THE USE GREEN TEA (*CAMELLIA SINENSIS*) LEAF FLAVAN-3-OLS 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 are necessary to shorten tea breeding and clonal selection programmes. Green leaf flavan-3-ols (catechins) and plain black tea quality of eleven Kenyan clones with wide genetic variations 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 were significantly correlated with black tea total theaflavin ($r = 0.749$, $P \leq 0.007$), theaflavin digallate equivalent ($r = 0.547$, $P \leq 0.078$), liquor brightness ($r = 0.899$, $P \leq 0.0001$) level and taster A ($r = 0.578$, $P \leq 0.092$), and taster B ($r = 0.595$, $P \leq 0.051$) sensory evaluations, and negatively with thearubigins ($r = -0.652$, $P \leq 0.027$), while epicatechin (EC) was positively correlated with thearubigins ($r = 0.694$, $P \leq 0.016$) and negatively with total theaflavins, ($r = -0.576$, $P \leq 0.061$), theaflavin digallate equivalent ($r = -0.751$, $P \leq 0.007$), and sensory evaluation by taster A ($r = -0.785$, $P \leq 0.003$) and taster B ($r = -0.679$. $P \leq 0.02$). Thus high levels of EGCg and low levels of EC are green leaf indicators of black tea quality potential of tea plants in Kenya. For related flavan-3-ols, the sum of gallated flavan-3-ols were significantly positively correlated with theaflavin digallate equivalent ($r = 0.669$, $P \leq 0.022$) levels, and sensory evaluation by taster B ($r = 0.522$, $P \leq 0.096$), but negatively with the black tea thearubigins ($r = -0.585$, $P \leq 0.052$) levels. The sum of trihydroxy flavan-3-ols (gallocatechins) was positively correlated with total theaflavins ($r = 0.525$, $P \leq 0.094$), brightness ($r = 0.75$, $P \leq 0.007$) and sensory evaluation by taster A ($r = 0.656$, $P \leq 0.026$), but negatively with thearubigins ($r = -0.641$, $P \leq 0.031$) levels. However, the sum of dihydroxy flavan-3-ols (simple catechins) levels were positively correlated with thearubigins ($r = 0.716$, $P \leq 0.012$) and negatively with total theaflavins ($r = -0.669$, $P \leq 0.022$), theaflavin digallate equivalent ($r = -0.631$, $P \leq 0.035$), brightness ($r = -0.843$, $P \leq 0.001$) and taster B ($r = -0.638$, $P \leq 0.032$) sensory evaluation. The ratios of trihydroxy to dihydroxy flavan-3-ols were positively correlated with brightness ($r = 0.678$, $P \leq 0.02$) and sensory evaluation of taster A ($r = 0.667$, $P \leq 0.023$) but negatively with thearubigins ($r = -0.697$, $P \leq 0.015$). In addition to green leaf high levels of EGCg and low levels of EC, high levels of sum of gallated catechins, trihydroxy-flavan-3-ols and ratio of trihydroxy to dihydroxy flavan-3-ols are also useful parameters in green tea leaf that can be used to predict black tea quality potential of Kenyan tea clones.

INTRODUCTION

World production of different beverages from the young tender shoots of tea (*Camellia sinensis* (L) O. Kuntze) has continued to rise[1] despite lack of commensurate increase in consumption. In Kenya, for example, production rose to 273.0 thousand metric tonnes of made tea in 2000 from 18.1 thousand metric tonnes in 1963[1] due to improvement in production technologies and large increase in land under tea. However, the price of tea has not improved with time[1] and sometimes is declining despite the continued increase in the costs of production. Lack of extensive expansion in tea consumption implies that the tea market will 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. High quality tea can only be obtained from raw material with correct quality potential[2]. But quantifiable breeding or selection criteria for quality have been elusive. Past tea breeding/clonal selection in Eastern Africa put emphasis mainly on yields[3] and whatever high quality material in production now might not have been selected for this attribute. Traditional selection/breeding methods in tea relied on a combination of morphological characteristics, which are slightly empirical, slow, and laborious[4]. Many high yielding and good quality planting materials have been developed by chance. Instead sensory evaluation was the only method of assessing the black tea after processing. Sensory evaluation is however subjective and is influenced by many factors outside quality[5,6]. Development of reliable and quantifiable selection criteria is therefore necessary.

A lot of time has been spent on the development of black tea quality parameters, which have only worked on limited tea samples. For example, theaflavins levels significantly correlated with sensory evaluation/prices of Central African[7-9], and North East Indian[10] black teas. However, the correlations were not significant for Kenyan[11,12] and Sri Lankan[13] black teas. The relative astringencies of the four predominant theaflavins in black tea (Figure 1), i.e. theaflavin digallate, theaflavin-3-gallate, theaflavin-3'-gallate and theaflavin, had been shown to be 6.4:2.2:2.2:1, respectively[14]. The lack of significant relationship between total theaflavins levels and sensory evaluation or prices of black tea produced in some countries[11-14], could be partly due to the fact that the total theaflavins might not have been a good measure for quality and taste of tea. With improvements in analytical procedures, it is now possible to partition the individual theaflavins and to calculate an astringency normalizing factor (theaflavin digallate equivalent)[15-17] of the various black teas. Using this factor, a better relationship was observed between the gallated theaflavins or theaflavins digallate equivalent and sensory evaluations[17] in teas where previous regressions using total theaflavins were insignificant[11,12]. In a recent study, this factor better correlated with the sensory evaluation of Eastern, Central and Southern African black teas[18], than the total theaflavins showing that this factor is a better measure of plain black tea quality irrespective of the country of production.

