from the cooled tea infusion prepared above, 1 mL was pipette into a 25 mL volumetric flask and 8 mL of distilled water was added before adding 1 mL of aqueous 10 % oxalic acid. Methanol was used to top up to mark and E3 was obtained ready for optical density measurement.

Optical densities (absorbance) of E1, E3 at wavelength of 380nm and E1, E2 at 460 nm using the 10 mm cell were measured.

Calculation of thearubigins: TR % =7.06 (4E3-E1)*DM%…… (9)

Total colour was calculated as shown; at 460 nm: Total colour = 6.25*4E2*DM% (Roberts method)……... (10)

% Brightness = E1/4E2*100 …… (11)

3.9.4 Determination of individual theaflavins

High performance liquid chromatography (HPLC) was used to determine the ratios of individual theaflavins (Mcdowell e*t al*., 1991). The individual theaflavins being refered to here are; simple theaflavins, theaflavin-3-monogallate, theaflavin-3’-monogallate and theaflvavin-3-3’-digallate. In the preparation of the samples, a 475 mL thermos flask was used to put 4 g of made tea followed by adding 200 mL boiling distilled water to make an infusion. The infusion was shaken for ten minutes prior to filtering through a cotton wool into a 250 mL conical flask. Dilution of 1:1 with distilled water was done then 20 µL was injected onto HPLC C18 ODS column. The wavelength was set at 375 nm and flow rate was set at 1.5 mL/min. Solvent A was 1% citric acid while acetonitrile was solvent B. A linear gradient from 8 % to 31 % solvent B over 60 minutes was used (Bailey e*t al*., 1990). The individual amount of theaflavin was determined using theaflavin standards as determined by HPLC under similar analytical conditions.

3.9.5 Assay of antioxidant activity of aerated (black) teas

The procedure described in section 3.3.3 of Brand-Williams *et al.* (1995) was used to determine the scavenging of free radicals of aerated teas from both clones.

3.9.6 Total soluble solids

The procedure described in 3.5.4 was used to determine the total soluble solids in black tea liquor for both studied clones.

3.10 Sensory evaluation of aerated (black) teas

Five grams of the coded sample was transferred to a 250 mL infusion cup and boiling water added, covered and left for five minutes to steep. This was then filtered into an infusion bowl and the residue (infusion) collected on the infusion cup lid. Both the liquors and infusions were described accordingly by the tasters. The researcher studied the descriptions and scored the samples using parameters and scale which was described by Kilel e*t al.* (2012) with modifications (Appendix II). The highest score this time round denoted the best. The scale was used for CTC teas. For orthodox teas, the researcher found the description of liquors of orthodox teas narrowed to liquor colour and liquor flavour. A scale of 1-5 with 5 being the best and 1 being the least (Appendix III and Appendix IV) for TRFK 306 and TRFK 6/8 orthodox teas, respectively were then developed . This is the first ever used scale for aerated orthodox teas and further research need to be done to come up with more comprehensive and agreed terminologies for orthodox teas.

3.11 Data analyses

The tea leaves for the study were obtained from the Timbilil estate of TRI, Kericho. Data was collected in triplicate and the means were analysed using General Linear Model (GLM) procedure of Statistical Analysis Software (SAS, 9.1.3). When significant differences ($p< 0.05$) were detected, Least Significant Difference was used to separate means.

CHAPTER FOUR
RESULTS AND DISCUSSIONS

4.0 Introduction

Results of non-processed purple tea, non-aerated (green) both orthodox and CTC and aerated (black) both orthodox and CTC will be presented in that order respectively. TRFK 306 and TRFK 6/8 teas were coded in figures as 306ORTHO/306CTC and 68ORTHO/68CTC denoting orthodox/CTC teas, respectively. Tables were also used to present the results and 3OR/3CT and 6OR/6CT denoted orthodox and CTC teas of TRFK 306 and TRFK 6/8, respectively unless specified otherwise.

4.1 Non-processed purple-leafed tea leaves

These include; total monomeric anthocyanins, antioxidant activity and effect of citric acid on the anthocyanins levels, antioxidant activity and sensory evaluation of the liquor from non-processed purple-leafed teas.

4.1.1 Total monomeric anthocyanins and antioxidant activity

The results of total momomeric anthocyanins and antioxidant activity are presented (Table 2). There were significant differences ($p< 0.05$) within and between time/temperature combinations. Liquors brewed at 70 ^{0}C irrespective of the timing had less total monomeric anthocyanins than liquors brewed at boiling temperature. The highest level recorded at 70 ^{0}C brewing temperature was 27.72 mg/L which was lower than the lowest recorded using boiling temperature, 37.54 mg/L, though not significantly different ($p< 0.05$). The highest anthocyanin levels were recorded in liquors brewed for five minutes using boiling drinking water with 92.02 mg/L and the levels decreased with increase in brewing time.

There was no significant difference ($p< 0.05$) in antioxidant activity of the liquor obtained by brewing at 92 ^{0}C timed at 5, 10 and 15 minutes. The liquors brewed at ten minutes however, had relatively higher antioxidant activity than the rest, with 92 %. There was a slight increase in antioxidant activity with increase in time. The lowest antioxidant activity recorded in the current study was 90.7 %.

Table 2: **Total monomeric anthocyanins (TA) and antioxidant activity (AA) in tea brew from non-processed purple-leafed tea leaves**

Temperature (^{0}C)	Brewing time (min.)	TA (mg/L)	AA (%)
70	5	27.7±4.5^{cd}	N/A
	10	17.9±3.7^{de}	N/A
	15	9.5±1.7^{e}	N/A
92	5	92.0±3.6^{a}	90.7±4.7^{a}
	10	63.7±7.6^{b}	92.0±3.9^{a}
	15	37.5±3.6^{c}	91.3±0.9^{a}

Means followed by the same letter (s) along the column are not significantly different at p< 0.05. n= 18.N/A means the time/temperature was not applicable in the affected row and column.

The results showed that, anthocyanins were detected in the liquors brewed from fresh non-processed Kenyan purple-leafed teas. The results agree with findings of Kerio *et al*. (2012). The tea leaves were subjected to heat treatment using hot water thus inactivating the enzyme polyphenol oxidase inherent in tea leaves which can degrade anthocyanins (Liu, *et al*., 2007). The study also confirmed that anthocyanins in tea leaves can be extracted using hot water (He *et al*., 2016). Brewing with boiling water at 5 minutes had relatively higher anthocyanins than brewing for 10 and 15 minutes. The findings corroborate the findings of Kerio *et al*. (2012) where they found higher anthocyanins when brewing was done between 5 and 7 minutes. Anthocyanins can also be extracted using methanol, ethanol, acetone, water or a mixture of these solvents (Welch *et al*., 2008). Anthocyanins are relatively unstable pigments, and temperature is the main factor that triggers the degradation of the anthocyanins (Liazid *et al*., 2014). Otherwise other factors including their chemical structure, pH, and presence of light, ions, enzymes or oxygen can trigger their degradation (Sui *et al*., 2014). Liquors brewed at 70 ^{0}C, had relatively less anthocyanins even at 15 minutes timing compared to liquors brewed with boiling water. This could be because high extraction temperatures improve extraction efficiency. The high heat renders the cell walls more permeable facilitating extraction and increasing the solubility and diffusion coefficients of the tea components (Vuong *et al*., 2011c). The experiment was mimicking how people currently brew non-processed purple tea leaves, but having the knowledge of anthocyanins degradation at high temperatures (Sharif *et al*., 2010), the study wanted to find out if water at a

temperature of 70 ^{0}C could be sufficient for extraction. Anthocyanins levels irrespective of brewing regime, decreases with increase in time taken. The decrease in anthocyanins concentration with increase in brewing time could be because of degradation of anthocyanins with prolonged boiling which agrees with what was found by Surh and Koh, (2014).

The DPPH radical scavenging method was used in the current study because it is a relatively quick and widely used method for evaluating free radical scavenging activity. The effects of antioxidants on DPPH radical scavenging is thought to be due to their hydrogen-donating ability (Biswas *et al*., 2010; Gangwar *et al*., 2014). Otherwise there are many methods that can be applied in determination of antioxidant potential of single compound or their mixtures (Abramovic *et al*., 2018). The researchers mentioned the use of chromogenic radicals; 2, 2-azino-bis-3-ethylbenzotiazolin-6-sulfonic acid ($ABTS^{+}$) and Folin- ciocalteu (FC) reagent apart from 2, 2-diphenyl-1-picrylhydrazyl (DPPH). The antioxidant activity results showed that the Kenyan non-processed purple tea clone has higher antioxidant potency than even some green teas found in other parts of the world displayed by Pal *et al*. (2012). In their research, they found antioxidant activity ranging from 60 % to 70% lower than what this study found. This study showed, there was no significant difference at ($p< 0.05$) (Table 2), in brewing time, though 10 or 15 minutes, resulted in better liquors with higher antioxidant activity than brewing for five minutes. This could be because of better extraction of polyphenols. Total polyphenols are water soluble and has antioxidant activity (Serpen *et al*., 2012). The current findings corroborate the findings of Liu *et al.* (2018). The researchers found out that, higher brewing temperature and longer brewing time yield an increase in antioxidant activity of green tea infusions. Recently, Perez- Burillo *et al*. (2017) also assessed the effect of time-temperature brewing conditions on the overall antioxidant capacity of white tea. Their results showed that the anti radical capacity of white tea gradually grew in a linear manner with infusion time and water temperature. There is need for more research on this to know exactly which compounds are contributing to high antioxidant activity at 10 and 15 minutes.

4.1.2 Effect of citric acid on total monomeric anthocyanins

The results of total anthocyanins at various citric acid levels are presented (Figure 12). The least amount of 0.1 % had higher total monomeric anthocyanins than the brew without citric acid, respectively. The highest recorded total anthocyanins was 92.0 mg/L, from brew without citric acid while a brew with 0.1 % had 103.36 mg/L. The content of total

anthocyanins increased with increase of citric acid amount up to 0.3 % before it marginally dropped (Figure 12).

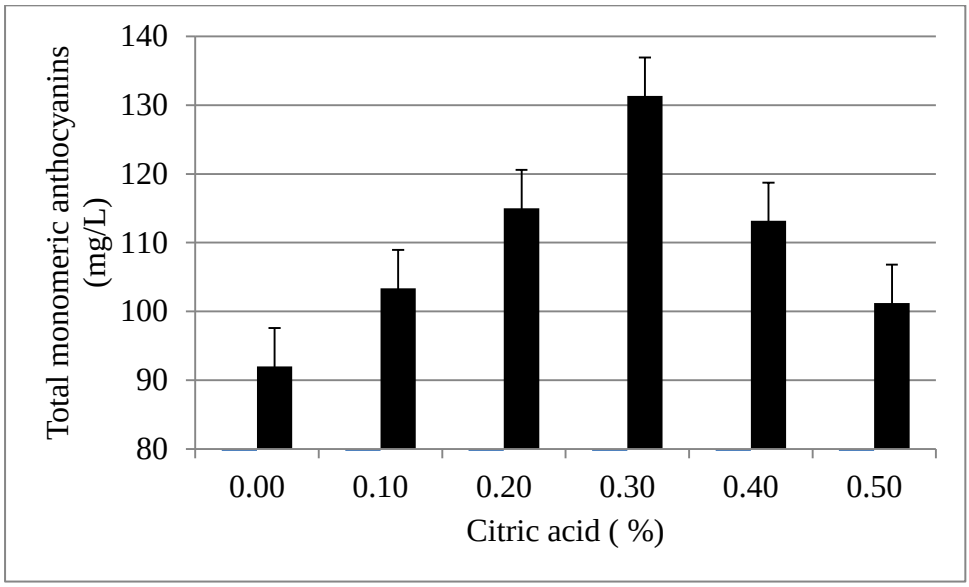


Figure 12: **Effect of citric acid concentration on total monomeric anthocyanins**

Citric acid used in the study was able to cause an effect on the liquor because of its high solubility in water (Vandenberghe *et al*., 2017). The total monomeric anthocyanins increased with increasing citric acid amount up to 0.3 %. The pH values also decreased from 5.3 (without citric acid) to 2.5 (with 0.5 % citric acid) (Table 3), with addition of citric acid. This is because the acid increased the hydrogen ion concentrations in the solution lowering pH values. The increase in total monomeric anthocyanins with addition of citric acid up to some point concurs with the findings of Brenes *et al*. (2005). These researchers found out that presence of ascorbic acid aided to retain higher anthocyanin content than the control. They also argued that copigmentation with ascorbic acid aided to retain a higher anthocyanin content than the control. According to Brenes *et al*. (2005) anthocyanin copigmentation reactions result from the association of metal ions or colourless polyphenols to anthocyanins under acidic conditions and the complexes formed serve to enhance the colour and stability characteristics of anthocyanins in low acid conditions. In another study by Anggraini *et al*. (2018), they found out that addition of citric acid to Jamblang peel increased the total phenol and anthocyanins content of the jam in a dose dependent manner. A recent study by Akkarachaneeyakorn and Tinrat (2015) found that decreasing the pH increased the anthocyanin content in pure mulberry juice. They justified the observation by saying that structurally, anthocyanins are more stable under acidic conditions than neutral or alkaline

conditions. In the current study, the results showed the decline of total monomeric anthocyanins with addition of 0.4 % citric acid and 0.5 % though the colour intensity improved (Plate 4). This could be because more citric acid increases the release of other compounds which has the counter effect of lowering the purity of the extract and decreasing the anthocyanin content (Alcazar-Alay *et al*., 2017).

4.1.3 Effect of citric acid on antioxidant activity

Since the results of boiling point (92 ^{0}C) timed at ten minutes showed higher antioxidant activity, the investigation on effect of citric acid was timed as such. The results are presented (Figure 13). The brew with no citric acid added (plain) had an antioxidant activity of 88.5 % which was higher than the rest. The antioxidant activity decreases with increasing citric acid concentration where 0.5 % had the lowest antioxidant activity of 39.96 %. There was a significant difference at ($p<0.05$) in the antioxidant activity at various citric acid concentrations.

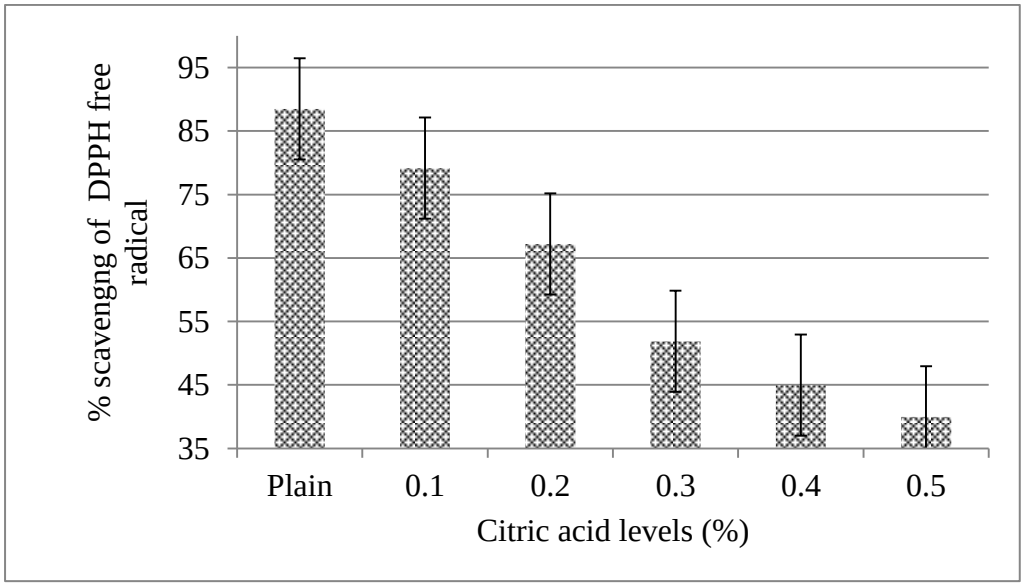


Figure 13: Effect of citric acid concentration on antioxidant activity

Antioxidant activity is defined as ‘ an inhibition of the oxidation of lipids, proteins, DNA or other molecules that occurs by blocking the propoagation step in oxidative chain reactions’ and primary antioxidants directly scavenge free radicals, while secondary antioxidants indirectly prevent the formation of free radicals through Fenton’s reaction (Huang *et al*., 2005). Hot water was used in the current study to extract the phenolic compounds because most are soluble in water (Yang and Liu, 2012). The results showed that there was a significant decrease in antioxidant activity when the amount of citric acid was increased. This could be so because when water is used in the extraction, like in this case, the

addition of citric acid decreases the antioxidant activity unlike when alcohol is used in the extraction (Halee *et al*., 2018; Anggraini *et al*., 2018). Earlier studies (Ammar *et al*., 2015; Bridgers *et al*., 2010; Rayle and Cleland, 1992) had explained this phenomenon by arguing that, the free form of phenolic compounds obtained from water extraction might have been destroyed by acid and hence reduction in the antioxidant activity. The results of the current study corroborate the findings of Bartoszek *et al.* (2018), who found out that addition of lemon to tea brew reduces its antioxidant activity. Citric acid is a weak organic acid that occurs naturally in various fruits and vegetables, especially citrus fruits including lemon (Kanse *et al*., 2017). The current findings contradict (Anggraini *et al*., 2018; Akaranta and Akaho, 2012) where they argued citric acid being a secondary antioxidant is often added to foods in combination with primary antioxidants therefore yielding synergistic effect to increase the activity of primary antioxidants.

4.1.4 Sensory evaluation of freshly brewed non-processed purple teas leaves

The results of sensory evaluation of fresh purple tea leaves brewed for five and ten minutes with/without citric acid added at varying quantities are presented (Table 3). Taste and colour were evaluated because the two parameters are the most varied especially when citric acid is added. Five - point hedonic scale was used to evaluate the freshly brewed non-processed purple tea leaves where forty panellists participated in the study. Colour scores ranged from 4.4 to 2.8, as shown in (Table 3), both timings. The highest colour score was the liquor with 0.5 % added citric acid and the lowest score was shown by the liquor without citric acid added. There was no significant difference ($p<0.05$) in liquors with 0.3, 0.4 and 0.5 % citric acid in both timings. Plain brews from both timings scored relatively low with 2.9 and 2.8 for 5 and 10 minutes, respectively. The results (Table 3 and Plate 4) shows that the colour improved with increased citric acid levels evident by the increase in the scores recorded.

The scores of the taste of non-processed purple tea leaves are presented (Table 3). The trend is the same as in the colour scores, where plain brews scored the lowest and the taste scores increased with increase in addition of citric acid. The highest score recorded in five minutes brewing were the brew with 0.3 % citric acid added with 3.8 while for ten minutes brewing, 0.5 % citric added had the highest score of 3.9. The brew with 0.4 and 0.5 % citric acid added scored relatively lower than those with 0.3 % citric acid in 5 minutes timings though better than plain and those with 0.1 and 0.2 % citric acid added.

The pH values of the brews were also measured. The results are shown (Table 3). Brews without citric acid had higher values in both timings and did not differed significantly ($p<0.05$). The pH values decreased with increasing citric acid concentration.

Table 3: pH values; taste and colour scores of brew from fresh non-processed purple-leafed teas

Time (minutes)	Amount of citric acid (%)	colour score	Taste score	pH value
5	0.0	2.9±0.2 ^{fg}	2.4±0.2 ^{c}	5.3±0.14 ^{a}
	0.1	3.3±0.2 ^{ef}	**3.2±0.2 ^{b}**	2.8±0.01 ^{b}
	0.2	3.8±0.2 ^{bcd}	3.5±0.2 ^{ab}	2.7±0.05 ^{c}
	0.3	3.9±0.2 ^{abc}	3.8±0.2 ^{a}	2.5±.0.02 ^{de}
	0.4	4.3±0.1^{ab}	3.6±0.2 ^{ab}	2.4±0.02 ^{e}
	0.5	4.2±0.2 ^{ab}	3.7^{ab}±0.2 ^{ab}	2.5±0.03 ^{e}
10	0.0	2.8±0.2 ^{g}	2.5±0.2 ^{c}	5.4±0.07 ^{a}
	0.1	3.4±0.2 ^{def}	3.3±0.2 ^{ab}	2.9±0.03 ^{b}
	0.2	3.6±0.2 ^{cde}	3.6±0.2 ^{ab}	2.6^{cd}±0.01^{cd}
	0.3	4.3±0.1^{ab}	3.8±0.1^{a}	2.5±0.01^{de}
	0.4	4.3±0.15 ^{ab}	3.4±0.22 ^{ab}	2.4^{e}±0.02 ^{e}
	0.5	4.4±0.15 ^{ab}	3.9±0.25 ^{ab}	2.4^{e}±0.01 ^{e}

Colour score, taste score and pH values means and standard error along a column with same letter (s) are not significantly different at ($p<0.05$). n = 40 (panellists).

Colour scores showed that addition of citric acid improved the appearance of the brew (Plate 4 and Table 3). The colour shifted to a more intense red coloration with decreasing pH values. The finding agrees with Truong *et al*. (2019) findings where the hue and chroma of the juice increased with increase in acid concentration. Purple-leafed Kenyan teas have anthocyanins (Table 2) and earlier studies done by Kerio *et al*. (2012) had found out the clone has anthocyanins. Seven anthocyanins were isolated from Kenyan purple tea by Kerio *et al*. (2012). They found malvidin as most predominant otherwise, peonidin, pelargonidin, cyanidin, delphinidin, cyanidin-3- *O*- glucoside and cyanidin-3- *O*- galactoside was also isolated. Malvidin is an anthocyanin which appears red in colour in acidic condition and has been found to give red wine its appealing colour (Tomankova *et al*., 2016). Malvidin has excellent antioxidant properties (Huang *et al*., 2016). Anthocyanins are naturally occurring pigments of red and purple where the red pigments are mostly used in food industries as colorants (Khoo *et al*., 2017; Wahyuningsih *et al*., 2017). They are also used as food and pharmaceutical ingredients and have potential health benefits (Khoo *et al*., 2017). As it can

be observed from (Plate 4), the sample with no acid added had faded colour but colour changes to reddish colour and got brighter red with increased addition of citric acid. This could be because of the change of pH where the brew changed from basic to acidic, (Table 3) and with increased addition of citric acid since anthocyanins appear red in acidic conditions (Wahyuningsih *et al*., 2017). These results show that the colour of anthocyanins is actually dependent on the pH of the solution and this could be because of the ionic nature of their molecular structure (Turturica *et al*., 2015). Literature shows that those red-coloured pigments of anthocyanins are predominantly in the form of flavylium cations which appears red (Bakowska- Barczak, 2005) and these anthocyanins are more stable at the lower pH. The brew with no citric added scoring low may be because those with added acid appeared more appealing because of the increase in the intensity of the red hue. This increase in the intensity of the red hue could be because, acidic conditions maintains the stability of flavylium ion and increase the intensity of the red hue in the anthocyanin pigment (Khoo *et al.,* 2017).

Taste score are presented (Table 3) where the brew with no citric acid added scored low and the scores increased with increase in addition of citric acid up to a point. Citric acid was used here to mimic the usual way of utilizing fresh non-processed purple tea leaves where lemon fruits juices are added. The scores increased in both timings demonstrating that citric acid increased the organoleptic property of the food product as was found by Reddy *et al*. (2016) and contributes a sour gustatory flavour quality (Veldhuizen, *et al*., 2018). Citric acid being water soluble enhances the flavour of citrus-based foods (Jensen and Hansen, 2005).

The pH value of a food is a direct function of the free hydrogen ions present in that food (McGlynn, 2010). This author defined and demonstrated clearly the meaning of pH. The author stated that the pH is a measure of free acidity and precisely defined it as 'pH is a negative log of the hydrogen ion concentration'. Therefore if a food has a pH value of 3, then the concentration of hydrogen ion equals to 10^{-3} (0.001) moles/liter. If the pH value is 6, then the concentration of hydrogen ion equals to 10^{-6} (0.000001) moles/liter. He explained that the examples he gave above, shows the concentration of hydrogen ions decreases as the pH value of the food increases and this explains why a low pH food is a high-acid food and a high pH food is a low-acid food (basic). The pH values presented (Table 3) shows that, there is a decrease in pH values with increase in citric acid addition because the acidity increased. Brew without citric acid had a pH of 5.3 and 5.4 in 5 minutes and ten minutes, respectively, while the brew with 0.5 % had 2.5 and 2.4 in that order. The pH values will always increase

(basic) or decrease (acidic) depending on the changes of hydrogen ions concentrations whether decreasing or increasing, respectively.

4.2 Non-aerated (green) orthodox and CTC teas

Total polyphenols, total catechins, non-gallated, gallated catechins, gallic acid, caffeine, antioxidant activity and sensory evaluation results are presented and discussed. These parameters are considered the key quality parameters in non-aerated (green) teas.

4.2.1 Total polyphenols

The results of total polyphenols are presented (Figure 14). There were significant differences ($p<0.05$) within and between the two clones in terms of type of leaf disruption and withering duration. Most TRFK 306 orthodox teas had relatively higher total polyphenols compared to CTC teas of the same clone with orthodox teas withered for five hours having 25.32 % total polyphenols. TRFK 306 teas irrespective of the processing technique had lower total polyphenols than TRFK 6/8 teas. TRFK 6/8 orthodox teas had relatively higher total polyphenols than CTC teas of the same clone. Highest total polyphenol was observed in orthodox teas from TRFK 6/8 which was withered for fifteen hours, with 27.48 % total polyphenols.

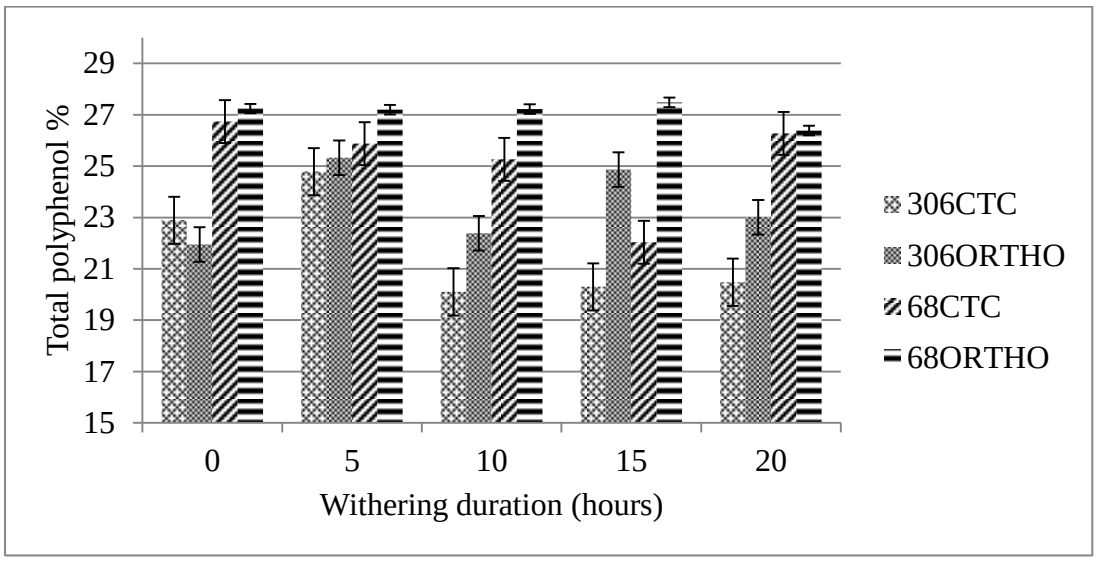


Figure 14: Effect of leaf disruption method and withering duration on total polyphenols. Each value is a mean of three replicates. Bar means ± standard error are shown. The legend shows, 306CTC, 306ORTHO, 68CTC and 68ORTHO representing, TRFK 306 CTC teas, TRFK 306 orthodox teas, TRFK 6/8 CTC teas and TRFK 6/8 orthodox teas, respectively.

