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The use of green tea (*Camellia sinensis*) leaf flavan-3-ol composition in predicting plain black tea quality potential

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Abstract

Reliable and quantifiable green leaf parameters for predicting black tea quality potential of tea bushes at the single bush stage are necessary to shorten tea breeding and clonal selection programmes. Green leaf flavan-3-ols (catechins) and plain black tea quality of 11 popular Kenyan clones were determined. The individual green leaf flavan-3-ols, and all the black tea quality parameters and sensory evaluations, except total flavan-3-ols were significantly different. The green leaf epigallocatechin gallate (EGCg) levels significantly correlated with black tea total theaflavin ($r = 0.749$, $P \leqslant 0.007$), liquor brightness ($r = 0.898$, $P \leqslant 0.0001$) levels and Taster B ($r = 0.595$, $P \leqslant 0.051$) sensory evaluations, and negatively with thearubigins ($r = -0.652$, $P \leqslant 0.027$), while green leaf epicatechin (EC) levels correlated positively with thearubigins ($r = 0.694$, $P \leqslant 0.016$) and negatively with theaflavin digallate equivalent ($r = -0.751$, $P \leqslant 0.007$), and sensory evaluation by Taster A ($r = -0.785$, $P \leqslant 0.003$) and Taster B ($r = -0.679$, $P \leqslant 0.020$). Thus, high levels of EGCg and low levels of EC in tea green leaf can be used as indicators of plain black tea quality potential of tea plants. The sum of gallated flavan-3-ols correlated significantly and positively with theaflavin digallate equivalent ($r = 0.669$, $P \leqslant 0.022$) levels, but negatively correlated with the black tea thearubigins ($r = -0.582$, $P \leqslant 0.052$). The sum of trihydroxyflavan-3-ols (gallocatechins) positively correlated with brightness ($r = 0.750$, $P \leqslant 0.007$), and sensory evaluation by Taster A ($r = 0.656$, $P \leqslant 0.026$), but negatively with thearubigins ($r = -0.641$, $P \leqslant 0.031$). However, the sum of dihydroxyflavan-3-ols (simple catechins) correlated positively with thearubigins ($r = 0.716$, $P \leqslant 0.012$) and negatively with total theaflavins ($r = -0.6694$, $P \leqslant 0.022$), theaflavin digallate equivalent ($r = -0.631$, $P \leqslant 0.035$), brightness ($r = -0.843$, $P \leqslant 0.001$), Taster A ($r = -0.775$, $P \leqslant 0.004$) and Taster B ($r = -0.638$, $P \leqslant 0.032$) sensory evaluation. The ratios of trihydroxyflavan-3-ols to dihydroxyflavan-3-ols correlated positively with brightness ($r = 0.678$, $P \leqslant 0.020$) and sensory evaluation of Taster A ($r = 0.667$, $P \leqslant 0.023$), but negatively with thearubigins ($r = -0.697$, $P \leqslant 0.015$). In addition to green leaf high levels of EGCg and low levels of EC, high levels of the sum of gallated catechins, trihydroxyflavan-3-ols, and the ratio of trihydroxyflavan-3-ols to dihydroxyflavan-3-ols are parameters in green tea leaf that may be used in predicting plain black tea quality potential of Kenyan tea clones.

Keywords: Clonal tea; Flavan-3-ols; Theaflavins; Brightness; Colour; Sensory evaluation; Kenya

1. Introduction

World production of different beverages from the young tender shoots of tea (*Camellia sinensis* (L) O. Kuntze) has continued to rise (Anon., 2004) despite lack of commensurate increase in consumption. In Kenya, for example, production rose to 324.6 thousand metric tonnes of made tea, in 2004, from 18.1 thousand metric tonnes in 1963 (Anon., 2005) due to improvement in production technologies and large increase in land under tea. However, the price of black tea has not improved with time (Anon., 2004) and sometimes is declining, despite the continued increase in the costs of production due to lack of commensurate increase in tea consumption. Lack of extensive expansion in tea consumption implies that the tea market will

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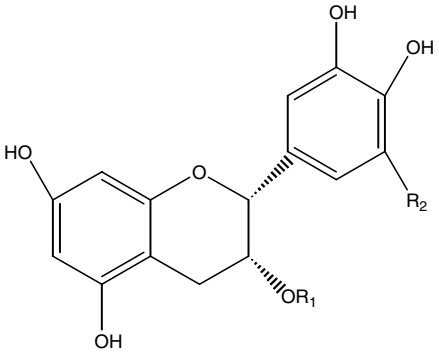
continue to be selective and only producers of high quality tea are likely to survive in the market. But tea is a major player in the economies of many producing countries such that, despite the declining or stagnant prices, growing tea remains a viable economic activity, creating employment and generating hard currencies. One way of improving the profitability of tea production is by planting high-yielding clones with excellent quality. But, high-quality tea can only be obtained from raw material with the correct quality potential. However, quantifiable breeding or selection criteria for quality have been elusive. Past tea breeding/clonal selection put emphasis mainly on yield (Seurei, 1997) and whatever high-quality material is in production now might not have been selected for this attribute. Traditional selection/breeding methods for tea have relied on a combination of morphological characteristics, which are somewhat empirical and slow, and laborious to assess (Njuguna, 1984). Much high-yielding and good quality planting material has evolved by chance. Sensory evaluation has been the only method of assessing tea routinely but is applied after processing. Sensory evaluation is, however, subjective and is influenced by many factors outside quality (Biswas, Biswas, & Sarkar, 1971, 1973). Development of reliable and quantifiable selection criteria for quality is therefore necessary.

