RESEARCH ARTICLE

Influence of Geographical Area of Production on the Caffeine and Flavan-3-ol Profiles of Selected Clonal Green Tea Leaves from Smallholder Tea Farms in Kenya

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Abstract

Despite the use of similar cultivars in the smallholder tea sector in Kenya, resultant black tea quality varies. These variations could in part be arising from the formation of varying quantities and ratios of the black tea quality precursor compounds with agro-ecological zones of production. This study evaluated the variations in the caffeine and flavon-3-ol profiles of three cultivars (SFS 150, TRFK 31/8 and TRFK 303/577) of tea from smallholder tea farms in three agro-zones in Kenya. Overall, there were significant variations ($p \leq 0.05$) in the levels of caffeine and flavan-3-ols due to cultivars and agro-ecological zones of production. In the same cultivar, the components varied ($p \leq 0.05$) with an ecological area of production. The patterns in the changes were not systematically leading significant ecological zone x cultivar interactions effects in gallic acid, catechin, and epicatechin gallate. This demonstrated that it may not be predictable how the quality of clonal tea may vary when produced in different agro-ecological zone. Consequently, a high-quality clone in one agro-ecological zone may not replicate the same characteristics in the different agro-ecological zone. It is, therefore, necessary to test new clones in new environments before they are extensively exploited in these environments. The current findings suggest that flavon-3-ols may not be potential factors in the discrimination of tea quality within the ecological zones of Kenya.

Keywords: *Agro-ecological zones, Camellia sinensis*, Clonal variations, Flavan-3-ols

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Introduction

The tea plant, *Camellia sinensis* (L.) O. Kuntze, contains a bioactive polyphenols,[1] mainly the catechins, which influence the quality of black tea product.[2] Other active components include the alkaloids (theobromine, caffeine, theophylline),[3,4] amino acids, polysaccharides, lipids, and inorganic salts.[5-7]

Tea is an important economic crop generating income, employment and structural development.[8,9] The tea growing areas in Kenya are broadly sub-divided into two regions defined by the Great Rift Valley. In the east of the Rift Valley tea is grown around the Aberdare Highlands, Mt. Kenya regions and Nyambene Hills, while in the west of the Rift Valley tea is grown in Nandi Hills, Kericho, Mt. Elgon, and Kisii Highlands. Over 60% of Kenyan tea is produced by about 300,000 smallholder tea farmers managed by the Kenya Tea Development Agency Ltd (KTDA). These farmers live in rural areas where economic opportunities are rare.[10] The rest of the tea is produced by the estate's sector. Tea is a source of livelihood to over 5 million Kenyans.[11] In terms of economic contribution, tea is the country's leading export earner, accounting for 4% GDP and 20% total export earnings.[12] Smallholder (KTDA) black teas are known for high quality.[13,14] However, despite the use of uniform agronomic and cultural practices,[15] there are differences in the prices that the tea fetch. Often the prices seem to follow geographical patterns. Although the tea growing areas stride the equator, the regions differ in soil characteristics and environmental factors that influence growth[16] and biochemical composition[17] of tea leaves. These factors include rainfall amounts and distribution and temperatures. The rainfall in the west of the Rift Valley is largely influenced by close proximity to Lake Victoria, the largest inland water mass in Africa. The region receives mainly conventional rain that is well distributed throughout the year, although there are peaks in March-May and September-October.[18] In the east of the Rift Valley, rainfall is relief type and is controlled by proximity to Mt Kenya and Aberdare Highlands. Again rainfall is bimodal peaking between March–May and September-October.[15] The areas in the west of the Rift Valley tend to be warmer due to relatively low altitudes. 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 tends to be faster than 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 in the three ecological zones.