Despite the development of reliable black tea quality parameters that can be used successfully in breeding/clonal selection for quality programs, 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 areas, long durations and is expensive with no guarantee for success. Generally, from identification to first testing takes at least six years[19]. Consequently, the future of tea breeding/clonal selection lies in making assessment at 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 contain 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-ols composition of the clonal tea leaves vary[20]. For Kenyan black teas, when the individual polyphenols were partitioned, there was a positive significant relationship between epicatechin gallate and/or epigallocatechin with theaflavin digallate equivalent[21] while in another study epicatechin gallate and epigallocatechin gallate showed best relationship with sensory evaluation[20]. However, for Central and Southern African black teas, Wright *et al*[19] showed that the epicatechin levels correlated best with sensory evaluation, followed by the non-gallated catechins, and then the dihydroxy (simple) catechins. Whereas the observed differences between the Kenyan[20,21] and Central and Southern Africa[19] black teas, could partly be due to geographical area of production[22], it is also possible that the black teas used in each study had a very narrow genetic base[23] making discrimination difficult. This study was done to re-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 variabilities[24].

The theaflavins, or their attributes, especially theaflavin digallate equivalent, have dominant effect in the quality of black teas[7-12,15,17,18,25]. 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 Figure 1 and 2 respectively. The ratio of the dihydroxy to trihydroxy flavan-3-ol in the green leaf may therefore have a major influence on the ultimate amounts of the theaflavins in black tea. The previous studies relating the green leaf flavan-3-ol composition to black tea quality did not assess this aspect. This study also evaluated the possible relationship between the trihydroxy to dihydroxy flavan-3-01 ratios on formed theaflavins and sensory evaluations.

The amounts of the individual theaflavins formed are largely influenced by the amounts of the precursor catechins in green leaf, the redox potential and/or polyphenol oxidase preference of the individual catechins and activity. Since the level of the astringency is largely dependent on the extent of esterification of the theaflavins, more gallated theaflavins can only be formed if the flavan-3-ol gallate esters occur in reasonable quantities in green tea leaf. Whereas Wright *et al*[19] showed that sum of non-gallated flavan-3-ol levels had significant effect on black tea quality, there was no estimation of how the ratio of the gallated to non gallated catechins influence the ultimate black tea quality. This study also evaluated the possible role of the ratio of gallated to non-gallated flavan-3-ol ratios on the quality of black tea.

Although the thearubigins are thought of as polymeric materials from the polyphenols, their structures are not well documented. However, high thearubigins levels in black tea reduce the brightness[18]. This study also assessed how the individual flavan-3-ols levels in green leaf affect thearubigins formation.

EXPERIMENTAL

Leaf

Leaf was obtained from 11 clones in Clonal Field Trials planted at the Tea Research Foundation of Kenya, Timbilil Estate, 2180m 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 trihydroxy to dihydroxy flavan-3-ol their leaves[24] or wide variations in the individual theaflavins distribution in their black teas[15]. Except for clones 301/6 and 378/1 used in the study all clones had been released to the industry and are being used commercially. Clone 301/6 is a *Camellia sinensis* var *assamica* ssp *lasiocalyx*, while clone 378/1 is a *Camellia sinensis* var *assamica* polyploid (triploid) plant. It was hoped with the use of clones with wide genetic variation/diversity[24], a criteria developed would have wider application compared to limited clones used in previous studies[19-21]. The plants were receiving uniform agronomic and fertilizer at 150 Kg N ha^{-1} $year^{-1}$ as NPKS 25:5:5:5. Plucking was done at 10 to 14 days intervals depending on leaf availability. One kilogram of leaf comprising mostly of 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 to 18 hours to achieve 70% physical wither. The leaf was then miniature CTC macerated. Fermentation was done for 90 minutes and terminated using bench top fluid bed dryer (Teacraft, U.K.). The unsorted black teas were subjected to chemical analyses and sensory

evaluation as explained below.

Reagents

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

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 especially Kenyan teas, which they auction regularly.

Chemical analysis

Total theaflavins were determined by the Flavognost method[26] while the individual ratios were determined by HPLC[22,27,28]. Liquors were prepared by adding 4g of black tea to 195 ml deionised water that had just reached boiling and shaking was done for 10 min in 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 prior to HPLC analysis. The liquor was analysed on a Cecil Series 1000 HPLC with a 20 μ l sample loop and a Hypersil 5 μ ODS column (25cmx4.6mm). 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 per minute was used[22,27]. The theaflavin ratios calculated from the HPLC data and the Flavognost (total) theaflavins 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[28.]

The black tea thearubigins, liquor colour and brightness were determined as described by Roberts and Smith[29].

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Extraction and HPLC analysis of catechins in green tea leaf

The flavan-3-ols were analysed as outlined earlier[21]. Part of the leaf for manufacture (100 g) was steamed for one minute, then vacuum oven dried and crushed to powdery form. About 125 mg of the powder was extracted for each clone, in 25 ml acetonitrile water (1:1 v/v) mixture at room temperature for 30 minutes with constant shaking. Each clone was sampled four times.

The extract was filtered through a filter cartridge (DIS MIC 13HP, Advantec Toyo, Tokyo, Japan) and diluted five - fold with water before HPLC analysis on a Shiseido Capcell C18 UG 120 A 5 µm 4.6 x 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 to 5 min, 10% B; 5 to 25 min, 25% B; 25 to 26 min, 100% B; 26 to 35 min, 10% B; 35 to 45 min, 10% B. At flow rate 1.0 ml/min, 10 µl injection volume and monitored at 270 nm. Authentic flavanol standards ((+) catechin C; (-) epicatechin EC; (-) epigallocatechin EGC; (-) epicatechin gallate ECG; (-) epigallocatechin gallate EGCG) were used to identify and calculate the concentration of flavanols in green tea leaf clonal samples.

Analysis of Variance and Regressions

The results were subjected to analysis of variance using MSTAT statistical package. The means were

used to do linear regressions between black tea quality parameters and sensory evaluations and green tea leaf flavan-3-ol..