The Tea Research Institute of Kenya, through continuous research, has established that teas can be categorized as follows: high quality teas have 24.8–27.1 % total polyphenols; medium high quality have 22.5–24.4 % total polyphenols; medium quality have 19.6–22.3 % total polyphenols; low quality teas have 17.5–19.2 % total polyphenols. In this study the first category of high quality teas included, all TRFK 6/8 orthodox and CTC teas except CTC teas of the same withered for fifteen hours which had 22.04 % falling under medium quality teas. High quality teas from TRFK 306 included orthodox teas withered for five and fifteen hours and CTC teas withered for five hours, with 25.32 %, 24.86 % and 24.78 % total polyphenols respectively (Figure 14). Medium high quality teas included TRFK 306 orthodox and CTC teas which were withered for 20 hours and non-withered, respectively. Medium quality teas were mostly TRFK 306 CTC teas except those which were not withered. It also included TRFK 306 orthodox teas withered for ten hours and those non-withered. TRFK 6/8 CTC teas withered for 15 hours also fall in this category of medium quality teas. The two studied clones had high levels of total polyphenols and this could be because these two clones are Kenyan clones. Kenyan tea-breeding programme has indirectly and consistently selected tea germplasm for high total polyphenols content, to produce black teas with high levels of TFs and TRs (Anonymous, 2013; Kerio *et al*., 2013). Orthodox teas had higher total polyphenols- which mainly constitute the catechins - than CTC teas, this could be because, for CTC teas they have higher surface area to volume ratio because of full maceration hence most of the catechins can easily be lost through oxidation. TRFK 306 teas from both processing methods had lower total polyphenols than TRFK 6/8. This could be because TRFK 6/8 is a standard tea clone bred to have high total polyphenols. The current findings on distinct differences on the studied clones agree with the findings of Muthiani *et al*. (2016). These researchers had observed that the differences in polyphenolic composition were due to individual clone's genotype. The effect of withering on total polyphenols in the current study could not be concluded since there was no clear pattern on the same, save for TRFK 306 CTC teas which display decreased total polyphenols with longer withering duration.

4.2.2 Total catechins

Total catechins results are presented (Figure 15). There were significant differences ($p<0.05$) within and between the clones studied, in the type of leaf disruption and withering duration. TRFK 306 orthodox teas whose leaves had been withered for 5 hours had relatively higher mean of 12.95 % with CTC teas whose leaves had been withered for 15 hours had the lowest mean of 7.96 % total catechins. TRFK 6/8 teas both orthodox and CTC teas had

relatively higher total catechins than TRFK 306. TRFK 6/8 orthodox teas which were not withered and those withered for five hours had relatively higher total catechins with 20.53 % and 20.27 % respectively, though not significantly different at ($p<0.05$). Except for non-withered, all orthodox processed teas showed higher mean value of total catechins than the corresponding CTC processed counter parts. There was general decline in total catechins with withering time except for orthodox teas from the purple clone.

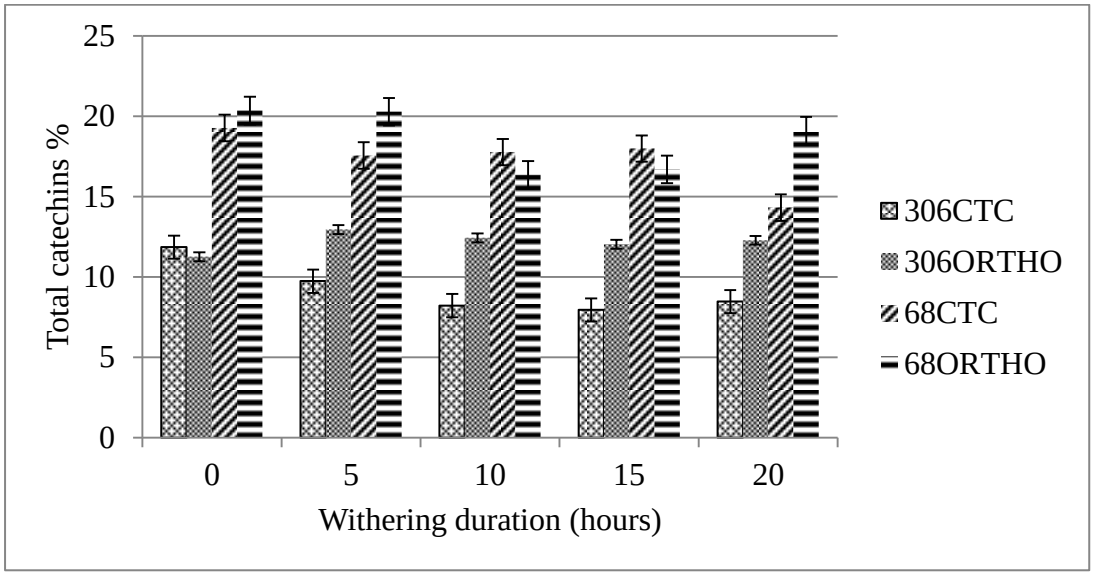


Figure 15: Effect of leaf disruption method and withering duration on total catechins. Each value is a mean of three replicates. Bar means ± standard error are presented. The legend shows, 306CTC, 306ORTHO, 68CTC and 68ORTHO representing, TRFK 306 CTC teas, TRFK 306 orthodox teas, TRFK 6/8 CTC teas and TRFK 6/8 orthodox teas, respectively.

There was a significant difference ($p<0.05$) between the clones studied where TRFK 6/8 both CTC and orthodox teas had higher total catechins than teas from TRFK 306 (Figure 15). This agrees with former researchers findings (Cherotich *et al*., 2013; Kerio *et al*., 2013; Lai *et al*., 2016) where they found the purple clone had lower catechins than the green clones. TRFK 6/8 having relatively higher total catechins, demonstrates that, this clone can make good quality non-aerated teas. The results agree with the findings of Ochanda *et al*. (2012) where they found the clone having realtively higher total catechins and therefore concluded the clone can be used to make quality green tea. The total catechins were observed to be decreasing with increasing withering time especially in CTC type of manufacture, this could be because CTC teas have higher surface area and therefore faster oxidation and reduction of catechins than orthodox teas which are whole leaf; consequently there was a general decline in total polyphenols. This could be because catechins form most total polyphenols composition. The general decrease in catechins levels agrees with earlier researchers who

found out that, during chemical withering, complex compounds are broken down into simpler compounds and catechins levels decreases (Deb and Pou, 2016). The findings also corroborate the findings of Jiang *et al*. (2018) where they found the enzyme activity increased during withering and therefore resulting to less catechin since they have been acted up on by the enzymes. Catechins are substrates to polyphenol oxidase enzyme which is a major enzyme in the tea leaves.

4.2.3 Non-gallated catechins

Non-gallated catechins include, catechin (+C), Epicatechin (EC) and Epigallocatechin (EGC) and they are free catechins without the gallate moiety. The order of the non-gallated catechins in percent found in this study was EGC>EC>C in both clones (Appendix VI).

Catechin (+C)

The results of +C are presented (Figure 16). Significant difference at ($p<0.05$) of +C were observed both on the type of leaf disruption and withering time in TRFK 306 teas. TRFK 306 orthodox processed teas that had been withered for 15 hours had the highest mean value of 0.83 % of (+) C whereas CTC processed teas which had been withered for 15 hours had the lowest mean value of 0.44 %. There was no significant difference at ($p<0.05$) in +C % in all TRFK 6/8 teas though slight decrease was noted with longer withering time in CTC teas.

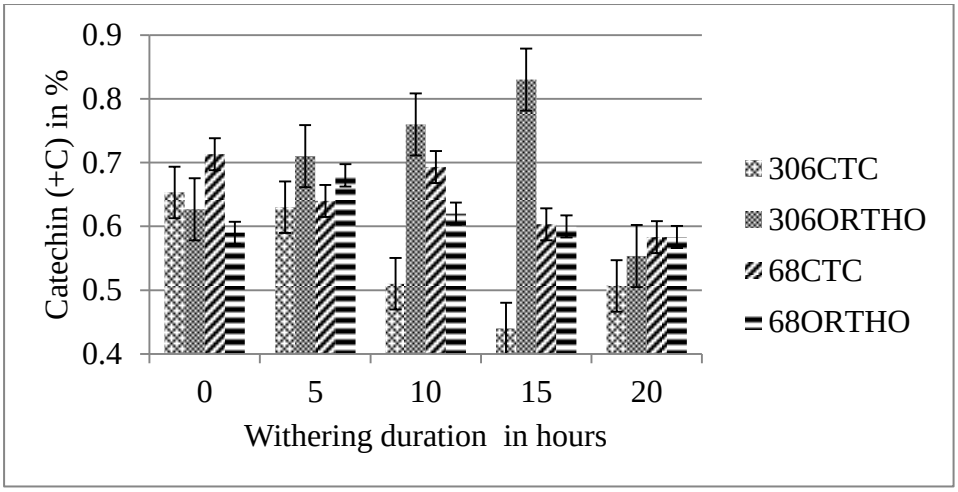


Figure 16: Effect of leaf disruption method and withering duration on (+) C.
Each value is a mean of three replicates. Bar means ± standard error are presented. The legend shows, 306CTC, 306ORTHO, 68CTC and 68ORTHO representing, TRFK 306 CTC teas, TRFK 306 orthodox teas, TRFK 6/8 CTC teas and TRFK 6/8 orthodox teas, respectively.

It can be noted (Figure 16) that, the +C are decreasing with increase in withering time in CTC manufacture while it seems to be increasing in orthodox manufacture with withering time for TRFK 306 teas. This can be explained by the fact that in CTC, because of full maceration and hence higher surface area for the enzyme polyphenol oxidase, there is more oxidation of the catechins and hence their decrease in CTC type of manufacture. Whereas in orthodox manufacture the leaves are not macerated and hence there is less oxidation of the catechins.

Epicatechin (EC)

The results (Table 4), depicts the effect of withering on EC in TRFK 306 teas and TRFK 6/8. There was significant differences ($p<0.05$) in the mean values of EC in most of these teas and TRFK 306 CTC teas which were withered for 20 hours which had a low content of 0.56 %.

Table 4: Epicatechin (EC) content (%) of TRFK 306 and TRFK 6/8 manufactured via orthodox and CTC

TRFK 306 (orthodox and CTC)		TRFK 6/8 (orthodox and CTC)	
Treatment	EC (%)	Treatment	EC (%)
OR10	1.12±0.08 [a]	OR20	1.93±0.16 [a]
OR5	1.07±0.1 [a]	CT10	1.92±0.01 [a]
OR15	1.07±0.15 [a]	OR0	1.89±0.11 [a]
CT5	1.05±0.07 [a]	OR5	1.84±0.13 [ab]
CT0	1.04±0.06 [a]	CT15	1.84±0.39 [ab]
CT15	0.88±0.05 [ab]	CT0	1.66±0.17 [abc]
CT10	0.88±0.23 [ab]	CT5	1.52±0.16 [bc]
OR0	0.88±0.05 [ab]	OR15	1.51±0.27 [bc]
OR20	0.84±0.02 [ab]	OR10	1.47±0.3 [c]
CT20	0.56±0.25 [b]	CT20	1.46±0.05 [c]

EC mean (%) and standard error followed by the same letter (s) along a column are not significantly different at $p< 0.05$. n= 30. OR0 and CTC0, OR5 and CTC5, OR10 and CT10, OR15 and CT15, OR20 and CT20 represent orthodox teas and CTC teas withered for 0, 5, 10, 15 and 20 hours, respectively.

Both manufacture depicts the teas of TRFK 306 having low levels than TRFK 6/8 teas. TRFK 6/8 orthodox teas which were withered for 20 hours had a relatively higher mean of EC with 1.93 % while the lowest recorded EC mean for TRFK 6/8 was 1.46 % in CTC teas withered same hours (20 hours). The lowest mean value among TRFK 6/8 CTC teas was relatively higher than the highest recorded mean among TRFK 306 CTC teas which had EC of 1.05%. TRFK 6/8 orthodox teas which were withered for 10 and 15 hours recorded low

mean values with 1.47 % and 1.51 %, respectively. TRFK 6/8 teas in both manufacture and all withering regime recorded higher mean values of EC than TRFK 306 teas. The effect of withering on EC was not convincingly significant ($p<0.05$) in the current study.

It can be noted (Table 4) that, and EC are decreasing with increase in withering time in CTC manufacture while it seems to be increasing in orthodox manufacture with withering time for TRFK 306 teas. This can be explained by the fact that in CTC, because of full maceration and hence higher surface area for the enzyme polyphenol oxidase, there is more oxidation of the catechins and hence their decrease in CTC type of manufacture. Whereas in orthodox manufacture the leaves are not macerated and hence there is less oxidation of the catechins. Other literature (UoN, n.d) had found out that, EC increased after withering. They argued that it could be because of degradation of EGCG. TRFK 6/8 teas had higher EGC and EC from the study. This could be because the green clone, TRFK 6/8 has more catechins than the purple tea clone (Cherotich *et al*., 2013).

Epigallocatechin (EGC)

The results are presented (Table 5). TRFK 306 CTC teas which had been withered for 20 hours had relatively higher mean value of EGC with 2.09 % while CTC processed teas that had been withered for 5 hours showed the lowest mean value with 0.97 % EGC (Table 5). TRFK 306 CTC teas which were withered for 20 and 15 hours had relatively higher EGC and did not differ significantly at ($p<0.05$) with orthodox teas which were withered for 10 and 15 hours. TRFK 306 CTC teas which were not withered and those which were withered for 5 and 10 hours did not differed significantly ($p<0.05$) in EGC and had low values compared to long withered teas. TRFK 6/8 teas withered for short duration, had higher EGC than those teas withered for long (Table 4) in both type of manufacture. The highest mean was 7.52 % of orthodox teas withered for 5 hours and this did not differed significantly at ($p<0.05$) with CTC teas withered for 5 hours and those not withered of both manufacture. The lowest mean was 4.07 %, for CTC teas which were withered for 20 hours and did not differed significantly at ($p<0.05$) with orthodox teas withered for 10 hours. Comparing both orthodox and CTC teas from both clones (Table 4), it was observed that all TRFK 6/8 teas had relatively higher EGC than TRFK 306 teas with orthodox teas having relatively higher levels.

Table 5: Epigallocatechin (EGC) content (%) of TRFK 306 and TRFK 6/8 manufactured via orthodox and CTC

TRFK 306 (orthodox and CTC)		TRFK 6/8 (orthodox and CTC)	
Treatment	EGC (%)	Treatment	EGC (%)
CT20	2.09±0.21 [a]	OR5	7.52±0.42 [a]
CT15	1.94±0.07 [ab]	OR0	7.37±0.54 [a]
OR15	1.64±0.23 [ab]	CT0	7.28±0.99 [a]
OR10	1.57±0.11 [abc]	CT5	7.04±0.83 [ab]
OR5	1.45±0.11 [bcd]	OR20	5.99±0.29 [bc]
OR0	1.08±0.14 [cd]	CT15	5.47±0.42 [c]
OR20	1.04±0.04 [cd]	CT10	5.31±0.93 [c]
CT0	1.03±0.12 [d]	OR15	5.28±0.84 [c]
CT10	1.02±0.41 [d]	OR10	5.22±0.83 [cd]
CT5	0.97±0.03 [d]	CT20	4.07±0.11 [d]

EGC mean (%) and standard error followed by the same letter (s) along a column are not significantly different at $p< 0.05$. n= 30. OR0 and CTC0, OR5 and CTC5, OR10 and CT10, OR15 and CT15, OR20 and CT20 represent orthodox teas and CTC teas withered for 0, 5, 10, 15 and 20 hours, respectively.

Non-gallated catechins are catechins without galloyl moiety in their structure (Kim *et al*., 2016). EGC is noted to be increasing with withering time in TRFK 306 teas and this can be attributed to degradation of EGCG which is decreasing with withering time, (Figure 17). EGCG might degrade with time after plucking resulting in formation of EGC and the liberation of free gallic acid. TRFK 6/8 teas had higher EGC at short withering duration than long withered teas in both type of manufacture. EGC is inherent in the teas leaves and this it is depleted with time in this clone. EGC catechin is a substrate of polyphenol oxidase enzyme which is also inherent in the tea leaves therefore reduction of EGC upon withering is expected. TRFK 6/8 teas both orthodox and CTC had higher EGC than TRFK 306 teas. These findings corroborate the findings of earlier researchers (Kerio *et al*., 2013; Cherotich *et al*., 2013). They found most purple clones had lower EGC than green clones.

4.2.4 Gallated catechins

The results of effect of processing and withering duration on gallated catechins levels of both clones are presented (Figure 17and 18). The gallated catechins (EGCG and ECG) are the predominant catechins among the catechins. The discussions on the same are explained together, though the results are presented individually.

Epigallocatechin gallate (EGCG)

TRFK 6/8 teas had relatively higher EGCG than TRFK 306 teas with orthodox teas having higher though not significantly varying with withering time, that is there was no significant difference ($p< 0.05$) amongst TRFK 6/8 orthodox teas. TRFK 306 teas had EGCG levels varying significantly at ($p < 0.05$) being influenced by the type of leaf disruption and withering duration. The highest mean value of EGCG content was noted in orthodox processed teas withered for 5 hours which had 5.05 % EGCG and CTC processed teas withered for 15 hours recorded the lowest mean with 1.65 %.

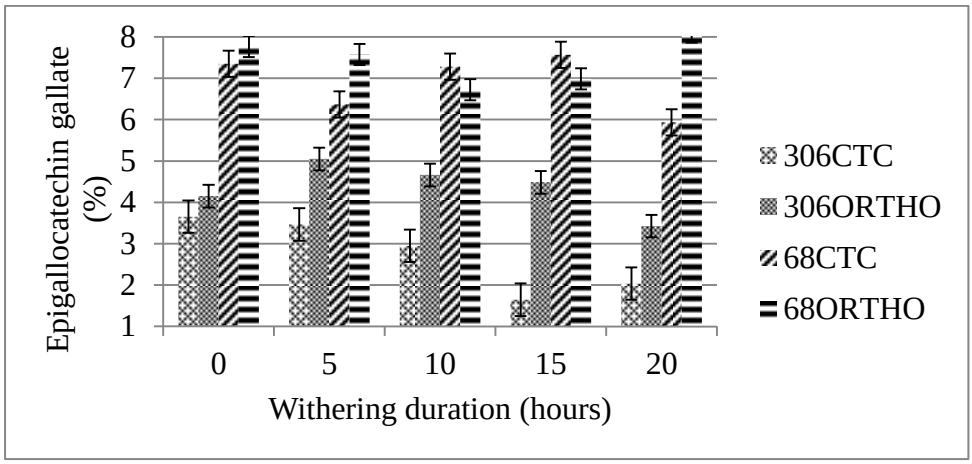


Figure 17: Effect of leaf disruption method and withering duration on EGCG.

Each value is a mean of three replicates of both TRFK 306 and TRFK 6/8. Bar means ± standard error are shown. The legend shows, 306CTC, 306ORTHO, 68CTC and 68ORTHO representing, TRFK 306 CTC teas, TRFK 306 orthodox teas, TRFK 6/8 CTC teas and TRFK 6/8 orthodox teas, respectively.

Epicatechin gallate (ECG)

Whereas TRFK 6/8 teas had relatively higher EGCG levels, it had relatively lower ECG levels than TRFK 306 teas where orthodox teas from TRFK 306 had higher levels than even CTC teas from the same clone. There were significant differences ($p< 0.05$) in ECG percentage with regard to the type of clone. There was, however, no significant difference ($p< 0.05$) on TRFK 6/8 teas on the type of leaf disruption and the withering duration (Figure 18). Most TRFK 306 teas had relatively higher mean values of ECG with the highest mean value being observed in orthodox processed teas whose leaves had been withered for twenty hours with 6.43 % ECG and the lowest mean from the same clone was noted in CTC processed teas withered for 10 hours which had 2.85 % ECG.

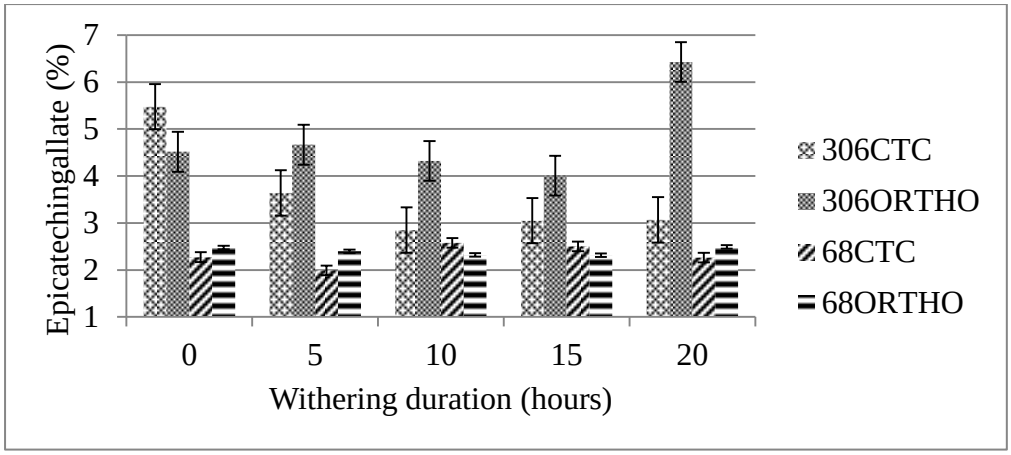


Figure 18: Effect of leaf disruption method and withering duration on ECG.
Each value is a mean of three replicates of both TRFK 306 and TRFK 6/8. Bar means ± standard error are shown. The legend shows, 306CTC, 306ORTHO, 68CTC and 68ORTHO representing, TRFK 306 CTC teas, TRFK 306 orthodox teas, TRFK 6/8 CTC teas and TRFK 6/8 orthodox teas, respectively.

Gallated catechins have galloyl moiety in their structure (Kim *et al*., 2016). (Figure 17) shows TRFK 6/8 teas had higher EGCG levels than TRFK 306 teas. These results are in agreement with Joshi *et al.* (2015) findings where they found green shoots having more EGCG than purple tea shoots. There was however a noted decline of EGCG with prolonged withering with exception on orthodox teas from TRFK 6/8 withered for twenty hours which shows exceptionally high mean values of EGCG with 8.11 %. The decline of EGCG with withering is expected since there is oxidation and degradation of EGCG. The current findings agrees with Yin *et al.* (2008) results where they found, EGCG, GCG, ECG and total ester-catechins gradually decreasing with longer withering duration (Figure 17) it can be observed that, with withering for 5 hours onwards, there is a consistent trend where the orthodox type of manufacture displays a higher mean value of both EGCG and ECG (Figure 17 and 18), respectively. Moreover, ECG and EGCG are decreasing with withering time for both CTC and orthodox teas of both clones, save for the noted outlier in TRFK 6/8 orthodox teas withered for twenty hours which showed a higher value of EGCG (Figure 17). This is because during withering stage, the content of catechins is mostly influenced by polyphenol oxidase and peroxidase enzymes. The oxidation of catechins under the action of the enzymes results in the decrease of catechins (Theppakorn, 2016; Deb and Pou, 2016). Longer withering duration leads to an increase in moisture removal leading to an increase in concentration of cell sap (Deb and Pou, 2016). This results in the increase of enzyme concentration and formation of high molecular units from low molecular sub units, activating the enzymatic activity of polyphenol oxidase. The activity of polyphenol oxidase was also

found to increase during withering (Tram *et al*., 2015). The fact that TRFK 306 orthodox teas had higher ECG (Figure 18) than orthodox teas from TRFK 6/8 shows that the purple clone can synthesize more of that catechin than TRFK 6/8 as was found by Lai *et al*. (2016). They found out that the 'Ziyan' purple clone exhibits a preference for synthesizing B-ring trihydroxylated catechins and therefore their accumulation than the green clones. Anthocynins and catechins share the biosynthetic pathways and this can lead to competition between these compounds (Lai *et al*., 2016) affecting the catechins levels of anthocyanin-rich clones.

4.2.5 Gallic acid content

There are other trihydrobenzoic acis but gallic acid (3, 4, 5-trihydroxybenzoic acid) is more popular among them (Erukainure *et al.*, 2018). Gallic acid occurs in certain red fruits, black radish, onions and tea containing up to 4.5 g/Kg fresh weight in tea leaves (Tomas-Barberan and Clifford, (2000). The acid consists of three hydroxyl groups and a carboxylic acid group attached to a benzene ring and the bonding of the hydroxyl groups in an ortho position results in a coplanar and bent configuration, which is favourable for antioxidant activities (Sroka and Cisowski, 2003; Badhani *et al*., 2015). Gallic acid results are presented (Figure 19; Appendix VI) shows that, there was a significant difference ($p< 0.05$) with the type of clone with TRFK 306 teas having relatively higher gallic acid than TRFK 6/8 teas. TRFK 6/8 teas had relatively lower gallic acid than TRFK 306 teas though there was no significant difference ($p< 0.05$) in the type of leaf disruption and withering time. The highest mean value of gallic acid among purple teas was noticed in orthodox type of leaf disruption at five and ten hours wither with 1.28 % and 1.24 %, respectively, though not significantly different ($p< 0.05$). In TRFK 306 clone, the lowest mean value of gallic acid was observed in CTC purple teas withered for ten hours with 0.74 %. It is worth noting here that, the average gallic acid in the non-withered (0 wither hour) purple tea both CTC and orthodox and that subjected to 20 hours of withering for both types of leaf disruption, though not varying significantly ($p<0.05$), those withered for 20 hours had slightly higher average values.