In recent times, tea prices have stagnated and at times reduced.[14] This has been against the background of increased costs of production and farm inputs resulting in decreased profitability in tea production. Small-scale tea farmers in different tea growing zones in the country face financial constraints that in the long term shall threaten the tea farming enterprise. The problem is exacerbated

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by the fact that within the same sector farmers get differing rates of income from tea sales, making regions with low rates contemplating venturing into alternative activities. Although the differences in prices have been explained in terms of differences in quality.[19,20] the objectivity of the explanation has been questioned by tea farmers. To offer further objective explanation there is need to assess and ascertain the variations in the biochemical compositions in tea leaves due to ecological zones and illustrate how the differences (if any) may affect the quality and therefore pricing. The objective of this study was to determine the influence of clones and geographical area of production on caffeine and flavan-3-ol profiles in tea leaf. Caffeine and flava-3-ols are the major green tea leaf precursors that are responsible for plain black tea quality.

Materials and methods

Green Leaf Tea Samples

Leaf (two leaves and a bud) were collected from three randomly selected KTDA managed factory catchments: Imenti, and Nyankoba, in three different tea agro-ecological zones in Kenya[5] of Murang'a, Meru and Kisii respectively. Three farms with requisite clones were randomly selected form each factory catchments. Farms selected had three popular tea clones (TRFK 31/8, SFS 150 and TRFK 301/577). Each farm represented a replicate. From each farm, 2000g green young shoots comprising of over at least 75% two leaves and a bud were collected. The samples were appropriately labeled and transported to the laboratory in a cool ice-box at 0°C where they were immediately microwave-dried to stop any oxidative enzymatic activity. The dry samples were ground into a fine homogenous powder using an electric blending device (Moulinex AR 1043, China). Representative samples were drawn from the crushed leaf and subjected to various analyses.

Determination of Dry matter content

Before each analysis, the dry matter contents of the ground tea samples were determined as described earlier.[21] Briefly, 15 ± 0.001 g of each ground tea sample was accurately weighed using a digital analytical balance (BL-3200 HL, Shimadzu, Japan) in separate, clean, moisture-free pre-weighed dishes and oven-dried (Memmert, 854 Schwabach, Germany) at 103 ± 2°C for at least 16 hours to constant weight. The percent dry matter in each sample was then calculated from the weight differences. These values were used in the calculation of the caffeine and flavan-3-ol contents on a dry weight basis.

Standards and Reagents

Authentic catechin standards [(+)-catechin (C), (–)-epicatechin (EC), epigallocatechin (EGC), (–)-epicatechin gallate (ECG) and (-)-epigallocatechin gallate (EGCG)] and caffeine (Caff.) were purchased from Sigma Aldrich, Germany. All chemicals used (methanol (≥ 99.5 %), ethylenediaminetetraacetic acid (EDTA, ≥ 99.1 %), acetonitrile (HPLC grade), ascorbic acid (≥ 99.0 %) were "Analar" grade. Nitrogen "white spot" gas (≥ 99.9 %) was used. Double distilled water was used in all dilutions.

Extraction of Caffeine and flavan-3-ols

Extracts used for the quantitation of polyphenols and catechins in the tea samples were obtained following the method by Zou et al.[22] with slight modifications [23]. Each milled tea sample (0.2 ± 0.001 g) was weighed into a separate 10 mL graduated extraction tube. Five (5.0) mL of a 70% aqueous methanol mixture at of 70°C, was dispensed into the extraction tubes using a bottle top dispenser (Stuart BD10, Bibbypet, Germany), stoppered and mixed using a vortex mixer (VM-1000, Digisystem Laboratory Instruments Inc., Taiwan). The extraction tubes were then incubated in a water bath (GFL, Typ. 1013, Germany) at 70°C for 10 minutes and vortexed at the 5th and 10th minutes, respectively. The tubes were removed from the water bath, allowed to cool, then centrifuged for 10 minutes at 3500 revolutions per minute (rpm), using a digital high-speed universal centrifuge (HSCEN-204, M.R.C Ltd., Israel) fitted with a rotor (RA-1512S, M.R.C Ltd., Israel). The supernatant was decanted into a graduated tube and the sediment subjected to a second extraction, as above; the extracts were then combined and made up to 10 mL with cold, 70% methanol extraction mixture and thoroughly mixed using a vortex mixer.

