RESEARCH ARTICLE

Catechins and Plain Black Tea Parameters Variations in Selected Tea Growing Agro-Ecological Zones in Kenya

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Abstract

Over 60% of tea in Kenya is produced by smallholder tea farmers distributed in the East and West of the Rift Valley. With all smallholder tea farmers using the same production technologies including planting materials, the quality of tea from different agro-ecological zones should be the same. However, black teas attract different prices. This study compared levels of leaf quality, catechins, and polyphenols in green leaf and quality parameters of black tea from twelve factories in three different agro-ecological zones in Kenya and assessed the relationships between the green leaf quality parameters and black tea quality parameters. The catechins contents, the black tea quality parameters, and sensory evaluations changed ($p \leq 0.05$) between the factories and with an agro-ecological zone of production. All black teas had high antioxidant activities, which differed ($p \leq 0.05$) across the factories and zones. Despite the use of same agronomic inputs in the smallholder tea growing zones in Kenya, the quality of resultant black teas was largely influenced by the environmental factors in the different regions. It is therefore not possible to produce black teas of the same quality across the country. Despite the variations, all regions exhibited high levels of black tea quality parameters and sensory evaluation, re-affirming the Kenyan smallholder black teas are of high quality. The high antioxidant activities suggest possible beneficial pharmacological activities from consumption the teas. The green leaf quality (leaf count) were significantly ($p \leq 0.05$) correlated with black tea brightness and all sensory evaluation parameters, demonstrating high benefits from good plucking standards. Green leaf gallic acid, epigallocatechin, epicatechin, epigallocatechin gallate, and total catechins levels were related ($p \leq 0.05$) to black tea total polyphenols and all sensory evaluation parameters. In addition, the green leaf caffeine, epicatechin, epigallocatechin gallate, and total catechins influenced ($p \leq 0.05$) theaflavins in black tea. These green leaf parameters are therefore key drivers of Kenyan plain black tea quality.

Keywords: Agro-ecological zones, Black tea quality parameters, Camellia sinensis, Catechins, Kenya

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Introduction

Tea, *Camellia sinensis* (L.) O. Kuntze is an important crop in Kenya. Approximated over 76% of tea consumed globally is black tea.[1] Kenya is the third leading producer and the leading exporter of tea, mainly black tea. Quality of "Kenya tea", therefore, has major impact on the quality of black tea consumed worldwide. The tea in Kenya is grown in the east and west of the Great Rift Valley, close to the equator at altitudes between 1300 and 2700 m above sea level.[2] Despite striding the equator, these regions differ in soil characteristics and environmental parameters that influence growth.[3,4] Such factors cause changes in the biochemical composition of tea leaves and consequently the quality of resultant teas. Tea leaves contain flavan-3-ols (catechins), which are transformed into theaflavins and thearubigins during black tea processing. The theaflavins and thearubigins are responsible for the quality of plain black tea. Other green leaf components, xanthines (mainly caffeine), other polyphenols and their glycosides, amino acids, polysaccharides, lipids, and inorganic elements. Some of these components have beneficial pharmacological activities, making tea the most widely consumed fluid after water.

Over 60% of the Kenyan tea is produced by the smallholder farmers under the management of the Kenya Tea Development Agency (KTDA) Ltd. These farmers use the same agronomic and cultural technologies, including cultivars. The quality of the KTDA tea is therefore expected to be similar. However, the produced black teas attract different prices. The variations in pricing have caused many inconclusive debates. There is, therefore, need to objectively establish the possible causative factors to the price variations. This study evaluated the green leaf chemical components and black tea quality parameters from selected factories in different agro-ecological zones to determine if these parameters vary.

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Materials and methods

Tea Samples

Samples were obtained from three agro-ecological zones, Meru and Murang'a in the east and Kisii in the west of the Rift Valley, (Figure 1). Four factories (Kinoro, Kionyo, Imenti, and Githongo) from Meru; (Ngere, Kanyenya-ini, Gatunguru, and Kiru) from Murang'a and (Nyansiongo, Nyankoba, Tombe and Eberege) from Kisii were randomly selected from each of the three zones. From each factory, green leaves and CTC black tea samples were obtained.