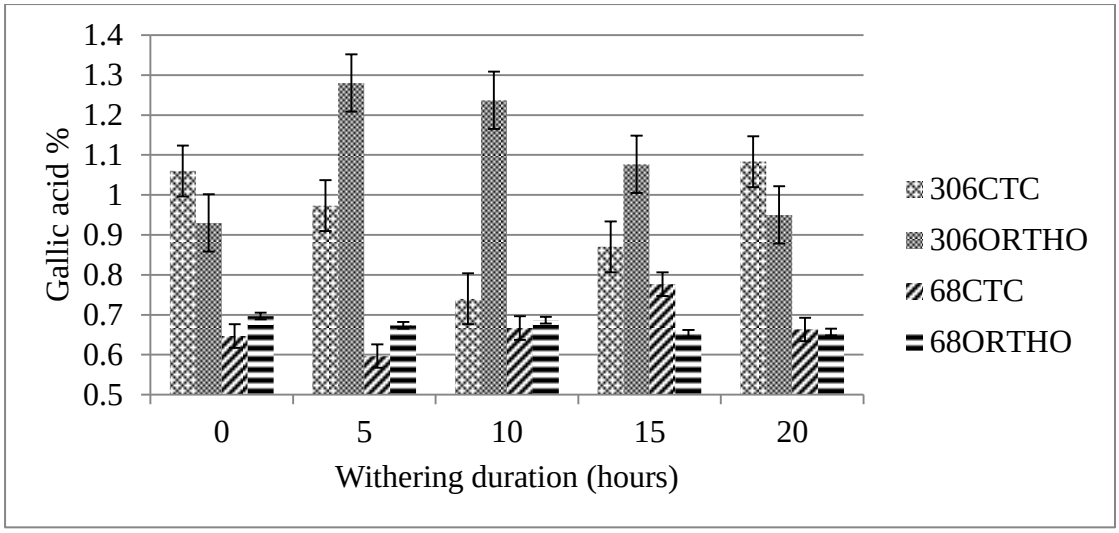


Figure 19: Effect of leaf disruption method and withering duration on gallic acid. Each value is a mean of three replicates of both TRFK 306 and TRFK 6/8. Bar means ± standard error are presented. The legend shows, 306CTC, 306ORTHO, 68CTC and 68ORTHO representing, TRFK 306 CTC teas, TRFK 306 orthodox teas, TRFK 6/8 CTC teas and TRFK 6/8 orthodox teas, respectively.

TRFK 6/8 had lower gallic acid than TRFK 306 in all manufacturing conditions, (Figure 19); this explains why there is high levels of EGC and EGCG in TRFK 6/8 teas. The catechins (EGC and EGCG) are oxidatively degallated to form gallic acid. These results confirms TRFK 6/8 as a clone with low gallic acid values as was discovered by Kilel, (2013) thesis where TRFK 6/8 had lower gallic acid than studied purple clones. Gallic acid alongside catechins and caffeine influences green tea quality. In the current study, the results showed that there were significant differences ($p< 0.05$) in the type of manufacture and in withering time among the TRFK 306 teas with orthodox tea showing increase of gallic acid with withering time. This could be because of oxidative degallation of catechin especially EGCG where free gallic acid is released and cleavage of galloyl groups (Theppakorn, 2016). The increase in gallic acid with increase in withering time also agrees with the findings of Ye *et al*. (2018). These researchers found gallic acid contents increasing with increasing time of withering and temperature. Gallic acid exists in plant material in the form of free acids, esters, catechins derivatives and hydrolysable tannins. Some studies have shown that gallic acid and its derivatives have antioxidant activity (Karamaæ *et al.,* 2005, Gramza *et al*., 2006). Gallic acid formation pathways have been shown to include the hydration of epigallocatechin gallate and degradation from the dimer of epigallocatechin gallate (Chen *et al*., 2013). The researchers concluded so since epigallocatechin gallate was decreasing with increasing gallic acid. Otherwise other three methods had been proposed (Saijo, 1983).

4.2.6 Caffeine content

Caffeine (1, 3, 7-trimethylxanthine) is a purine alkaloid present in high concentration in tea and coffee and has also been found in coca cola beverages (Misako and Kouichi, 2004). The caffeine levels results are presented (Figure 20), were significantly different ($p<0.05$) with regard to the type of clone, type of leaf disruption and withering time. TRFK 6/8 teas had relatively higher caffeine levels than TRFK 306 teas. The teas withered for twenty hours (orthodox and CTC) and CTC teas withered for ten hours had higher values, which did not differed significantly ($p< 0.05$) with 3.27 %, 3.13 % and 3.33 % caffeine levels, respectively. The highest mean value of caffeine in TRFK 306 teas was observed in CTC teas which had been withered for twenty hours (CTC 20 hours) with 2.83 % and the lowest mean value observed in teas processed via CTC and withered for ten hours wither with 1.55 % caffeine.

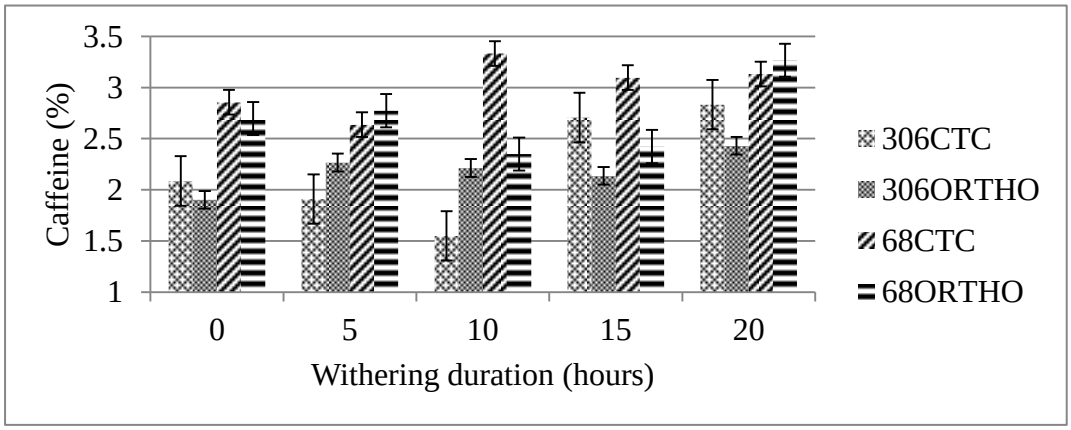


Figure 20: Effect of leaf disruption method and withering duration on caffeine. Each value is a mean of three replicates of both TRFK 306 and TRFK 6/8. Bar means ± standard error are shown. The legend shows, 306CTC, 306ORTHO, 68CTC and 68ORTHO representing, TRFK 306 CTC teas, TRFK 306 orthodox teas, TRFK 6/8 CTC teas and TRFK 6/8 orthodox teas, respectively.

TRFK 6/8 teas had relatively higher caffeine levels than TRFK 306 teas, these results concurs with those found by earlier researchers (Cherotich *et al*., 2013; Kerio *et al*., 2013; Joshi *et al*., 2015), that purple clones has lower caffeine levels than green clones. (Figure 20) depicts that the caffeine content increased with withering time in both clones. Previous studies had also found caffeine increasing with increasing duration of withering (Deb and Pou, 2016; 2018; Kalidass *et al*., 2019). The increase in caffeine with longer withering time might be due to higher rate of respiration with release of water molecule and carbondioxide. This explanation is not satisfactory and further research need to be done to demystify the scenario. Caffeine is one of the most widely consumed, naturally occurring, mild and central nervous system stimulant (Gonzalez- Caldenon *et al*., 2015). Caffeine is important in the

quality of tea since it is responsible for the briskness of the tea liquor and also good for human health as it prevents tumorgenesis (Lin and Liang, 2000). Caffeine is also known as a tea plant ingredient with considerate functionality, including its ability to promote wakefulness and cardiotonic action (Nesumi *et al*., 2012).

4.2.7 Antioxidant activity

The results of antioxidant activity are presented in (Figure 21). There were significant differences ($p<0.05$) noted in the type of clone, leaf disruption and withering duration. Most TRFK 6/8 orthodox teas had relatively higher percent inhibition against DPPH than CTC teas of the same clone. The highest antioxidant activity in TRFK 306 teas was observed in teas withered for twenty hours manufactured via CTC, whereas the lowest mean value was noted in orthodox teas withered for five hours with 92.07 % and 89.99 %, respectively. There was significant difference ($p< 0.05$) in inhibition against DPPH of purple teas on the type of leaf disruption and withering time and those non-withered and withered for five hours orthodox teas had lower DDPH inhibition percent.

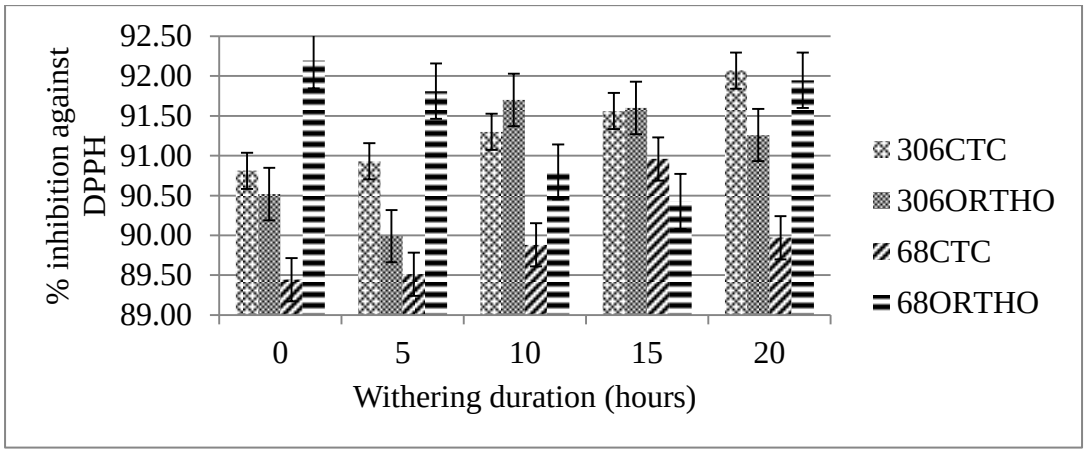


Figure 21: Effect of leaf disruption method and withering duration on antioxidant activity.
Each value is a mean of three replicates of both TRFK 306 and TRFK 6/8 clones. Bar means ± standard error are shown. The legend shows, 306CTC, 306ORTHO, 68CTC and 68ORTHO representing, TRFK 306 CTC, TRFK 306 orthodox, TRFK 6/8 CTC and TRFK 6/8 orthodox teas, respectively.

Non-aerated teas are commonly known as green tea because the final product (dry leaf) is greenish in colour since it does not undergoes tea oxidation during processing. The results are presented as the percentage inhibition against the radical DPPH, and this is equivalent to the antioxidant activity of the teas. The 2,2-diphenyl- 1- picrylhydrazyl (DPPH) is a stable free radical that accepts an electron or hydrogen radical to become a stable diamagnetic molecule (Biswas *et al*., 2010). Tea catechins can act as antioxidants by donating

a hydrogen atom, as acceptors of free radicals and interrupters of chain oxidation reactions or by chelating metals (Saklar *et al*., 2015). There are several findings confirming tea has antioxidant properties (Kosinska and Aldlauer, 2014; Sharma and Borua, 2017; Prasanth *et al*., 2019). TRFK 6/8 orthodox teas had higher antioxidant activity than CTC teas of the same clone. This could be because of higher total polyphenols, (Figure 14) and EGCG levels (Figure 17), than the CTC teas, since polyphenols has antioxidant activity (Cooper, 2011). Polyphenols are the most abundant bioactive compounds and are known for their health benefits (Anyasi *et al*., 2013). The scavenging activities of polyphenols have been reported to be mainly due to their oxidation and reduction properties, thus enabling them to act as singlet oxygen collectors and hydrogen donors (Babbar *et al*., 2011). The increase in the inhibition against DPPH with increase in withering time, (Figure 21) could be because of the increase in gallic acid (Figure 19), since gallic acid have been found to be a strong antioxidant (Inoue *et al.,* 1994; Karamaæ *et al*., 2005; Gramza *et al*., 2006; Kongpichitchoke *et al*., 2016). TRFK 6/8 orthodox teas had higher percent inhibition against DPPH than CTC teas, the results agrees with Sharma and Borua, (2017) findings that orthodox teas has higher antioxidant activity than CTC teas.

4.2.8 Total soluble solids in non-aerated teas of both clones

The results of total soluble solids in non-aerated teas of both clones manufactured using the two methods are presented (Table 6). There was a significant difference ($p< 0.05$) between and within clones in terms of manufacturing and withering time. Orthodox teas of both clones had more total soluble solids than CTC teas. TRFK 6/8 teas had more total soluble solids than TRFK 306 teas in both manufacture. There was a general decrease of total soluble solids with increasing withering time.

Table 6: Total soluble solids of green teas from TRFK 306 and TRFK 6/8

TRFK306 (Orthodox and CTC)		TRFK 68 (Orthodox and CTC)	
Treatment	TSS (mg/mL)	Treatment	TSS (mg/mL)
OR20	3.5±0.09 [a]	OR0	4.29±0.25 [a]
CT0	3.43±0.09 [ab]	CT15	4.27±0.36 [a]
OR15	3.39±0.04 [abc]	OR10	4.18±0.06 [ab]
OR10	3.39±0.02 [abc]	CT0	4.09±0.09 [abc]
OR5	3.3±0.07 [bcd]	OR5	4.08±0.04 [abc]
CT20	3.23±0.08 [cde]	CT5	3.83±0.05 [abcd]
OR0	3.2±0.02 [de]	OR15	3.63±0.09 [bcd]
CT5	3.19±0.04 [de]	OR20	3.62±0.08 [cd]
CT10	3.12±0.04 [e]	CT10	3.51±0.27 [d]
CT15	3.09±0.01 [e]	CT20	3.51±0.25 [d]

TSS mean (mg/mL) and standard error followed by the same letter (s) along a column are not significantly different at $p< 0.05$. n= 30. OR0 and CTC0, OR5 and CTC5, OR10 and CT10, OR15 and CT15, OR20 and CT20 represent orthodox teas and CTC teas withered for 0, 5, 10, 15 and 20 hours, respectively.

The fact that, orthodox teas from both clones had more total soluble solids than their CTC counter parts could be because, most orthodox teas were found to have more total polyphenols and total catechins, (Figure 14 and 15), respectively. Polyphenols in tea refers to various catechins, catechin gallates, phenolic acids, anthocyanins and flavonoids glycosides (Joshi *et al*., 2015). Green tea catechins accounts for 10- 25 % of dry mass of the leaf (Kuhnert *et al*., 2010) or even 30 % (Prasanth *et al*., 2019) and they are water soluble (Bharadwaz and Bhattacharjee, 2012). The results also showed that most TRFK 6/8 teas had higher total soluble solids than TRFK 306 teas from both manufacturing processes. This can also be attributed to higher total polyphenols and total catechins of TRFK 6/8 as shown in (Figure 14 and 15). The low total soluble solids content in the purple clone than the green clone agrees with the findings of Lai *et al*. (2016). There was a general decrease in total soluble solids with increase in withering time. The results contradict with Mahanta and Baruah (1989) findings where they found increased total soluble solids with longer withering duration.

4.2.9 Total monomeric anthocyanins of non-aerated TRFK 306 teas

The results of total monomeric anthocyanins of processed TRFK 306 are presented (Table 7). There were relatively higher total monomeric anthocyanins in teas which were

processed immediately without withering than withered teas. There was a significant ($p < 0.05$) reduction of total anthocyanins in both type of manufacture with increased withering time. CTC teas had more drastic reduced total anthocyanins after ten hours of withering than orthodox type of manufacture.

Table 7: **Total monomeric anthocyanins of TRFK 306 non-aerated teas**

Manufacture	Withering hours	Total monomeric Anthocyanin (mg/L)
CTC	0	93.5±0.9 [a]
	5	77.4±1.2 [b]
	10	26.7±0.8 [c]
	15	24.7±0.5 [c]
	20	24.4±0.1 [c]
Orthodox	0	93.1±1.1 [a]
	5	51.8±0.7 [b]
	10	50.6±0.5 [b]
	15	47.1±0.6 [b]
	20	23.6±0.4 [c]

Means of total monomeric anthocyanins and standard error followed by the same letter(s) along a column are not significantly different at $p < 0.05$. n= 30.

The results showed that processed teas from TRFK 306 have anthocyanins whether processed via CTC or orthodox type of manufacture. Anthocyanins are powerful antioxidants with high nutraceutical value due to their anti-inflamatory and anti-carcinogenic effects on human health (Liu *et al*., 2017; Tsuda, 2012). Malvidin was found to be the most predominant anthocyanin in Kenyan purple tea (Kerio *et al*., 2012). Total monomeric anthocyanins were higher in freshly plucked none withered teas in both type of manufacture. This could be because of less time was allowed for the enzyme polyphenol oxidase which is known to degrade the anthocyanins (Liu *et al.*, 2007). The action of polyphenol oxidase is augmented by increasing gallic acid with withering. This is so because degradation of anthocyanins by the enzyme requires presence of gallic acid among other substrates (Welch *et al.*, 2008). There was more rapid reduction in CTC type of manufacture than orthodox type of manufacture. This could be because of higher surface area to volume ratio of CTC teas compared to orthodox teas and therefore faster reaction and degradation of anthocyanins.

4.2.10 Sensory evaluation of non-aerated (green) teas

These include non-aerated orthodox and non-aerated CTC teas.

Non-aerated orthodox teas

The terms used in the score sheet of orthodox teas were based on description of the tasters who participated in the sensory evaluation. The tasters were allowed to describe the teas where the descriptions were found to be narrowed to the liquor colour and flavour thus the development of the score sheet (Appendix V). Liquor colour refers to the brightness of the liquor, where the liquor can be light which is not desirable and therefore scores the least. The liquor colour could be purplish or purple (TRFK 306) it could be yellowish or yellow for (TRFK 6/8) and could score as shown in (Appendix V). Bright liquors are desirable and scored the highest; (Appendix V). Withering was varied with an interval of five hours from non- withered to twenty hours. The results of liquor colour of orthodox teas are presented (Figure 22 and Appendix V). There was a significant difference ($p<0.05$) in the liquor colour. TRFK 306 orthodox teas withered for fifteen and twenty hours however had relatively better liquors where they were bright. Non-withered TRFK 6/8 teas had the lowest score in the liquor colour.The current results reveals that, the liquor colour of orthodox teas from both clones improved with withering time. Orthodox teas from TRFK 306 had better liquor colour than teas from TRFK 68.

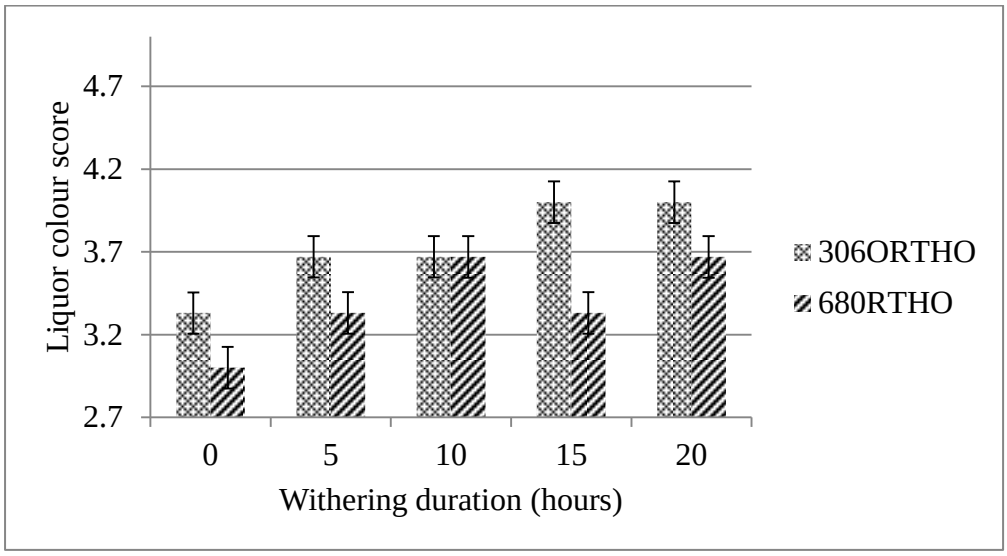


Figure 22: Green orthodox tea liquor colour scores. 306ORTHO and 680RTHO represents TRFK 306 orthodox teas and TRFK 6/8 orthodox teas, respectively.

The liquor colour of both clones is noted to be improving with wthering time.These results agrees with some earlier researchers' findings (Singhal *et al*., 1997; Fard *et al*., 2015) who found longer withering time improved the liquor colour and strength. The increase in liquor colour of TRFK 6/8 orthodox teas with longer withering duration could be because of

the decrease in total soluble solids. (Table 6), depicts the results of total soluble solids, where there was a general decline in total soluble solids with withering time and this might have led to a general increase in liquor colour of TRFK 6/8 orthodox teas. TRFK 6/8 orthodox teas had higher total soluble solids than TRFK 306 teas, (Table 6) and consequently had lower liquor colour (Figure 22).

Liquor flavour scores are presented (Figure 23). There was significant difference ($p<0.05$) in liquor flavour of both clones across the withering regime. It can however be noted that the liquor flavour of TRFK 306 green orthodox teas marginally increased with withering while TRFK 6/8 increased up to ten hours wither then decrease. Liquor flavour score sheet is shown (Appendix V).

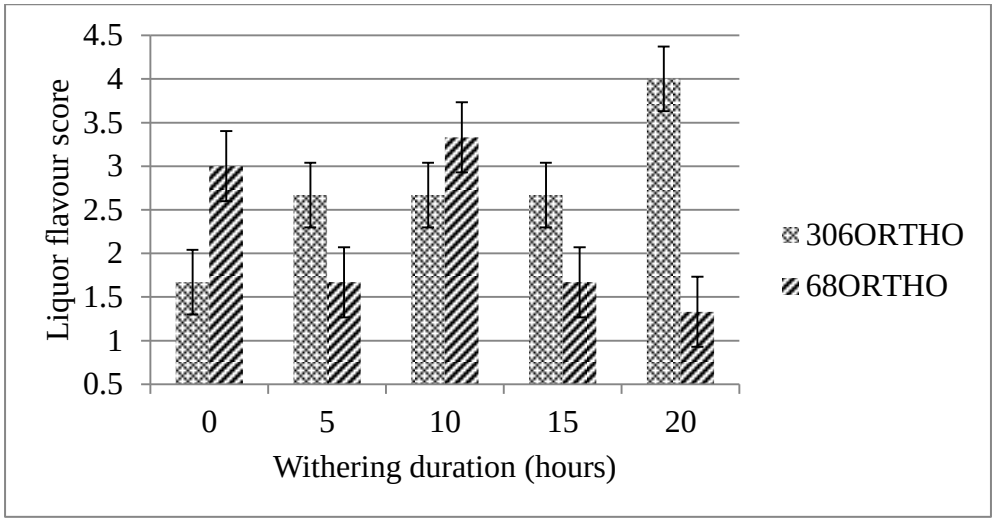


Figure 23: Green orthodox tea liquor flavour scores. 306ORTHO and 68ORTHO represents TRFK 306 orthodox teas and TRFK 6/8 orthodox teas, respectively.

The liquor flavour of orthodox TRFK 306 teas were increasing with withering time and this could be because of increase in flavour compounds especially group II volatile compounds (Panda, 2011; Sanyal, 2011; Zheng *et al*., 2016). Group II volatile flavour compounds include linalool and geraniol and they influences the liquor aroma positively. TRFK 306 orthodox green teas had pungent liquors with briskness. This could be because of relatively higher ECG since ECG has been found to show the strongest taste in green tea liquors (Narukawa *et al*., 2010).

Non-aerated CTC teas

The tasters who participated in tasting the CTC samples were allowed to describe the coded CTC green teas from both clones and from their description; the score sheet for the same (Appendix I) was developed. They described the liquor colour, liquor body and

briskness. Regarding liquor colour, all the samples were described as bright and therefore the liquor colour were then ignored in the presentation and discussions. The results of liquor body are presented (Figure 24). Liquor body results shows that, some liquors were light others were fairly thick or thick. Non- withered teas of both clones were light in their liquors like most TRFK 6/8 teas and scored lowest in liquor body. TRFK 306 teas scored relatively better in liquors compared to teas from TRFK 6/8 clone (Figure 24) except those teas which were not withered.

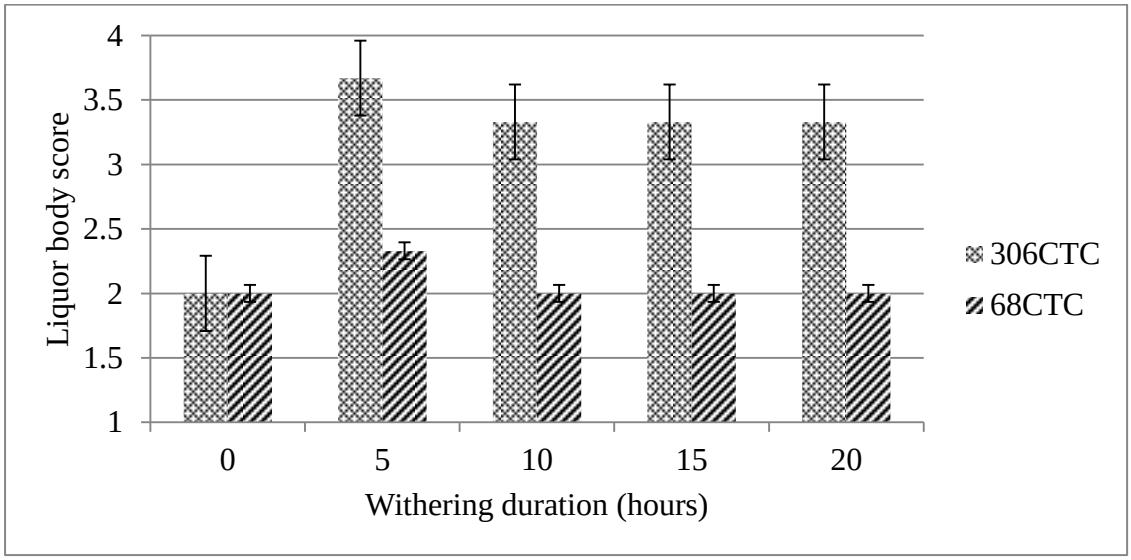


Figure 24: Green CTC teas liquor body scores. 306CTC and 68CTC represents TRFK 306 CTC teas and TRFK 6/8 CTC teas, respectively.

The liquor body of TRFK 306 scored better than teas from TRFK 6/8, meaning the teas were thicker. This might be attributed to the inherent colour, purple which on processing appear more or less green than teas from green clone, giving the liquors more colour. Total soluble solids influence positively the thickness of tea liquor. (Table 6), shows TRFK 6/8 CTC teas having relatively higher total soluble solids than TRFK 306 CTC teas and therefore, we expected TRFK 6/8 CTC teas to have thicker liquors, though it is not so in the current findings. This is revealing the need to have separate description of terms of teas from purple-leafed teas and green tea clones.

The results of liquor briskness of CTC teas are presented (Figure 25). The tasters described the liquor briskness where some were plain as others were harsh while others were pungent with some in between. TRFK 306 CTC teas were more brisk than TRFK 6/8 teas as presented (Figure 25). TRFK 306 teas withered for twenty hours had a highest score of 4 in briskness. The highest score in TRFK 68 teas had a score of 2 and the lowest was 1.33.