Chromatographic analysis of caffeine and flavan-3-ols

The caffeine and flava-3-ol content in the tea samples were quantified using reverse-phase high-performance liquid chromatography (RP-HPLC).[24] Methanolic extract (1.0 mL) obtained in the previous section was diluted to 5.0 mL with a stabilizing solution comprising of 10 % v/v acetonitrile, 500 μgmL^{-1} EDTA and 10 mg mL^{-1} ascorbic acid in the ratio 2:1:1, diluted five times. The solution was filtered through a 0.45 µm nylon membrane filter and put in sample vials.

The RH-HPLC set up comprised of a Shimadzu LC 20 AT HPLC fitted with a SIL 20A auto-sampler, an SPD-20 UV-Visible detector, a class LC10 chromatography workstation and a Gemini C_6 ODS column, 250 mm × 4.6 mm i.d. (Phenomenex, Inc. Torrance CA, USA), fitted with a Gemini C_6 ODS, 4.0 mm × 3.0 mm i.d. (Phenomenex, Inc. Torrance CA, USA) guard column. The binary gradient elution solutions and conditions were as follows; 100 % of mobile phase A comprising of acetonitrile/acetic acid/bidistilled water (8:2:90, v/v/v) for 10 min, then over 15 min, a linear gradient to 68 % mobile phase A and 32% mobile phase B comprising of acetonitrile/acetic acid/bidistilled water (80:2:18, v/v/v) and held at this composition for 10 min. The condition was then reset to 100 % mobile phase A and allowed to equilibrate before the next injection. The flow rate, injection volume, column temperature, and λ_{max} were 1.0 $\mu Lmin^{-1}$, 20 µL, 35 ± 0.5 °C and 278 nm respectively.

Individual flavan-3-ol fractions were identified by comparing the retention times of the sample solution peaks against those obtained from authentic falavan-3-ol standards. Catechin and caffeine quantitation was done using a caffeine calibration curve ($r^2 = 0.9992$), together with the consensus relative response factors (RRFs) with respect to caffeine on a dry matter basis. Caffeine content was quantified as follows:

$$\%\ \text{Caff} = \frac{(A - B) \times \text{RRFstd} \times V \times d \times 100}{S \times m \times 1000 \times DM}$$

Where A is the peak area of the individual component in the test sample, B is the peak area at the point of interception on Y-axis, S is the slope of the caffeine calibration curve, V is the sample extraction volume, d is the dilution factor, m is the mass in g of test sample and DM is the dry matter content of test sample earlier determined.

Data Analysis

Data obtained were subjected to a three-way analysis of variance (ANOVA) using MSTAT statistical package version 2.10. The least significant difference (LSD, $p \leq 0.05$) test was used in mean separation.

Results and discussion

The order of elution of the analytes was; gallic acid, epigallocatechin, catechin, caffeine, epicatechin, epigallocatechin gallate, and

Table 1: Changes (% dry weight) in gallic acid, caffeine, individual flavan-3-ol and the total flavan-3-ol content in young fresh green tea shoots in the three clones of tea obtained from the three agro-zones (± the standard deviation, M ± SD[#])