Triplicate samples of green leaves (400 g) were randomly selected from leaf arriving in the factory. Samples were obtained when the leaf arrived in the factory at intervals of one hour between the replicates. Half of each sample (200 g) was subjected to leaf count and the other half (200 g) was heated in a microwave for two minutes to deactivate the oxidative enzymes, then cooled

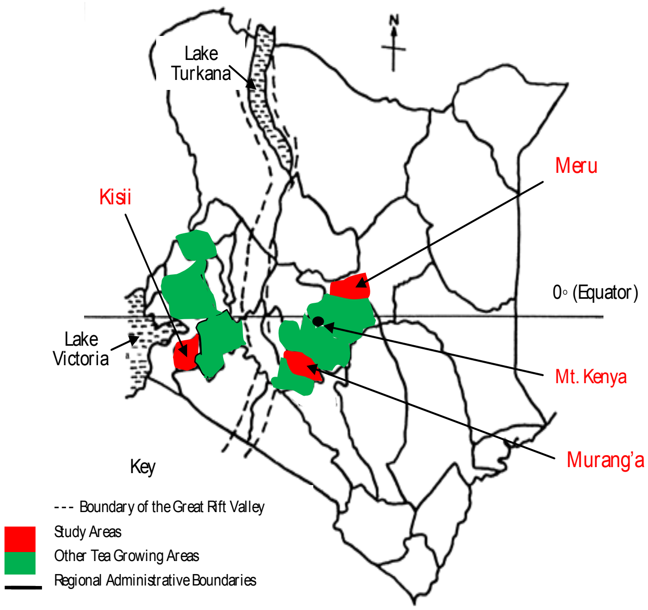

Fig. 1: Tea growing areas of Kenya and sampling sites

to room temperature. The samples were oven dried at 103 ± 2°C to a constant weight. The dry, samples were ground into a fine powder using an electric blender. The homogenous samples were subjected to various green leaf chemical parameters analyses. Drier mouth samples of black tea (500 g) were taken from each factory in triplicate. Samples were taken in a space of 1 hour between the replicates. The black tea samples were used in the determination of plain black tea quality parameters and sensory evaluations.

Leaf Quality Determination

The leaf count method was used to determine leaf quality of leaf. Approximately 100 loose green tea leaves were shuffled and sorted into different plucking standards of one leaf and a bud, two leaves and a bud, three leaves and a bud, four leaves and a bud and loose leaf. The leaf quality was expressed as a percentage of the acceptable leaf of up to two leaves and a bud.

Green Leaf Chemical Analyses

Green leaf extracts for the quantitation of polyphenols and catechins in the tea samples were obtained following the method by Zuo *et al.*[5] with slight modifications.[6] The catechins content in the samples were analyzed by reverse phase high-performance liquid chromatography (RP-HPLC) using ISO method.[7] Individual catechins were identified on the HPLC chromatogram by comparing the retention times with authentic standards that included catechin (+C), epicatechin (EC), gallocatechin (GC), epigallocatechin (EGC), epicatechin gallate (ECG) and epigallocatechin gallate (EGCG), and caffeine (Sigma Aldrich, UK). Total phenolic content was determined using Folin–Ciocalteu's phenol reagent.[8,9]

Black tea chemical analyses and sensory evaluations

The total theaflavin content of the tea samples was assayed using Flavognost method.[10] The total thearubigins content, total, coluor, and brightness of the tea samples were determined using the Roberts method.[11] The free radical scavenging activity of the black CTC tea extracts was determined by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical assay,[12] with slight modifications. Five (5.0) g of tea was infused in 100 mL of boiling distilled water, stirred with a magnetic stirrer and steeped for 30 minutes at room temperature. The extracts were strained through a nylon mesh (120 µm) followed by a filter paper (Whatman No. 54). Aliquots of the extract were frozen at –18 ± 2°C until further use. The soluble solid extracts were standardized to give stock solutions of 50 mg soluble solids per 100ml of 50% aqueous methanol. A 50 µL methanolic solution of tea sample was placed in a curvette and 2.0 mL of 6.0×10^{-5} M of 80% methanolic solution of DPPH added. The decrease in absorbance at 517 nm was determined using a UV-Vis spectrophotometer (UV-1800 ENG 240V Soft, Shimadzu, Japan) until the absorbance stabilized. Readings were recorded at between 15 and 30 minutes interval until the reaction reached a plateau phase. The percentage inhibition of the DPPH radical was then estimated. The % inhibition against DPPH= (AB-AA)/ (AB/100)

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

The drier mouth CTC black tea samples were randomly numbered and subjected to blind organoleptic evaluation by two professional tea tasters from a tea broking firm in Mombasa, Kenya. The tea liquors were assessed for brightness, briskness, strength, aroma each on a scale of 0–12 and overall quality as a summation of the individual parameters.