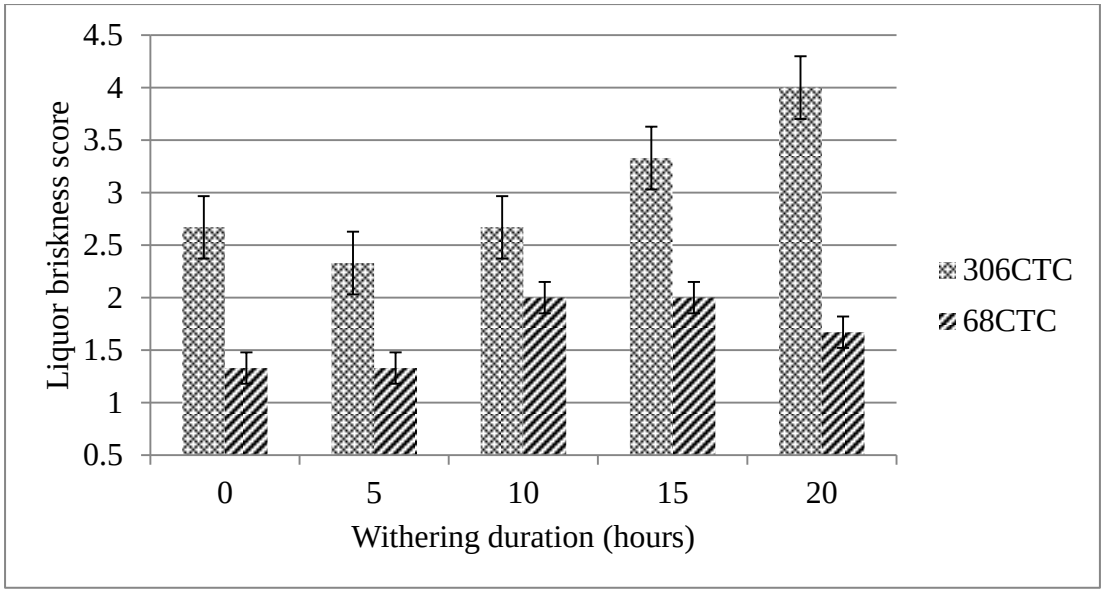


Figure 25: Green CTC teas liquor briskness score. 306CTC and 68CTC represents TRFK 306 CTC teas and TRFK 6/8 CTC teas, respectively.

Most TRFK 6/8 CTC teas were described as harsh and therefore scoring low in liquor briskness. Harsh tea is bitter and is not enjoyable. Harsh is associated with a biting sensation, a little rough, caused by tannins (Palais, n.d). The harshness of TRFK 6/8 teas could be because of high polyphenols (tannins) (Figure 14) and total catechins (Figure 15). Catechins which constitute 50 % of polyphenols are water soluble and colourless contributing to astringency and bitterness in green tea (Yoshida *et al*., 1999; Gramza and Korczak, 2005). TRFK 306 teas which were withered for twenty hours had a highest score of 4 in briskness and this could be because of high levels of caffeine (Figure 20) though TRFK 6/8 had high caffeine too but scored low in briskness. TRFK 6/8 had higher total polyphenols and total catechins making the liquors taste harsh.

4.3 Aerated (black) orthodox and CTC teas chemical analysis

Total theaflavins, thearubigins, total colour, brightness, individual theaflavins, antioxidant activity, total soluble solids and sensory evaluation results are presented and discussed.

4.3.1 Total theaflavins

The results of theaflavins (TFs) expressed in µmoles/g are presented in (Figure 26). The results showed that, black tea from both clones processed through orthodox method (rolling) has lower theaflavins than teas processed through CTC maceration. Black orthodox teas from TRFK 306 showed higher theaflavins than orthodox teas from TRFK 6/8. CTC teas from TRFK 306 also showed higher theaflavins especially with shorter withering time. The

effect of withering duration was noticeable between the clones. TRFK 306 had higher theaflavins for shorter withering duration and reduces with increasing withering duration especially for CTC teas as the thearubigins slightly increases. TRFK 6/8 had generally increasing theaflavins with increasing withering duration and aeration time. Within the same withering duration there were three aeration timing, 30, 60 and 90 minutes, but aeration of 90 minutes recorded highest theaflavins content. Black CTC teas from TRFK 306 had higher theaflavins than teas from TRFK 6/8 in all the withering and aeration duration except in 20 hours withering and in all aeration of 90 minutes.

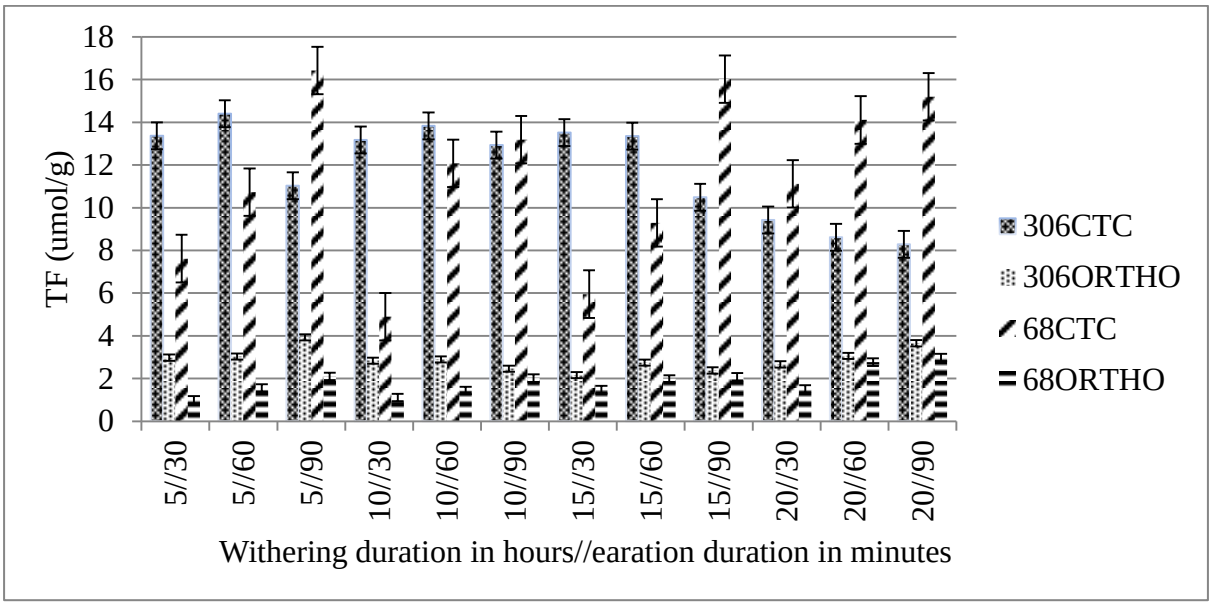


Figure 26: Effect of processing method, withering duration and aeration time on theaflavins.
Where; 5//30,5//60,5//90 denotes 5 hours withering time and 30, 60 and 90 minutes aeration time, respectively; 10//30, 10//60, 10//90 represent 10 hours withering time with 30, 60 and 90 minutes aeration time, respectively, 15//30, 15//60, 15//90 represent 15 hours withering time with 30, 60 and 90 minutes aeration time, respectively while 20//30, 20//60 and 20//90 represent 20 hours withering time with 30, 60 and 90 minutes aeration time, respectively. The legend, 306CTC, 306ORTHO, 68CTC and 68ORTHO represent TRFK 306 CTC teas, TRFK 306 orthodox teas, TRFK 68 CTC teas and TRFK 6/8 orthodox teas, respectively.

Orthodox teas had lower theaflavins than CTC teas; this could be because during CTC maceration there is full maceration hence more surface area for reaction and oxidation of catechins (Owuor and Reeves, 1986) and consequently more theaflavins. On the other hand, in rolling (orthodox), the tea leaves are left almost whole and there is less release of biochemicals for oxidative reaction and hence less theaflavins. The findings that black orthodox teas from TRFK 306 showed higher theaflavins than orthodox teas from TRFK 6/8, agrees with Joshi *et al*. (2015) findings where they found out that, higher levels of theaflavins

were recorded in orthodox black tea from purple shoots compared to black tea made from green shoots. Though the current study never investigated the residual catechins in aerated teas, the levels of catechins can still be used to explain the formation of theaflavins. Wilson and Clifford, (1992), had explained that high concentration of catechin gallates like EGCG, may inhibit polyphenol oxidase activity affecting the formation of theaflavins. The results shown (Figure 17), depicts TRFK 6/8 teas having relatively higher levels of EGCG than TRFK 306 teas and this may have contributed to TRFK 6/8 having lower TFs than TRFK 306 teas. The current study found out CTC teas from TRFK 306 also showed higher theaflavins especially with shorter withering time. These are good findings especially for Kenya who is leading in black CTC tea export. The purple clone can still be processed into black tea. The effect of withering duration was noticeable between the clones where TRFK 306 had higher theaflavins for shorter withering duration and reduces with increasing withering duration especially for CTC teas as the thearubigins slightly increase. This could be because of oxidative degradation of theaflavins to form thearubigins (Robertson, 1992). TRFK 6/8 had generally increasing theaflavins with increasing withering duration and aeration time. Within the same withering duration there was three aeration timing, 30, 60 and 90 minutes, but aeration of 90 minutes recorded highest theaflavins content. Most black CTC teas from TRFK 306 had higher theaflavins than from TRFK 6/8 in all the withering and aeration duration except in 20 hours withering and 90 minutes aeration. These confirms the above findings even in orthodox processing and the findings of Joshi *et al*. (2015) that purple-leaf coloured teas produces more theaflavins than green coloured teas.

4.3.2 Thearubigins

The results for thearubigins content are presented (Figure 27). The CTC teas from both clones had higher level of thearubugins than the orthodox teas. There was a gradual increase in thearubigins with increase in aeration time in CTC teas from TRFK 6/8 and TRFK 306 and orthodox teas from TRFK 6/8. TRFK 306 CTC teas had relatively higher thearubigins than CTC teas from TRFK 6/8 and decreases with increase in withering time. There was a noticeable increase in thearubigins in orthodox teas of both clones with increase on withering hours.

The results of black orthodox teas from both clones having lower levels of thearubigins (TRs) than black CTC teas, is expected because there is more surface area for oxidative reaction in CTC teas than in orthodox teas. Thearubigins are among major products of oxidative enzymatic action during aeration. CTC teas from TRFK 6/8 and TRFK 306 and

orthodox from TRFK 6/8 showed noticeable effect on aeration duration where there was a gradual increase in thearubigins with increase in aeration time, (Figure 27). This might have led to increased liquor body (thickness) in CTC teas of both clones (Figure 35).

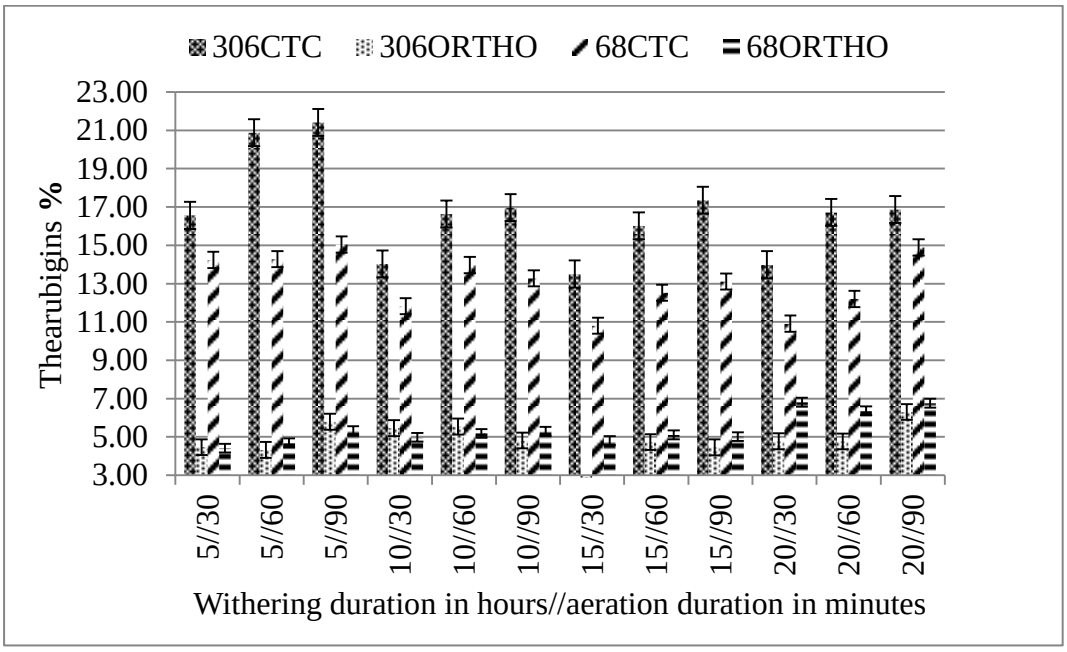


Figure 27: Effect of processing method, withering duration and aeration time on thearubigins.
Where; 5//30,5//60,5//90 denotes 5 hours withering time and 30, 60 and 90 minutes aeration time, respectively. 10//30, 10//60, 10//90 represent 10 hours withering time with 30, 60 and 90 minutes aeration time, respectively, 15//30, 15//60, 15//90 represent 15 hours withering time with 30, 60 and 90 minutes aeration time, respectively. 20//30, 20//60 and 20//90 represent 20 hours withering time with 30, 60 and 90 minutes aeration time, respectively. The legend, 306CTC, 306ORTHO, 68CTC and 68ORTHO represent TRFK 306 CTC teas, TRFK 306 orthodox teas, TRFK 68 CTC teas and TRFK 6/8 orthodox teas, respectively.

The increase in thearubigins with aeration time concurs with other researchers (Ngure, *et al*., 2009; Robertson, 1992; Teshome, 2019). The increase in thearubigins in orthodox teas agrees with the earlier findings (Ullah, *et al*., 1984; Fard *et al*., 2015) where they had found out that thearubigins increases with increase in withering time up to a certain level. TRFK 306 CTC teas were found to have relatively higher thearubigins than TRFK 6/8 counter part teas, this agrees with an earlier study (Kilel *et al*., 2013b) where, it was found out that some purple clones had higher thearubigins than TRFK 6/8 teas. The results (Figure 26), depicts TRFK 306 CTC teas having consistently relatively higher theaflavins than TRFK 6/8 CTC teas. This might have as well led to higher thearubugins in CTC teas of TRFK 306 since theaflavins are prescursors of thearubigins (Yassin *et al*., 2015a).

4.3.3 Total colour

The results of total colour are presented (Figure 28). There was a noticeable effect on aeration time where longer aeration time of ninety minutes showed better colour and short aeration time of thirty minutes had low total colour percentage. TRFK 306 CTC teas had better colour than TRFK 6/8 CTC teas though all CTC teas of both clones had better total colour than all orthodox teas.

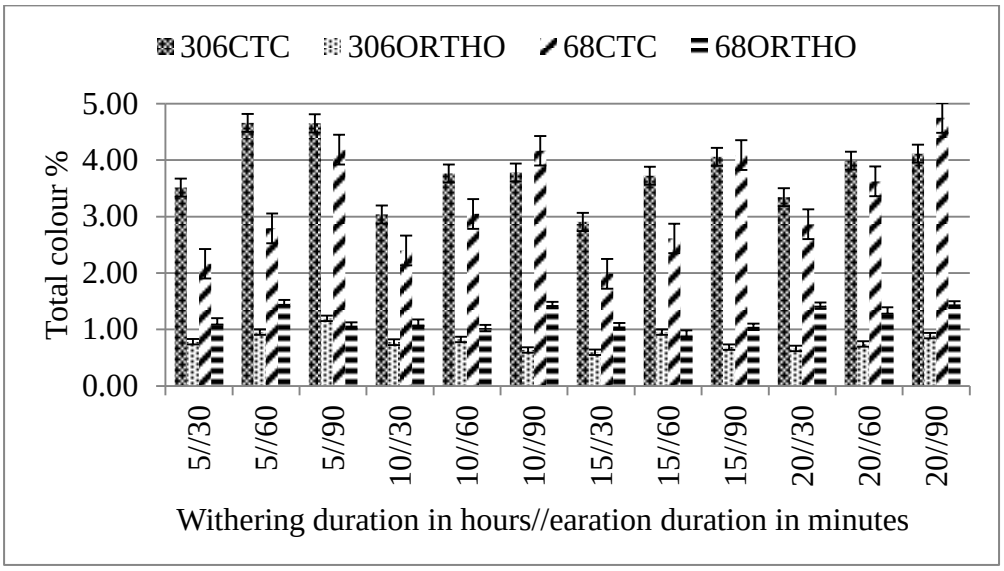


Figure 28: Effect of processing method, withering duration and aeration time on total colour.

Where; 5//30,5//60,5//90 denotes 5 hours withering time and 30, 60 and 90 minutes aeration time, respectively. 10//30, 10//60, 10//90 represent 10 hours withering time with 30, 60 and 90 minutes aeration time, respectively, 15//30, 15//60, 15//90 represent 15 hours withering time with 30, 60 and 90 minutes aeration time, respectively. 20//30, 20//60 and 20//90 represent 20 hours withering time with 30, 60 and 90 minutes aeration time, respectively. The legend, 306CTC, 306ORTHO, 68CTC and 68ORTHO represent TRFK 306 CTC teas, TRFK 306 orthodox teas, TRFK 68 CTC teas and TRFK 6/8 orthodox teas, respectively.

Longer aeration time had better colour than short aerated teas (Figure 28), this could be because of the trend in the theaflavins where theaflavins increased with longer aeration time, (Figure 26). This is because the higher the theaflavins the better the liqour colour (Kilel *et al*., 2013b). Orthodox teas had lower total colour than CTC teas and this could be because of the trend in theaflavins as well (Figure 26), where orthodox teas had lower levels of theaflavins than CTC teas. The findings concurs with Hafezi *et al*. (2006) results where they found CTC teas having higher total colour than orthodox teas.

4.3.4 Brightness percentage

Brightness percentage results are presented (Figure 29). The brightness percentage of TRFK 306 processed by CTC method showed significant effect ($P < 0.05$) on aeration time where long aeration time decreases the brightness percentage. TRFK 306 CTC and orthodox teas had generally higher brightness in all the 30 minutes aeration time in all the withering duration relative to longer aeration time except for five hours withering. The brightness for CTC teas from TRFK 306 increases after ten hours wither and was highest at fifteen hours wither and thirty minutes aeration but decline on subsequent longer withering time. Orthodox teas from TRFK 306 had a similar trend except for five hours wither with thirty minutes aeration which showed relatively lower brightness percentage. Black CTC teas from TRFK 6/8 aerated for 30 minutes had relatively lower brightness percentage with respect to other longer aeration time except for teas withered for twenty hours and aerated for thirty minutes (20//30). Black orthodox teas from TRFK 6/8 showed increasing brightness with increase in aeration time within a specific withering time.

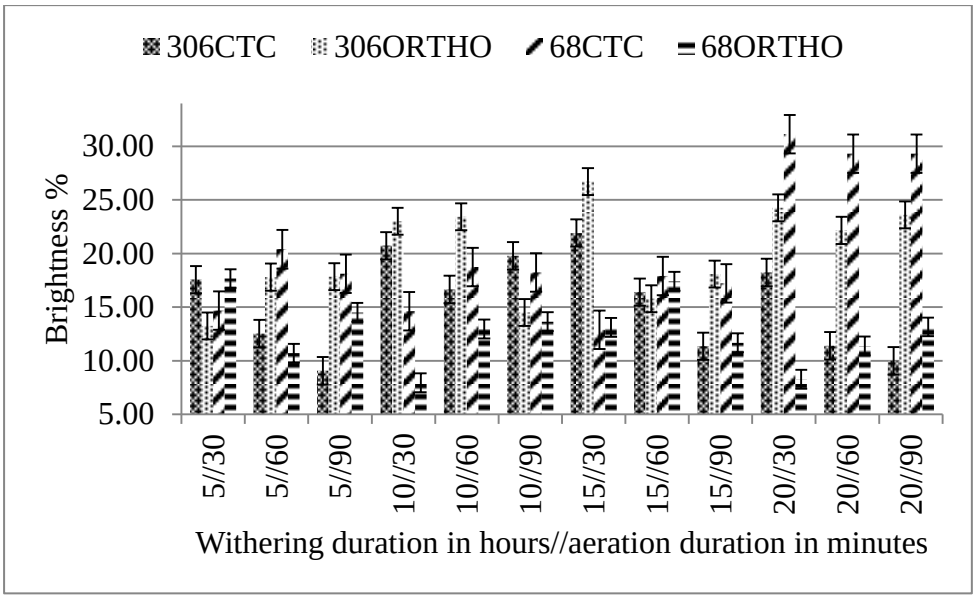


Figure 29: Effect of processing method, withering duration and aeration time on brightness

Where; 5//30,5//60,5//90 denotes 5 hours withering time and 30, 60 and 90 minutes aeration time, respectively; 10//30, 10//60, 10//90 represent 10 hours withering time with 30, 60 and 90 minutes aeration time, respectively, 15//30, 15//60, 15//90 represent 15 hours withering time with 30, 60 and 90 minutes aeration time, respectively. 20//30, 20//60 and 20//90 represent 20 hours withering time with 30, 60 and 90 minutes aeration time, respectively. The legend, 306CTC, 306ORTHO, 68CTC and 68ORTHO represent TRFK 306 CTC teas, TRFK 306 orthodox teas, TRFK 68 CTC teas and TRFK 6/8 orthodox teas, respectively.

Liquor brightness indicates the ability of the liquor to reflect light from the surface (Teshome *et al*., 2013). The brightness percentage of TRFK 306 processed by CTC method showed significant effect (P< 0.05) on aeration where the brightness decreases as aeration time increases. TRFK 306 CTC and orthodox teas had generally higher brightness in all the 30 minutes aeration time in all the withering duration relative to longer aeration time except for five hours withering. This could be because of noticeable lower levels of thearubigins in 30 minutes aeration especially in CTC teas. The brightness for CTC teas from TRFK 306 increases after ten hours wither and was highest at fifteen hours wither and thirty minutes aeration but decline on subsequent longer withering time. These results concurs with those of Owuor *et al*. (2008), who noted that theaflavins contribute to brightness and long fermentation times produced more colour in black tea at the expense of brightness. Orthodox teas from TRFK 306 had a similar trend except for five hours wither with thirty minutes aeration which showed relatively lower brightness percentage. The decrease in brightness with increase in aeration time for CTC teas from TRFK 306 could be due to the increase in thearubigins with increase in aeration time and decrease in theaflavins with increase in aeration time. Theaflavin is responsible for black tea brightness and briskness (Borah *et al*., 2012). Black CTC teas from TRFK 6/8 aerated for 30 minutes had relatively lower brightness percentage with respect to other longer aeration time except for teas withered for twenty hours and aerated for thirty minutes (20//30).This is could be because of lower theaflavins in thirty minutes aeration time for teas processed through CTC relative to longer aeration time. Black orthodox teas from TRFK 6/8 showed increasing brightness with increase in aeration time within a specific withering time. This could be because of the increase in theaflavins level with increase in aeration time (Figure 26).

4.3.5 Individual theaflavins

Theaflavins 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 (Tüfekci and Güner, 1997; Yoshino *et al*., 2010). These theaflavins accounts for 2-6 % of the solids in brewed black tea and are responsible for the unique colour and flavour of black tea (Sharangi *et al*., 2014). A recent study by Wu *et al*. (2018) concluded that topical application of theaflavins may reduce inflammation and bone resorption in experimental periodontits showing theaflavins have therapeutic potential in the treatment of periodontal disease. Simple theaflavins, TF-3-

monogallate, TF-3'- monogallate and theaflavin-3-3'-digallate are the four major theaflavins which mostly contribute to the quality and bioactivity of black tea (Xu *et al*., 2010).

Simple theaflavins

Simple theaflavins results are presented (Figure 30). The orthodox teas had lower levels of simple theaflavins than CTC teas of both clones. There was a significant difference ($p<0.05$) in simple theaflavins in method of manufacture and between the studied clones. TRFK 6/8 teas (both CTC and orthodox) had higher simple theaflavins than all TRFK 306 teas.

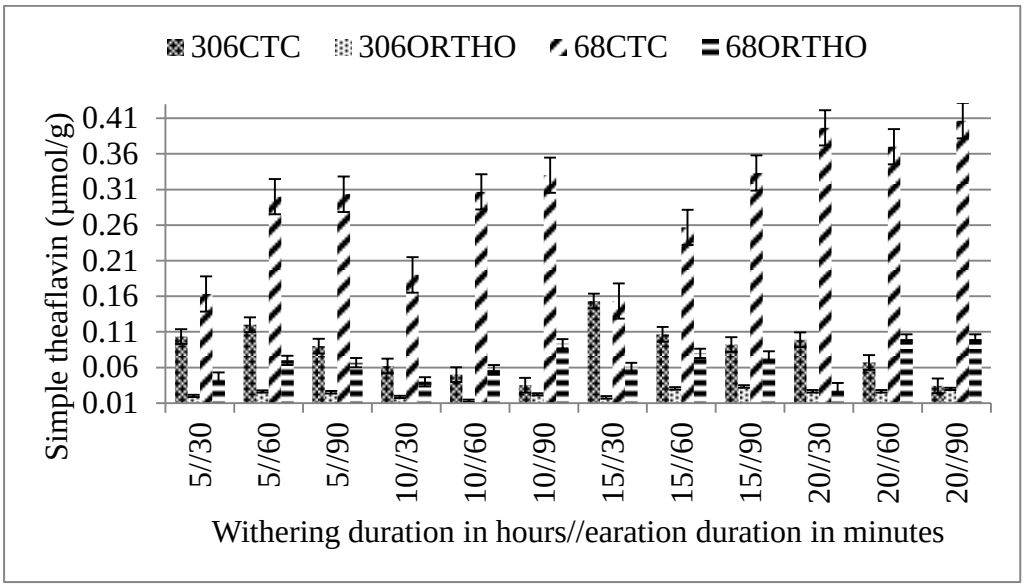


Figure 30: Effect of processing method, withering time and aeration on simple theaflavin.
Where; 5//30,5//60,5//90 denotes 5 hours withering time and 30, 60 and 90 minutes aeration time, respectively; 10//30, 10//60, 10//90 represent 10 hours withering time with 30, 60 and 90 minutes aeration time, respectively, 15//30, 15//60, 15//90 represent 15 hours withering time with 30, 60 and 90 minutes aeration time, respectively. 20//30, 20//60 and 20//90 represent 20 hours withering time with 30, 60 and 90 minutes aeration time, respectively. The legend, 306CTC, 306ORTHO, 68CTC and 68ORTHO represent TRFK 306 CTC teas, TRFK 306 orthodox teas, TRFK 68 CTC teas and TRFK 6/8 orthodox teas, respectively.

Simple theaflavins are among the four major theaflavins formed during enzymatic oxidation of catechins (Yoshino *et al*., 2010). Simple theaflavins are formed from two catechins, Epicatechin (EC) and Epigallocatechin (EGC). Though residual catechins were not assayed in the current study, the results of non-aerated teas can be used to explain the patterns in theaflavins formation. TRFK 6/8 teas had higher simple theaflavins than all TRFK 306 teas. This could be because of higher EC and EGC of TRFK 6/8 teas than TRFK 306 teas, (Table 4 and Table 5), respectively. Orthodox teas had lower simple theaflavins than CTC

teas in both clones. This could be because of the surface area to volume ratio differences in the teas. Orthodox teas have smaller surface area to volume ratio than CTC teas and therefore slower enzymatic reaction rates, ultimately affecting products formation. TRFK 6/8 teas had higher simple theaflavins than TRFK 306 teas from both manufacture. This could be because of relative higher EGC in TRFK 6/8. This also contributed to the trend in theaflavins where orthodox teas had lower theaflavins than CTC (Figure 26).