Much time has been spent on the development of plain black tea quality parameters, but they have only been applicable to a limited range of tea samples. For example, theaflavin levels significantly correlated with sensory evaluation/prices of Central African (Hilton & Ellis, 1972; Hilton & Palmer-Jones, 1975), and North East Indian (Deb & Ullah, 1968) plain black teas. However, the correlations were not significant for Kenyan (Owuor, Reeves, & Wanyoko, 1986) and Sri Lankan (Roberts & Fernando, 1981) black teas. The lack of a significant relationship between total theaflavin levels and sensory evaluation, or prices of black tea produced in some countries (Owuor et al., 1986; Roberts & Fernando, 1981), could be due, partly, to the fact that the total theaflavins might not have been a good measure for quality of black teas. The relative astringencies of the four predominant theaflavins in black tea (Fig. 1), namely, theaflavin digallate, theaflavin-3-gallate, theaflavin-3′-gallate and theaflavin, had been shown to be 6.4:2.2:2.2:1, respectively (Sanderson et al., 1976). With improvements in analytical procedures, it is now possible to partition the individual theaflavins and to calculate an astringency-normalizing factor (theaflavin digallate equivalent) (Owuor & McDowell, 1994; Owuor & Obanda, 1995, 1997) of the various black teas. Using this factor, a better relationship was observed between the gallated theaflavins or theaflavin digallate equivalent and sensory evaluations (Owuor & Obanda, 1995) in plain teas, where previous regressions using total theaflavins were not significant (Owuor et al., 1986). In a recent study, this factor cor-



(-) -Epicatechin(EC)

$R_1 = R_2 = H$

(-) -Epicatechin-3-gallate (ECg)

R_1 = 3,4,5-Trihydroxybenzoyl

$R_2 = H$

(-) -Epigallocatechin (EGC)

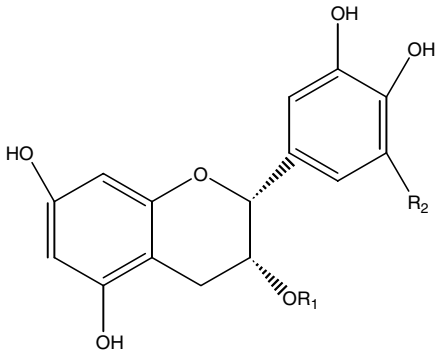
$R_1 = H$

$R_2 = OH$

(-) -Epigallocatechin -3-gallate (EGCg)

R_1 = 3,4,5-Trihydroxybenzoyl

$R_2 = OH$



(+) -Catechin (C)

$R_1 = R_2 = H$

Fig. 1. The flavan-3-ols (catechins) in fresh tea leaves.

related better with the sensory evaluation of eastern, central, and southern African black teas (Owuor et al., submitted), than with the total theaflavins, showing that it is a better measure of plain black tea quality, irrespective of the country of production.

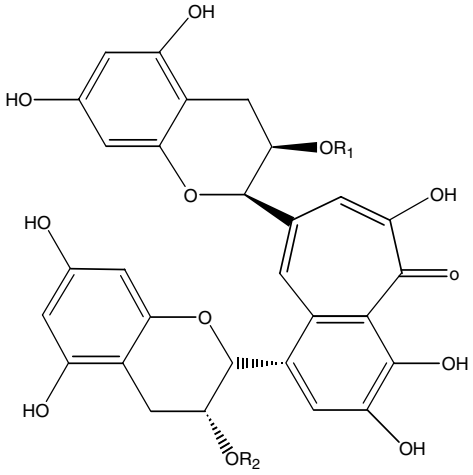
Despite the development of reliable plain black tea quality parameters that can be used successfully in breeding/clonal selection for quality programmes, there are problems with assessing clones for quality after tea processing. The process requires that there is adequate leaf for at least miniature processing. Consequently, plants to be evaluated must be multiplied and produced in large enough numbers to generate adequate leaf for processing. This requires large land areas, long durations, and is expensive with no guarantee of success. Usually, from identification to first testing takes at least six years (Wright, Mphangwe, Nyirenda, & Apostolides, 2000). Consequently, the future of tea breeding/clonal selection lies in making the assessment at the single-bush stage. It is therefore necessary to develop methods that can predict black tea quality potential of tea plants at a single bush stage.

Green tea leaves have high levels of polyphenols mainly flavan-3-ols (catechins), that are responsible for the formation of theaflavins and thearubigins in black tea. The flavan-3-ol composition of the clonal tea leaves varies (Obanda, Owuor, & Taylor, 1997). For central and southern African black teas, Wright et al. (2000) showed that the epicatechin levels correlated best with sensory evaluation, followed by the non-gallated catechins, and then the dihydroxy (simple) catechins. This study was done to evaluate the relationship between the fresh green leaf flavan-3-ol composition and sensory evaluation of some Kenyan tea clones, using plants with wide genetic variability (Magoma, Wachira, Obanda, Imbuga, & Agong', 2000).

The total theaflavins (Deb & Ullah, 1968; Hilton & Ellis, 1972; Hilton & Palmer-Jones, 1975; Owuor et al., 1986; Wright, Mphangwe, Nyirenda, & Apostolides, 2002), or derived theaflavin digallate equivalents (Owuor and Obanda, 1997, 1995; Owuor et al., submitted), have a dominant effect on the quality of black teas. The formation of a single theaflavin molecule requires a dihydroxy and a trihydroxy flavan-3-ol, as follows:-

Epicatechin (EC) + Epigallocatechin (EGC)	→	Simple theaflavin (TF).
EC + Epigallocatechin gallate (EGCg)	→	Theaflavin-3-gallate (TF-3-g)
Epicatechin gallate + EGC	→	Theaflavin-3-gallate (TF-3′-g)
ECG + EGCg	→	Theaflavin-3, 3′- digallate (TF dg)

The structures of the flavan-3-ols and theaflavins are presented in Figs. 1 and 2, respectively. The ratio of the dihydroxyflavan-3-ol to trihydroxyflavan-3-ol in the green leaf may therefore have a major influence on the ultimate amounts of the theaflavins in black tea. The present study evaluates the possible relationship between this ratio and the theaflavins formed and the sensory evaluation.



I Simple theaflavin (TF)
$R_1 = R_2 = H$

II Theaflavin-3-gallate (TF-3-g)
R_1 = 3,4,5-Trihydroxybenzoyl
R_2 = H

III Theaflavin-3'-gallate (TF-3'-g)
R_1 = H
R_2 = 3,4,5-Trihydroxybenzoyl

IV Theaflavin-3,3'-digallate (TF-3,3'-dg)
$R_1 = R_2$ = 3,4,5-Trihydroxybenzoyl

Fig. 2. The major individual theaflavins in black teas.

The amounts of the individual theaflavins formed are largely influenced by the amounts of the precursor catechins in green leaf, their redox potentials and/or polyphenol oxidase preference of the individual catechins and activity. Since the level of the astringency increases with the extent of esterification of the theaflavins, the flavan-3-ol gallates need to occur in reasonable quantities in green tea leaf. Whereas Wright et al. (2000) showed that sum of non-gallated flavan-3-ol levels had a significant effect on black tea quality, there was no assessment of how the ratio of the gallated to non-gallated catechins influences the ultimate black tea quality. The present study evaluates the possible role of the ratio of gallated to non-gallated flavan-3-ols in the quality of black tea.