Statistical Analysis

Data obtained from the various analyses and sensory evaluations were subjected to analysis of variance (ANOVA) using MSTAT C statistical package.

Results

Green Leaf Quality

Results of the assessment of the quality of leaf delivered to the different factories for the processing of black CTC tea are presented in Table 1. The mean leaf count obtained of good leaf were not significantly different ($p \leq 0.05$) between the agro-ecological zones. Although there were significant ($p \leq 0.05$) differences in leaf quality between the factories, within the same agro-ecological zones the levels were not different. Farmers in the Murang'a and Meru agro-ecological zones delivered leaf of better quality indicative of superior plucking standards.

Green Leaf Chemical Parameters

The analyses of the green leaf samples showed the presence of epigallocatechin, catechin, epicatechin, caffeine, epigallocatechin gallate and epicatechin gallate (Table 1). The levels of the parameters differed ($p \leq 0.05$) across the zones. Generally the total and individual catechins were lower ($p \leq 0.05$) in Kisii than in Murang'a and Meru. The mean caffeine levels in Murang'a and Kisii agro-ecological zones were higher ($p \leq 0.05$) than the levels in Meru zone. The individual factories differed ($p \leq 0.05$) in their levels of total catechins, individual catechins, and caffeine.

Black tea quality parameters

The variations in the black tea quality parameters are presented in Table 2. The total polyphenols, theaflavins, brightness, and anti-oxidant activities were higher ($p \leq 0.05$) in Murang'a black teas than Meru and Kisii black teas. However, the thearubigins and total color levels were not different across the agro-ecological zones. The order was Murang'a > Meru > Kisii. The black tea quality parameters varied ($p \leq 0.05$) with factories. The changes were not, however, systematic and were not wholly dependent on agro-ecological zones of

Table 1: Variations in leaf quality and green leaf precursor chemical quality parameters of leaf delivered to the factories with agro-ecological zones and factories

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

#, means within a column preceded with the same superscript letter(s) are not statistically significantly different ($p > 0.05$); NS, not significant; LQ, leaf quality; GA, gallic acid; EGC, epigallocatechin gallate; C, catechin; Caff, caffeine; EC, epicatechin; EGCG, epigallocatechin galllate; ECG, epicatechin gallate; TCat, total catechins

Table 2: Changes in total polyphenols, total theaflavins, total thearubigins, total color, liquor brightness and antioxidant capacity levels in CTC black tea with agro-ecological areas and factories

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

#, means within a column preceded with the same superscript letter(s) are not statistically significantly different ($p > 0.05$); NS, not significant; TP, total polyphenols; TF, total theaflavins; TR, total thearubigins; TCol, total colour; Brigt, liquor brightness; AC, antioxidant capacity

Table 3: The influence of agro-ecological zone of production and factories on sensory evaluation of CTC black teas