RESULTS AND DISCUSSION

Several studies have been conducted to determine green leaf biochemical compounds, which can be used to predict quality of black tea. The carotenoids and chlorophyll content were negatively correlated[30] while caffeine content was positively correlated with quality [20,31]. However, the biochemical parameter influencing plain black tea quality is the green leaf are polyphenols, especially the flavanols, which are responsible for the formation of the black tea thearubigins and theaflavins[8]. Whereas the total polyphenols correlated with black tea quality[32], conflicting results have been given 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 of some tea clones[20]. The trihydroxy flavan-3-ol best predicted the black tea brightness potential while ECg correlated best with theaflavin digallate equivalent[21]. However, Wright *et al*[19] showed EC to be the critical flavan-3-ol in predicting the quality potential of Central and Southern African tea cultivars. Despite the significant relationship observed between total polyphenols and black tea quality[32] , some polyphenols do not contribute to the formation of any black tea quality parameter. Only flavan-3-ols are critically in the biosynthesis of the black tea quality parameters.

The distribution of the flavan-3-ol in the green tea leaves of the clones used in this study is presented in Table 1. There were clones in the study in which epigallocatechin was the dominant flavan-3-ol, while in some epicatechin gallate dominated. Figure 3 demonstrates the kind of variations that were exhibited. These results demonstrate that the clones used in this study had wide distribution of the flavan-3-ols suggesting large genetic variations[23,24]. Indeed there were significant ($P \leq 0.05$) differences in the levels of all individual flavan-3-ols suggesting that the quality potentials of these clones were different[18].

The astringency of the resultant theaflavins biosynthesised is largely influenced by the amounts of the esterified flavan-3-ols[14]. The sum of the gallated flavan-3-ols and ratio of gallated to non-gallated flavan-3-ols are presented in Table I. These parameters were significantly ($P \leq 0.05$) different in the clones used demonstrating further that the astringency potential of the black teas from the study were likely to be different.

Magoma *et al*[24] demonstrated that the genetic variability of tea clones can be determined, in part by the ratios of the trihydroxy and dihydroxy flavan-3-ols. The total trihydroxy and dihydroxy flavan-3-ols in the green leaf used in the study are also presented in Table 1. These parameters were significantly ($P \leq 0.05$) different in the green leaf of the clones used. The ratio of trihydroxy to dihydroxy flavan-3-ols varied from 0.40 in clone 301/6 to 2.10 in clone 31/11 demonstrating the large genetic variations in the clones used in the study. The formation of theaflavin requires a combination of a trihydroxy flavan-3-ol a dihydroxy flavan-3-ol[33,34]. A correct balance and amount of the trihydroxy and dihydroxy flavan-3-ols are therefore necessary to ensure maximum formation of the theaflavins, which are the key chemical quality parameters of black tea[7-10,12,13,17-19,25]. However, the redox potentials of the flavan-3-ols[35] and their affinity to polyphenol oxidase vary with the trihydroxy flavan-3-ols having lower redox potentials. The trihydroxy flavan-3-ols are therefore oxidised faster during the fermentation phase of black tea processing. Although in clones used in this study their levels were higher, they could be limiting factor to theaflavins formation as they run out faster. However, the flavan-3-ol gallate esters have a higher substrate inhibition property on polyphenol oxidase than the non-gallated flavan-3-ols[34], due to increased complexation to proteins, as a result of higher molecular weights and flexibility[36]. The situation is therefore complicated by several factors. However, in our fermentation trials, we have observed a fast disappearance of the trihydroxy flavan-3-ols. The levels of trihydroxy and dihydroxy flavan-3-ols and their ratios were significantly ($P \leq 0.05$) different. This further demonstrated the possible

differences in the black tea quality potential in the clones used in the study.

Despite the large differences in the individual flavan-3-ols composition, the total flavan-3-ol levels in the clones were not significantly ($P \leq 0.05$) different. The results were not surprising as the clones had all passed chloroform test[37] which is the only biochemical test traditionally and routinely used in assessing the potential of new cultivars to make black teas. It was shown that this test is not reliable in selecting tea plants for black tea quality[25].

All the plain black tea quality parameters and sensory evaluations (Table 2) were significantly ($P \leq 0.05$) different, demonstrating that the composition of the individual flavan-3-ols is more critical in black tea quality than the total flavan-3-ols *per se*. Provided the thresh hold level of flavan-3-ols is present, the total may not be important. There is need to work out this thresh hold level. Generally the Kenyan tea clones make black teas with very high total theaflavins levels[11] suggesting presence of adequate amounts of total flavan-3-ols in green leaf. A survey of even rejected clones as being of poor quality showed that their black tea theaflavins levels were higher than even good quality clones planted in Central Africa[11]. Further breeding work is therefore necessary to produce clones with much lower or wider range of flavan-3-ols to facilitate the determination of the critical thresh hold level of flavan-3-ols in green leaf 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 green leaf parameters significantly influencing black tea quality. The total (Flavognost) theaflavin had positive and significant correlation with EGCg ($r = 0.749$, $P \leq 0.007$), and sum of trihydroxy flavan-3-ols ($r = 0.525$, $P \leq 0.094$), but negative and significant correlation with the sum of dihydroxy flavan-3-ols ($r = -0.669$, $P \leq 0.022$) and EC ($r = -0.576$, $P \leq 0.061$). The positive and significant correlation shown here with EGCg confirms the earlier observations of Obanda *et al*[21] using tea clones of narrow genetic base. Thus for Kenyan clones in the production of plain black teas, selection can be done at single bush or green leaf level using EGCg and sum of trihydroxy flavan-3-ols and/or ratio of the trihydroxy to dihydroxy flavan-3-ol. These results are at variance with studies from Central and Southern Africa in which EC had the best correlation with black tea theaflavins[19]. 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[23]. This may be responsible for the wide variations in the polyphenol profiles in their black teas[22]. However the differences could also be caused by the difference in the environments these teas were planted. Indeed, in the Central and Southern Africa green leaf EGCg highly dominated the flavan-3-ols composition[19], while in this study there was no catechin with such dominant composition effects. The major catechin varied with clones. 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 and *vice versa*.