Although the thearubigins are thought of as polymeric material derived from polyphenols, their structures are not well documented. However, high thearubigin levels in black tea reduce the brightness (Owuor et al., submitted). This study also assessed how the individual flavan-3-ol levels in green leaf affect thearubigin formation.

2. Materials and methods

2.1. Leaf

Leaf was obtained from 11 clones, part of Clonal Field Trials, planted at the Tea Research Foundation of Kenya, Timbilil Estate, 2180 m a.m.s.l., latitude 0°22′S and longitude 35°21′E. Clones were selected which had shown good quality potential, but had either shown wide variation in the ratio of trihydroxyflavan-3-ol to dihydroxyflavan-3-ol in their leaves (Magoma et al., 2000) or wide variations in the individual theaflavin distributions in their black teas (Owuor & Obanda, 1997). Except for clones 301/6 and 378/1, all clones used in this study had been released to the industry and are being used commercially. Clone 301/6 is a *C. sinensis* var *assamica* ssp *lasiocalyx*, while clone 378/1 is a *C. sinensis* var *assamica* polyploid (triploid) plant. It was hoped that, with the use of clones with wide genetic variation/diversity (Magoma et al., 2000), the criteria developed would have wide application. The plants were receiving uniform agronomic treatment, with fertilizer at 150 kg N ha^{-1} $year^{-1}$ as NPKS 25:5:5:5. Plucking was done at 10–14 day intervals, depending on leaf availability. One kilogramme of leaf, comprising mostly two leaves and a bud, was plucked from each clone. The harvesting and manufacture were done in four replicates.

In each manufacture, leaf was withered for 14–18 h to achieve 70% physical wither. The leaf was then miniature CTC-macerated. Fermentation was done for 90 min and terminated using a bench top fluid bed dryer (Tea craft, UK). The unsorted black teas were subjected to chemical analyses and sensory evaluation as explained below.

2.2. Reagents

The isobutyl methyl ketone (IBMK), Flavognost reagent (diphenylboric acid 2-aminoethyl ester), HPLC grade acetonitrile and catechin standard samples were obtained from Aldrich Chemicals. The rest of the solvents and reagents were of analytical grade, while water was double-distilled.

2.3. Sensory evaluation

Experienced professional tea tasters, at two tea broking firms in Mombasa, evaluated the black teas. The Mombasa Tea Auction Centre is now the second largest in the world after Colombo. The tasters have expert knowledge of black teas, especially of Kenyan teas, which they auction regularly.

2.4. Chemical analysis

Total theaflavins were determined by the Flavognost method (Hilton, 1973), while the individual theaflavin ratios were determined by HPLC (Bailey, McDowell, & Nursten, 1990; McDowell, Feakes, & Gay, 1991; Steinways & Engelhard, 1989). Liquors were prepared by adding 4 g of black tea to 195 ml deionised water that had just reached boiling and shaking was done for 10 min in a 475 ml capacity Thermos flask. Clean liquor was obtained by filtration through cotton wool. The hot liquor was cooled to room temperature by placing the flask containing the liquor under a cold water tap (1–3 min). The liquor was diluted (1:1) with double-distilled water before HPLC analysis. The liquor was analysed on a Cecil Series 1000 HPLC with a 20 µl sample loop and a Hypersil 5 µ ODS column (25 cm × 4.6 mm). The UV monitor was set at 365 nm and results were recorded and analysed using a JCL 6000 Cecil data system. Solvent A was 1% aqueous acetic acid and Solvent B was acetonitrile. A linear gradient from 8% to 31% solvent B over 60 min, with a flow rate of 1.5 ml/min, was used (Bailey et al., 1990; McDowell et al., 1991). The theaflavin ratios, calculated from the HPLC data and the Flavognost (total) theaflavin data, were used to calculate the amounts of the individual theaflavins, since the molar absorption coefficients of the four theaflavins are similar at 365 nm (Steinways & Engelhard, 1989).

The black tea thearubigins, liquor colour and brightness were determined as described by Roberts and Smith (1963).

2.5. Extraction and HPLC analysis of catechins in green tea leaf

The flavan-3-ols were analysed as outlined earlier (Obanda, Owuor, & Rutto, 1999). Part of the leaf for manufacture (100 g) was steamed for 1 min, then vacuum-oven dried and crushed to a powder. About 125 mg of the powder were extracted for each green leaf sample in 25 ml acetonitrile water (1:1 v/v) mixture at room temperature for 30 min with constant shaking. Each clone was sampled 4 times.

The extract was filtered through a filter cartridge (DIS MIC 13HP, Advantec Toyo, Tokyo, Japan) and diluted fivefold with water before HPLC analysis on a Shiseido Capcell C18 UG 120 A 5 µm 4.6 × 250 mm column maintained at 35 °C. The mobile phase (A) was 0.1% phosphoric acid in water and mobile phase (B) was acetonitrile. The HPLC running programme was 0–5 min, 10% B; 5–25 min, 25% B; 25–26 min, 100% B; and monitored at 270 nm. The flow rate was 1.0 ml/min and the injection volume 10 µl. Authentic flavanol standards (Sigma Chemicals) ((+)-catechin C; (−)-epicatechin EC; (−)-epigallocatechin EGC; (−)-epicatechin gallate ECG; (−)-epigallocatechin gallate EGCG) were used to identify and calculate the concentrations of flavanols.

2.6. Analysis of variance and regressions

The results were subjected to analysis of variance using the MSTAT statistical package. The means were used in the linear regressions between black tea quality parameters and sensory evaluations and green tea leaf flavan-3-ols.