Agro-zone	*Factory*	*Aroma*	*Briskness*	*Brightness*	*Strength*	*Taster's score*	*Ranking*
Murang'a	Ngere	10.0 ± 1.0^{ab}	10.3 ± 1.2^{a}	9.7 ± 1.2^{a}	10.3 ± 1.2^{a}	40.3 ± 0.3^{ab}	3
	Kanyenyaini	11.0 ± 0.0^{a}	10.3 ± 1.2^{a}	9.7 ± 1.5^{a}	10.7 ± 0.6^{a}	41.7 ± 0.6^{ab}	2
	Gatunguru	11.0 ± 0.0^{a}	11.0 ± 0.0^{a}	11.0 ± 0.0^{a}	11.0 ± 0.0^{a}	44.0 ± 0.0^{a}	1
	Kiru	9.3 ± 1.5^{bc}	10.7 ± 0.6^{a}	10.0 ± 1.0^{a}	9.7 ± 1.2^{ab}	39.7 ± 0.6^{b}	4
	Mean	10.3 ± 0.8	10.6 ± 0.3	10.1 ± 0.6	10.4 ± 0.6	41.4 ± 1.9	
Meru	Kinoro	5.0 ± 1.0^{ef}	5.3 ± 1.5^{cd}	5.3 ± 1.5^{cd}	6.0 ± 1.0^{c}	21.7 ± 0.4^{de}	7
	Kionyo	5.3 ± 0.6^{de}	5.0 ± 1.0^{de}	5.3 ± 0.6^{cd}	6.0 ± 1.0^{c}	21.7 ± 0.6^{de}	7
	Imenti	4.0 ± 8.7^{f}	3.5 ± 0.5^{ef}	5.5 ± 1.3^{cd}	4.8 ± 1.8^{c}	17.8 ± 0.9^{e}	10
	Githongo	8.7 ± 1.8^{c}	7.3 ± 1.8^{b}	7.5 ± 1.0^{b}	8.5 ± 1.3^{b}	32.0 ± 0.7^{c}	5
	Mean	5.8 ± 2.0	5.3 ± 1.6	5.9 ± 1.1	6.3 ± 1.6	23.3 ± 6.1	
Kisii	Nyansiongo	6.3 ± 0.8^{d}	7.0 ± 1.3^{bc}	7.0 ± 1.3^{bc}	4.8 ± 0.6^{c}	25.2 ± 1.0^{d}	6
	Nyankoba	5.2 ± 1.3^{def}	4.8 ± 0.8^{de}	5.0 ± 1.0^{d}	5.3 ± 1.5^{c}	20.3 ± 0.2^{e}	9
	Tombe	2.7 ± 0.6^{g}	3.0 ± 0.0^{f}	1.7 ± 1.2^{e}	3.0 ± 0.0^{d}	10.3 ± 0.6^{f}	11
	Eberege	1.2 ± 0.3^{h}	1.0 ± 0.0^{g}	3.0 ± 0.0^{e}	1.5 ± 0.9^{d}	6.7 ± 0.9^{f}	12
	Mean	3.8 ± 2.4	4.0 ± 2.6	4.2 ± 2.3	3.7 ± 1.7	15.6 ± 8.6	
Coefficient of variation (%)		11.6	15.5	15.3	15.2	9.2	
LSD ($p = 0.05$)	Agro-zone	1.4	1.8	1.8	1.8	4.3	

#, means within a column preceded with the same superscript letter(s) are not statistically significantly different ($p > 0.05$); NS, not significant

Table 4: The relationships (r) be black tea chemical quality parameters and sensory evaluations with green leaf characteristics

	Parameter	*Leaf quality (%)*	*Gallic acid (%)*	*Epigallocatechin (%)*	*Cactechin (%)*	*Caffeine (%)*	*Epicatechin (%)*	*Epigallocatechin gallate (%)*	*Epicatechin gallate (%)*	*Total catechins (%)*
Black tea chemical parameters	Total polyphenols (%)	0.53	0.75** .	0.72*	0.50	0.67*	0.82**	0.78**	0.52	0.77**
	Theaflavins (%)	0.58	0.59	0.58	0.12	0.68*	0.74*	0.61*	0.18	0.60*
	Thearubigins (%)	0.27	0.07	0.20	–0.11	0.50	0.34	0.03	-0.18	0.19
	Total colour (%)	0.16	0.08	0.21	–0.15	0.60*	0.28	-0.01	-0.34	0.16
	Brightness (%)	0.68*	0.36	0.43	0.23	–0.14	0.35	0.40	-0.10	0.39
	Antioxidant activity (%)	0.11	0.22	0.56	0.77*	–0.12	0.35	0.22	0.71	0.55
Sensory evaluative	Aroma	0.69*	0.42	0.67*	0.37	0.22	0.79**	0.82**	0.35	0.72*
	Briskness	0.64*	0.43	0.69*	0.39	0.23	0.78**	0.79**	0.38	0.72*
	Brightness	0.70*	0.35	0.65*	0.33	0.01	0.75**	0.77**	0.40	0.69*
	Strength	0.73**	0.46	0.73**	0.41	0.16	0.80**	0.79**	0.37	0.76**
	Taster's score	0.70*	0.42	0.70*	0.38	0.16	0.79**	0.81**	0.38	0.73**

production. The black tea aroma, briskness, brightness, strength and total evaluations (Table 3) were higher ($p \leq 0.05$) for Murang'a zone black teas. The Meru zone black teas were also evaluated higher ($p \leq 0.05$) than Kisii zone black teas. There were significant ($p \leq 0.05$) variations in sensory evaluations of black teas from different factories within a single agro-ecological zone and across the zones.

