Flavanol Composition and Caffeine Content of Green Leaf as Quality Potential Indicators of Kenyan Black Teas

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Abstract: The flavanol composition and caffeine content of green tea leaf, black tea quality parameters of theaflavins, thearubigins, liquor brightness and total colour varied more among clones than with time of the year. In green leaf, either (−)-epicatechin gallate or (−)-epigallocatechin gallate was the dominant flavanol present. Regression analysis of tasters’ preferences for black teas against green leaf chemical components showed positive and significant correlations for (−)-epicatechin gallate ($r = 0.498, P < 0.05$ for taster A; $r = 0.665, P < 0.01$ for taster B, and $r = 0.678, P < 0.01$ for both tasters’ overall ranking), (−)-epigallocatechin gallate ($r = 0.513, P < 0.05$ for taster B; $r = 0.532, P < 0.05$ for both tasters’ overall ranking and caffeine ($r = 0.523, P < 0.05$ for taster A; $r = 0.657, P < 0.01$ for taster B; and $r = 0.686, P < 0.01$ for both tasters’ overall ranking). Similar regressions against black tea theaflavins, thearubigin content, liquor brightness and total colour were not significant. The results suggest that the green leaf chemical components, (−)-epicatechin gallate, (−)-epigallocatechin gallate and caffeine could be used as quality potential indicators during clonal selection and propagation.

Key words: flavanols, (−)-epicatechin, (−)-epigallocatechin gallate, (−)-epicatechin gallate, theaflavin, thearubigin, astringency, brightness, caffeine.

INTRODUCTION

To increase economic returns (profits) black tea producers have tended to focus on increasing yields per unit land area through improved agronomic and cultural practices rather than on clonal selection for high quality. The latter has received little attention due to the lack of objective indicators for the quality potential of leaf at the clonal selection stage in tea production. Recently, attempts have been made to remedy the situation. Owuor et al (1986b) have developed an index based on the volatiles composition of black tea which has shown reasonable relationship with tasters’ evaluation. Similarly Taylor et al (1992) reported that the carotenoid and chlorophyll composition of green leaf had variable influence on the tasters’ preference of black teas. At present these methods are at different stages of evaluation and adoption by black tea producers and the development of further clonal selection methods for quality continues. Clones have differing abilities to produce caffeine. The important role that caffeine has in black tea quality characteristics has been acknowledged by several workers. Bhatia (1963), Millin et al (1969), Deb and Ullah (1968) observed that caffeine contributes towards the briskness of black tea. Caffeine complexes with the polyphenols in tea, mainly theaflavins (Roberts 1962; Collier et al 1972). This complex modifies the taste characteristics of both caffeine and theaflavins.
Plain black teas are evaluated for their briskness, brightness, strength, body and total colour of liquors (Roberts and Smith 1963). These black tea quality attributes result from the presence of flavanols in the green leaf and the oxidation and condensation of these flavanols during the fermentation stage in black tea manufacture (Bhatia 1961; Millin et al 1969; Hilton and Palmer-Jones 1973). The main flavanols found in green leaf include (+)gallocatechin, (+)catechin, (−)epicatechin, (−)epigallocatechin, (−)epigallocatechin gallate and (−)epicatechin gallate. The major products formed from the oxidation of the flavanols are theaflavins and thearubigins which are responsible for most of the plain black tea quality attributes (Roberts and Smith 1963; Takino et al 1964; Brown et al 1966; Berkowitz et al 1971; Robertson 1983). Thus, Ellis and Cloughley (1981) found that the prices of Central African black teas were highly dependent on the theaflavin content. And in a related study, Owuor et al (1986) showed that the correlation between theaflavin content and tasters’ evaluations of Kenyan black teas gave positive, though statistically non-significant, correlation coefficients. More recently, Owuor and Obanda (1995) have observed that the levels of theaflavin-3′,3′-digallate and the theaflavin digallate equivalents of black teas show a better relationship with sensory evaluation than did total (flavognost) theaflavins.