3. Results and discussion

Several studies have determined green leaf compounds, which can be used to predict the quality of black tea. The carotenoid and chlorophyll contents correlated (Taylor et al., 1992), while the caffeine content correlated positively with quality (Millin, Crispin, & Swaine, 1969; Obanda et al., 1997). However, the biochemical parameter influencing plain black tea quality in the green leaf consists of polyphenols, especially the flavanols, which are responsible for the formation of the black tea thearubigins and theaflavins (Hilton & Palmer-Jones, 1973). Whereas the total polyphenols correlated with black tea quality (Obanda, Owuor, & Njuguna, 1992), some polyphenols do not contribute to the formation of any black tea quality parameter. Only flavan-3-ols are critical in the biosynthesis of the black tea quality parameters. Conflicting results have been obtained on the role of the individual flavan-3-ols in predicting black tea quality potential of different cultivars. The ECg and EGCg in green leaf correlated with black quality, as measured by total theaflavins of some tea clones (Obanda et al., 1997). However, Wright et al. (2000) showed EC to be the critical flavan-3-ol in predicting the quality potential of central and southern African tea cultivars.

The composition, in terms of the flavan-3-ols of the green tea leaves of the clones used in this study, is presented in Table 1. There were variations in the composition of flavan-3-ols in the clones used in the study. Fig. 3 demonstrates the kinds of variations that were exhibited. These results demonstrate that the clones used in this study had wide differences in flavan-3-ol composition, suggesting large genetic variations (Magoma et al., 2000). Indeed, there were significant ($P \leqslant 0.05$) differences in the levels of all individual flavan-3-ols, suggesting that the quality potentials of these clones could be different. This led to large variations in the individual theaflavins composition in the black teas (Fig. 4).

The astringency of the resultant theaflavins biosynthesised is largely influenced by the amounts of the esterified flavan-3-ols (Sanderson et al., 1976). The sum of the gallated flavan-3-ols and ratio of gallated to non-gallated flavan-3-ols are presented in Table 1. These parameters were significantly ($P \leqslant 0.05$) different in the clones used, implying that the astringency potential of the black teas in this study could be different. However, more recently, Scharbert, Holzmann, and Hofmann (2004) observed that the flavan-3-ol glycosides also confer astringency at levels 16,000 times lower than theaflavins. In this study, the variations in the flavan-3-ol glycosides in the different clones were not established.

Magoma et al. (2000) demonstrated that the genetic variability of tea clones can be seen, in part in the ratios of the trihydroxyflavan-3-ols to dihydroxyflavan-3-ols. The total trihydroxyflavan-3-ols and dihydroxyflavan-3-ols in the green leaf used in this study is also presented in Table 1. These parameters were significantly ($P \leqslant 0.05$) different in the green leaf of the clones used. The ratio of trihydroxyfl-

Table 1
The composition of flavan-3-ols (catechins), the sum of related flavan-3-ols and flavan-3-ols ratios in different tea clones

Clone	EGC (μmol/g)	+C (μmol/g)	EC (μmol/g)	EGCG (μmol/g)	ECG (μmol/g)	Gallated (μmol/g)	Non-gallated (μmol/g)	Gallated/non-gallated ratio	Trihydroxy flavan-3-ols (μmol/g)	Dihydroxy flavan-3-ols (μmol/g)	Trihydroxy/dihydroxy flavan-3-ols	Total flavan-3-ols (μmols/g)
6/8	86.3	12.8	36.2	64.3	49.0	113	135	0.84	151	98.0	1.54	247
S15/10	118	14.7	18.2	68	55.8	124	151	0.85	186	88.8	2.08	275
Ejulu	63.9	30.9	28.9	56.0	61.5	117	124	0.95	120	121	0.99	241
31/11	107	6.55	8.97	68.0	58.9	127	122	1.05	175	74.5	2.36	249
301/6	13.9	5.60	110	43.9	61.7	106	130	0.81	57.8	177	0.33	235
303/35	86.3	15.7	37.4	67.5	50.1	118	139	0.87	154	103	1.49	257
303/216	92.7	16.6	34.5	63.8	51.1	115	144	0.81	156	102	1.55	259
347/314	104	13.0	29.4	68.9	52.4	121	147	0.85	173	94.9	1.83	268
378/1	56.0	17	28.9	68.7	61.4	130	102	1.28	125	108	1.16	232
F7/346	82.8	6.98	30.7	71.24	58.4	130	121	1.10	154	96.7	1.59	251
PMC 61	54	15.5	15	70.9	67.5	138	85.5	1.66	125	98.7	1.27	224
CV (%)	19.3	20.3	17.8	10.4	8.59	8.88	15.1	16.1	12.3	9.04	11.2	9.76
LSD, $P \leqslant 0.05$	21.9	4.14	8.82	9.72	7.09	15.6	27.8	0.23	25.7	13.8	0.24	NS