The relationships between green leaf parameters and black tea quality parameters are presented in Table 4. The black tea sensory evaluation parameters were significantly ($p \leq 0.05$) associated with green leaf quality (plucking standards), epigallocatechin, epicatechins, epigallocatechin gallate, and total catechins. Theaflavins were related ($p \leq 0.05$) to levels of caffeine, epicatechin and epigallocatechin gallate. The total phenolic components of black tea were related ($p \leq 0.05$) to gallic acid, epigallocatechin, caffeine, epicatechin, epigallocatechin gallate, and total catechins levels. The total color was associated ($p \leq 0.05$) with caffeine level. The black tea brightness was associated ($p \leq 0.05$) with leaf quality, while the anti-oxidant activity related ($p \leq 0.05$) to catechin level. Surprisingly, there was no green leaf parameter that was significantly ($p \leq 0.05$) associated with thearubigins levels.

Discussion

Variations in tea quality in Kenya due to the geographical area of production had been reported in previous studies.[14-16] Generally, smallholder tea factories from the east of the Rift Valley have realized better tea prices than the factories in the west of the Rift Valley. These had been attributed to climatic differences. Quality of tea depends on several factors including cultivars and climatic conditions. The climatic factors influence biochemical pathways that ultimately change the chemical composition and quality tea.[17] The three agro-ecological areas under study, *viz.,* Kisii, Murang'a and Meru differ in a number of ways including temperatures, rainfall total, and distribution, agronomic practices, and altitudes.

The good quality leaf is regarded as one that constitutes over 70% acceptable leaf of up to two leaves and a bud.[18,19] All the factories produced leaf above this standard. Leaf quality is largely a reflection of plucking standards. Coarse plucking standards reduce black tea quality,[20-25] due to a decline in catechin levels[26] and changes in polyphenol oxidase iso-enzymes composition and activities.[27] These reduce the levels of total theaflavins[15,22-25] in black tea. Also, coarse plucking standards increased levels of unsaturated fatty acids in the leaf[15] resulting in the production of less aromatic black teas.[25] Apart from plucking standards, harvesting rounds also influence black tea quality.[14,15,21,24,28] Discussion with factory personnel revealed that whereas farmers in the Murang'a and Meru agro-zones were plucking between three to four rounds per month, Kisii farmers harvested two rounds per month. Despite using identical plucking standard, the quality of resultant black tea could vary as a previous study two leaves and a bud from long plucking interval produced inferior black tea compared to that from short plucking interval.[14,24,28] Although the leaf quality was slightly lower in Kisii zone than Murang'a and Meru zones, the difference was insignificant. The variations in quality observed could, therefore, be due to differences in plucking rounds. Factors that affect the growth rate of the tea plant normally lead to variations in biochemical composition and quality of black tea.[29] One such factor is the altitude.[21,30] Kisii in the west of the Rift Valley has on an average lower altitude ranging from 1500 to about 1800 m above mean sea level (amsl). Also, Kisii has mean ambient temperatures average between 20 and 25°C at lower altitudes. Rainfall in this area is mainly conventional due to proximity to Lake Victoria, and is well distributed throughout the year resulting in evenly distributed tea production but with peaks between April and May and October and November.[3] Murang'a and Meru both in the east of Rift Valley are in close proximity to Mt Kenya, Aberdare highlands and Nyambene Hills at altitudes above 1800 m amsl. Temperatures in the two zones are usually low especially between July and September, (locally referred to as the "Kathano"). During the period suppressed growth rates of tea shoots are observed. Although rainfall is relief type, Murang'a is on the windward side while Meru is on the leeward side of Mt Kenya making the two areas ecologically different. With relatively lower altitudes, warmer temperatures and evenly distributed rainfall, the growth rate of tea in Kisii are expected to be faster compared to Murang'a and Meru. Therefore the variations noted in biochemical composition of teas grown and processed in these zones would be attributed to the difference in environmental conditions prevalent in the three ecological zones. Similar variations had been observed in the green leaf[31-34] and black tea[14,15,28,35,36] chemical parameters in clonal tea in Kenya. The results demonstrate that despite the use of the same agronomic and cultural practices across the smallholder tea farming system in Kenya, it may not be possible to produce black tea of exactly the same quality. Such variations can be large in the teas produced in locations further away from each other.[37,38]

The catechin, polyphenol and caffeine profiles tea of leaves had been proposed for use to classify the geographic origin of the teas[39] (Eetu Makela., 2012). In this study, there were variations in individual catechins and black tea parameters with both agro-ecological zones of production and factories. The individual catechins and total theaflavins were lower in Kisii zone and factories, while caffeine levels in Murang'a and Kisii zones were higher than levels in Meru zone. However, the data did not present a clear pattern that can be used in the geographic origin classification of the teas.