Due to their significant role in the manufacture of black tea and on its quality, attempts have been made to correlate the levels of flavanols and other polyphenols present in green leaf with the quality of black tea. Hilton and Palmer-Jones (1973) reported that the concentration of (−)epigallocatechin in the fresh shoots is highly correlated with the levels of theaflavins and hence with the pricing of black tea. Recently, Obanda et al (1992) using the phenol reagent method (Dev Choudhury and Goswami 1983), reported that the total polyphenol content in the fresh leaf positively correlated with the plain black tea quality parameters of brightness, thearubigin contents and total colour, and with black tea organoleptic evaluation by three professional tea tasters. However, only one taster’s evaluation was statistically significant. The present paper reports on the further development of this work in which a wider range of clonal materials growing in the Nandi Hills (West Rift Valley) tea growing region of Kenya were used. The variations between individual flavanols and caffeine content in the green leaf, black tea chemical quality parameters and tasters’ evaluations are discussed.

MATERIALS AND METHODS

Leaf

Two leaves and a bud portions of fresh green shoots from 15 high yielding clones (BB35, 6/8, C182/40, D99/10, C12, D69/99, H81/22, TN14/3, D8/44, D1-32, TN15/23, EJULU, C8/27, D8/55 and S15/10) planted in Sitoi Estate (altitude 2060 m amsl), Eastern Produce Kenya Limited, situated in the Nandi Hills tea-growing region, West Rift Valley of Kenya, and under the same agronomic and cultural management, were plucked on twelve separate occasions between October 1993 and September 1994 for manufacture and chemical analysis.

Green leaf flavanol extraction

A portion of the two leaves and a bud fresh tea was steamed for 30 s immediately after plucking and then dried in a vacuum oven at 100°C for at least 6 h. The sample from the vacuum oven was allowed to cool to room temperature before being placed in a plastic satchet, flushed with pure nitrogen and sealed to wait for extraction. Dried samples for each clone collected over a period of 4 months were bulked and ground to fine powder before extraction as follows. About 200 mg of finely ground leaf tea samples were weighed in duplicate to the nearest 0.1 mg and put into glass tubes. Aqueous methanol (5 ml of 700 ml litre⁻¹) at 70°C was added to each tube and the tubes placed in a water bath set at 70°C for 10 min, with mixing after each 5 min. The tubes were then removed from the water bath, allowed to cool for a few minutes and centrifuged at 3000 × g for 10 min. The supernatant was carefully decanted into clean graduated glass tubes and the extraction procedure repeated on the residue. The two extracts for each sample were combined and the volume made to 10 ml with aqueous methanol.

One millilitre aliquots of the green tea leaf flavanol extracts were put into separate tubes and diluted to 5 ml with mobile phase A described below. The contents were mixed and then filtered through 0.2 μm filters (Millipore Ltd, UK). This solution was then used for HPLC analysis.

Total polyphenol content in green leaf

The total polyphenol content in two leaves, bud and the connecting stem parts for each clone was determined by the phenolic reagent method first described by Dev Choudhury and Goswami (1983). In this method, 20 g fresh tea shoots are refluxed with 400 ml distilled deionised water for 1 h. The extract is filtered and the residue is washed with distilled deionised water and filtered as before. After cooling, the volume of the extract is made
up to 500 ml and 0.5 ml of the filtrate is diluted to 50 ml in a volumetric flask. One millilitre of the solution is pipetted into a test tube containing 1 ml of Folin-Ciocalteu phenol reagent (BDH Ltd, UK, one volume of the phenol reagent was diluted to three volumes with distilled water before use). Two ml sodium carbonate solution (350 g dissolved in 1 litre distilled water, filtered after overnight storage) are added to the mixture, shaken thoroughly and diluted to 6 ml by adding 2 ml of water. The mixture is allowed to stand for 0-5 h for completion of the reaction and the blue colour formed is measured at 700 nm using a spectrophotometer (CE 393 Digital Grating Spectrophotometer, Cecil Instruments, Cambridge, UK). The optical densities are converted into concentrations from a standard curve previously made by using 0-60 µg of (+)catechin with phenol reagent and sodium carbonate in a similar manner. In these experiments, the standard curve obtained had a minimum \( r^2 \) value of 0.996 and passed through the origin.