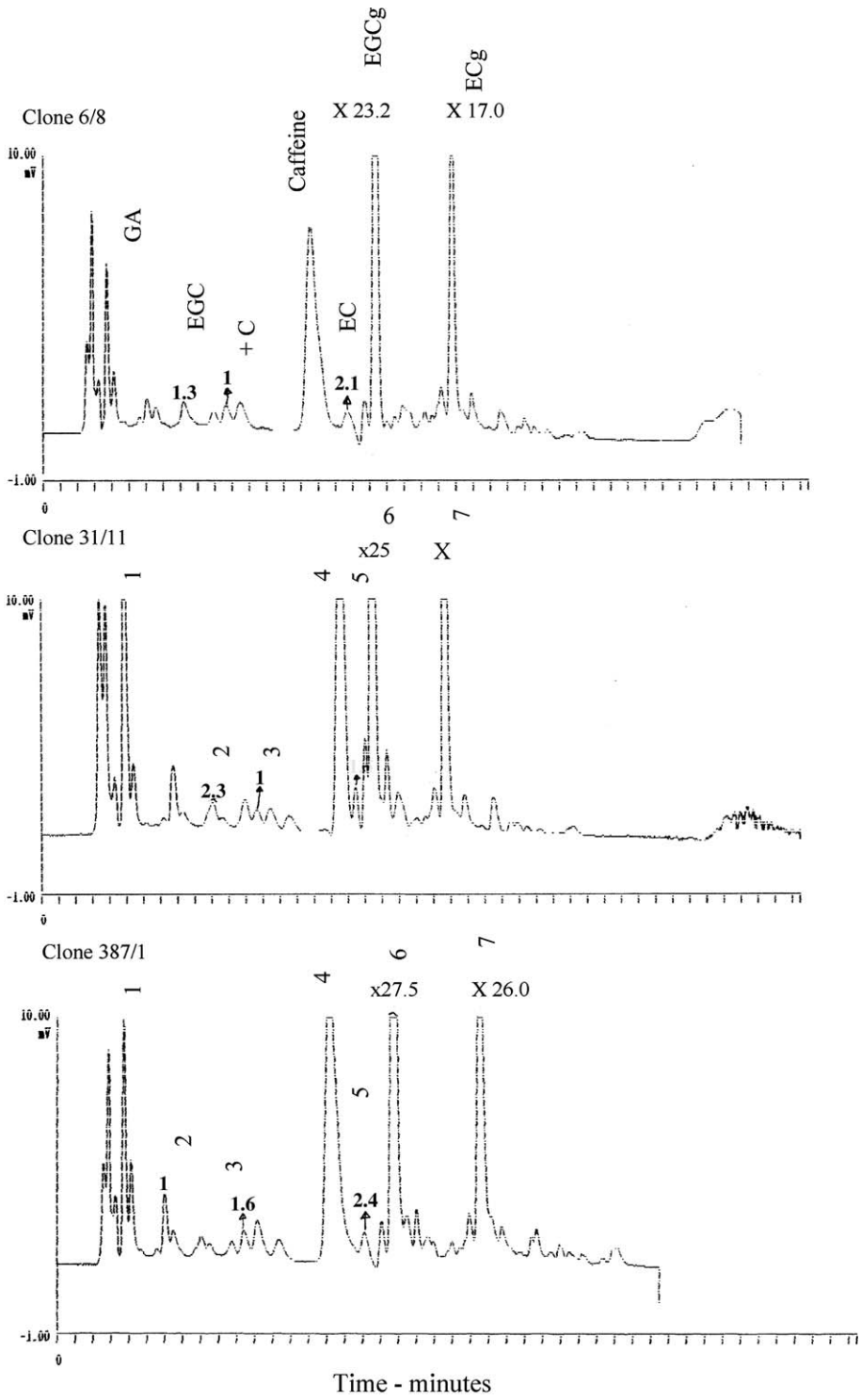


Fig. 3. The green leaf catechins HPLC profiles (278 nm) in some clones: 1 = GA, 2 = EGC, 3 = +C, 4 = Caffeine, 5 = EC, 6 = EGCg, 7 = ECg.

avan-3-ols to dihydroxyflavan-3-ols varied from 0.33 in clone 301/6 to 2.36 in clone 31/11 emphasising the large genetic variation in the clones used in this study. The formation of theaflavin requires a reaction between a trihydroxyflavan-3-ol and a dihydroxyflavan-3-ol (Nakagawa & Torii, 1965; Robertson, 1983). The correct balance and amount of the trihydroxyflavan-3-ols and dihydroxyflavan-3-ols are therefore necessary to ensure maximum formation of the theaflavins, one of the key chemical quality parameters of black tea (Deb & Ullah, 1968; Hilton & Ellis, 1972; Hilton & Palmer-Jones, 1975; Owuor et al., 1986, submitted; Wright et al., 2000, 2002). However, the redox potential of the flavan-3-ols (Bajaj, Anan, Tsushida, & Ikegaya, 1987) and their affinity for polyphenol oxidase vary, with the trihydroxyflavan-3-ols having the lower redox potentials. The trihydroxyflavan-3-ols are therefore oxi-

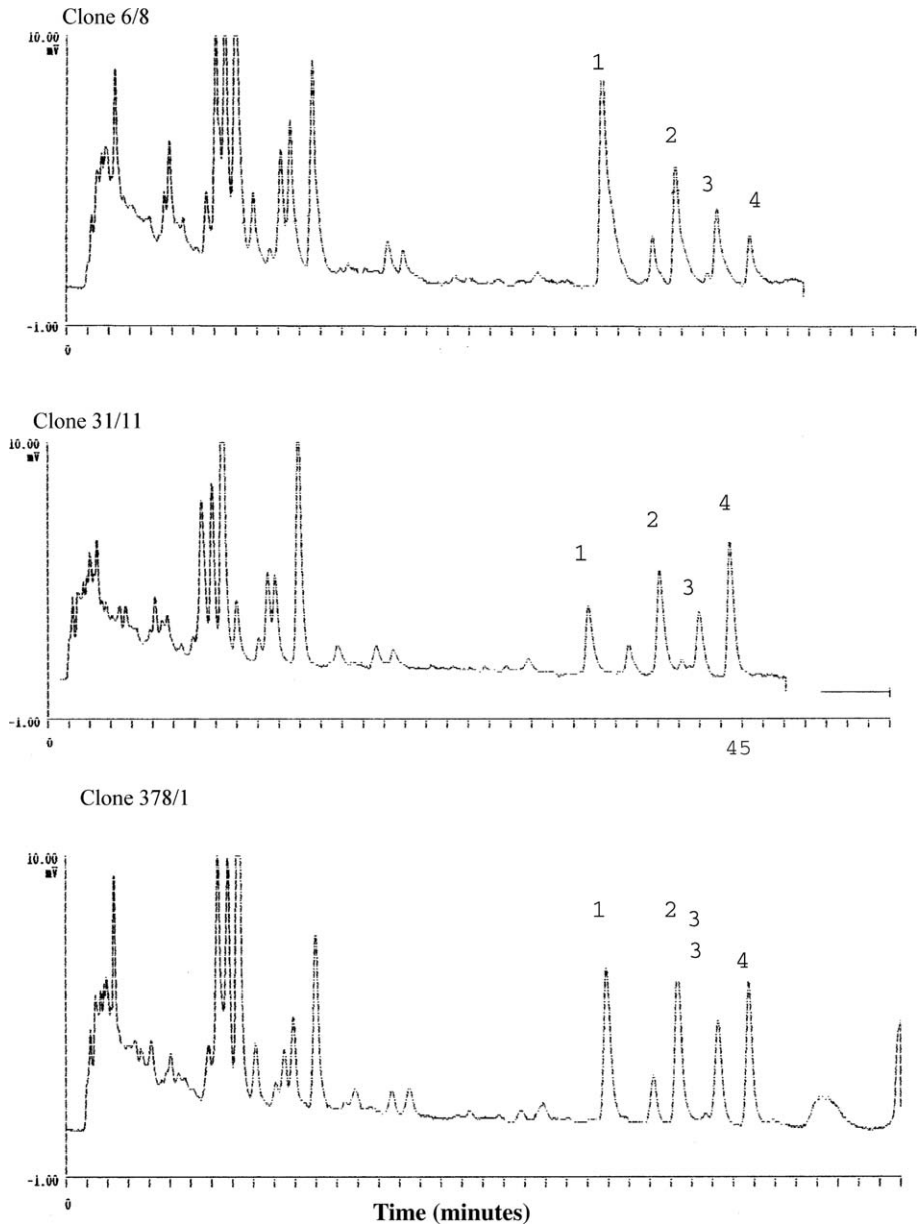


Fig. 4. The HPLC patterns (at 365 nm) of individual theaflavins in different clonal black teas: 1 = TF, 2 = TF-3-g, 3 = TF-3′-g, and 4 = TF-3,3′-dg.