Comparison of the flavan-3-ols data presented here and those on tea clones produced in Central and Southern Afraca[19] reveals a large difference that had not been observed before. From the higher total theaflavins in Kenyan black teas than the Central African black teas[11], it had been assumed that Kenyan green tea leaves have higher flavan-3-ols than those from Central Africa. Although the differences in the theaflavins between Kenyan teas used here and those used in a recent Central African black tea study[25] reaffirm the earlier observations, the Central African tea clones had on the average about twice the amounts of flavan-3-ols[19] compared to the Kenya tea clones used in this study. Thus the earlier speculation that the total amounts of flavan-3-ols in green leaf largely influenced the resultant total theaflavins in black tea is incorrect. The distribution of the individual flavan-3-ols in green leaf is more critical to theaflavins formation, not total flavan-3-ols *pre se.*

On the average about 50% of the Central and Southern African green tea leaf flavaan-3-ols

composition is EGCg[19]. 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 biosynthesised black teas with high amounts of TF-3-g and TF-3,3'-dg (Table 2) whose one of the precursors is EGCg than the Central and Southern African clonal green leaf[25]. It is speculated that the very high levels of EGCg in the Central and Southern African clonal green leaf cause a flooding effect of EGCg quinones during fermentation leading to formation of other products, possibly thearubigins[25]. 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. EGCg is therefore not limiting in the Central and Southern African clonal green leaf, consequently becoming less important as a quality determinant.

In earlier fermentation trials, Cloughley[38] showed that the Optimum fermentation duration for the Central African clonal green leaf was 40 to 50 minutes. The range of optimal duration for Kenyan clonal teas was however 75 t0 125 minutes[39]. The present results and those presented for the Central and Southern African clonal green leaf[22] explain in part the large differences between fermentation duration for the Central African[38] and Kenya[39] clonal green leaves.. The Central African clonal green tea leaves are dominated with EGCg[19] that has lower redox potential[34] and therefore ferments faster. The presence of high amounts of EGCg in the Central African clonal green leaf deactivates polyphenol oxidase faster[35],[36] shortening fermentation process.

Although several studies had used total theaflavins to determine quality, the success was variable[7-13]. Some of the problems leading to less success of the correlations was due to the variable contribution of the individual theaflavins to tea taste[17] and hence quality vary. A normalising factor (theaflavin digallate equivalent), was recently demonstrated to significantly correlate with sensory evaluation of all black teas irrespective of geographical area of production[18]. 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[19].

The theaflavin digallate equivalent linearly and significantly correlated with green leaf EGCg ($r = 0.547$, $P \leq 0.078$), sum of gallated flavan-3-ols ($r = 0.669$, $P \leq 0.022$) and the ratio of gallated to non-gallated flavan-3-ols ($r = 0.527$, $P \leq 0.093$). However, there were significant and inverse relationship between theaflavin digallate equivalent and green leaf EC ($r = -0.751$, $P \leq 0.007$), and sum of dihydroxy flavan-3-ols ($r = -0.631$, $P \leq 0.035$). These results confirm the earlier results that gallated flavan-3-ols levels largely influence plain Kenyan black tea quality[20,21]. The high EGCg and/or sum of gallated flavan-3-ols and low levels of EC are therefore reliable parameters in selection of Kenyan clones for black tea quality potential.

The high levels of theaflavin digallate equivalent are largely influenced by the presence of high amounts of gallated theaflavins. The TF-3-g (Table 3) formation was largely enhanced by EGCg ($r = 0.648$, $P \leq 0.024$), sum of trihydroxy flavan-3-ols ($r = 0.611$, $P \leq 0.043$), and the ratio of gallated to non-gallated flavan-3-ols ($r = 0.538$, $P \leq 0.071$), but inhibited by high amounts of EC ($r = -0.544$, $P = 0.081$) and sum of dihydroxy flavan-3-ols ($r = -0.563$, $P \leq 0.068$). The TF-3'-g formation was increased by EGCg ($r = 0.881$, $P \leq 0.0001$), sum of gallated flavan-3-ols ($r = 0.662$, $P \leq 0.024$), sum of trihydroxy flavan-3-ols ($r = 0.581$, $P \leq 0.058$), and high trihydroxy to dihydroxy flavan-3-ol ratio ($r = 0.545$, $P \leq 0.08$), but decreased with high amounts of EC ($r = -0.734$, $P \leq 0.009$), and sum of dihydroxy flavan-3-ols ($r = -0.77$, $P \leq 0.005$). TF-3, 3'-dg formation was enhanced by high amounts of the sum of gallated flavan-3-ols ($r = 0.523$, $P \leq 0.096$), and declined with high levels of EC ($r = -0.612$, $P \leq 0.043$). These results demonstrate that selecting Kenyan clones with high amounts of EGCg, and sum of gallated flavan-3-ols, and/or low levels of EC and sum of dihydroxy flavan-3-ol lead to black teas with high TFdg equivalent and hence quality.

Thearubigins are also an important black tea quality parameter[29] and their high levels reduce black tea

liquor brightness[18]. The levels of thearubigins were enhanced by high EC (r = 0.694, P≤0.016) levels. However, high amounts EGC (r = -0.591, P≤0.053), sum of trihdroxy flavan-3-ols (r = -0.641, P≤0.031), and the ratio of trihydroxy to dihydroxy flavan-3-ols (r = -0.697, P≤0.015) significantly reduced the thearubigins formation.

There was no green leaf biochemical parameter that was significantly correlated with black tea liquor colour in this study. However, the liquor brightness improved with high amounts of EGC (r = 0.654, P≤0.027), EGCg (r = 0.899, P≤0.0001), sum of trihydroxy flavan-3-ols (r = 0.75, P≤0.007), and the trihydroxy to dihydroxy flavan-3-ols ratio (r = 0.678, P≤0.02), but declined with high amounts of EC (r = -0.731, P≤0.009), and sum of dihydroxy flavan-3-ol (r = -0.843, P≤0.001). These results further demonstrate the importance high amounts of trihydroxy flavan-3-ols and low levels of dihydroxy flavan-3-ols in Kenya green tea leaf for the production of black teas of high quality.