Antioxidant Activity

There were high anti-oxidant activities in the black teas across the zones. The activities were higher in Murang'a and Kisii than in Meru black teas. Several studies have associated the antioxidant activities in tea to presences of polyphenols,[40,41] the polyphenols, especially catechins in green leaf[40,42,43] and theaflavins in black tea.[41,44,45] Indeed, the black tea theaflavins have higher antioxidant activity than epigallocatechin gallate, the strongest antioxidant among catechins.[44] High levels of residual catechins were observed in the Kenyan black tea,[46] suggesting positive benefits of the activities of catechins in drinking the Kenyan black teas. The results presented in this study further demonstrate the benefits of accruing from black tea consumption.

Relationship between Green Leaf Parameters and Black Tea Quality Parameters

The quality of black tea is dependent on processing conditions and the quality of green leaf used in its manufacture. In black tea processing, good green leaf quality is thought to be a precursor to high black tea quality.[32-34] This situation is observed when processing conditions and leaf handling are optimal. The good quality leaf is usually defined as plucked leaf comprising of over 70% two leaves and a bud.[18,19] Several studies have shown black tea quality improvement with fine plucking standards. Few studies have related the green leaf components to the black tea quality parameters.[47,48] Surprisingly, there was no green leaf parameter that was related to black tea thearubigins. The significant ($p \leq 0.05$) relationship between the leaf quality (plucking standard) with all sensory evaluation parameters demonstrates the importance of fine plucking standards to good black tea quality. Similarly, the significant ($p \leq 0.05$) relationship between black tea sensory evaluations with epigallocatechin, epicatechins, epigallocatechin gallate, and total catechins demonstrated the importance of these parameters in the green leaf to black tea quality. These results re-affirm the earlier observations that these catechins are the critical plain black tea quality precursors.[47-49] The theaflavins levels were significantly ($p \leq 0.05$) related to greenleaf epicatechin and epigallocatechin gallate. The epicatechins had been demonstrated to be key black tea precursor in Central African black teas,[48, 49] while epigallocatechin gallate was critical for Kenyan black tea.[49,50] These results demonstrate the need to use agronomic and cultural practices that promote the production of high levels of catechins in the green leaf to produce high-quality black tea.

In conclusion, no two factories or regions had a green leaf or black tea parameters of identical levels. It is therefore expected that prices of the black teas from different agro-ecological zones and factories will vary. Despite the variations, the smallholder factories produced high-quality black teas with high antioxidant properties. Such teas should attract good prices. Plucking leaf of good quality was demonstrated to be key to the production of high-quality black teas. Such tea was superior when the plucking intervals were short. Efforts should be directed in shortening harvesting intervals in areas where the intervals are still long.

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References

1. Cabrera C, Giménez R and López MC (2003), Determination of tea components with antioxidant activity. *Journal of Agricultural and Food Chemistry* 51:4427-4435.
2. TRFK., *Tea Research Foundation of Kenya* (2002). *Tea Grower's Handbook*. Kericho, Kenya.
3. Ng'etich WK, Stephens W and Othieno CO (2001), Responses of tea to environment in Kenya. 3. Yield and yield distribution. *Experimental Agriculture* 37:361-372.
4. Carr MKV and Stephens W (1992), Climate weather and the yield of tea in *Tea Cultivation to Consumption*, ed. by Willson KC and Clifford MN. Chapman and Hall, London, pp 87-135.
5. Zuo Y, Chen H and Deng Y (2002), Simultaneous determination of catechins, caffeine and gallic acids in green, oolong, black and pu-erh teas using HPLC with a photodiode array detector. *Talanta* 57:307–316.
6. Kerio LC, Wachira FN, Wanyoko JK and Rotich MK (2013), Total polyphenols, catechin profiles and antioxidant activity of tea products from purple leaf coloured tea cultivars. *Food Chemistry* 136:1405-1413.
7. ISO, ISO 14502-2-2005E. Determination of substances characteristic of green and black tea. Part 2 (2005). Contents of catechins in green tea: method using High Performance Liquid Chromatography, Ed.
8. ISO, ISO 14502–1-2005E: Tea; Methods for determination of substances characteristic of green and black tea. Part 1 (2005), determination of total polyphenols in tea: Colorimetric method using High Performance Liquid Chromatography, Ed.
9. Pourmorad F, Hosseinimehr SJ and Shahabimajd N (2006), Antioxidant activity, phenol and flavonoid contents of some selected Iranian medicinal plants. *African Journal of Biotechnology* 5.
10. Hilton PJ (1973), Tea, in *Encyclopaedia of Industrial Chemical Analysis*, ed. by Snell FD and Ettre LS. John Wiley and Sons Inc, New York, pp 455–518.
11. Roberts EAH and Smith RF (1961), Spectrophotometric determination of theaflavins and thearubigins in black tea liquors in assessments of quality in tea. *The Analyst* 86:94–98.