dised faster during the fermentation phase of black tea processing. Thus, although, in clones used in this study, their levels were higher, they could be the limiting factor in theaflavin formation because they run out faster. However, the flavan-3-ol gallates have a higher substrate inhibition property on polyphenol oxidase than the non-gallated flavan-3-ols (Robertson, 1983), because of higher molecular weights and flexibility (Spencer et al., 1988). The situation is therefore complicated. However, in our fermentation trials, we observed rapid disappearance of the trihydroxyflavan-3-ols (Owuor, Orchard, & McDowell, 1994). The levels of trihydroxyflavan-3-ols and dihydroxyflavan-3-ols and their ratios were significantly ($P \leqslant 0.05$) different. This indicates possible differences in the black tea quality potential in the clones used in this study.

Despite the large differences in the levels of the individual flavan-3-ols, the total flavan-3-ol levels were not significantly ($P \leqslant 0.05$) different. The results were not surprising, as the clones had all passed the chloroform test (i.e. exposing young tea leaves to chloroform vapour to assess how fast it forms brown colouration) (Sanderson, 1963), which is the only biochemical test traditionally and routinely used in assessing the potential of new cultivars to make black teas. However, this test is not reliable in selecting tea plants for black tea quality (Obanda et al., 1999).

All the plain black tea quality parameters and sensory evaluations (Table 2), were significantly ($P \leqslant 0.05$) different, demonstrating that the composition, in terms of the individual flavan-3-ols (Table 1) in green leaf, is more critical in black tea quality than is the total flavan-3-ols per se. Provided a threshold level of flavan-3-ols is present, the

Table 2
The plain black tea quality parameters of different clones

Clone	Total theaflavins (μmol/g)	TF (μmol/g)	TF-3-g (μmol/g)	TF-3′-g (μmol/g)	TF-3, 3′-dg (μmol/g)	TF dg eq (μmol/g)	Thearubigins (%)	Total colour (%)	Brightness (%)	Taster A	Taster B
6/8	26.4	12.5	6.86	4.16	2.64	8.42	18.6	5.43	31.3	51	20
S15/10	19.2	7.58	5.20	3.19	3.33	7.42	15.6	4.21	27.6	33	15
Ejulu	18.0	4.00	5.14	2.56	6.31	10.0	18.4	5.32	21.7	50	20
31/11	22.2	4.06	6.47	3.81	7.88	12.1	15.2	5.02	29	59	21
301/6	14.8	7.71	4.58	1.25	1.20	4.44	19.6	4.53	16.0	8	13
303/35	21.4	9.36	5.43	3.88	3.03	7.47	17.2	4.36	30.5	32	17
303/216	20.0	10.2	4.98	2.95	1.87	6.21	15.2	4.61	29.7	46	18
347/314	25.4	12	6.49	3.90	3.00	8.48	18.1	5.49	29.9	44	20
378/1	24.9	7.82	6.91	4.70	5.44	10.7	17.2	5.57	31.2	35	20
F7/346	22.6	8.94	5.93	3.72	3.77	8.60	16.7	4.92	29.2	41	21
PMC	22.7	7.27	6.71	4.01	4.10	9.54	16.0	4.70	28.4	38	19
C.V.%	13.0	19.8	13.4	17.7	24.4	15.0	8.16	13.3	15.1	33.5	8.16
LSD, $P \leqslant 0.05$	4.06	2.37	1.16	0.89	1.36	2.01	2.01	0.95	6.03	19	2

total may not be important. There is a need to determine this threshold level. Generally, the Kenyan tea clones make black teas with very high total theaflavin levels (Owuor et al., 1986), suggesting presence of adequate amounts of total flavan-3-ols in green leaf. A survey, even of clones rejected as being of poor quality, showed that their black tea theaflavin levels were higher than those of good quality clones planted in central Africa (Owuor et al., 1986). It may be necessary for breeders to develop clones with much lower, or a wider range of, flavan-3-ols to facilitate the determination of the threshold level of flavan-3-ols in green leaf critical for high quality.

The plain black tea quality parameters and sensory evaluations were regressed against the green leaf flavan-3-ols (Table 3) to establish which green leaf flavan-3-ols significantly influenced the parameters. The total theaflavins (Flavognost) had a positive and significant correlation with EGCg ($r = 0.749$, $P \leqslant 0.007$) and with the sum of trihydroxyflavan-3-ols ($r = 0.525$, $P \leqslant 0.094$), but a negative and significant correlation with the sum of dihydroxyflavan-3-ols ($r = -0.669$, $P \leqslant 0.022$) and EC ($r = -0.576$, $P \leqslant 0.061$). The positive and significant correlation shown here demonstrates that, in the production of plain black teas, clones of superior quality tea, according to total theaflavins as a quality measure (Ellis & Cloughley, 1981), can be selected at the single bush or green leaf level using EGCg and sum of trihydroxyflavan-3-ols. These results are at variance with studies from central and southern Africa, in which EC had the best correlation with black tea theaflavins (Wright et al., 2000). The difference in response between the Kenyan clones and the central and southern African clones are not unique, as it had also been shown that the tea plants from different regions have large genetic variations (Wachira, Tanaka, & Takeda, 2001). This may be responsible for the wide variations in the polyphenol profiles in their black teas (McDowell et al., 1991). However, the differences could also be caused by the difference in the environments in which these teas were planted. Indeed, in central and southern Africa, green leaf EGCg greatly dominated the flavan-3-ol composition (Wright et al., 2000), while, in the present study, no particular catechin has such a dominant position. That notwithstanding, it is important to note that the key flavan-3-ol for predicting formation of high amounts of total theaflavins in central and southern African black teas reduces the total theaflavins in Kenya black teas, while the key flavan-3-ol for predicting formation of high amounts of total theaflavins in Kenya black teas reduces the total theaflavins in central and southern African black teas.