Despite the use of the biochemical parameters in black tea, the tea trade still relies mostly on sensory evaluation to determine quality and value. The correlation between black tea sensory evaluations 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 = 0.65, P≤0.03), EGCg (r = 0.528, P≤0.092), sum of trihydroxy flavan-3-ols (r = 0.656, P≤0.026) and the ratio of trihydroxy to dihydroxy flavan-3-ols (r = 0.667, P≤0.023), but negative correlation with EC (r = -0.785, P≤0.003), and sum of dihydroxy flavan-3-ols (r = -0.775, P≤0.004). For taster B the correlation was linear and significant with EGCg (r = 0.595, P≤0.051), and sum of gallated flavan-3-ols (r = 0.522, P≤0.096), but inverse and significant for EC (r = -0.679, P≤0.02) and sum of dihydroxy flavan-3-ols (r = -0.638, P≤0.032).

The results presented here demonstrate that the green leaf biochemical parameters which enhanced the 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 and *vice versa*. It is therefore suggested that high levels of EGCg, sum of gallated catechins and ratio of trihydroxy to dihydroxy flavan-3-ols and/or low levels of EC and sum dihydroxy flavan-3-ol in green tea leaf can be used to predict quality of Kenyan black tea.

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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 (µmoles/g)	+C (µmoles/g)	EC (µmoles/g)	EGCG (µmoles/g)	ECG (µmoles/g)	Gallated (µmoles/g)	Non-gallated (µmoles/g)	Gallated/ non gallated ratio	Trihydroxy *flavan-3-ols* (µmoles/g)	Dihydroxy *flavan-3-ols* (µmoles/g)	Trihydroxy/ dihydroxy *flavan-3-ols*	Total *flavan-3-ols* (µmoles/g)
6/8	86.28	12.79	36.21	64.30	49.04	113.34	135.28	0.84	150.58	98.04	1.54	247.12
S15/10	117.57	14.74	18.19	68.23	55.83	124.06	150.50	0.85	185.80	88.76	2.08	274.56
Ejulu	63.89	30.87	28.88	55.95	61.48	117.43	123.63	0.95	119.84	121.23	0.99	241.07
31/11	106.70	6.55	8.97	67.96	58.94	126.89	122.22	1.05	174.66	74.45	2.36	249.11
301/6	13.89	5.60	110.00	43.89	61.71	105.60	129.50	0.81	57.78	177.32	0.33	235.09
303/35	86.28	15.69	37.42	67.52	50.06	117.58	139.38	0.87	153.80	103.17	1.49	256.96
303/216	92.65	16.55	34.49	63.76	51.08	114.83	143.69	0.81	156.40	102.12	1.55	258.52
347/314	104.33	13.02	29.40	68.88	52.44	121.31	146.74	0.85	173.21	94.85	1.83	268.01
378/1	55.97	17.33	28.88	68.72	61.37	130.10	102.17	1.28	124.69	107.58	1.16	232.27
F7/346	82.84	6.98	30.69	71.24	58.40	130.33	120.52	1.10	154.08	96.67	1.59	250.75
PMC 61	54.33	15.52	15.61	70.91	67.53	138.44	85.46	1.66	125.24	98.66	1.27	223.90
CV (%)	19.29	20.26	17.76	10.41	8.59	8.88	15.14	16.14	12.32	9.04	11.16	9.76
LSD, P ≤0.05	21.90	4.14	8.82	9.72	7.09	15.61	27.80	0.23	25.69	13.79	0.24	NS

Table 2: The plain *black tea` quality parameters of different clones*

Clone	Total theaflavins (µmoles/g)	TF (µmoles/g)	TF-3-g (µmoles/g)	TF-3'-g (µmoles/g)	TF-3,3'-dg (µmoles/g)	TF dg eq (µmoles/g)	Thearubigins (%)	Total colour (%)	Brightness (%)	Taster A	Taster B
6/8	26.38	12.47	6.86	4.16	2.64	8.42	18.62	5.43	31.29	51	20
S15/10	19.23	7.58	5.20	3.19	3.33	7.42	15.63	4.21	27.58	33	15
Ejulu	18.00	4.00	5.14	2.56	6.31	10.03	18.40	5.32	21.65	50	20
31/11	22.22	4.06	6.47	3.81	7.88	12.08	15.19	5.02	29.01	59	21
301/6	14.75	7.71	4.58	1.25	1.20	4.44	19.60	4.53	15.97	8	13
303/35	21.44	9.36	5.43	3.88	3.03	7.47	17.23	4.36	30.54	32	17
303/216	19.95	10.16	4.98	2.95	1.87	6.21	15.21	4.61	29.71	46	18
347/314	25.35	11.95	6.49	3.90	3.00	8.48	18.06	5.49	29.93	44	20
378/1	24.87	7.82	6.91	4.70	5.44	10.69	17.22	5.57	31.20	35	20
F7/346	22.60	8.94	5.93	3.72	3.77	8.60	16.68	4.92	29.16	41	21
PMC	22.66	7.27	6.71	4.01	4.10	9.54	16.03	4.70	28.44	38	19
C.V.%	13.03	19.78	13.41	17.73	24.36	15.02	8.16	13.31	15.09	33.54	8.16
LSD, P≤0.05	4.06	2.37	1.16	0.89	1.36	2.01	2.01	0.95	6.03	19	2

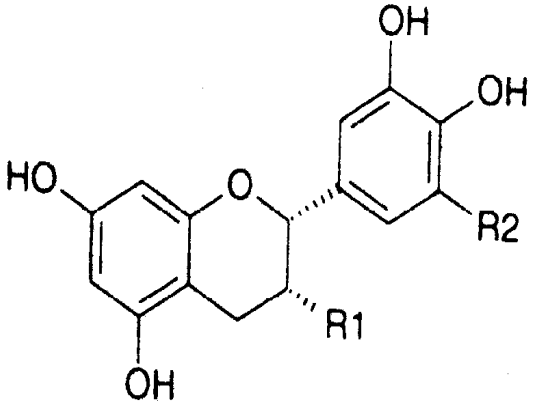
Table 3: Linear regression coefficients and significant levels between plain black tea quality parameters and catechins or catechin ratios of different clones