Theaflavin-3- monogallate

The results of TF-3- monogallate are presented (Figure 31). The theaflavin is a product of the two catechins, EC and EGCG. Orthodox teas of both clones had lower levels than CTC teas. TRFK 6/8 CTC teas had higher levels than TRFK 306 CTC teas and more sensitive to aeration duration where the levels were higher at longer aeration time. TRFK 6/8 CTC teas are fairly increasing in TF-3-monogallate with increasing withering time. TRFK 306 CTC teas are fairly stable with aeration time but is decreasing with longer withering duration.

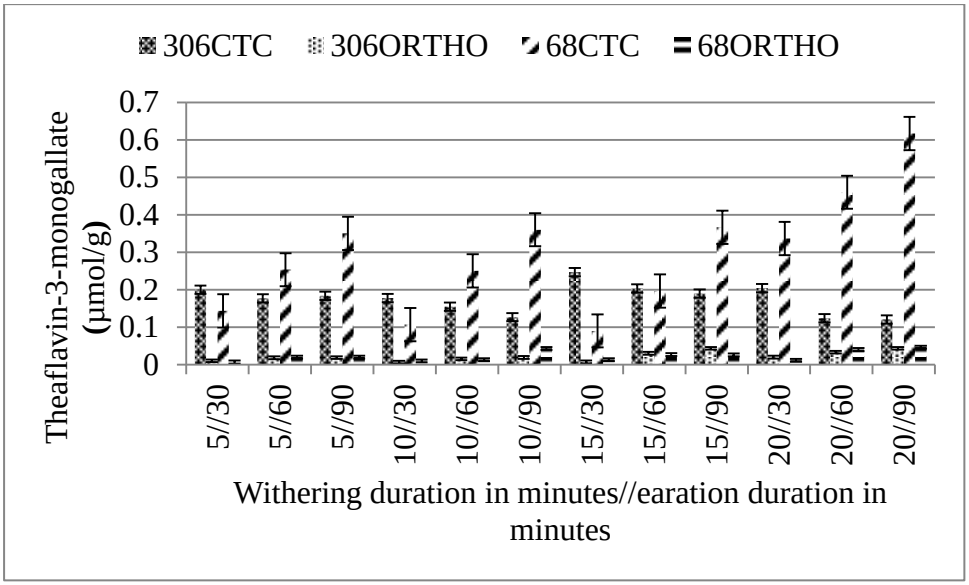


Figure 31: Effect of processing method, withering time and aeration on TF-3-monogallate.
Where; 5//30,5//60,5//90 denotes 5 hours withering time and 30, 60 and 90 minutes aeration time, respectively; 10//30, 10//60, 10//90 represent 10 hours withering time with 30, 60 and 90 minutes aeration time, respectively, 15//30, 15//60, 15//90 represent 15 hours withering time with 30, 60 and 90 minutes aeration time, respectively. 20//30, 20//60 and 20//90 represent 20 hours withering time with 30, 60 and 90 minutes aeration time, respectively. The legend, 306CTC, 306ORTHO, 68CTC and 68ORTHO represent TRFK 306 CTC teas, TRFK 306 orthodox teas, TRFK 68 CTC teas and TRFK 6/8 orthodox teas, respectively.

TF-3-monogallate is product from two catechins, a dihydroxy flava 3-ol and a tri hydroxyl flava-3-ol, Epicatechin (EC) and Epigallocatechin gallate (EGCG), respectively. Orthodox teas had lower levels of this theaflavin than CTC teas of both clones. This is expected because the formation of theaflavins is an enzymatic reaction affected by the level of tea leaves maceration. The more the tea leaves are macerated, the higher and faster the reaction and therefore the more the products formed. Maceration is a term used in leaf disruption process to refer to the process of bringing together the substrates (catechins) and the enzymes to facilitate the enzymatic oxidation reaction leading to theaflavins formation. The orthodox teas were hand-rolled and the tea leaves are usually left whole while CTC teas are fully ruptured leaving the tea leaves in smaller reduced sizes (Plate 1). The CTC teas therefore have higher surface area to volume ratio and therefore higher and faster reactions. The fact that TRFK 6/8 CTC teas had relatively higher levels than TRFK 306 CTC teas could be because of relatively higher EC than TRFK 306 teas, (Table 4). Moreover, TRFK 6/8 CTC teas had relatively higher EGCG than TRFK 306 (Figure 17). TRFK 6/8 CTC teas showed higher TF-3-monogallate with longer aeration time because sufficient time was allowed for the enzymatic reaction to take place. TRFK 6/8 CTC teas showed an increasing trend in the level of this theaflavin with increasing withering time. This could be because of increasing EGCG with increasing withering time, (Figure 17). TRFK 306 CTC teas never showed a noticeable effect on aeration time the way TRFK 6/8 CTC teas showed but the levels were decreasing with time. The decrease in TF-3-monogallate with increasing wither time, can be explained by the pattern depicted (Table 4 and Figure 17), where the catechins are also decreasing with longer withering time.

Theaflavin-3'- monogallate

Results of TF-3'-monogallate are shown (Figure 32) and the trend is similar to TF-3-monogallate. CTC teas of both clones had higher values than orthodox teas. TRFK 6/8 CTC teas had relatively higher TF-3'- monogallate than TRFK 306 CTC teas. TRFK 6/8 had relatively higher levels of the theaflavin at longer aeration time than at shorter aeration time. There was a noticeable trend where TRFK 6/8 CTC teas had the theaflavin was increasing with increasing withering time as TRFK 306 CTC teas had the same theaflavin decreasing.

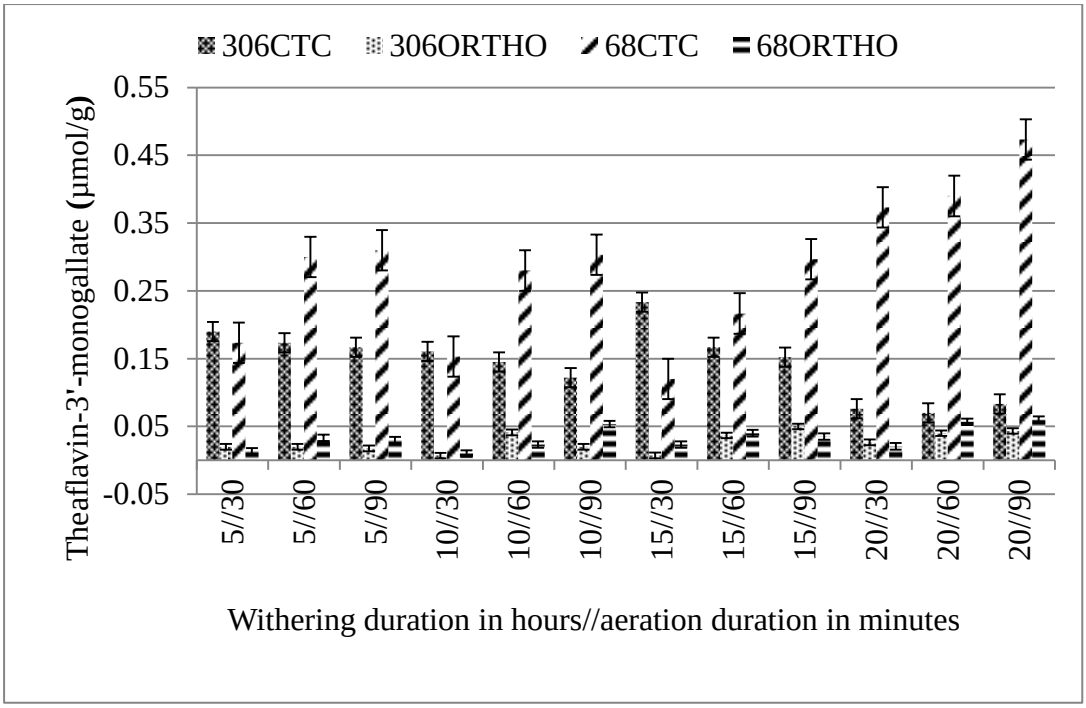


Figure 32: Effect of processing method, withering time and aeration on TF-3'-monogallate.

Where; 5//30,5//60,5//90 denotes 5 hours withering time and 30, 60 and 90 minutes aeration time, respectively; 10//30, 10//60, 10//90 represent 10 hours withering time with 30, 60 and 90 minutes aeration time, respectively, 15//30, 15//60, 15//90 represent 15 hours withering time with 30, 60 and 90 minutes aeration time, respectively. 20//30, 20//60 and 20//90 represent 20 hours withering time with 30, 60 and 90 minutes aeration time, respectively. The legend, 306CTC, 306ORTHO, 68CTC and 68ORTHO represent TRFK 306 CTC teas, TRFK 306 orthodox teas, TRFK 68 CTC teas and TRFK 6/8 orthodox teas, respectively.

TF-3'- monogallate is a product of two catechins also, Epigallocatechin (EGC) and Epicatechin gallate (ECG). The fact that CTC teas had relatively higher TF-3'- monogallate than the orthodox teas is related to the exposed area for the enzymatic reaction. CTC teas have more area exposed than the orthodox teas because of their relatively higher surface area to volume ratio than the orthodox teas. TRFK 6/8 CTC teas had higher levels of the theaflavins than TRFK 306 CTC teas. This could be because of the relatively higher levels of EGC, (Table 5) it had than TRFK 306 CTC teas. TRFK 6/8 CTC teas however had lower levels of ECG, (Figure 18), than TRFK 306 teas. TRFK 6/8 CTC teas depicted a noticeable effect on aeration time. The longer the aeration time, the higher the theaflavins formed and shorter aeration time led to less formation of the theaflavin. This could be because longer aeration time allows for more sufficient time for the enzymatic reaction to take place and therefore more theaflavins formed. The fact that TRFK 6/8 CTC teas had TF-3'-monogallate increasing with withering while TRFK 306 CTC teas were decreasing (Figure 32) could be

because of the trend in ECG (Figure 18) and EGC (Table 5). The results on (Figure 18) show TRFK 6/8 CTC teas had ECG marginally increasing with increasing withering time while TRFK 306 CTC teas were decreasing. As shown (Table 5), EGC in TRFK 306 CTC teas increased with withering time but since they are in lesser amount than ECG their effect is less. TRFK 6/8 CTC teas (Table 5) showed EGC decreasing with withering time and in higher amount than ECG. The current findings call for more investigations to clearly know and understand the formation of theaflavins from the catechins especially per cultivar.

Theaflavin-3-3'-digallate

The results of TF-dg are presented (Figure 33). TF-dg is a product of two catechins, Epicatechin gallate (ECG) and Epigallocatechin gallate (EGCG). CTC teas of both clones had realtively higher TF-dg than the orthodox teas. TRFK 306 CTC teas had relatively higher levels of theaflavins-3-3'-digallate than TRFK 6/8 CTC teas at shorter withering time and the levels were higher at shorter aeration time.

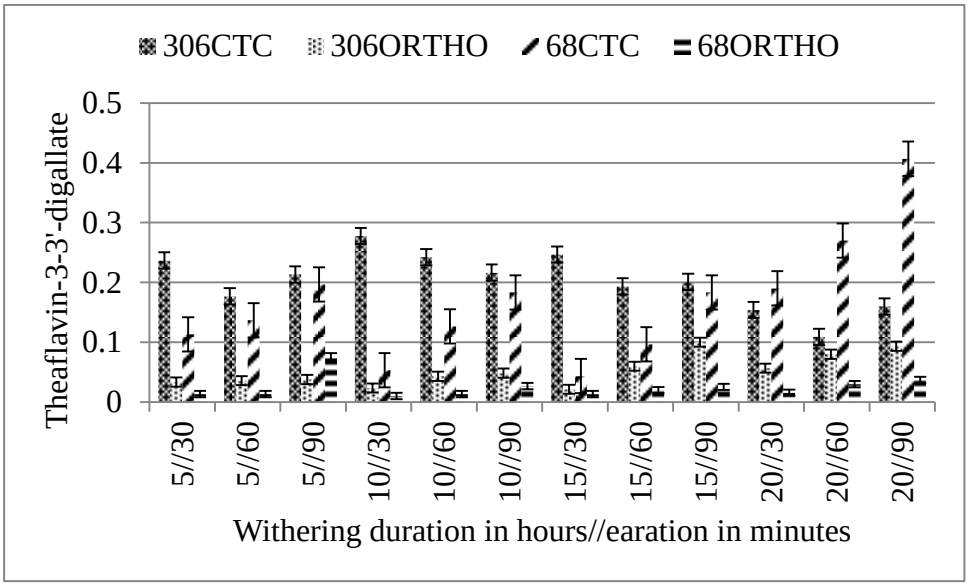


Figure 33: Effect of processing method, withering time and aeration on TF-dg.

Where; 5//30,5//60,5//90 denotes 5 hours withering time and 30, 60 and 90 minutes aeration time, respectively; 10//30, 10//60, 10//90 represent 10 hours withering time with 30, 60 and 90 minutes aeration time, respectively, 15//30, 15//60, 15//90 represent 15 hours withering time with 30, 60 and 90 minutes aeration time, respectively. 20//30, 20//60 and 20//90 represent 20 hours withering time with 30, 60 and 90 minutes aeration time, respectively. The legend, 306CTC, 306ORTHO, 68CTC and 68ORTHO represent TRFK 306 CTC teas, TRFK 306 orthodox teas, TRFK 68 CTC teas and TRFK 6/8 orthodox teas, respectively.

TRFK 306 orthodox teas had relatively higher values of this theaflavin than TRFK 6/8 orthodox teas. TRFK 306 CTC teas had the theaflavin decreasing with withering time as

TRFK 6/8 CTC teas increases. TRFK 6/8 CTC teas showed remarkable effect of aeration on TF-dg where longer, 90 minutes aeration time showed higher levels than shorter aeration time. All orthodox teas had lower levels than CTC teas and TRFK 306 had relatively higher than TRFK 6/8 othodox teas.

Theaflavin-3-3'digallate (TF-dg) influences black tea quality more than the rest of the known theaflavins (Owuor and Obanda, 2001) and many studies have been done on the same (Aneja *et al*., 2004; Krishman and Maru, 2004; Karori *et al*., 2007; Kimutai *et al*., 2015). There was a significant difference ($P< 0.05$) between and within the two studied clones, where TRFK 306 CTC teas had higher theaflavins at shorter withering time than TRFK 6/8 teas. This could be because of higher ECG (Figure 18) in TRFK 306 CTC teas than TRFK 6/8. It can also be observed that, TRFK 306 CTC teas were decreasing in TF-dg levels with increasing withering time as TRFK 6/8 CTC teas increases. This could be because, TRFK 306 CTC teas were also decreasing in EGCG and in ECG (Figure 17 and 18), respectively, with longer withering time. TRFK 6/8 CTC teas on the other hand had ECG (Figure 18) marginally increasing with longer withering time. Theaflavins are dimeric compounds that possess a benzotropolone skeleton that is formed from co-oxidation of selected pair of catechins (Kosinska and Andlauer, 2014). TRFK 6/8 CTC teas showed noticeable effect with aeration time (Figure 33) where TF-dg was higher at 90 minutes aeration time across the entire withering regime. Teas withered for twenty hours and aerated for ninety minutes had higher TF-dg. Most TRFK 306 orthodox teas had relatively higher TF-dg than TRFK 6/8 orthodox teas. The findings agrees with the findings of Joshi *et al.* (2015) where they found purple clone teas had higher TF-dg than green clone teas. This could be because TRFK 306 orthodox teas had relatively higher ECG (Figure 18) than TRFK 6/8 orthodox teas. Teas aerated for ninety minutes had higher TF-dg across the entire withering regime this also contributed to the trend seen in total theaflavins (Figure 26) where they increase with increase in aeration time. The fact that CTC teas of both clones had higher TF-dg than orthodox teas is also expected because of the difference in surface area exposed for the enzymatic reaction. CTC teas have higher surface area to volume ratio hence more products are expected.

4.3.6 Antioxidant activity of aerated teas

The antioxidant activities of aerated made teas from TRFK 306 and TRFK 6/8 are presented (Table 8). The results of each clone, TRFK 306 and TRFK 6/8, were analysed comparing both CTC and orthodox aerated teas and both clones were compared when processed via CTC and orthodox methods.

Table 8: Antioxidant activity of aerated teas of TRFK 306 and TRFK 6/8

TRFK 68 orthodox and CTC teas		TRFK 306 orthodox and CTC teas	
Treatment	Antioxidant activity (%)	Treatment	Antioxidant activity (%)
OR//20/90	92.05±0.55 [a]	OR//5/30	92.64±1.75 [a]
OR//20/30	92.01±0.10 [a]	CT//20/30	92.64±0.11 [a]
CT//15/90	91.94±0.30 [ab]	OR//10/60	92.87±0.57 [a]
CT//20/90	91.90±0.37 [abc]	OR//15/90	92.59±0.25 [a]
CT//20/60	91.82±0.38 [abc]	OR//10/90	92.38±1.68 [a]
CT//15/30	91.56±1.12 [abcd]	OR//15/60	92.17±1 [ab]
OR//15/30	91.39±0.36 [abcde]	OR//20/30	92.12±1.4 [ab]
OR//15/90	91.38±0.24 [abcde]	OR//20/60	92.28±1.89 [ab]
OR//20/60	91.27±0.24 [abcde]	OR//20/90	91.47±2.68 [ab]
CT//10/30	91.21±0.74 [abcdef]	OR//5/90	91.12±2.53 [abc]
OR//15/60	91.03±0.31 [abcdef]	OR//10/30	91.83±2.54 [abc]
CT//5/30	91.03±0.43 [abcdef]	OR//15/30	91.17±0.57 [abc]
CT//15/60	90.9±0.28 [abcdef]	CT//10/30	91.22±1.69 [abc]
CT//20/30	90.75±0.39 [bcdef]	CT//5/90	90.58±0.28 [abcd]
CT//5/60	90.72±0.65 [cdef]	CT//15/90	90.58±3.19 [abcd]
CT//5/90	90.72±0.53 [cdef]	CT//20/90	90.51±2.43 [abcd]
OR//5/90	90.71±0.24 [cdef]	OR//5/60	90.90±2.09 [abcd]
OR//10/60	90.44±0.66 [def]	CT//10/60	90.57±1.83 [abcd]
OR//10/90	90.38 ±0.06 [def]	CT//5/30	89.32±0.89 [bcde]
OR//5/30	90.32±0.56 [ef]	CT//15/60	89.30±0.82 [bcde]
OR//5/60	90.21±0.33 [ef]	CT//5/60	88.90±0.69 [cde]
OR//10/30	90.01±0.18 [f]	CT//10/90	87.87±2.12 [de]
CT//10/90	88.78±0.44 [g]	CT//15/30	86.95[e]±3.71 [e]
CT//10/60	87.52±2.78 [h]	CT//20/60	86.95±1.75 [e]

Means and standard error are shown. Means followed by same letter (s) in a column are not significantly different at p< 0.05. n= 72. OR and CT corresponds to orthodox and CTC manufacture, respectively. 5//30,5//60,5//90 denotes 5 hours withering time and 30, 60 and 90 minutes aeration time, respectively; 10//30, 10//60, 10//90 represent 10 hours withering time with 30, 60 and 90 minutes aeration time, respectively, 15//30, 15//60, 15//90 represent 15 hours withering time with 30, 60 and 90 minutes aeration time, respectively. 20//30, 20//60 and 20//90 represent 20 hours withering time with 30, 60 and 90 minutes aeration time, respectively.

The main aim was to check the effect of withering and aeration durations on the inhibition against DPPH in percentage of the teas from both clones using those two manufacturing methods (CTC and orthodox). There were significant differences between and within the clones in terms of the type of manufacture, withering and aeration time. TRFK 306 teas showed a significant difference ($P< 0.05$) in the type of type of manufacture where most orthodox teas had higher inhibition percentage against DPPH than CTC teas. TRFK 6/8 teas depicted a significant difference ($P< 0.05$) in withering time irrespective of manufacturing method where longer withering time resulted in higher inhibition percentage against DPPH. TRFK 306 orthodox teas had higher inhibition against DPPH than TRFK 6/8 orthodox teas whereas TRFK 6/8 CTC teas had higher inhibition percentage than TRFK 306 CTC teas.

Antioxidant activity is expressed as the capacity of a molecule or ion to avoid oxidative reactions of other molecules (Lorenzo and Munekata, 2016). Antioxidants are man-made and natural substances that may prevent or delay some types of cell damage (Yadav *et al*., 2016). Black tea is the most consumed type of tea and has been found to have antioxidant activity (Kiran and Kumar, 2018). The type of manufacture influences the antioxidant activity (Table 8) where orthodox teas showed higher values than CTC teas, especially for TRFK 306 teas. The results concurs with the findings of Pal *et al*. (2012) where they found out that the antioxidant activity of black orthodox teas were higher than that of CTC teas analysed from commercially available products. Carloni *et al*. (2013) also found out in their study that, CTC teas had lower catechin content and antioxidant activity compared to orthodox teas. More over, many researchers have found out that bigger sized teas have higher antioxidant activity than smaller sized teas. (Anesini *et al*., 2008; Shrestha *et al*., 2010; Serpen *et al*., 2012). Bigger sized teas are relatively less processed than smaller teas and therefore the more the antioxidant activity as was found by Kosińska and Andlauer, (2014). TRFK 6/8 teas had antioxidant activity increasing with increase in withering time, this can be attributed to increase in theaflavins (Figure 26) since theaflavins have antioxidant properties (Luczaj and Skrzydlewska, 2005) bacause they have hydroxyl groups in their structure. The general scavenging reaction between DPPH and an antioxidant, like the theaflavins in this case can be written as (Thamaraiselvi *et al*., 2012);

$$\underset{\text{(purple)}}{\text{DPPH + (H-A)}} \longrightarrow \underset{\text{(yellow)}}{\text{DPPH – H+ A}} \quad \text{.......................................}(12)$$

The colour of the radical, DPPH before scavenging is purple and when it is scavenged, by the tea compound like the theaflavins in this case, it changes the colour to yellow, that is, it

looses the purple colour. The degree of discoloration indicates the scavenging potential of the antioxidant compounds in terms of hydrogen donating ability.

4.3.7 Total soluble solids of aerated teas

The results of aerated total soluble solids (TSS) are presented (Table 9). There was a significant difference ($p < 0.05$) in the type of manufacture and the type of clone in total soluble solids. Orthodox teas of both clones had higher total soluble solids then their CTC teas counterparts. It can also be observed that TRFK 6/8 teas had higher total soluble solids than teas from TRFK 306 in both type of manufacture. Comparing the effect of withering and aeration, longer aeration affects total soluble solids negatively. This is evident from the results where all 30 minutes aerated teas had the highest total soluble solids as compared to teas aerated for 90 minutes. Short aeration of 30 minutes had higher total soluble solids than 90 minutes in most tea products.

Total soluble solids refers to the water extract from the tea products, both orthodox and CTC teas. Orthodox teas of both clones had relatively higher total soluble solids than CTC teas (Table 9). Other researchers had found similar results (Someswararao *et al*., 2013) where Lipton Darjeeling tea, an orthodox tea had higher total soluble solids than Red label tea, a CTC tea. Some findings had found opposite results where CTC teas had higher total soluble solids than orthodox (Mahanta and Baruah 1989). Total soluble solids in tea liquor are mainly from the polyphenols (catechins) and thearubigins. Though orthodox teas have less thearubigins (Figure 27), they could be having more residual catechins because of less surface area to volume ratio for aeration compared to CTC teas. The current findings opens a door for more investigations to demystify the scenario. Though the current researcher did not quantified the residual catechins in black teas, other literature (Kimutai *et al*., 2016) shows that black tea has residual catechins and TRFK 6/8 had more residual catechins than purple clones. This could be the reason why TRFK 6/8 teas have higher total soluble solids than TRFK 306 teas.

Table 9: **Total soluble solids of aerated teas from TRFK 306 and TRFK 6/8**

TRFK 6/8 orthodox and CTC teas		TRFK 306 orthodox and CTC teas	
Treatment	TSS (mg/mL)	Treatment	TSS (mg/mL)
OR//5/30	4.08±0.08 [a]	OR//5/30	3.47±0.059 [a]
OR//15/30	4.03±0.1 [ab]	OR//10/30	3.42±0.02 [a]
OR//15/90	4.01±0.01 [ab]	OR//15/60	3.36±0.02 [abc]
OR//15/60	3.99±0.04 [abc]	OR//10/60	3.31±0.09 [abc]
OR//20/60	3.91±0.04 [abcd]	OR//20/30	3.26±0.06 [bc]
OR//5/90	3.9±0.05 [abcd]	OR//10/90	3.25±0.1 [bc]
OR//10/30	3.89±0.1 [abcd]	OR//20/90	3.23±0.13 [bc]
OR//5/60	3.86±0.04 [bcd]	OR//5/90	3.23±0.06 [bc]
OR//10/60	3.85±0.12 [bcd]	OR//15/30	3.23±0.03 [bcd]
OR//10/90	3.8±0.16 [cd]	OR//15/90	3.21±0.08 [cde]
OR//20/90	3.78±0.02 [cde]	OR//5/60	3.18±0.03 [cdef]
OR//20/30	3.77±0.04 [de]	OR//20/60	3.17±0.1 [cdef]
CT//5/30	3.57±0.08 [ef]	CT//20/30	3.03±0.04 [defg]
CT//15/30	3.54±0.06 [fg]	CT//10/30	3.03±0.09 [efg]
CT//2030	3.49±0.03 [fgh]	CT//15/30	3.01±0.07 [fgh]
CT//10/30	3.47±0.07 [fghi]	CT//15/60	3±0.06 [fgh]
CT//10/60	3.45±0.02 [fghi]	CT//10/60	2.93±0.01 [ghi]
CT//15/60	3.37±0.1 [fghij]	CT//20/90	2.93±0.03 [ghi]
CT//20/60	3.37±0.12 [fghijk]	CT//20/60	2.91±0.04 [ghi]
CT//10/90	3.33±0.05 [ghijk]	CT//5/30	2.9±0.07 [ghi]
CT//5/60	3.32±0.01 [hijk]	CT//15/90	2.86±0.06 [ghi]
CT//5/90	3.26±0.08 [ijk]	CT//5/60	2.85±0.05 [ghi]
CT//15/90	3.19±0.04 [jk]	CT//10/90	2.81±0.12 [hi]
CT//20/90	3.16±0.06 [k]	CT//5/90	2.79±0.04 [i]

Means and standard error are shown. Means followed by same letter (s) in a column are not significantly different at p< 0.05. n= 72. OR and CT corresponds to orthodox and CTC manufacture, respectively. 5//30,5//60,5//90 denotes 5 hours withering time and 30, 60 and 90 minutes aeration time, respectively; 10//30, 10//60, 10//90 represent 10 hours withering time with 30, 60 and 90 minutes aeration time, respectively, 15//30, 15//60, 15//90 represent 15 hours withering time with 30, 60 and 90 minutes aeration time, respectively. 20//30, 20//60 and 20//90 represent 20 hours withering time with 30, 60 and 90 minutes aeration time, respectively.