**Black tea manufacture**

Plucked tea shoots were subjected to withering under ambient conditions to achieve 720 g kg\(^{-1}\) moisture content over a period of 18-21 h. After withering the leaf was macerated four times using the Crush, Tear and Curl (CTC) machine followed by fermentation at 22°C for 90 min. Fermentation was terminated by drying Curl (CTC) machine followed by fermentation at 22°C

**Measurement of black tea chemical quality parameters**

The total theaflavin contents (µmols g\(^{-1}\)) were measured by the Flavognost method (Hilton 1973), while total colour, thearubigins content and percent brightness were determined by the Roberts and Smith method (1963).

**Sensory analysis of black tea**

Each month black tea samples were manufactured from each of the fifteen clones being tested in this study and sent to two broking firms based in Mombasa for professional tasting. The tasters (A and B) scored the black teas for briskness, brightness, colour, thickness, overall quality and infusion to give a total score.

**Statistical methodology**

The total score obtained for each black tea sample was considered a direct reflection of the tasters’ order of preference for the clonal material for the relevant month of study.

However, in different months the tasters would score the various attributes of black tea quality on different scales thus making statistical analysis difficult. To reduce the effects of such variations, and while focusing on the tasters’ preferences for the individual black teas, the total scores obtained for each black tea sample were ranked from 1 to 15 and then matched on a descending but arbitrary points scale as follows:

**Tasters’ ranking and order of preference:**

<table>
<thead>
<tr>
<th>Rank</th>
<th>Tasters’ Scores</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1 2 3 4 5 6 7 8 9 10 11 12 13 14 15</td>
</tr>
</tbody>
</table>

**Arbitrary points:**

15 14 13 12 11 10 9 8 7 6 5 4 3 2 1

For each clone, the arithmetic mean obtained for the sum of the monthly score points over the 1 year study period was calculated. The arithmetic means so obtained were then regressed against the level of each flavanol in the corresponding clonal green tea leaf and against the mean values determined for theaflavins, thearubigins, brightness and total colour levels of the corresponding black te. The correlation coefficients, \( r^a \) and \( r^b \), obtained for tasters’ A and B mean scores against individual components are given in Table 1. The correlation coefficient of the regression of the sum of tasters’ A and B mean scores against individual components is given as \( r^f \).

**Chromatographic system and conditions**

Chromatography was carried out with an ACS 352 pump (ACS, Macclesfield, UK) and a photodiode-array detector (HP 1040A Hewlett-Packard, UK) set at 278 nm. Injection was performed with a Rheodyne 7125 injector (Jones Chromatography, Cardiff, UK) fitted with a 10 µl loop. The column (150 × 4.6 mm id) was packed with 5 µm silica based reverse phase material Nucleosil 5 C18 100A (batch 109517) obtained from Phenomenex Ltd (UK). The column was maintained at 35 ± 0.5°C. Mobile phase A was 100 ml acetonitrile and 20 ml acetic acid diluted to 1 litre with deionised water. Mobile phase B was pure acetonitrile. The conditions for HPLC elution were as follows: A for 10 min, then in 5 min to 75A : 25B, hold for 8 min, then in 3 min to B only, hold for 3 min, then in 1 min to A only, hold for 5 min before the next injection.

**HPLC standards and calibration**

Standard samples of epigallocatechin (EGC), epicatechin (EC), (+)catechin (+C), epigallocatechin gallate (EGCG) and epicatechin gallate (ECG) were used to make three mixed working standard solutions of the following concentrations (µg ml\(^{-1}\)), for calibration:
# TABLE 1

Flavanol composition and caffeine content in the green leaf, black tea chemical quality parameters and taster's mean scores and overall ranking