1

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	0.189	0.195	0.457	0.206	0.291	-0.591	-0.035	0.654	0.643	0.344
	NS	NS	NS	NS	NS	NS	0.053	NS	0.027	0.03	NS
Catechin	-0.075	-0.244	-0.118	0.028	0.288	0.224	0.082	0.23	-0.023	0.266	0.174
	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Epicatechin	-0.576	0.153	-0.544	-0.734	-0.612	-0.751	0.694	-0.203	-0.731	-0.785	-0.679
	0.061	NS	0.081	0.009	0.043	0.007	0.016	NS	0.009	0.003	0.02
Epigallocatechin gallate	0.749	0.2	0.648	0.881	0.305	0.547	-0.652	0.118	0.898	0.528	0.595
	0.007	NS	0.029	0.0001	NS	0.078	0.027	NS	0.0001	0.092	0.051
Epicatechin gallate	-0.305	-0.683	0.074	-0.168	0.407	0.305	-0.022	0.02	-0.45	-0.238	-0.002
	NS	0.019	NS	NS	NS	NS	NS	NS	NS	NS	NS
Gallated catechins	0.461	-0.256	0.611	0.662	0.523	0.669	-0.582	0.115	0.499	0.303	0.522
	NS	NS	0.043	0.024	0.096	0.022	0.052	NS	NS	NS	0.096
Non gallated catechins	-0.187	0.385	-0.504	-0.307	-0.42	-0.501	0.061	-0.263	-0.022	-0.012	-0.349
	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Gallated/Non gallated ratio	0.287	-0.312	0.558	0.453	0.399	0.527	-0.302	0.133	0.212	0.072	0.36
	NS	NS	0.071	NS	NS	0.093	NS	NS	NS	NS	NS
Gallo catechins	0.525	0.203	0.31	0.581	0.241	0.367	-0.641	-0.002	0.75	0.656	0.422
	0.094	NS	NS	0.058	NS	NS	0.031	NS	0.007	0.026	NS
Simple catechins	-0.669	-0.062	-0.563	-0.77	-0.459	-0.631	0.716	-0.139	-0.843	-0.775	-0.638
	0.022	NS	0.068	0.005	NS	0.035	0.012	NS	0.001	0.004	0.032
Gallo/simple catechins ration	0.483	0.049	0.363	0.545	0.37	0.46	-0.697	-0.017	0.678	0.667	0.421
	NS	NS	NS	0.08	NS	NS	0.015	NS	0.02	0.023	NS
Total catechins	0.055	0.35	-0.261	0.017	-0.224	-0.233	-0.267	-0.256	0.283	0.182	-0.123
	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