4.3.8 Sensory evaluation of aerated (black) teas

Aerated CTC teas sensory evaluation

Aerated teas terminologies are so elaborate and many studies on the same have been done, (Chaturvedula and Prakash, 2011). During the current study, each tea product was tasted and the liquor colour, liquor body, liquor strength, liquor briskness and infusion colour were described by every taster then scoring was done using a score scale developed by Kilel *et al*. (2013b) with modification. The highest score denoted the best and vice versa, using a scale of 1-5 (Appendix II). This was necessary since during sensory evaluation of black tea, the tasters' judgment is usually based on the colour, strength, briskness, flavour and overall quality of the tea (Hilton, 1973). The teas were coded such that the taster could not know which clone or the treatment the teas have been subjected to for objective results.

Liquor colour scores of aerated CTC teas are presented (Figure 34). There was a significant difference ($P< 0.05$) between and within the clones in terms of withering time and aeration duration. TRFK 6/8 teas had better colour than TRFK 306 teas. The effect of withering was not as noticeable as length of aeration time. Shorter aeration in most teas especially TRFK 306 resulted in better colour than teas aerated for longer time.

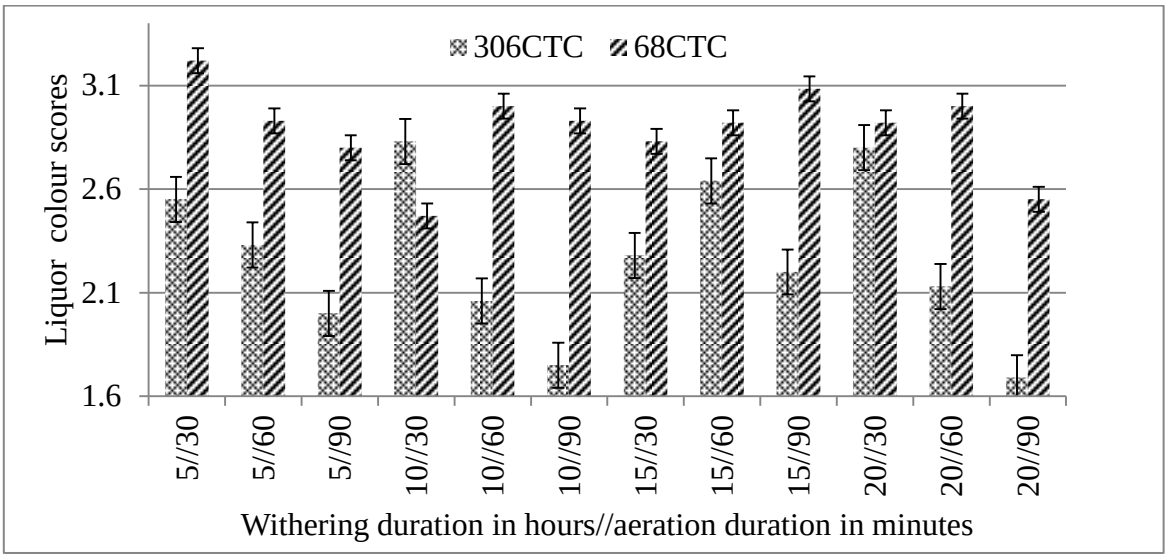


Figure 34: Effect of withering and aeration time on liquor colour of CTC teas. The legend, 306CTC and 68CTC represent TRFK 306 CTC teas and TRFK 6/8 CTC teas, respectively.
Where; 5//30,5//60, 5//90 denotes 5 hours withering time with 30, 60 and 90 minutes aeration time, respectively; 10//30, 10//60, 10//90 represent 10 hours withering time with 30, 60 and 90 minutes aeration time, respectively, 15//30, 15//60, 15//90 represent 15 hours withering time with 30, 60 and 90 minutes aeration time, respectively. 20//30, 20//60 and 20//90 represent 20 hours withering time with 30, 60 and 90 minutes aeration time, respectively.

Liquor colour denotes the brightness of the liquor that is the brew itself. Liquor colour can be coppery bright as in TRFK 6/8 if optimum manufacturing conditions are applied, it can also be dull which is not desirable. The levels of theaflavins and thearubigins influences the liquor brightness such that the higher the theaflavins the brighter the teas and the higher the thearubigins the duller the teas since theaflavins are known to contribute to briskness and brightness of tea liquor while thearubigins are responsible for the colour and body of the liquor (Kilel *et al*., 2013b). TRFK 306 results showed there was a significant difference at ($p< 0.05$) among the teas produced by varying withering and aeration time. Teas withered for ten hours and aeration for thirty minutes (CTC10//30) had liquor colour score of 2.83 and the highest while withering for twenty hours and aerating for ninety minutes (CTC 20//90) had the least liquor colour score of 1.69. It was not conclusive here that the significant difference ($p< 0.05$) was due to withering duration, it is however noticeable that aeration time affects the liquor colour. Within each withering regime (5, 10, 15 and 20 hours), the liquor colour decreases with increasing aeration time such that teas aerated for thirty (30) minutes had better liquor colour than teas aerated for ninety (90) minutes, (Figure 34). This could be because the thearubigins influences liquor colour hence brightness negatively (Obanda *et al*., 2001) and from (Figure 27) it can be noted that teas aerated for ninety minutes had more thearubigins than teas aerated for thirty minutes. Effect of withering and aeration time on liquor colour was observed in black CTC teas from TRFK 6/8 with significant difference at ($p< 0.05$) (Figure 34). Liquor colour was found to improve with increasing aeration time and withering hours especially after ten hours of wither up to withering hours of twenty and aeration time of sixty minutes (20//60). The liquor colour declined after 20//60 and teas withered for twenty hours and aerated for ninety minutes (20//90) recorded second lowest liquor colour score of 2.55. Teas withered for ten hours and aerated for thirty minutes (10//30) had the lowest score of 2.47. This could be because it had the lowest theaflavins with 4.89 µmol/g (Figure 26). Theaflavins contribute to liquor colour, strength, briskness though other components such as thearubigins, caffeine and volatile compounds also have some effects according to (Teshome *et al*., 2013). Theaflavins improved with increasing aeration time from these results, however, high thearubigins, affects the liquor colour negatively, (Ngure *et al*., 2009; Kilel *et al*., 2013b). Teas withered for twenty hours and aerated for ninety minutes from TRFK 6/8 showed low liquor colour because it had high thearubigins, even teas withered for five hours and aerated for ninety minutes had high thearubigins and consequently lower liquor colour.

The results of liquor body are presented (Figure 35). There was a significant difference ($p < 0.05$) within and between the studied clones. Teas which were aerated for long had better body than those aerated for shorter time for both clones. TRFK 6/8 teas had better liquor body than TRFK 306 teas.

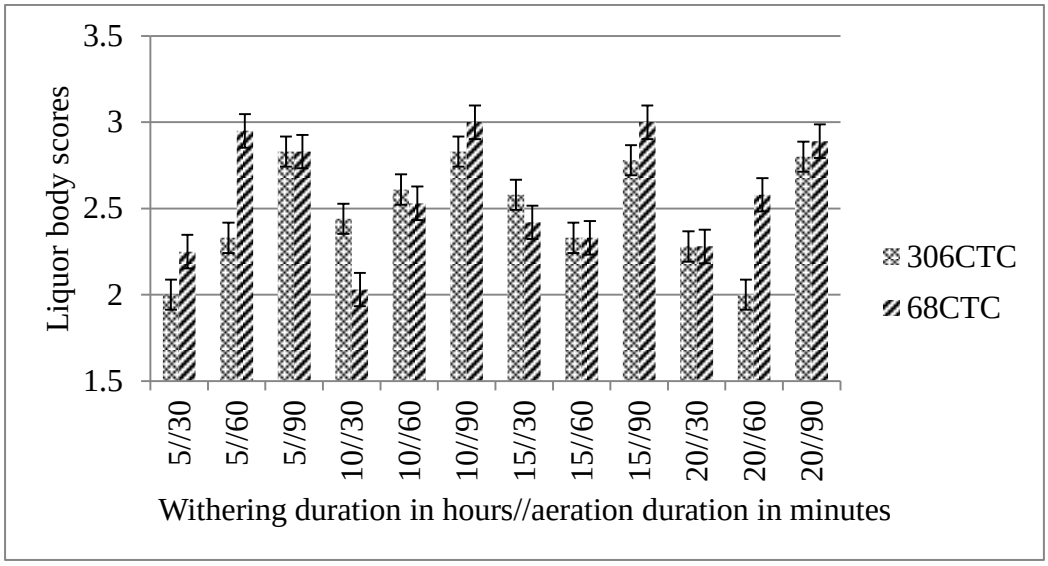


Figure 35: Effect of withering time and aeration time on liquor body of CTC teas.
Where; 5//30,5//60,5//90, denotes 5 hours withering time and 30, 60 and 90 minutes aeration time, respectively; 10//30, 10//60, 10//90 represent 10 hours withering time with 30, 60 and 90 minutes aeration time, respectively, 15//30, 15//60, 15//90 represent 15 hours withering time with 30, 60 and 90 minutes aeration time, respectively. 20//30, 20//60 and 20//90 represent 20 hours withering time with 30, 60 and 90 minutes aeration time, respectively. The legend, 306CTC and 68CTC represent TRFK 306 CTC teas and TRFK 6/8 CTC teas, respectively.

Liquor body indicates the thickness of the liquor, the thicker the liquor the better the quality and light thin liquors are not preferred. Thearubigins formed during fermentation contribute to the mouth feel (thickness) and colour (reddish brown) of the tea (Teshome *et al.*, 2013). Liquor body results of black CTC teas from TRFK 306 and TRFK 6/8 are presented in (Figure 35). Though there was no significant difference in liquor body at ($p < 0.05$), from the figure, it can be observed that there is noticeable effect on aeration time where the liquor body improves with increase in aeration time. Within every withering regime, ninety minutes aeration had better liquor body for teas from TRFK 306. This could be because of higher thearubigins at every ninety minutes aeration time since thearubigins are responsible for thickness and colour of both the liquor and infusion (Teshome *et al.*, 2013). Teas withered for five and ten hours and aerated for ninety minutes (5//90 and 10//90) had relatively higher scores of liquor body and 5//90 had the highest TR of 21.41 % (Figure 27). Liqour body for teas from TRFK 6/8 also shows improvement with increasing aeration time

irrespective of withering duration, with ninety minutes aeration time having better liquor body across the withering regime. Teas which had been withered for ten and fifteen hours and aerated for ninety minutes (10//90 and 15//90) had the highest score of 3 in liquor body. This trend is following the TR trend (Figure 27), where ninety minutes aeration time had relatively higher TR % across the entire withering regime. Teas from TRFK 6/8 had better liquor body than teas from TRFK 306 (Figure 35). This could be attributed to higher total soluble solids (Table 9) of the clone than TRFK 306 teas. Total soluble solids contribute to the thickness of the liquor because these are what dissolved in the liquor.

The results of CTC teas on liquor strength are presented (Figure 36). There was a significant difference in liquor strength at ($p< 0.05$) in both black CTC teas from TRFK 306 and TRFK 6/8 (Figure 36). Teas from TRFK 306 which were withered for five hours and aerated for thirty minutes had the highest liquor strength score of four (4) as shown (Figure 36).

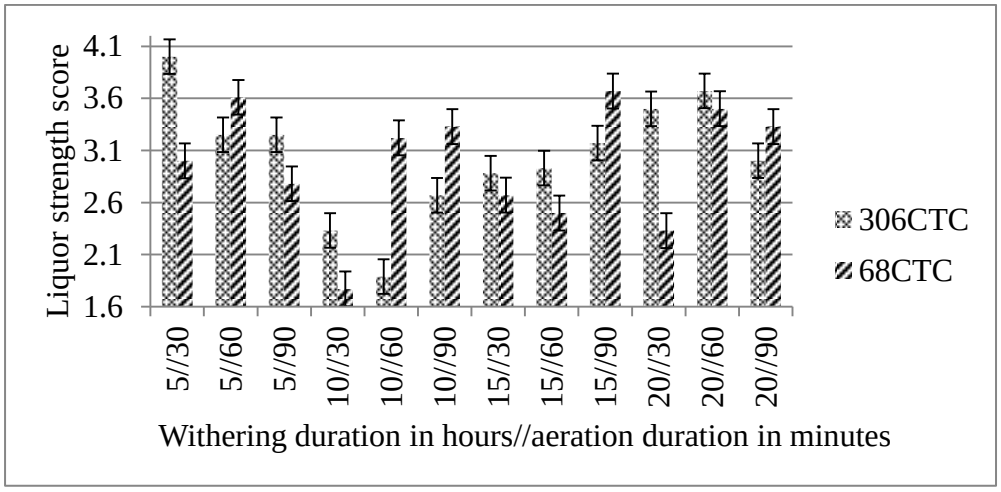


Figure 36: Effect of withering time and aeration time on liquor strength of CTC teas.

Where; 5//30,5//60,5//90 denotes 5 hours withering time and 30, 60 and 90 minutes aeration time, respectively; 10//30, 10//60, 10//90 represent 10 hours withering time with 30, 60 and 90 minutes aeration time, respectively, 15//30, 15//60, 15//90 represent 15 hours withering time with 30, 60 and 90 minutes aeration time, respectively. 20//30, 20//60 and 20//90 represent 20 hours withering time with 30, 60 and 90 minutes aeration time, respectively. The legend, 306CTC and 68CTC implies TRFK 306 CTC teas and TRFK 6/8 CTC teas, respectively.

There was a significant difference in liquor strength at ($p< 0.05$) in both CTC teas from TRFK 306 and TRFK 6/8 (Figure 36). Teas from TRFK 306 which were withered for five hours and aerated for thirty minutes had the highest liquor strength score of four (4) as shown (Figure 36). This could be due to the high level of theaflavins as shown (Figure 26) since theaflavins are responsible for liquor strength (Teshome *et al*., 2013). The strength, however, decreases sharply with increase in aeration time and withering up to ten hours

wither and sixty minutes aeration (10//60) time. This is unexpected and it calls for more research so as to demystify this. The strength however improves rapidly with increasing withering hours and aeration time up to twenty hours withering time and sixty minutes aeration time (20//60). This can be linked to the increase in theaflavins because the higher the theaflavins, the stronger the cup (Kilel *et al*., 2013b). The high liquor strength score of 20//60 treatment could be because of high level of thearubigins since they contribute to the strength and colour of tea liquor (Teshome *et al*., 2013; Tomlins *et al*., 1996). Teas from TRFK 6/8 showed significant difference at ($p< 0.05$) in liquor strength. Teas aerated for longer aeration, sixty and ninety minutes had better liquor strength irrespective of duration of withering than teas aerated for thirty minutes. Teas withered for fifteen minutes and aerated for ninety minutes had relatively higher theaflavins (Figure 26) and consequently relatively higher liquor strength score of 3.67 and those withered for ten hours and aerated for thirty minutes recorded the lowest theaflavins value of 4.89 µmol/g (Figure 26) and consequently the lowest score of 1.77 in liquor strength.

The results of liquor briskness of CTC teas are presented (Figure 37). TRFK 6/8 teas had better briskness than TRFK 306 teas. TRFK 306 teas aerated for sixty minutes had better score than those aerated for thirty and ninety minutes.

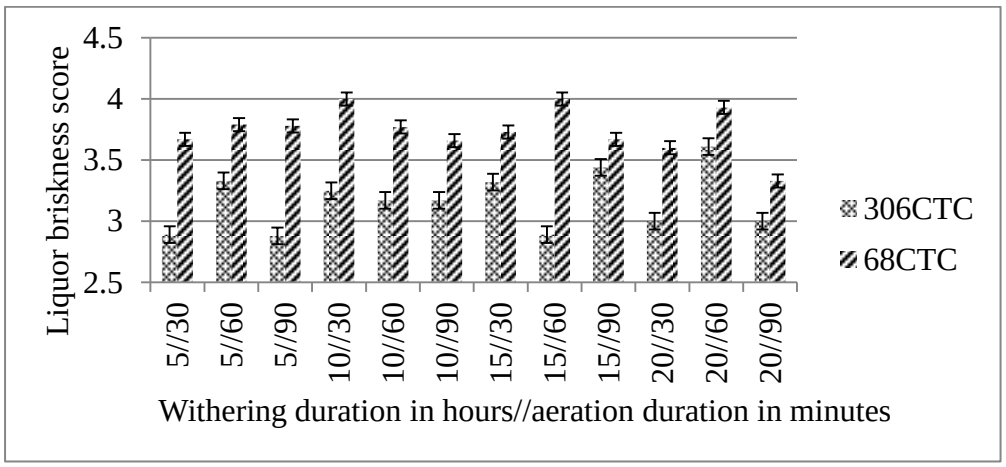


Figure 37: Effect of withering time and aeration time on liquor briskness of CTC teas. Where; 5//30, 5//60, 5//90 denotes 5 hours withering time and 30, 60 and 90 minutes aeration time, respectively; 10//30, 10//60, 10//90 represent 10 hours withering time with 30, 60 and 90 minutes aeration time, respectively, 15//30, 15//60, 15//90 represent 15 hours withering time with 30, 60 and 90 minutes aeration time, respectively. 20//30, 20//60 and 20//90 represent 20 hours withering time with 30, 60 and 90 minutes aeration time, respectively. The legend, 306CTC and 68CTC represents TRFK 306 CTC teas and TRFK 6/8 CTC teas, respectively.

There was no significant difference ($p< 0.05$) on liquor briskness with withering and aeration time, for both clones. TRFK 306 teas which were withered for twenty hours and

aerated for sixty minutes and teas withered for fifteen hours and aerated for ninety minutes had relatively higher liquor briskness score of 3.61 and 3.44, respectively, (Figure 37). Teas from TRFK 306 which were aerated for sixty minutes had relatively higher theaflavins and consequently relatively higher briskness. TRFK 6/8 teas withered for ten hours and aerated for thirty minutes and those which were withered for fifteen hours and aerated for sixty minutes had relatively higher liquor briskness score of 4 each (Figure 37). Liquor briskness increased with increasing withering and aeration time for TRFK 6/8 teas though not significant at ($p<0.05$). This could be because of higher theaflavins with increase in withering and aeration time (Figure 26).

The results of infusion colour are presented (Figure 38). TRFK 6/8 teas were more bright than TRFK 306 teas.

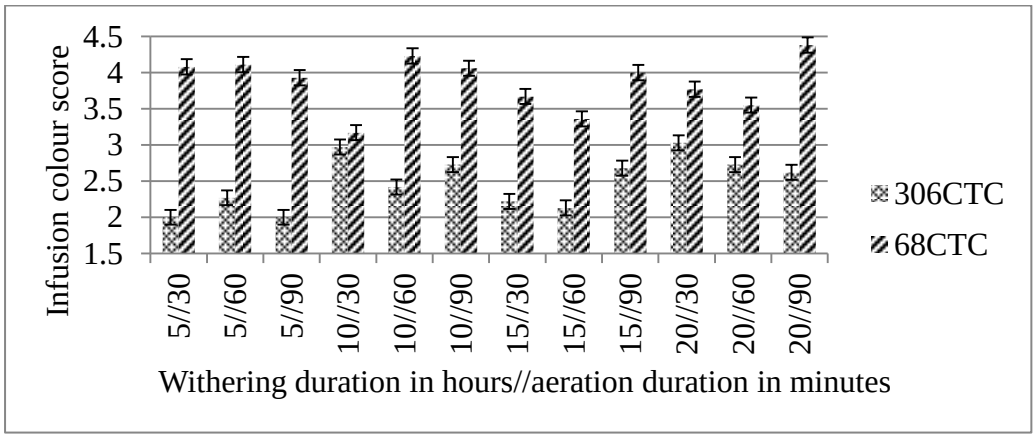


Figure 38: Effect of withering time and aeration time on infusion colour of CTC teas. Where; 5//30,5//60,5//90 denotes 5 hours withering time and 30, 60 and 90 minutes aeration time, respectively; 10//30, 10//60, 10//90 represent 10 hours withering time with 30, 60 and 90 minutes aeration time, respectively, 15//30, 15//60, 15//90 represent 15 hours withering time with 30, 60 and 90 minutes aeration time, respectively. 20//30, 20//60 and 20//90 represent 20 hours withering time with 30, 60 and 90 minutes aeration time, respectively. The legend, 306CTC and 68CTC represents TRFK 306 CTC teas and TRFK 6/8 CTC teas, respectively.

(Figure 38) depicts scores of infusion colour of black CTC teas from both TRFK 306 and TRFK 6/8. There was significant difference ($p< 0.05$) on infusion colour of teas from TRFK 306. Teas which were withered for shorter time had less bright infusions than teas withered for longer time. Teas from TRFK 306 withered for five hours and aerated for thirty and ninety minutes scored low in infusion colour, with a score of 2. This could be because of high thearubigins since thearubigins are responsible for thickness and colour of both the liquor and infusion (Teshome *et al*., 2013). Teas from TRFK 306 withered for twenty and ten hours and aerated for thirty minutes had relatively lower thearubigins as shown (Figure 27)

and consequently scored relatively higher in infusion colour with scores of 3 and 2.9, respectively. There was no significant difference ($p < 0.05$) for teas from TRFK 6/8 on the infusion colour. Teas from TRFK 6/8 aerated for ninety minutes had relatively higher theaflavins (Figure 26) and consequently scored high in infusion colour, Figure 38. Though theaflavins influences infusion colour positively (Kilel *et al*., 2013b), these results reveals that thearubigins has more effect on infusion colour of TRFK 306 while theaflavins have more effect on infusion colour on TRFK 6/8 teas.

Aerated orthodox teas sensory evaluation

The tasters were provided with coded samples in three replicates and were requested to describe the liquors as much as they could. The researcher found most descriptions were narrowed down to liquor colour and liquor flavour though the terms used for TRFK 6/8 and TRFK 306 were different (Appendix IV and III, respectively).

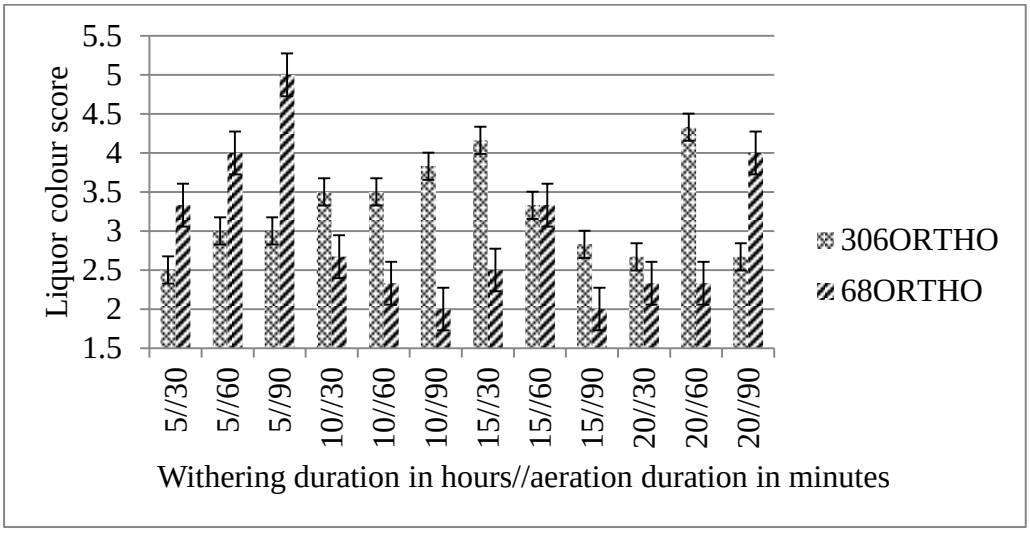


Figure 39: Effect of withering and aeration on liquor colour of orthodox teas.

Where; 5//30,5//60,5//90 denotes 5 hours withering time and 30, 60 and 90 minutes aeration time, respectively; 10//30, 10//60, 10//90 represent 10 hours withering time with 30, 60 and 90 minutes aeration time, respectively, 15//30, 15//60, 15//90 represent 15 hours withering time with 30, 60 and 90 minutes aeration time, respectively. 20//30, 20//60 and 20//90 represent 20 hours withering time with 30, 60 and 90 minutes aeration time, respectively. The legend, 306ORTHO and 68ORTHO represents TRFK 306 orthodox teas and TRFK 6/8 orthodox teas, respectively.

The score sheet developed by the current researcher is the first in orthodox tasting and it can be improved with further research on orthodox tea tasting terminologies. The results of

liquor colour for orthodox aerated teas of both clones are presented (Figure 39). There was a significance difference ($p< 0.05$) within and between the two clones in liquor colour. TRFK 6/8 orthodox teas withered for five hours and aerated for ninety minutes had the highest score of 5, meaning they were bright. TRFK 6/8 teas withered for five hours had better colour than TRFK 306 teas though increased withering time improved TRFK 306 teas in liquor colour.

The results of liquor flavour are presented (Figure 40). TRFK 6/8 orthodox aerated teas had better liquor flavour than TRFK 306 orthodox aerated teas. It can be observed that there is a general decline in liquor flavour with longer withering time especially in TRFK 6/8 teas.

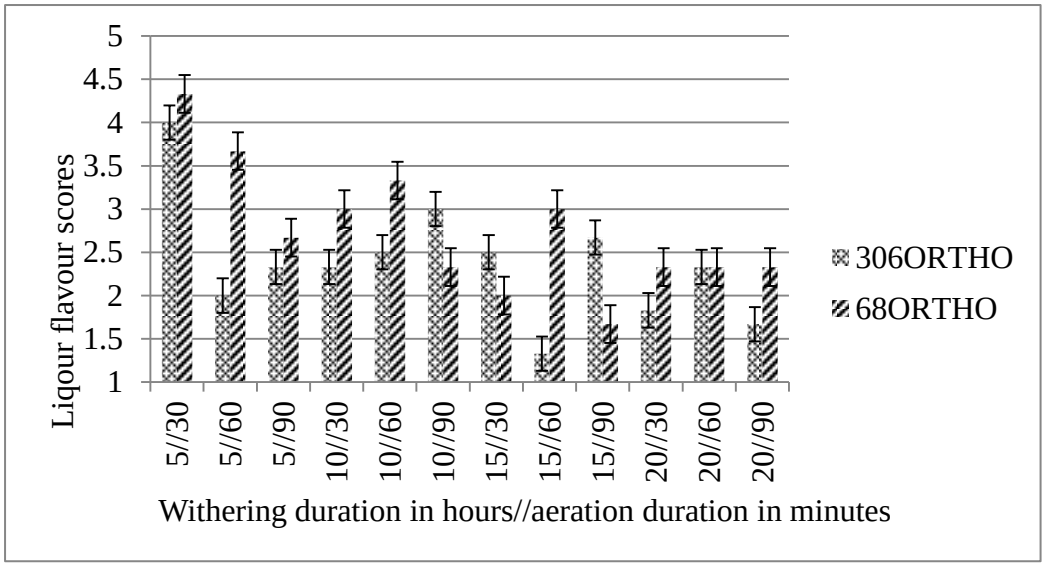


Figure 40: Effect of withering and aeration time on liquor flavour orthodox teas. Where; 5//30,5//60,5//90 denotes 5 hours withering time and 30, 60 and 90 minutes aeration time, respectively; 10//30, 10//60, 10//90 represent 10 hours withering time with 30, 60 and 90 minutes aeration time, respectively, 15//30, 15//60, 15//90 represent 15 hours withering time with 30, 60 and 90 minutes aeration time, respectively. 20//30, 20//60 and 20//90 represent 20 hours withering time with 30, 60 and 90 minutes aeration time, respectively. The legend, 306ORTHO and 68ORTHO represents TRFK 306 orthodox teas and TRFK 6/8 orthodox teas, respectively.