12. Brand-Williams W, Cuvelier M-E and Berset C (1995), Use of a free radical method to evaluate antioxidant activity. *LWT-Food science and Technology* 28:25-30.
13. Yen GC and Duh PD (1994), Scavenging effect of methanolic extracts of peanut hulls on free radical and active oxygen species. *Journal of Agriculture and Food Chemistry* 42:629–632.
14. Owuor PO, Kamau DM and Jondiko EO (2009), Response of clonal tea to location of production and plucking intervals. *Food Chemistry* 115:290-296.
15. Owuor PO, Kamau DM, Kamunya SM, Msomba SW, Uwimana MA, Okal AW and Kwach BO (2011), Effects of genotype, environment and management on yields and quality of black tea, in *Genetics, Biofuels and Local Farming Systems: Sustainable Agriculture Reviews*, ed. by Lichtfouse E. Springer, Heidelberg, pp 277-307.
16. Owuor PO and Othieno CO (1991), Response of black tea quality to locality in smallholder tea farms. *Tea* 12:41-45.
17. Kottur G, Venkatesan S, Kumar R and Murugesan S (2010), Influence of seasons on biochemical parameters of green shoots and quality parameters of made tea under south Indian conditions. *Journal of Bioscience Research* 1:74-82.
18. Owuor PO and Obanda M (1995), Clonal variation in the individual theaflavin levels and their impact on astringency and sensory evaluations. *Food Chemistry* 54:273-277.
19. Owuor PO and Obanda M (1999), The effects of blending clonal leaf on black tea quality. *Food Chemistry* 66:147-152.
20. Botheju WS, Abeysinghe ISB and Illeperuma CK (2001), Effect of plucking standard on quality and profitability of made tea produced in the up country Dimbulla region during the cropping season. *Tropical Agricultural Research* 13:90–99.
21. Mahanta PK, Baruah S, Owuor PO and Murai T (1988), Flavour volatiles of Assam black teas manufactured from different plucking standards and orthodox teas manufactured from different altitudes of Darjeeling. *Journal of the Science of Food and Agriculture* 45:317-324.
22. Obanda M and Owuor PO (1995), Clonal variations in the response of black tea quality due to plucking standards. *Food Chemistry* 53:381-384.
23. Obanda M, Owuor PO and Wekesa H (2002), The influence of clone and plucking standards on the formation of black tea chemical and sensory parameters. *Tea* 23:81-90.
24. Owuor PO, Ng'etich WK and Obanda M (2000), Quality response of clonal black tea to nitrogen fertilisers, plucking intervals and standards. *Journal of the Science of Food Agriculture* 80:439-446.
25. Owuor PO, Obanda MA, Othieno CO, Horita H, Tsushida T and Murai T (1987), Changes in chemical composition and quality of black tea due to plucking standards. *Agricultural and Biological Chemistry* 51:3383-3384.
26. Forrest GI and Bendall DS (1969), The distribution of polyphenols in the tea plant (*Camellia sinensis* L.). *Biochemical Journal* 113:741-755.
27. Őlmez H and Yilmaz A (2010), Changes in chemical constituents and polyphenol oxidase activity of tea leaves with shoot maturity and cold storage. *Journal of Food Processing and Preservation* 34:653-665.
28. Owuor PO, Kamau DM, Kamunya SM, Msomba SW, Jondiko EO and Uwimana MA (2013), The response of clone BBK 35 tea to nitrogen fertilizer rates and harvesting intervals in the Lake Victoria Basin of Kenya. *Journal of Agriculture, Food and Environment* 11:757-763.
29. Robertson AR (1983), Effects of catechin concentration on the formation of black tea polyphenols during *in vitro* oxidation. *Phytochemistry* 22:897-903.
30. Owuor PO, Obaga SO and Othieno CO (1990), The effects of altitude on the chemical composition of black tea. *Journal of the Science of Food and Agriculture* 50:9-17.
31. Cherotich L, Kamunya SM, Alakonya A, Msomba SW, Uwimana MA and Owuor PO (2014), Genotypic stability and adaptability of tea cultivars in relation to catechin levels across four environments. *Tea* 35:8-16.