*Numbers in bracket are significant levels, limit set at P = 0.10

2

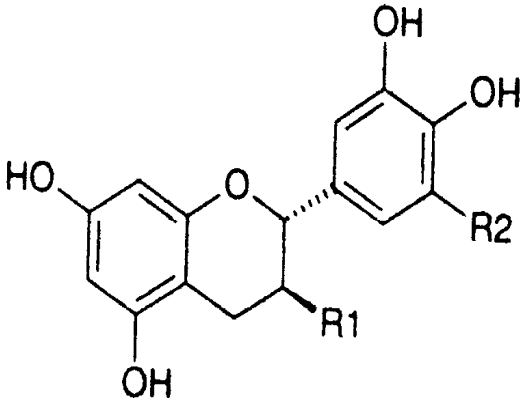


(-) Epicatechin (EC)
R1 = OH
R2 = H

(-) Epicatechin-3-gallate (ECg)
R1 = 3,4,5-trihydroxybenzoyl
R2 = H

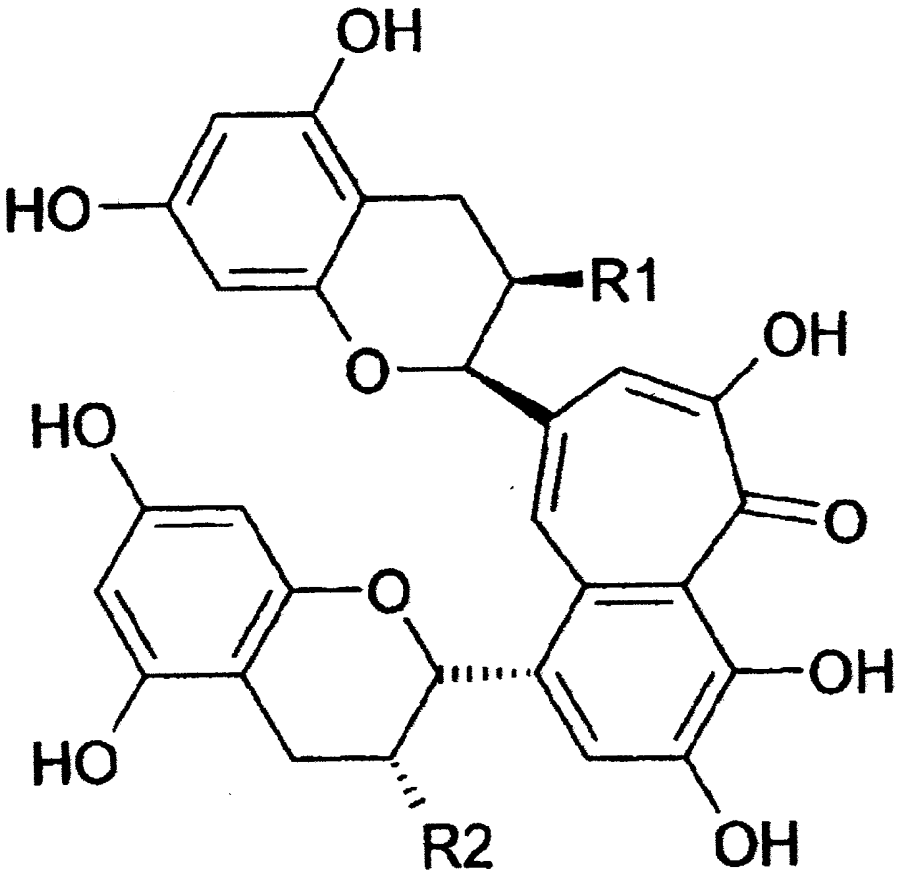
(-) Epigallocatechin (EGC)
R1 = R2 = OH

(-) Epigallocatechin-3-gallate (EGCg)
R1 = 3,4,5-trihydroxybenzoyl
R2 = OH



(+) Catechin (C)
R1 = OH
R2 = H

Figure 1: The flavan-3-ols (catechins) in fresh tea leaves



I Simple theaflavin (TF)
R1 = OH
R2 = OH

II Theaflavin-3-gallate (TF-3-G)
R1 = 3,4,5-trihydroxybenzoyl
R2 = OH

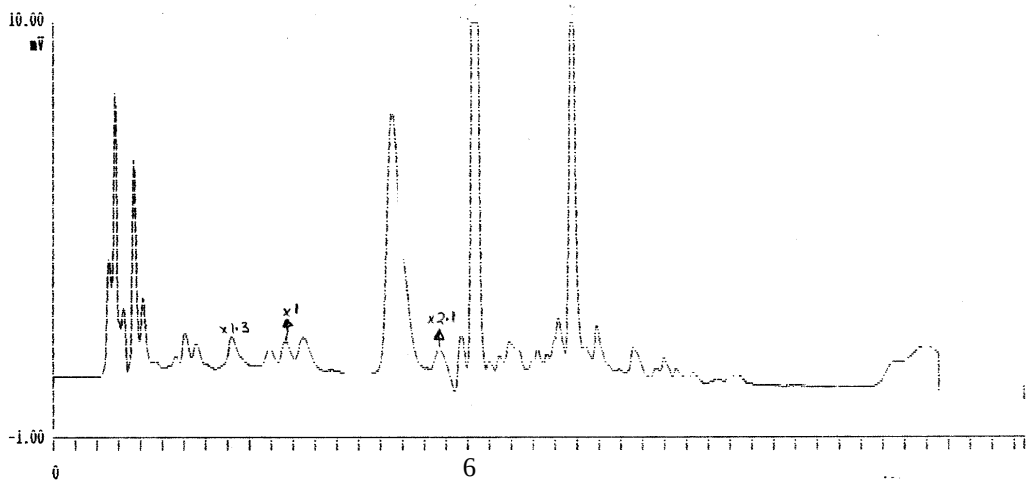
III Theaflavin-3'-gallate (TF-3'-G)
R1 = OH
R2 = 3,4,5-trihydroxybenzoyl

IV Theaflavin-3,3'-digallate (TF-3,3'-DG)
R1 = 3,4,5-trihydroxybenzoyl
R2 = 3,4,5-trihydroxybenzoyl

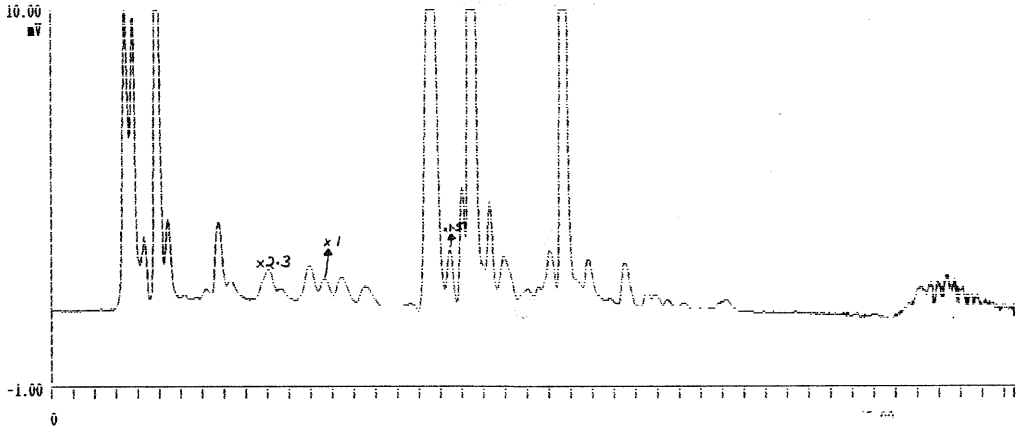
Figure 2: The major individual theaflavins in black teas