<table>
<thead>
<tr>
<th>Clone</th>
<th>Green leaf</th>
<th>Black tea</th>
<th>Taster A mean score</th>
<th>Taster B mean score</th>
<th>Overall rank</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>EGC (μmol g⁻¹ DM)</td>
<td>+C (μmol g⁻¹ DM)</td>
<td>EC (μmol g⁻¹ DM)</td>
<td>EGCG (μmol g⁻¹ DM)</td>
<td>ECG (μmol g⁻¹ DM)</td>
</tr>
<tr>
<td>BB 35</td>
<td>133.00</td>
<td>39.30</td>
<td>38.30</td>
<td>196.30</td>
<td>75.10</td>
</tr>
<tr>
<td>C182/40</td>
<td>93.30</td>
<td>12.10</td>
<td>42.40</td>
<td>142.10</td>
<td>41.90</td>
</tr>
<tr>
<td>D99/10</td>
<td>117.00</td>
<td>16.60</td>
<td>47.60</td>
<td>167.70</td>
<td>48.90</td>
</tr>
<tr>
<td>C12</td>
<td>182.70</td>
<td>44.50</td>
<td>59.30</td>
<td>174.70</td>
<td>67.60</td>
</tr>
<tr>
<td>D6/99/99</td>
<td>161.40</td>
<td>30.00</td>
<td>49.30</td>
<td>148.50</td>
<td>52.90</td>
</tr>
<tr>
<td>H8/12</td>
<td>213.40</td>
<td>29.70</td>
<td>57.90</td>
<td>132.50</td>
<td>43.20</td>
</tr>
<tr>
<td>TN14/3</td>
<td>166.30</td>
<td>27.20</td>
<td>37.60</td>
<td>132.30</td>
<td>41.40</td>
</tr>
<tr>
<td>D8/44</td>
<td>222.50</td>
<td>33.40</td>
<td>43.80</td>
<td>186.50</td>
<td>49.30</td>
</tr>
<tr>
<td>D1/32</td>
<td>215.40</td>
<td>37.60</td>
<td>57.30</td>
<td>206.30</td>
<td>53.20</td>
</tr>
<tr>
<td>TN15/23</td>
<td>164.40</td>
<td>28.30</td>
<td>57.60</td>
<td>256.60</td>
<td>75.30</td>
</tr>
<tr>
<td>EJULU</td>
<td>269.30</td>
<td>0.03</td>
<td>46.60</td>
<td>145.40</td>
<td>111.10</td>
</tr>
<tr>
<td>C8/27</td>
<td>121.20</td>
<td>24.80</td>
<td>36.20</td>
<td>214.60</td>
<td>72.90</td>
</tr>
<tr>
<td>D8/35</td>
<td>184.30</td>
<td>47.20</td>
<td>53.10</td>
<td>208.70</td>
<td>61.80</td>
</tr>
<tr>
<td>SI 5/10</td>
<td>208.20</td>
<td>42.40</td>
<td>57.90</td>
<td>170.70</td>
<td>55.20</td>
</tr>
<tr>
<td>CV%</td>
<td>15.0</td>
<td>15.7</td>
<td>8.88</td>
<td>9.62</td>
<td>7.90</td>
</tr>
<tr>
<td>SE</td>
<td>0.77</td>
<td>0.30</td>
<td>0.32</td>
<td>0.78</td>
<td>0.21</td>
</tr>
<tr>
<td>LSDP ≤ 0.05</td>
<td>1.66</td>
<td>0.27</td>
<td>0.07</td>
<td>1.67</td>
<td>0.45</td>
</tr>
</tbody>
</table>

Correlation coefficients:

- \( r^a \)
- \( r^b \)
- \( r^c \)

* mg equivalent (× katechin g⁻¹ fresh shoot wt.; \( TF \), the flavins; \( TR \), theaflavins. \( r^a \), correlation coefficient of the regression of taster A mean score against component; \( r^b \), correlation coefficient of the regression of taster B mean score against component; \( r^c \), correlation coefficient of the regression of the sum of taster's A and B mean scores against component. \(* * * \) Significant at \( P \leq 0.05 \) and \( P \leq 0.01 \), respectively.
Quality potential indicators of Kenyan black teas

RESULTS

Table 1 shows the flavanol, caffeine and black tea chemical quality parameter of each clonal leaf material and tasters’ mean and overall score rankings of the black teas. Depending on the clone either (−)epigallocatechin gallate or (−)epigallocatechin was the most abundant flavanol present in green leaf. The sum total for individual flavanols in green leaf varied from 332.3 μmol g⁻¹ dry matter (DM) corresponding to 128.1 g kg⁻¹ DM in clone C18/40 to 582.2 μmol g⁻¹ DM corresponding to 226.0 g kg⁻¹ DM in TN15/23. Ejulu was unique in having the highest amounts of (−)epigallocatechin and (−)epicatechin gallate. TN15/23 had the most (−)epigallocatechin gallate present in green leaf.