The colour of TRFK 6/8 teas were brighter than TRFK 306 teas when the teas were withered for five hours. This was unexpected since as we can see from (Figure 26), orthodox teas from TRFK 306 had higher theaflavins. Further work needs to done on sensory evaluation of orthodox teas and analysis done to reveal the compounds responsible for the quality.

The flavour of tea is the most important factor in determining the quality of tea (Joshi *et al*., 2015). Flavour usually involves both taste and aroma in food sensory. The taste is affected by the non-volatile water soluble components while aroma is affected by volatile

flavour components (Joshi *et al*., 2015). TRFK 6/8 teas scored better in liquor flavour than TRFK 306 teas. This could be attributed to the more non-volatile water soluble compounds witnessed in relatively higher total soluble solids (Table 9).

CHAPTER FIVE

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

From the findings of this study, all the hypotheses were rejected and the following conclusions can be drawn;

i. For maximum anthocyanins levels from unprocessed TRFK 306 tea leaves, making a brew using boiling drinking water timed at five minutes is the best.

ii. Fresh purple tea leaves when brewed using boiling water timed at 10 minutes has higher antioxidant activity using tea leaves: water ratio of 1:20. Addition of citric acid more than 0.3 % reduces the amount of anthocyanins in a brew made from fresh purple tea leaves. Addition of citric acid improves the colour and taste of the liquor brewed from fresh TRFK 306 tea leaves.

iii. TRFK 306 is best processed via orthodox method with not less than 15 hours withering time for optimum green tea quality teas. TRFK 306 is a fast fermenter and can be used to make aerated CTC teas with withering not exceeding 15 hours wither and not exceeding 60 minutes aeration time.

iv. Orthodox aerated teas have higher antioxidant activity than CTC teas.

v. Sensory evaluation terms describing both CTC and orthodox teas, aerated and non-aerated teas from purple-leafed tea leaves should be swiftly developed.

5.2 Recommendations

The following recommendations can be made after carrying out this study;

i. When hot water is used to brew liquor from fresh purple-leafed teas, for maximum extraction of anthocyanins, five minutes brewing time is the best.

ii. To get liquor with high antioxidant activity, brewing for ten minutes using boiling water is recommended and addition of any acidic additives should be avoided since it will lower the antioxidant activity, though the colour will be improved.

iii. For TRFK 306 teas, aeration should be done not longer than 60 minutes.

CHAPTER SIX

6.1 Summary of results

(i) Brewing TRFK 306 fresh tea leaves using boiling drinking water timed at five minutes gave maximum anthocyanins of 92.0 mg/L.

(ii) Brewing TRFK 306 fresh tea leaves using boiling drinking water timed at 10 minutes gave relatively higher levels of antioxidant activity of 92.0 % though addition of citric acid reduces the antioxidant activity. Water temperature of 70 ^{0}C extracted less anthocyanins from TRFK 306 fresh tea leaves than boiling water. Addition of 0.3 % citric acid increased the anthocyanins levels up to 133 mg/L, while liquor without acid had 92.0 mg/L.

(iii) Orthodox teas had higher, total polyphenols, total catechins than CTC tea in both studied clones. CTC teas of both clones had higher theaflavins and thearubigins than orthodox teas.

(iv) Non-aerated TRFK 306 CTC teas are brisker than non- aerated TRFK 6/8 CTC teas though for CTC aerated teas; TRFK 6/8 is more brisk than TRFK 306 CTC teas.

(v) Non- aerated TRFK 306 teas of both manufacture had lower antioxidant acivity than non-aerated TRFK 6/8 orthodox teas.

6.2 Suggestions for further research

(i) Further research should be done on effects of various handling practices of fresh purple tea leaves on the quality (withering, time of plucking, bruising of tea leaves) to guide farmers who may wish to sell the tea leaves for direct utilization without further processing.

(ii) More chemical quality characteristic analyses should be done on the brew made from fresh Kenyan teas with various additives like honey.

(iii) Similar investigations on fresh tea leaves and brewing regimes could be done on Kenyan green clones. This will inform on alternative use of the tea leaves grown in Kenya.

(iv) Further research on optimization of drying regimes on quality of purple tea is imminent; the current study optimized withering and aeration time.

(v) Research on effect of various processing conditions on anthocyanins should be done on both aerated and non-aerated teas

(vi) More research needs to be done on aerated orthodox teas from the purple tea clones to ensure higher quality parameters.

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APPENDICES

Appendix I: Score sheet used for CTC green tea, TRFK 306 and TRFK 6/8

Liquor body	Liquor briskness
1. Very light	1. Plain/soft/flat
2. Light	2. Harsh
3. Fairly thick	3. Fairly brisk/strength
4. Thick	4.Brisk/some flavour
5. Very thick	5. Astringent/Very brisk

Appendix II: Score sheet for CTC aerated (black) tea TRFK 306 and TRFK 6/8

Liqour colour	1. Dull 2. Fairly bright 3. Bright 4. Very bright 5. Coppery
Liqour body	1. Light/thin 2. Fairly thick 3. Thick 4. Very thick 5. Creamy
Liqour briskness	1. Flat/soft 2. Not Brisk 3. Fairly brisk 4. Brisk 5. Very brisk
Liqour strength	1. Harsh 2. Soft 3. Fair strength 4. Strong 5. Pungent/Very strong
Infusion colour	1. Greenish 2. Dull 3. Fairly bright 4. Bright 5. Very bright

Appendix III: Score sheet for liquors from black orthodox teas, TRFK 306

Liquor colour	Liquor flavour
1.Dull	1. Mild/relative flavour
2.Light	2. Sweet mouth feel/flavoury
3.Fairly light or fairly purplish	3. Pronounced flavour
4. Purplish	4. Deep /rich flavoury
5.Deep colour/bright	5. Astringent/ sharp striking flavour

Appendix IV: Score sheet for liquors from black orthodox teas TRFK 6/8

Liquor colour	Liquor flavour
1.Greenish	1.Remote/touch brisk
2. Light/thin	2. Fairly brisk/relative flavours
3.Yellowish	3. Useful flavours/flavoury
4.Yellow	4. Astringent
5. Bright	5. Sharp flavours

Appendix V: Score sheet for liquors from green orthodox teas, TRFK 306 and TRFK 6/8

Liquor colour	Liquor flavour
1. Light	1. Tending plain/plain
2. Purplish/yellowish	2. Little flavour
3. Purple/yellow	3. Some flavour
4. Bright	4. Fairly brisk
	5. Brisk/pungent/sharp flavour

Appendix VI: Summary of some green tea results of TRFK 306 and TRFK 6/8

TRFK 306	Ga	EGC	C	CAFF	EC	EGCG	ECG	TC	TPP	AA%
CTC 0	1.06±0.09	1.03±0.12	0.65±0.01	2.09±0.09	1.04±0.06	3.65±0.5	5.48± 1.49	11.85±1.02	22.89± 0.33	90.81±0.42
ORTHO 0	0.93±0.11	1.08±0.14	0.63±0.04	1.90±0.17	0.88±0.05	4.15±0.38	4.52± 0.82	11.26±0.83	21.95± 0.47	90.52±0.3
CTC 5	0.97±0.04	0.97±0.03	0.63±0.03	1.91±0.08	1.05±0.07	3.46±0.1	3.64± 0.15	9.74± 0.13	24.78± 0.38	90.93±0.52
ORTHO 5	1.28±0.12	1.45±0.11	0.71±0.07	2.27±0.21	1.07± 0.1	5.05±0.5	4.67± 0.44	12.95±1.18	25.32± 0.6	89.99±0.93
CTC 10	0.74±0.14	1.02±0.41	0.51±0.14	1.55±0.36	0.88±0.23	2.95±0.82	2.85± 0.69	8.22± 2.2	20.1± 0.19	91.30±0.05
ORTHO 10	1.24±0.07	1.57±0.11	0.76±0.04	2.21±0.12	1.12±0.08	4.66±0.2	4.32± 0.2	12.43±0.63	22.38± 0.79	91.70±0.06
CTC 15	0.87±0.01	1.94±0.07	0.44±0.01	2.71±0.09	0.88±0.05	1.65±0.07	3.05± 0.09	7.96± 0.14	20.3± 0.2	91.56±0.09
ORTHO 15	1.08± 0.1	1.64±0.23	0.83±0.11	2.14±0.22	1.07±0.15	4.48±0.37	4.01± 0.35	12.03± 1.2	24.86± 0.48	91.60±0.1
CTC 20	1.08±0.02	2.09±0.21	0.51±0.02	2.83±0.06	0.56±0.25	2.04±0.04	3.07± 0.07	8.47± 0.3	20.47± 0.09	92.07±0.29
ORTHO 20	0.95±0.02	1.04±0.04	0.55±0.00	2.43±0.03	0.84±0.02	3.43±0.08	6.43± 0.21	12.28±0.25	23.00± 0.95	91.26±0.17
TRFK 6/8	Ga	EGC	C	CAFF	EC	EGCG	ECG	TC	TPP	AA %
CTC0	0.65±0.06	7.28±0.99	0.71±0.07	2.86±0.29	1.66±0.17	7.35±0.43	2.27±0.19	19.27±0.25	26.74±0.65	89.44±0.50
ORTHO0	0.70±0.06	7.37±0.54	0.59±0.02	2.70±0.07	1.89±0.11	7.77±0.32	2.48±0.12	20.35±1.21	27.24±0.67	92.19±0.11
CTC5	0.60±0.02	7.04±0.83	0.64±0.0.06	2.64±00.28	1.52±0.16	6.37±0.54	1.99±0.14	17.56±1.72	25.87±0.35	89.51±0.85
ORTHO5	0.67±0.07	7.52±0.42	0.68±0.08	2.77±0.16	1.84±0.13	7.57±0.51	2.40±0.22	20.28±0.69	27.19±0.27	91.81±0.37
CTC 10	0.67±0.06	5.31±0.93	0.69±0.09	3.33±0.02	1.92±0.01	7.28±0.77	2.58±0.04	17.77±1.72	25.26±1.06	89.88±0.58
ORTHO10	0.69±0.1	5.22±0.83	0.62±0.21	2.35±0.33	1.47±0.3	6.72±0.94	2.32±0.35	16.36±2.62	27.22±0.41	90.80±0.78
CTC15	0.78±0.13	5.47±0.42	0.60±0.03	3.10±0.58	1.84±0.39	7.57±1.65	2.50±0.52	17.99±2.97	22.04±2.39	90.96±0.97
ORTHO15	0.65±0.14	5.28±0.84	0.60±0.15	2.42±0.48	1.51±0.27	6.99±1.43	2.31±0.47	16.69±3.14	27.48±0.6	90.43±0.27
CTC20	0.66±0.12	4.07±0.11	0.58±0.02	3.13±0.04	1.46±0.05	5.93±0.1	2.26±0.03	14.32±0.3	26.28±0.41	89.97±0.8
ORTHO20	0.66±0.04	5.99±0.29	0.58±0.04	3.27±0.19	1.93±0.16	8.11±0.52	2.49±0.25	19.11±0.96	26.39±0.38	91.95±0.38

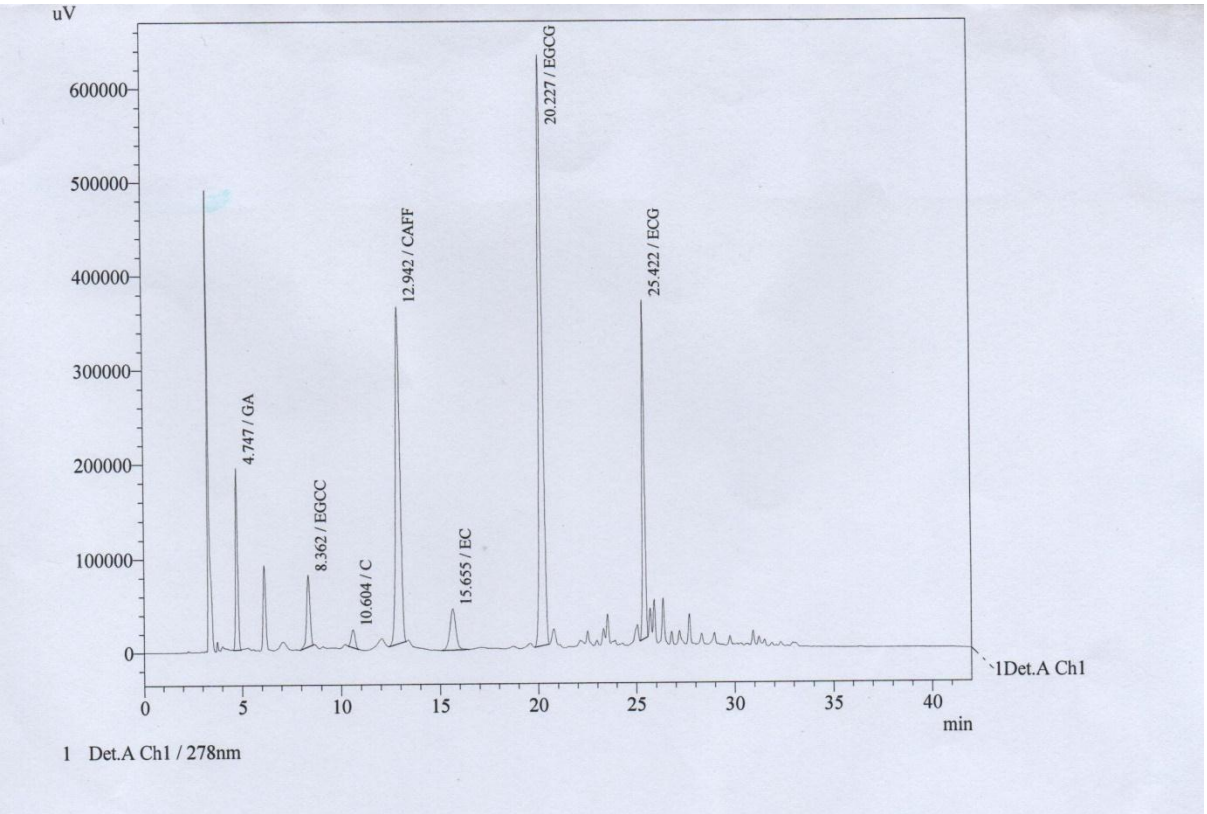
Appendix VII: Coding of fresh purple tea samples for sensory evaluation

Time (minutes)	Citric acid con. (g)	Code used
5	Plain	X456
	0.1	B109
	0.2	Y249
	0.3	M733
	0.4	N501
	0.5	F683
10	Plain	G992
	0.1	J743
	0.2	W582
	0.3	C386
	0.4	K113
	0.5	E704

Appendix VIII: 5- point hedonic scale sheet

Score	**Description**
1	Dislike extremely
2	Dislike slightly
3	Neither like nor dislike
4	Like slightly
5	Like extremely

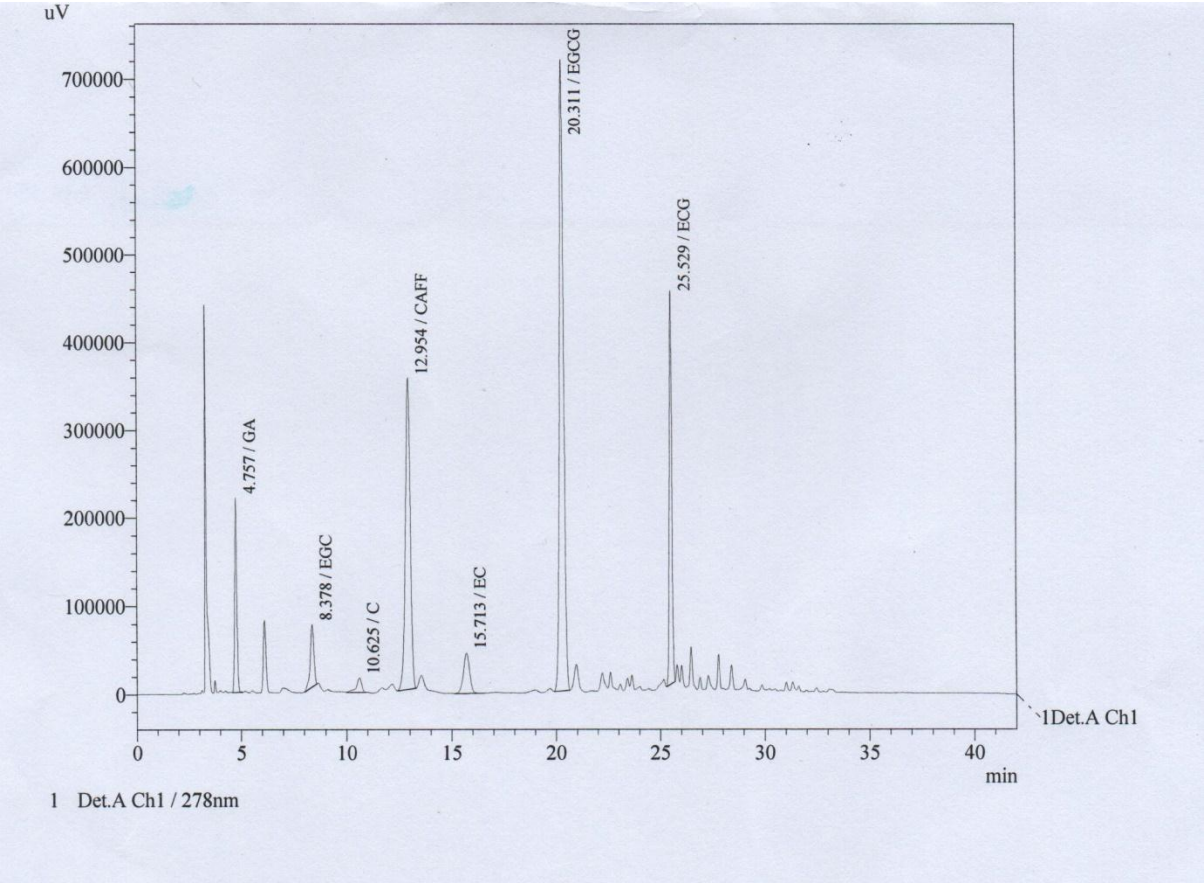
Appendix IX: HPLC elution profile of catechins in a sample of TRFK 6/8 non-aerated CTC teas



Key: X = Retention time in minutes

Y= Intensity, µV

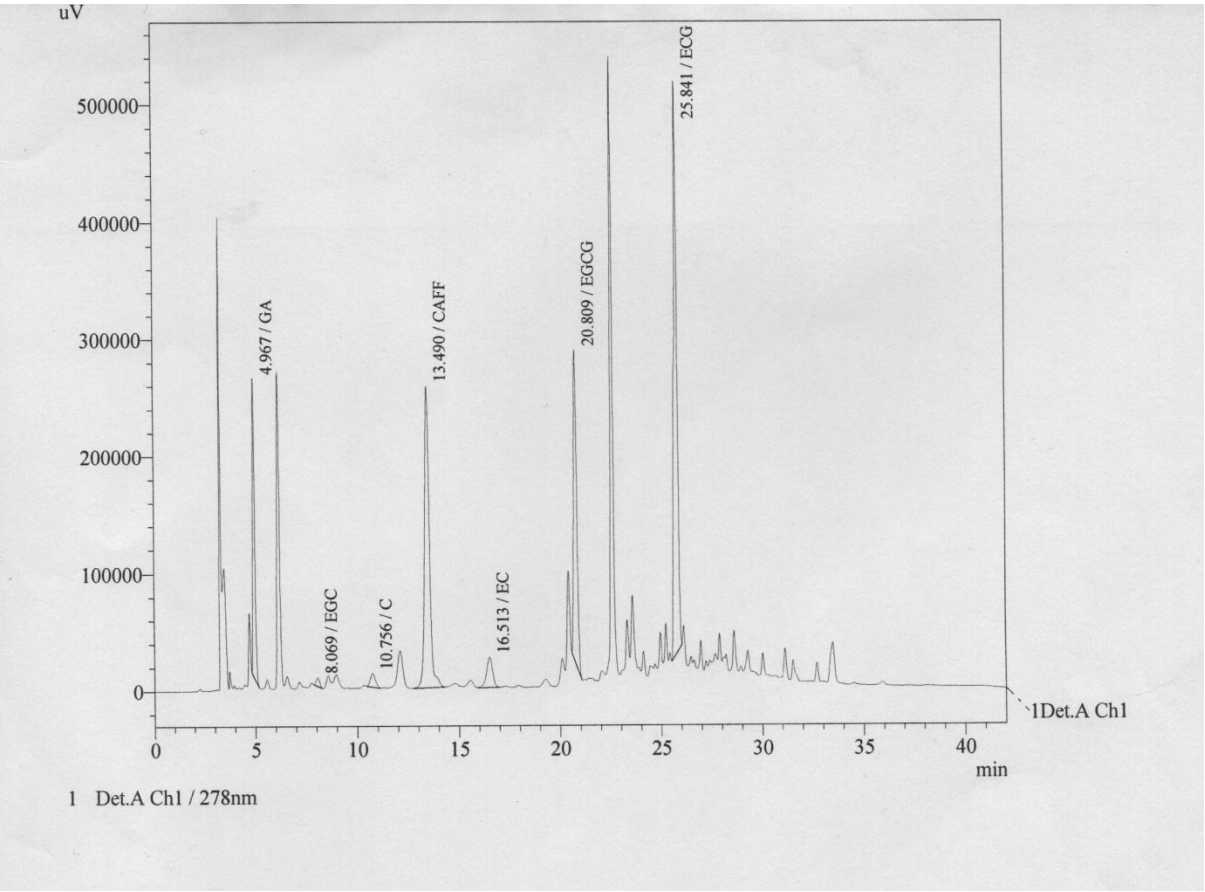
Appendix X: A representative of HPLC elution profile of catechins in TRFK 6/8 non-aerated orthodox teas



Key: X = Retention time in minutes

Y= Intensity, µV

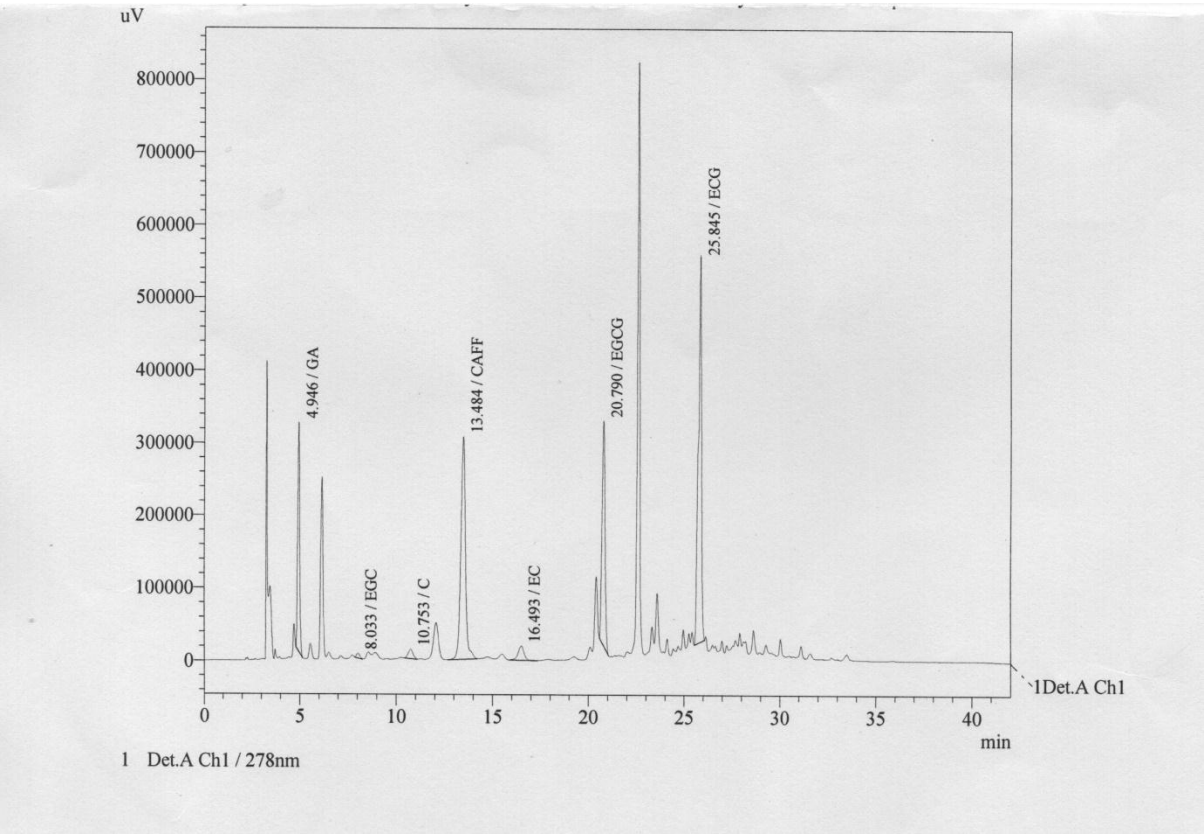
Appendix XI: A representative HPLC elution profile of catechins in TRFK 306 non-aerated CTC teas