32. Cherotich L, Kamunya SM, Alakonya A, Msomba SW, Uwimana MA, Wanyoko JK and Owuor PO (2013), Variation in catechin composition of popularly cultivated tea clones in East Africa (Kenya). *American Journal of Plant Sciences* 4:628-640.
33. Kwach BO, Owuor PO, Kamau DM, Wanyoko JK and Kamunya SM (2013), Influence of location of production, season and genotype on caffeine and flavan-3-ols in young green leaves of tea (*Camellia sinensis*) leaves in Kenya. *Journal of Agricultural Science and Technology* B3:557-574.
34. Kwach BO, Owuor PO, Kamau DM, Msomba SW and Uwimana MA (2016), Variations in the precursors of plain black tea quality parameters due to location of production and nitrogen fertilizer rates in Eastern African clonal tea leaves. *Experimental Agriculture* 52:266–278.
35. Owuor PO, Wachira FN and Ng'etich WK (2010), Influence of region of production on relative clonal plain tea quality parameters in Kenya. *Food Chemistry* 119:1168-1174.
36. Owuor PO, Kamau DM and Jondiko EO (2010), The influence of geographical area of production and nitrogenous fertiliser on yields and quality parameters of clonal tea. *Journal of Food Agriculture & Environment* 8:682-690.
37. Fernández PL, Pablos F, Martín MJ and González AG (2002), Study of catechin and xanthine tea profiles as geographical tracers. *Journal of Agricultural and Food Chemistry* 50:1833-1839.
38. Owuor PO, Obanda M, Nyirenda HE and Mandala WL (2008), Influence of region of production on clonal black tea characteristics. *Food Chemistry* 108:263-271.
39. McDowell I, Taylor S and Gay C (1995), The phenolic pigment composition of black tea liquors: part II: Discriminating origin. *Journal of the Science of Food and Agriculture* 69:475-480.
40. Amić D, Davidović-Amić D, Beš¡lo D and Trinajstić N (2003), Structure-radical scavenging activity relationships of flavonoids. *Croatica Chemica Acta* 76:55-61.
41. Frei B and Higdon JV (2003), Antioxidant activity of tea polyphenols in vivo: evidence from animal studies. *The Journal of Nutrition* 133:3275S-3284S.
42. Rao T, Lekh R and Takado Y (2006), Green tea catechins against oxidative stress of renal diseases, in *Protective Effects of Tea on Human Health*, ed. by Jain NK, Siddiqi M and Burger JW. CAB, London, pp 109-119.
43. Zhu N, Wang M, Wei GN, Lin J, Yang S and Ho I (2001), Identification of reaction products of epigallocatechin, epigallocatechingallate and pyragallol with 2,2-diphenyl-1-picrylhydrazylradical. *Food Chemistry* 73:345 -349.
44. Leung LK, Su Y, Chen R, Zhang Z, Huang Y and Chen Z-Y (2001), Theaflavins in black tea and catechins in green tea are equally effective antioxidants. *The Journal of nutrition* 131:2248-2251.
45. Miller NJ, Castelluccio C, Tijburg L and Rice-Evans C (1996), The antioxidant properties of theaflavins and their gallate esters - radical scavengers or metal chelators? *FEBS letters* 392:40-44.
46. Obanda M, Owuor PO and Taylor SJ (1996), Chemical composition of some Kenyan black teas and their probable benefits to human health. *Tea* 17:20-26.
47. Obanda M, Owuor PO and Taylor SJ (1997), Flavanol composition and caffeine content of green leaf as quality potential indicators of Kenyan black teas. *Journal of the Science of Food and Agriculture* 74:209-215.
48. Wright LP, Mphangwe NIK, Nyirenda HE and Apostolides Z (2000), Analysis of caffeine and flavan-3-ol composition in the fresh leaf of *Camellia sinesis* for predicting the quality of the black tea produced in Central and Southern Africa. *Journal of the Science of Food and Agriculture* 80:1823-1830.
49. Owuor PO, Obanda M, Nyirenda HE, Mphangwe NIK, Wright LP and Apostolides Z (2006), The relationship between some chemical parameters and sensory evaluations for plain black tea (*Camellia sinensis*) produced in Kenya and comparison with similar teas from Malawi and South Africa. *Food Chemistry* 97:644-653.
50. Owuor PO and Obanda M, The use of green tea (*Camellia sinensis*) leaf flavan-3-ol composition in predicting plain black tea quality potential. *Food Chemistry* 100:873-884 (2007).