Agro-zone	*Clone*	*% GA*	*% EGC*	*% C*	*% Caff*	*% EC*	*% EGCG*	*% ECG*	*% TC*
Murang'a (Kanyenyaini)	SFS 150	^{*a}0.97 ± 0.03	^{bc}4.11 ± 0.87	^{ab}0.60 ± 0.20	^{abc}3.93 ± 0.47	^{ab}1.69 ± 0.58	^{a}9.93 ± 1.85	^{c}2.63 ± 0.29	^{ab}18.96 ± 2.31
	TRFK 31/8	^{b}0.69 ± 0.03	^{cd}3.42 ± 0.31	^{cd}0.42 ± 0.07	^{a}4.30 ± 0.36	^{a}1.95 ± 0.57	^{a}9.90 ± 0.94	^{c}2.56 ± 0.12	^{abc}18.25 ± 1.65
	TRFK 303/577	^{b}0.69 ± 0.07	^{a}4.99 ± 0.65	^{bcd}0.45 ± 0.06	^{d}2.84 ± 0.32	^{a}1.95 ± 0.34	^{a}8.05 ± 0.15	^{c}2.69 ± 0.26	^{abc}18.13 ± 1.34
	Mean	0.79 ± 0.16	4.17 ± 0.79	0.49 ± 0.10	3.69 ± 0.76	1.86 ± 0.15	9.29 ± 1.08	2.63 ± 0.07	18.45 ± 0.45
Meru (Imenti)	SFS 150	^{b}0.69 ± 0.10	^{d}3.33 ± 0.37	^{e}0.23 ± 0.04	^{ab}4.03 ± 0.37	^{b}1.30 ± 0.03	^{a}8.37 ± 0.57	^{b}3.60 ± 0.04	^{bc}16.84 ± 0.44
	TRFK 31/8	^{a}1.04 ± 0.06	^{cd}3.50 ± 0.16	^{a}0.71 ± 0.12	^{a}4.04 ± 0.06	^{ab}1.47 ± 0.26	^{a}8.07 ± 1.13	^{a}4.10 ± 0.19	^{abc}17.85 ± 1.40
	TRFK 303/577	^{b}0.63 ± 0.18	^{bc}4.16 ± 0.12	^{de}0.30 ± 0.05	^{bcd}3.11 ± 0.29	^{ab}1.81 ± 0.07	^{ab}7.63 ± 0.62	^{c}2.36 ± 0.29	^{c}16.25 ± 0.44
	Mean	0.79 ± 0.22	3.66 ± 0.44	0.41 ± 0.26	3.73 ± 0.54	1.53 ± 0.26	8.02 ± 0.37	3.35 ± 0.90	16.98 ± 0.81
Kisii (Nyankoba)	SFS 150	^{a}1.04 ± 0.02	^{ab}4.53 ± 0.23	^{cde}0.38 ± 0.04	^{abc}3.80 ± 0.08	^{ab}1.44 ± 0.04	^{a}10.03 ± 0.31	^{b}3.54 ± 0.22	^{a}19.92 ± 0.53
	TRFK 31/8	^{a}1.15 ± 0.19	^{bc}4.12 ± 0.30	^{cde}0.39 ± 0.04	^{a}4.53 ± 0.67	^{ab}1.47 ± 0.05	^{a}9.23 ± 0.26	^{b}3.49 ± 0.09	^{ab}18.69 ± 0.29
	TRFK 303/577	^{b}0.57 ± 0.12	^{ab}4.70 ± 0.37	^{bc}0.52 ± 0.19	^{cd}3.07 ± 1.10	^{ab}1.57 ± 0.38	^{a}9.09 ± 0.28	^{c}2.55 ± 0.43	^{abc}18.43 ± 1.02
	Mean	0.92 ± 0.31	3.92 ± 0.58	0.43 ± 0.08	3.80 ± 0.73	1.49 ± 0.07	9.45 ± 0.51	3.19 ± 0.56	19.02 ± 0.80
Clones (mean)	SFS 150	0.90 ± 0.05	3.99 ± 0.49	0.40 ± 0.09	3.92 ± 0.31	1.48 ± 0.22	9.44 ± 0.91	3.26 ± 0.18	18.57 ± 1.09
	TRFK 31/8	0.96 ± 0.09	3.68 ± 0.26	0.51 ± 0.08	4.29 ± 0.36	1.63 ± 0.29	9.07 ± 0.78	3.38 ± 0.13	18.26 ± 1.11
	TRFK 303/577	0.63 ± 0.12	4.62 ± 0.38	0.42 ± 0.10	3.01 ± 0.57	1.78 ± 0.26	8.26 ± 0.35	2.53 ± 0.33	17.60 ± 0.93
Coefficient of Variation (%)		13.5	10.4	23.8	14.4	19.7	19.6	8.2	7.0
LSD ($p \leq 0.05$)	Agro-zone	0.23	0.86	NS	NS	NS	3.41	0.51	1.91
	CloneXSZ	0.23	0.86	NS	1.09	NS	NS	0.51	NS
	Interactions	0.25	NS	0.58	NS	NS	NS	0.57	NS