Key: X = Retention time in minutes

Y= Intensity, µV

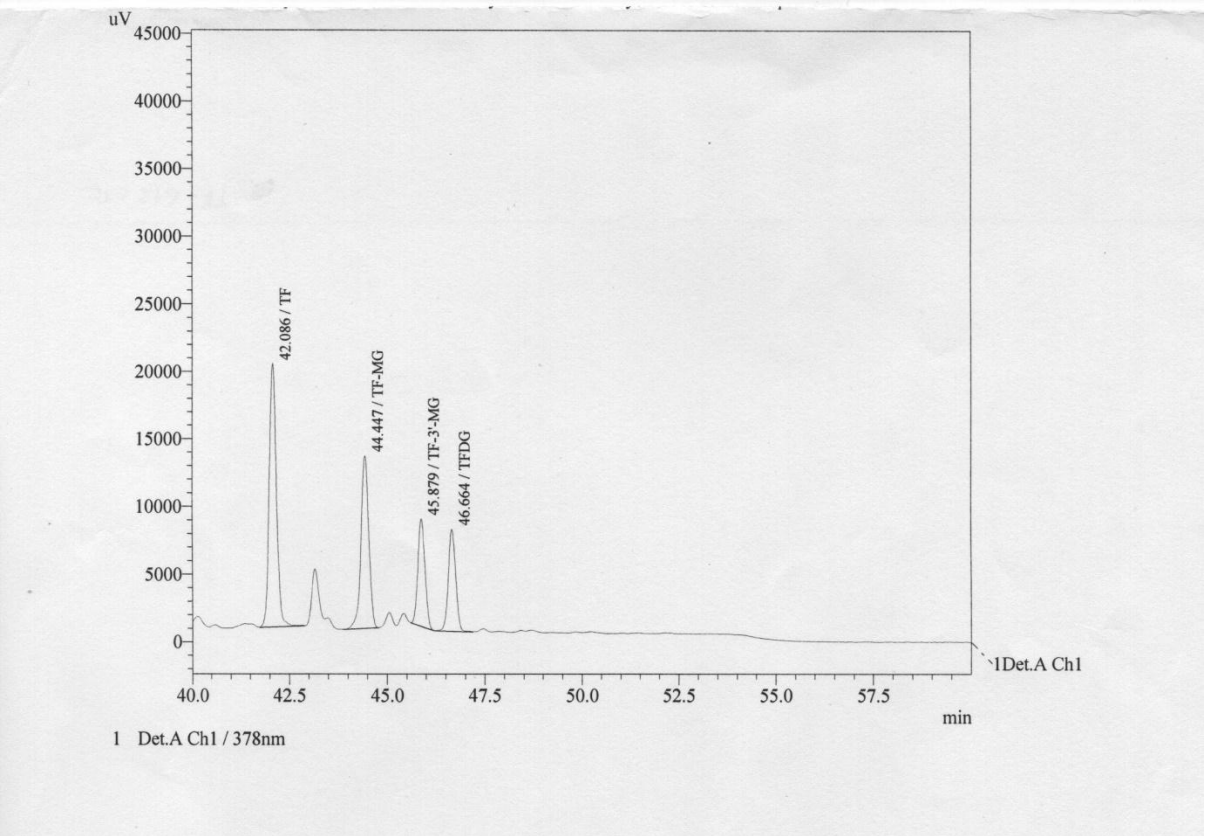
Appendix XII: A representative HPLC elution profile of catechins in TRFK 306 non-aerated orthodox teas



Key: X = Retention time in minutes

Y= Intensity, µV

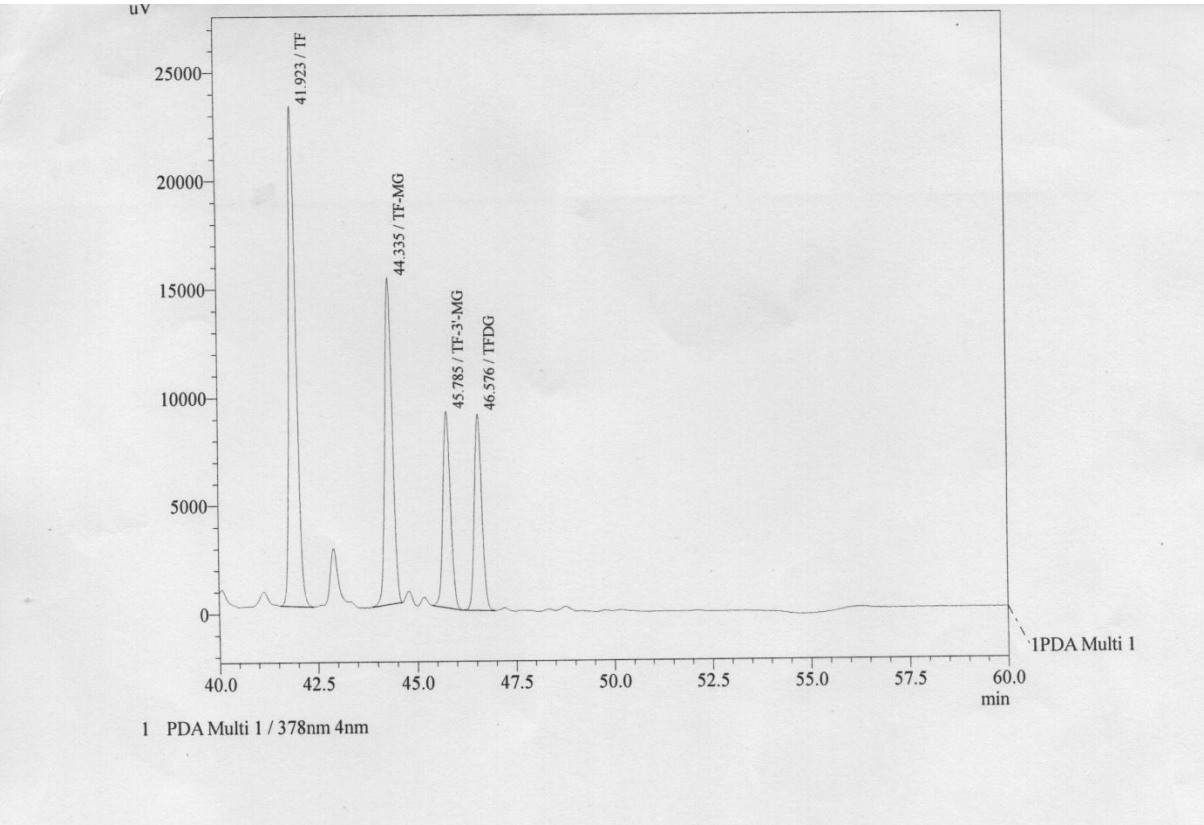
Appendix XIII: A representative HPLC elution profile of individual theaflavins in TRFK 6/8 aerated CTC teas



Key: X = Retention time in minutes

Y= Intensity, µV

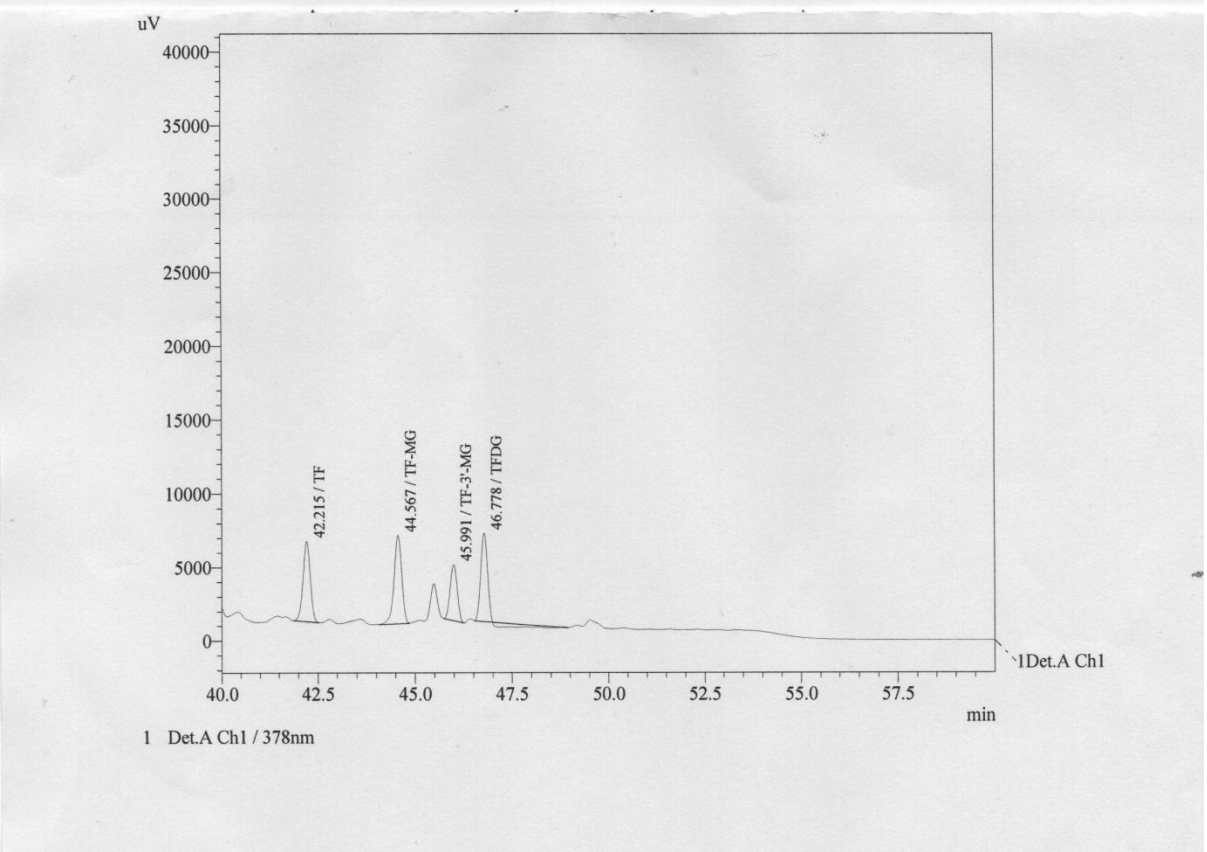
Appendix XIV: A representative of HPLC elution profile of individual theaflavins in TRFK 6/8 aerated orthodox teas



Key: X = Retention time in minutes

Y= Intensity, µV

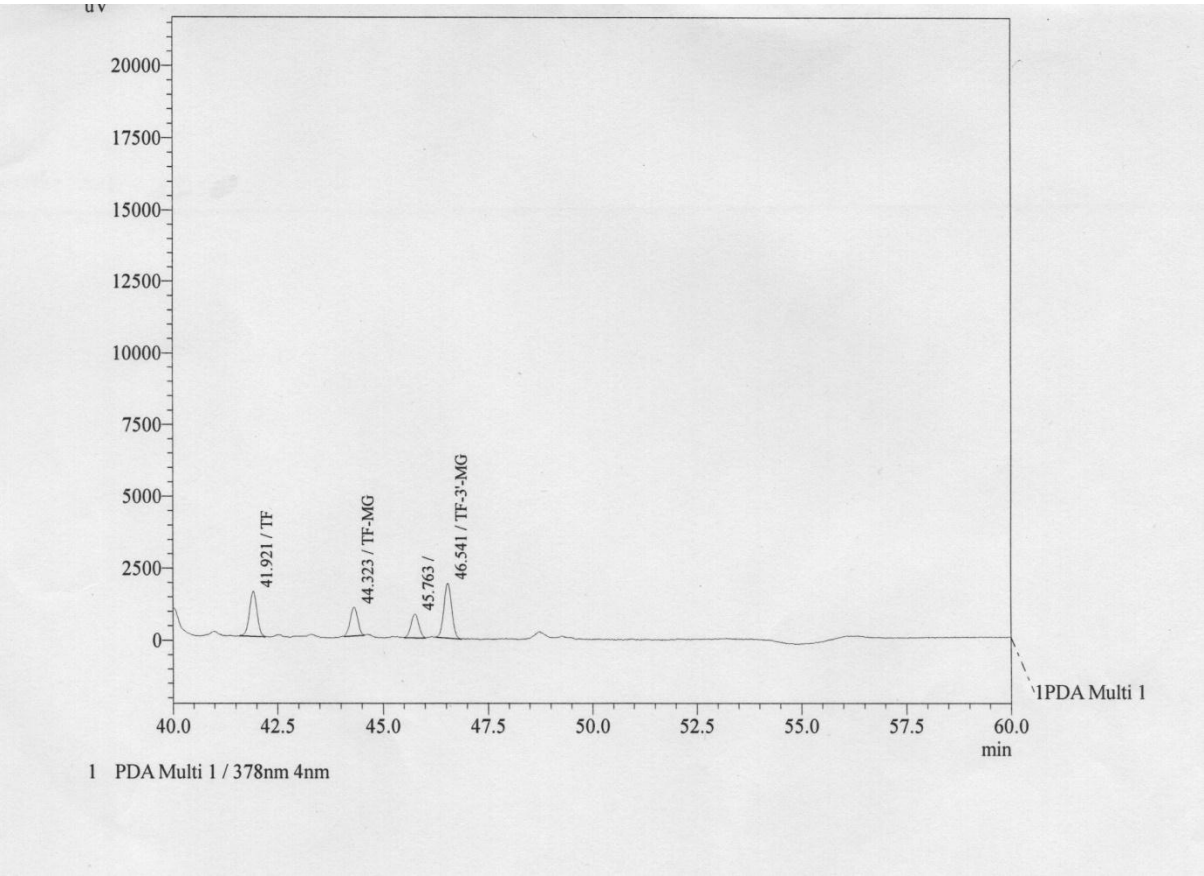
Appendix XV: A representative of HPLC elution profile of individual theaflavins in TRFK 306 aerated CTC teas



Key: X = Retention time in minutes

Y= Intensity, µV

Appendix XVI: A representative of HPLC elution of individual theaflavins in TRFK 306 aerated orthodox teas



Key: X = Retention time in minutes

Y= Intensity, µV

Appendix XVII: GML tables

Dependent Variable: Effect of antioxidant activity of liquor from fresh purple tea leaves

Source	DF	Sum of Squares	Mean Square	F value	Pr > F
Model	5	108.95	21.79	1.92	0.16
Error	12	136.13	11.34		
Corrected Total	17	245.08			

Source	DF	Type I SS	Mean Square	F Value	Pr> F
Citric acid (%)	5	108.95	21.79	1.92	0.16

Source	DF	Type III SS	Mean Square	F value	Pr > F
Citric acid (%)	5	10.8.95	21.79	1.92	0.16

Appendix XVIII

R-Square	Coeff Var	Root MSE	antioxidant activity Mean
0.444559	3.842036	3.368100	87.66444

Dependent Variable: Total Polyphenols

Source	DF	Sum of squares	Mean Square	F Value	Pr>F
Model	19	362.96	19.10	22.82	<.0001
Error	40	33.48	0.84		
Corrected Total	59	396.44			

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Clone	1	190.60	190.60	227.71	<.0001
ManuWithtime	18	172.35	9.57	11.44	<.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Clone	0	0.0000			
ManuWithtime	18	172.35	9.57	11.44	<.0001

R-Square	Coeff Var	Root MSE	TPP Mean
0.915544	3.751317	0.914896	24.38867

Dependent Variable: Total catechins

Source	DF	Sum of Squares	Mean Square	F Value	Pr>F
Model	19	984.65	51.82	16.13	<.0001
Error	40	128.54	3.21		
Corrected total	59	1113.19			

Source	DF	Type I SS	Mean Square	F Value	Pr>F
Clone	1	788.73	788.73	245.44	<.0001
ManuWithtime	18	195.92	10.88	3.39	0.0007

Source	DF	Type III SS	Mean Square	F Value	Pr> F
Clone	0	0.0000			
ManuWithtime	18	195.92	10.88	3.39	0.0007

R-Square	Coeff Var	Root MSE	Total catechin Mean
0.884527	12.49694	1.792645	14.34467

Dependent variable: TF (CTC aerated teas)

Source	DF	Sum of Squares	Mean Square	F value	Pr >F
Model	23	651.52	28.33	13.68	< 0.0001
Error	48	99.43	2.07		
Corrected total	71	750.94			
Source	DF	Type I SS	Mean Square	F value	Pr>F
Clone	1	4.17	4.17	2.02	0.16
manuwithtimeAeratime	22	647.34	29.42	14.21	0.0001
Source	DF	Type III SS	Mean square	F value	Pr> F
Clone	1	0.000			
manuwithtimeAeratime	22	647.34	29.42	14.21	0.0001

R-Square	CV	Root MSE	TF mean
0.87	12.37	1.44	11.63

Dependent variable: Thearubigins (CTC aerated teas)

Source	DF	Sum of Squares	Mean Square	F value	Pr >F
Model	23	506.66	22.03	14.53	< 0.0001
Error	48	72.78	1.52		
Corrected total	71				
Source	DF	Type I SS	Mean Square	F value	Pr>F
Clone	1	240.43	240.43	158.56	< 0.0001
manuwithtimeAeratime	22	266.23	12.1	7.98	< 0.0001
Source	DF	Type III SS	Mean square	F value	Pr> F
Clone	1	0.000			
manuwithtimeAeratime	22	266.23	12.1	7.98	< 0.0001

R-Square	CV	Root MSE	TR mean
0.87	8.26	1.23	14.92

Dependent Variable: Liqour briskness (aerated CTC teas)

Source	DF	Type I SS	Mean Square	F value	Pr > F
Model	17	7.84	0.46	1.52	0.12
Error	54	16.39	0.3		
Corrected Total	71	24.23			

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Clone	1	6.09	6.09	20.07	<.0001
manuwithaeration	16	1.75	0.1	0.36	0.98
Clone*manuwithaeration	0	0.00000			

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Clone	0	0.0000			
manuwithaeration	16	1.75	0.11	0.36	0.98
Clone*manuwithaeration	0	0.00000			

R-Square	Coeff Var	Root MSE	liquor briskness Mean
0.323672	15.95391	0.550853	3.452778

Table showing effect of interactions among the treatments on theaflavins (TF µmol/g)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	47	3777.527496	80.372925	71.70	<.0001
Error	96	107.608828	1.120925		
Corrected Total	143	3885.136324			

R-Square	Coeff Var	Root MSE	TFumol Mean
0.972302	15.13074	1.058738	6.997264

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Clone	1	21.432270	21.432270	19.12	<.0001
Manu	1	3085.265540	3085.265540	2752.43	<.0001
Withtime	3	6.434403	2.144801	1.91	0.1326
Aeratime	2	90.849276	45.424638	40.52	<.0001
Clone*Manu	1	3.025860	3.025860	2.70	0.1037

Clone*Withtime	3	96.374784	32.124928	28.66 <.0001
Clone*Aeratime	2	153.260897	76.630448	68.36 <.0001
Manu*Withtime	3	11.158172	3.719391	3.32 0.0231
Manu*Aeratime	2	34.858814	17.429407	15.55 <.0001
Withtime*Aeratime	6	6.714626	1.119104	1.00 0.4310
Clone*Manu*Withtime	3	77.289494	25.763165	22.98 <.0001
Clone*Withti*Aeratim	6	24.849359	4.141560	3.69 0.0024
Clone*Manu*Aeratime	2	121.184679	60.592339	54.06 <.0001
Manu*Withtim*Aeratim	6	16.531714	2.755286	2.46 0.0296
Clon*Manu*With*Aerat	6	28.297608	4.716268	4.21 0.0008

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Clone	1	21.432270	21.432270	19.12	<.0001
Manu	1	3085.265540	3085.265540	2752.43	<.0001
Withtime	3	6.434403	2.144801	1.91	0.1326
Aeratime	2	90.849276	45.424638	40.52	<.0001
Clone*Manu	1	3.025860	3.025860	2.70	0.1037
Clone*Withtime	3	96.374784	32.124928	28.66	<.0001
Clone*Aeratime	2	153.260897	76.630448	68.36	<.0001
Manu*Withtime	3	11.158172	3.719391	3.32	0.0231
Manu*Aeratime	2	34.858814	17.429407	15.55	<.0001
Withtime*Aeratime	6	6.714626	1.119104	1.00	0.4310
Clone*Manu*Withtime	3	77.289494	25.763165	22.98	<.0001
Clone*Withti*Aeratim	6	24.849359	4.141560	3.69	0.0024
Clone*Manu*Aeratime	2	121.184679	60.592339	54.06	<.0001

The GLM Procedure

Dependent Variable: TFumol

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Manu*Withtim*Aeratim	6	16.531714	2.755286	2.46	0.0296
Clon*Manu*With*Aerat	6	28.297608	4.716268	4.21	0.0008

Appendix XIX: Research permit

REPUBLIC OF KENYA

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Appendix XX: Abtracts of published papers

KILEL'S PAPER

IJRCE
ISSN 2248-9649
International Journal of
Research in Chemistry and Environment
Available online at: www.ijrce.org

Research Paper

Effect of Leaf Withering Duration, Maceration types and Aeration Duration on the Quality of Black Tea of Clone TRFK 306 (Kenyan Purple Tea)

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Abstract: *TRFK 306 is a tea clone rich in anthocyanin giving the leaves a purple coloration and it is of assamica cultivar. It was developed in Kenya by the Tea Research Foundation of Kenya, currently Tea Research Institute and has since been released for commercial utilization. This type of tea clone just like any other tea clone can be processed into any type of tea (green, black, Oolong, white, yellow) depending on choice and preference of the processor. Since the clone was released for commercial utilization in 2011, processors are still not sure how best to process this new tea clone. The current study investigated the effect of processing method, withering and aeration durations on the quality of black tea from TRFK 306. TRFK 6/8 was used as a reference standard because of its proven black tea quality. Orthodox and Cut Tear and Curl (CTC) methods of processing were used in both tea clones. Withering was done at room temperature and varied at an interval of five hours, starting at five hours up to twenty hours. Within each withering regime, aeration was varied at 30 minutes interval from 30-90 minutes. Experienced regular tea tasters did the sensory evaluation by describing the liquor colour, body, strength, briskness and infusion colour then the researchers analysed and interpreted the description using a predetermined scale. Theaflavins, thearubigins, total colour, brightness percentage was analysed using UV spectrophotometer. The results showed that CTC teas had relatively higher black tea quality parameters than the orthodox teas irrespective of the tea clone. Within each processing method, TRFK 306 had better quality than TRFK 6/8. Aeration time had more pronounced effect on quality than withering duration. TRFK 306 processed through CTC and withered for ten hours and aerated for 90 minutes produced the best black tea according to the analysed quality parameters, while TRFK 6/8 produced best tea at twenty hours withering and 90 minutes aeration. For orthodox teas, TRFK 306 produced best tea at twenty hours withering aerated for 90 minutes while TRFK 6/8 produced best tea at fifteen hours withering and 60 minutes aeration. It was concluded that TRFK 306 produces better black tea than TRFK 6/8 based on the chemical parameters analysed.*

Keywords: aeration time, Cut Tear and Curl, orthodox, TRFK 306, withering time.

Introduction

Tea is made from the tender shoots of *Camellia sinensis* L.O Kuntze. Tea was first introduced in Kenya from India in 1903 and in the 1930's commercial planting began [1]. Planted tea area in Kenya has grown [2] from a mere 21,448 hectares in 1963 to over 203, 006 hectares by 2014. Kenya being a major black tea exporter earns more foreign exchange from it than from other agricultural produce. Young shoots of tea leaves can be processed in different ways/methods depending on the processors choice. According to [3], there are three main types of teas; Green, Oolong and black teas. Kenya basically produces black Cut Tear and Curl (CTC) teas though product diversification is being promoted to enhance sales and consumption of tea.

KILEL'S PAPER

International Food Research Journal 25(2): 730-736 (April 2018)
Journal homepage: http://www.ifrj.upm.edu.my

Optimization of manufacturing conditions of the new purple leafed Kenyan teas (TRFK 306) – maceration style and withering duration

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Article history

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Keywords

Clone TRFK 306
CTC and orthodox
Withering
Green tea
Catechins
Antioxidant activity

Abstract

Purple tea in Kenya, TRFK 306, has received great attention due to its perceived health benefits though how it should be optimally manufactured has not been researched on exhaustively. The current study investigated on the two possible methods of manufacture – Cut Tear and Curl (CTC) and orthodox manufacture - with varying withering time of 0, 5, 10, 15 and 20 hours. Catechins, gallic acid, caffeine, total polyphenols and antioxidant activity were assayed as quality indicators. If gallic acid and caffeine are the main chemicals targeted, then orthodox type with longer withering, 15 – 20 hours, is usually recommended. When targeting catechins especially epigallocatechin gallate and high total polyphenols, orthodox type of manufacture whichever the withering time is proposed. CTC with withering hours of 15-20 produces teas with high antioxidant activity. It was concluded that TRFK 306 is best processed by orthodox or CTC but with longer withering time.

Introduction

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). Planted tea area in Kenya has grown from 21,448 hectares in 1963 to over 180,000 hectares in 2011 and 203, 000 hectares by 2014 (AFFA, 2014). Kenya is mostly known for its Cut Tear Curl (CTC) tea production, but the country also produces orthodox (ORTHO) tea. Kenya is among the few Orthodox tea producers worldwide accounting for 1.3 percent of total production. In 2012, Kenyan production of orthodox tea accounted for approximately 3.5% of total Kenyan production. Production of Kenyan orthodox tea showed an average annual increase of 4.3% in the period 2008-2012 (International Tea Committee, 2013). Kenya also produces low amount of green tea and with the introduction of purple tea, TRFK 306, Kenya is destined for mass production of green tea. World green tea production is expected to grow at a faster rate than black tea, 8.2 percent, reflecting the growth in China where production of green tea is expected to reach 2.97 million tonnes by 2023. (Chang, 2015).

Green tea is a non-aerated tea which is produced by steaming the plucked leaves to inactivate the enzyme polyphenol oxidase, which can oxidize tea polyphenols resulting in the formation of a brown colour. Green tea can be processed in various methods like CTC and orthodox. In CTC type of manufacture, there is full maceration of the tea leaves such that we have smaller sized teas whereas in orthodox manufacture, the tea leaves are rolled either by hand or by rollers to get relatively bigger sized teas. Green tea has naturally occurring catechins, including (-) epigallocatechin gallate (EGCG), (-)-epigallocatechin (EGC) and (-) epicatechin gallate (ECG), all in higher concentrations than other types of teas (Hung *et al.*, 2010). The quality of processed tea is basically determined by the chemical constituents in tea leaves (Cabrera *et al.*, 2006; Chaturvedula and Prakash, 2011). The compounds determining tea flavour and astringency include catechins (flavan-3-ols), including (-)-epigallocatechin gallate (EGCG), (+)-catechin (C), (+)-gallocatechin (GC) and their oxidation products (Liang *et al.*, 2006; Narukawa et al., 2010; Chaturvedula and Prakash, 2011).

Polyphenolic compounds are known to be responsible for the antioxidant properties in many plants (Wen *et al.*, 2013; Chiu *et al.*, 2013). Epigallocatechin-3-gallate among other catechins play a key role as antioxidants in prevention and treatment of many diseases (Khan and Mukhtar, 2007; Sinija

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KILEL'S PAPER

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Effect of Citric Acid on the Total Monomeric Anthocyanins and Antioxidant Activity of Liquor Made from Unprocessed Purple Leafed TRFK 306 Kenyan Tea Clone

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Abstract

A study was conducted to find out the effect of citric acid on total monomeric anthocyanins and antioxidant activity of liquor made from unprocessed purple-leafed TRFK 306 Kenyan tea, brewed at various time/temperature combinations. Most purple tea consumers usually brew the tea leaves then add some lemon juice before consuming it. Citric acid was used here to mimic the use of lemon juice. Documentation on the quality of such a brew is scanty and the effect of acid on its quality is equally scanty. The current study used brewing time/temperature combinations of 5, 10 and 15 minutes with 70˚C and 92˚C drinking water. Total monomeric anthocyanins were analysed using UV-1800 spectrophotometer (Shimadzu, Japan) while DDPH method was used to assay for antioxidant activity. Citric acid concentration of 0, 0.1% to 0.5% was used to check their effect on the brews with the highest total anthocyanins and antioxidant activity. The results showed that brewing temperature of 70˚C had 9.5 mg/L to 27.7 mg/L, with 5 minutes brewing time being the highest. Temperature of 92˚C had 37.5 mg/L to 92 mg/L with 5 minutes brewing time having the highest total monomeric anthocyanins. Antioxidant activity of 92˚C brewing temperature ranged from 90.7% to 92.0%. Total monomeric anthocyanins increased with increased citric acid concentration up to 0.3% before it decreases while for antioxidant activity, it decreased with addition of citric acid. It was concluded that for maximum anthocyanins extraction, 5 minutes brewing time is the best while ten minutes brewing time could give optimum antioxidant activity.