GA 1 1

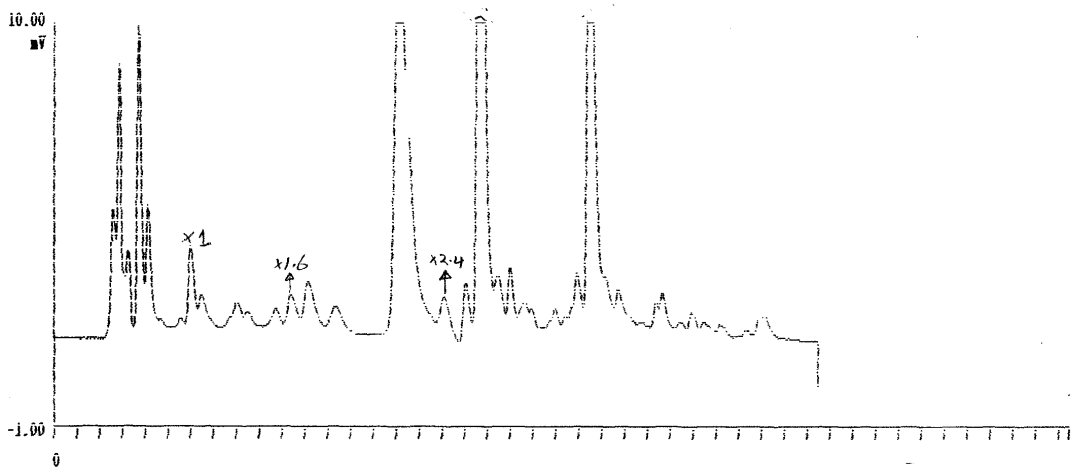
Clone 6/8 X 23.2 X 17.0



Clone 31/11 x25.5 X 26.3



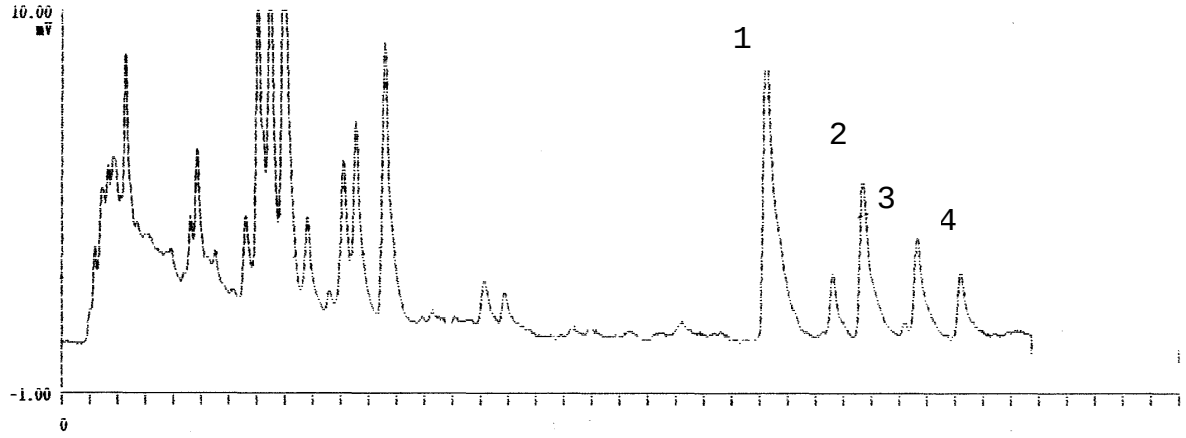
Clone 38//1 x27.5 X 26.0



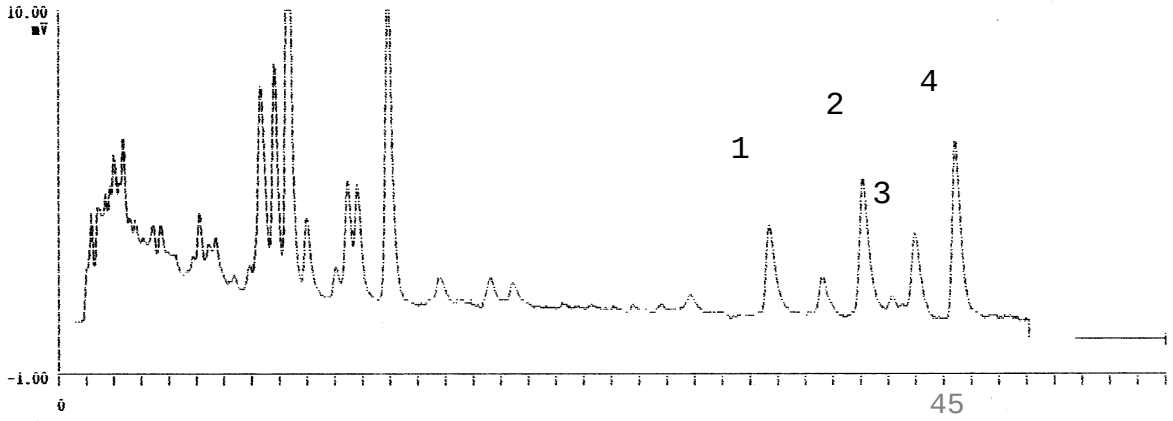
Time - minutes

Figure 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

Clone 6/8



Clone 31/11



Clone 378/1

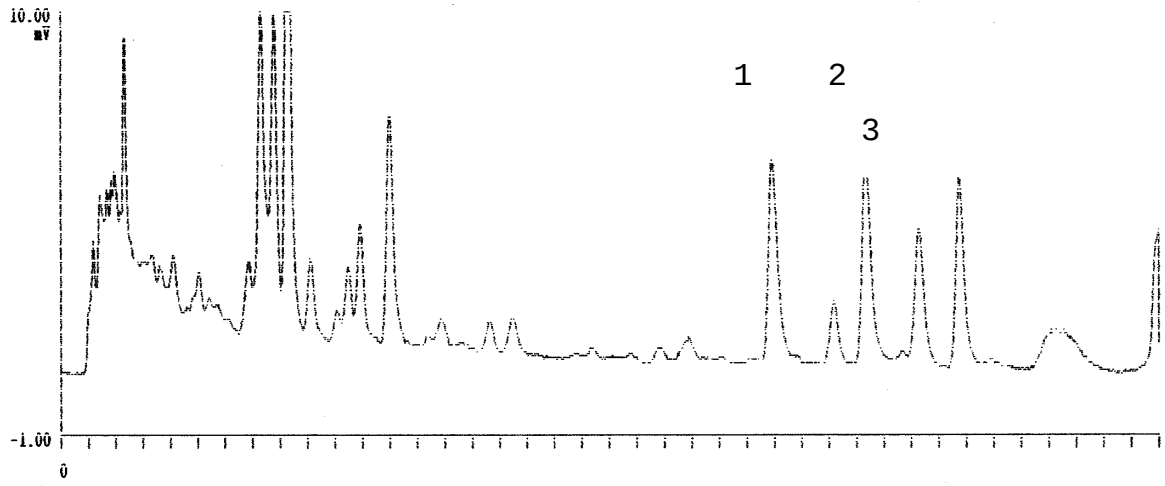


Figure 4: *The HPLC patterns (at 365 nm) of individual theaflavins in different clonal black teas*
1 = TF, 2 = TF-3-g, 3 = TF-3'-g, 4 = TF-3,3'-dg