± - Standard deviation; * - means within a column preceded with the same superscript letter(s) are not statistically significantly different ($p \leq 0.05$); NS - not significant; GA - Gallic acid; EGC -epigallocatechingallate; C -Catechin; Caff- Caffeine; EC - epicatechin; EGCG - epigallocatechingalllate; ECG - epicatechingallate; TC -total flvan-3-ol content

epicatechin gallate, with retention times of 5.8, 6.1, 6.8, 7.5, 9.8, 13.9 and 23.8 minutes, respectively. The total flavan-3-ol content of each tea, as a percentage by mass, was given as the summation of the individual catechin contents. The levels of catechins differed ($p \leq 0.05$) across the zones for the different cultivars. However, for each clone, the HPLC chromatographic patterns were similar. This confirmed the earlier observations that flavao-3-ols patterns could be used as clonal tea markers.[25] But dissimilar results have been recorded in a few studies.26,27 These disparities suggest that flavan-3-ols could be an unstable parameter to characterize clonal teas. The general accumulation pattern of the flavan-3-ols was EGCG > EGC > ECG > EC >C. Such patter was similar to other studies on Kenya[2,28] and Central African[29] clonal teas.

In previous studies, [2, 28], clonal tea leaves with high levels of gallated catechins and gallocatechins were observed to make superior plain black teas. In this study, there was no clear pattern of the variations in these flvan-3-ols suggesting the clones had the potential to make similar black tea quality. Such a pattern of variation persisted across the agro-ecological zones. The noted differences in tea prices across the agro-ecological zones could not, therefore, be explained by the flvan-3-ol and caffeine composition. However, the significant ($p \leq 0;05$) differences in the levels of the flavan-3-ols and caffeine between the clones within and between the agro-ecological zones suggest that plain black tea quality will vary within and between the agro-ecological zones. It is, therefore, necessary to test clones for quality in regions of extended exploitation before widespread cultivation.

Table 1: Changes (% dry weight) in gallic acid, caffeine, individual flavan-3-ol and the total flavan-3-ol content in young fresh green tea shoots in the three clones of tea obtained from the three agro-zones (±the standard deviation, M ± SD[#])Some studies had previously compared black tea quality variations east and west of the Rift Valley. Indeed, variations in tea quality were recorded in black tea from selected factories[19] and miniature manufactured teas using leaf from selected farms [20] in the east and west of the Rift Valley The differences in the plain tea quality parameters, though significant, did not follow a particular pattern. Results presented herein on the precursors of plain tea quality parameters are consistent with the earlier results. However, the black teas from the east of the Rift Valley tended to be more aromatic than those from the west of the Rift Valley.[19,30] The differences in the quality of black teas could possibly be partially explained by the variations in levels of activities and forms of the oxidative enzymes that convert flavan-3-ols to black tea quality parameters. Multiple forms of polyphenol oxidase have been observed during fermentation of black tea[31] and leaf maturation.[32] It is, therefore, possible that environmental factors change the forms and activities of polyphenol oxidase in different regions and clones, thereby varying the levels and composition of the possible plain black tea quality parameters.

In conclusion, the levels and patterns of flavan-3-ols and caffeine in clonal tea leaves could not discriminate the tea by agr-ecological zones. These factors, therefore, cannot be used to explain price differences of black tea from the different agro-ecological zones. This suggests that for production of high-quality black tea, cultivars should be tested in the regions of intended release or production as had been observed in previous studies.[33]

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