Comparison of the flavan-3-ol data presented here and those on tea clones produced in central and southern Africa (Wright et al., 2000), reveals a large difference that had not been observed before. From the higher total theaflavins in Kenyan black teas than in the central African ones (Owuor et al., 1986), it had been assumed that Kenyan green tea leaves had higher levels of flavan-3-ols than those from central Africa. Although the differences in the theaf-

Table 3
Linear regression coefficients and significant levels between plain black tea quality parameters and catechins or catechin ratios of different clones

Catechin	Total theaflavins	Theaflavin	Theaflavin-3-gallate	Theafalvin-3′-gallate	Theaflavin-3,3′-digallate	Theaflavin digallate equivalent	Thearubigins	Total colour	Brightness	Taster A	Taster B
Epigallocatechin	0.426 NS	0.189 NS	0.195 NS	0.457 NS	0.206 NS	0.291 NS	−0.591 0.053	−0.035 NS	0.654 0.027	0.643 0.030	0.344 NS
Catechin	−0.075 NS	−0.244 NS	−0.118 NS	0.028 NS	0.288 NS	0.224 NS	0.082 NS	0.230 NS	−0.023 NS	0.266 NS	0.174 NS
Epicatechin	−0.576 0.061	0.153 NS	−0.544 0.081	−0.734 0.009	−0.612 0.043	−0.751 0.007	0.694 0.016	−0.203 NS	−0.731 0.009	−0.785 0.003	−0.679 0.020
Epigallocatechin gallate	0.749 0.007	0.200 NS	0.648 0.029	0.881 0.0001	0.305 NS	0.547 0.078	−0.652 0.027	0.118 NS	0.898 0.0001	0.528 0.092	0.595 0.051
Epicatechin gallate	−0.305 NS	−0.683 0.019	0.074 NS	−0.168 NS	0.407 NS	0.305 NS	−0.022 NS	0.020 NS	−0.450 NS	−0.238 NS	−0.002 NS
Gallated catechins	0.461 NS	−0.256 NS	0.611 0.043	0.662 0.024	0.523 0.096	0.669 0.022	−0.582 0.052	0.115 NS	0.499 NS	0.303 NS	0.522 0.096
Non-gallated catechins	−0.187 NS	0.385 NS	−0.504 NS	−0.307 NS	−0.420 NS	−0.501 NS	0.061 NS	−0.263 NS	−0.022 NS	−0.012 NS	−0.349 NS
Gallated/non-gallated ratio	0.287 NS	−0.312 NS	0.558 0.071	0.453 NS	0.399 NS	0.527 0.093	−0.302 NS	0.133 NS	0.212 NS	0.072 NS	0.360 NS
Gallocatechins	0.525 0.094	0.203 NS	0.310 NS	0.581 0.058	0.241 NS	0.367 NS	−0.641 0.031	−0.002 NS	0.750 0.007	0.656 0.026	0.422 NS
Simple catechins	−0.669 0.022	−0.062 NS	−0.563 0.068	−0.770 0.005	−0.459 NS	−0.631 0.035	0.716 0.012	−0.139 NS	−0.843 0.001	−0.775 0.004	−0.638 0.032
Gallo/simple catechin ratio	0.483 NS	0.049 NS	0.363 NS	0.545 0.080	0.370 NS	0.460 NS	−0.697 0.015	−0.017 NS	0.678 0.020	0.667 0.023	0.421 NS
Total catechins	0.055 NS	0.350 NS	−0.261 NS	0.017 NS	−0.224 NS	−0.233 NS	−0.267 NS	−0.256 NS	0.283 NS	0.182 NS	−0.123 NS

* Significant levels, limit set at $P = 0.10$.

lavins between Kenyan teas used here and those used in a recent central African black tea study (Wright et al., 2002) reaffirm the earlier observations, the central African tea clones had, on average, about twice the amounts of flavan-3-ols (Wright et al., 2000) compared to the Kenya tea clones used in the present study. Thus, the earlier speculation that the total amounts of flavan-3-ols in green leaf largely influences the resultant total theaflavins in black tea is incorrect. The distribution of the individual flavan-3-ols in green leaf may be more critical to theaflavins formation than total flavan-3-ols per se.

On average, about 50% of flavan-3-ols in the central and southern African green tea leaf flavan-3-ols is EGCg (Wright et al., 2000). For Kenyan clonal teas, the EGCg comprised only about 25% of the flavan-3-ols composition. Despite the low levels, the Kenyan clonal green leaf led to black teas with higher amounts of TF-3-g and TF-3,3′-dg (Table 2), one of whose precursors is EGCg, than central and southern African clonal green leaf (Wright et al., 2002). It is speculated that the very high levels of EGCg in the central and southern African clonal green leaf causes a flooding effect of EGCg quinones during fermentation, leading to formation of other products, possibly thearubigins (Wright et al., 2002). Due to more equitable distribution of the individual flavan-3-ols in Kenya green tea leaf, no such flooding effect occurs, leading to formation of more diverse theaflavins. Because EGCg is not limiting in central and southern African clonal green leaf, it becomes less important as a quality determinant.

In earlier fermentation trials, Cloughley (1979) showed that the optimum fermentation duration, measured by total theaflavin formation, was 40–50 min for the central African clonal green leaf. However, the range of optimal duration for Kenyan clonal teas was 75–125 min (Owuor & Reeves, 1986). The present results and those for central and southern African clonal green leaf (Wright et al., 2000) explain, in part, the large differences between fermentation duration for the central African (Cloughley, 1979) and Kenyan (Owuor & Reeves, 1986) clonal green leaves. The central African clonal green tea leaves are dominated by EGCg (Wright et al., 2000) that has lower redox potential (Bajaj et al., 1987; Robertson, 1983) and therefore ferments faster. Also, the presence of high amounts of EGCg in the central African clonal green leaf deactivates polyphenol oxidase faster (Bajaj et al., 1987; Spencer et al., 1988), shortening fermentation process.

Although several studies had used total theaflavins to determine quality, the success was variable (Deb & Ullah, 1968; Hilton & Ellis, 1972; Hilton & Palmer-Jones, 1975; Owuor et al., 1986; Roberts & Fernando, 1981). Some of the problems leading to less marked correlations was due to the variable contribution of the individual theaflavins to tea taste (Owuor & Obanda, 1995) and hence to quality. A normalising factor (theaflavin digallate equivalent) has recently been demonstrated to correlate significantly with sensory evaluation of all black teas, irrespective of geographical area of production (Owuor et al., submitted). Theaflavin digallate equivalent is therefore a more reliable plain black tea quality indicator and can therefore be used to predict black tea quality, irrespective of the genetic make up and/or geographical area of production.

The theaflavin digallate equivalent linearly and significantly correlated with green leaf EGCg ($r = 0.547$, $P \leqslant 0.078$), sum of gallated flavan-3-ols ($r = 0.669$, $P \leqslant 0.022$) and the ratio of gallated to non-gallated flavan-3-ols ($r = 0.527$, $P \leqslant 0.093$). However, there were significant and inverse relationships between theaflavin digallate equivalent and green leaf EC ($r = -0.751$, $P \leqslant 0.007$), and sum of dihydroxyflavan-3-ols ($r = -0.631$, $P \leqslant 0.035$). These results confirm the earlier results that gallated flavan-3-ol levels largely influence plain Kenyan black tea quality (Obanda et al., 1997, 1999). The high EGCg and/or sum of gallated flavan-3-ols and low levels of EC are therefore potentially useful parameters in selection of Kenyan clones for plain black tea quality potential.

The high levels of theaflavin digallate equivalent are based on high amounts of gallated theaflavins. TF-3-g (Table 3) formation was largely enhanced by EGCg ($r = 0.648$, $P \leqslant 0.029$), gallated catechins ($r = 0.611$, $P \leqslant 0.043$), and the ratio of gallated to non-gallated flavan-3-ols ($r = 0.558$, $P \leqslant 0.071$), but inhibited by high amounts of EC ($r = -0.544$, $P \leqslant 0.081$) and sum of dihydroxyflavan-3-ols ($r = -0.563$, $P \leqslant 0.068$). TF-3′-g formation was increased by EGCg ($r = 0.881$, $P \leqslant 0.0001$), sum of gallated catechins ($r = 0.662$, $P \leqslant 0.024$), sum of trihydroxyflavan-3-ols ($r = 0.581$, $P \leqslant 0.058$), and high trihydroxyflavan-3-ol to dihydroxyflavan-3-ol ratio ($r = 0.545$, $P \leqslant 0.08$), but decreased with high amounts of EC ($r = -0.734$, $P \leqslant 0.009$), and sum of dihydroxyflavan-3-ols ($r = -0.770$, $P \leqslant 0.005$). TF-3,3′-dg formation was enhanced by high amounts of the sum of gallated flavan-3-ols ($r = 0.523$, $P \leqslant 0.096$), and declined with high levels of EC ($r = -0.612$, $P \leqslant 0.043$). It is not clear why EGCg levels influenced the formation of TF-3′-g very significantly, yet it is involved in the TF-3′-g biosynthesis. This aspect needs further study.

Thearubigins are also an important plain black tea quality parameter (Roberts & Smith, 1963) and their high levels reduce black tea liquor brightness (Owuor et al., submitted). The levels of thearubigins were enhanced by high EC ($r = 0.694$, $P \leqslant 0.016$) levels. However, high amounts of EGC ($r = -0.591$, $P \leqslant 0.053$), sum of trihydroxyflavan-3-ols ($r = -0.641$, $P \leqslant 0.031$), and the ratio of trihydroxyflavan-3-ol to dihydroxyflavan-3-ols ($r = -0.697$, $P \leqslant 0.015$) significantly reduced thearubigins formation.

There was no green leaf biochemical parameter that was significantly correlated with black tea liquor colour in this study. However, liquor brightness improved with high amounts of EGC ($r = 0.654$, $P \leqslant 0.027$), EGCg ($r = 0.899$, $P \leqslant 0.0001$), sum of trihydroxy flavan-3-ols ($r = 0.750$, $P \leqslant 0.007$), and the trihydroxyflavan-3-ol to dihydroxyflavan-3-ols ratio ($r = 0.678$, $P \leqslant 0.020$), but declined with high amounts of EC ($r = -0.731$, $P \leqslant 0.009$), and sum of dihydroxyflavan-3-ol ($r = -0.843$,

$P \leqslant 0.001$). These results further demonstrate the importance high amounts of trihydroxyflavan-3-ols and low levels of dihydroxyflavan-3-ols in Kenya green tea leaf for the production of black teas of high quality.

Despite the development of the biochemical parameters for plain black tea evaluation, the tea trade still relies on sensory evaluation to determine quality and value. This is due to sensory evaluation being fast and practical. The correlations between black tea sensory evaluation and green leaf biochemical parameters are also presented in Table 3. For Taster A, there was linear and significant correlation between the sensory evaluations and EGC ($r = 0.643$, $P \leqslant 0.030$), EGCg ($r = 0.528$, $P \leqslant 0.092$), sum of trihydroxyflavan-3-ols ($r = 0.656$, $P \leqslant 0.026$) and the ratio of trihydroxyflavan-3-ol to dihydroxyflavan-3-ols ($r = 0.667$, $P \leqslant 0.023$), but negative correlation with EC ($r = -0.785$, $P \leqslant 0.003$) and the sum of dihydroxyflavan-3-ols ($r = -0.775$, $P \leqslant 0.004$). For Taster B, the correlation was linear and significant with EGCg ($r = 0.595$, $P \leqslant 0.051$) and the sum of gallated flavan-3-ols ($r = 0.522$, $P \leqslant 0.096$), but inverse and significant for EC ($r = -0.679$, $P \leqslant 0.020$) and sum of dihydroxy flavan-3-ols ($r = -0.638$, $P \leqslant 0.032$).

The results presented here demonstrate that the green leaf biochemical parameters which enhanced the pain black tea quality, as shown by high amounts of theaflavin digallate equivalent, and brightness and low levels of thearubigins, also enhance black tea sensory evaluations. It is therefore suggested that high levels of EGCg, sum of gallated catechins and ratio of trihydroxyflavan-3-ol to dihydroxyflavan-3-ols and/or low levels of EC and sum dihydroxy flavan-3-ol in green tea leaf be used to predict quality of Kenyan plain black tea.

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