Total polyphenol content in green leaf determined by the phenol reagent method showed minimal variations within the same clone but significant differences among clones. Clone C12 had the lowest levels (52.25 mg equivalent (+)catechin g⁻¹ fresh weight) while Ejulu had the highest polyphenol content (73.75 mg equivalent (+)catechin g⁻¹ fresh weight). Caffeine content varied from 27.6 g kg⁻¹ DM for TN14/3 to 49.7 g kg⁻¹ DM for TN15/23. Regression analysis of the mean value for the individual chemical parameters both in the green leaf and black teas with means and overall tasters’ rankings produced correlations some of which were positive with statistically very significant coefficients. The chemical components in green leaf with notable negative correlations with tasters’ preferences of black teas included (+)catechin and (−)epicatechin. (−)Epicatechin gallate content correlated positively with the black tea preferences of taster A (r = 0.498, P ≤ 0.05), taster B (r = 0.665, P ≤ 0.01) and the overall tasters’ ranking (r = 0.678, P ≤ 0.01). (−)Epigallocatechin gallate content correlated positively but non-significantly with the preferences of taster A but the correlation was quite significant for taster B (r = 0.513, P ≤ 0.05) and for the overall tasters’ ranking (r = 0.532, P ≤ 0.05). The response to caffeine content was similar to that for (−)epicatechin gallate while total polyphenol content (mg equivalent (+)catechin g⁻¹ fresh wt) gave relationships with tasters’ preferences similar to (−)epigallocatechin gallate. None of the black tea quality parameters, theaflavin content, thearubigin content, brightness and total colour, had a statistically significant correlation with tasters’ preferences.

DISCUSSION

Phenolic substances known to be present in green tea leaf include flavanols, flavanol glycosides, gallic acid, amino acids and their condensation products (Sanderson et al 1976) which are all detectable by the Folin–Ciocalteu phenol reagent. Proportionally, flavanols are the predominant polyphenols in tea leaf (Sanderson et al 1976). The phenol reagent method does not discriminate among the contributions of individual phenolic substances but instead gives the sum of all the polyphenols present in the green leaf. The minimal changes with time of year displayed by the plain black tea parameters, theaflavins, thearubigins, percent brightness and total colour of black tea liquors for each clone, imply that the levels of the various precursors present in green tea leaf also did not vary widely with time of the year. For total polyphenol content in green leaf the small variations observed within the same clone with time of the year suggest that clonal materials were either of high or low polyphenol content irrespective of time of year, under the growth conditions of the Nandi Hills tea-growing region of West Rift Valley in Kenya. The seasonal variations frequently observed in the quality of black tea in this region (Owuor 1992, 1994) are apparently caused by factors other than changes in the total polyphenol content. Indeed, Owuor (1994) has reported that it is the aroma components of black tea which show larger changes with the time of year than the plain black tea quality components. Theaflavins and thearubigins together contribute to the brightness, astrigency, body, mouthfeel and colour of black teas (Roberts and Smith 1963; Takino et al 1964; Brown et al 1966, 1969; Berkowitz et al 1971; Robertson 1983). Tasters mainly use these attributes to rank the quality of Kenyan black teas. The positive correlations between total polyphenol content in the green tea leaf and tasters’ preferences of the black tea are therefore logical when the roles of polyphenols in the quality of black tea are considered. It is during fermentation that enzyme catalysed oxidation of flavanols occurs to give theaflavins and thearubigins (Sanderson et al 1972). It is therefore expected that as the level of total polyphenols in green tea leaf rises, the chemical quality parameters of black tea will also rise and that would translate into better tasters’ evaluation of the black teas produced. However, there are cases when a rise in green leaf total polyphenol content may not necessarily translate into
better ranking by black tea tasters and vice versa. For instance, consider the tasters’ ranking of clone BB35 (Table 1). In terms of green leaf total polyphenol content BB35 was much lower than a number of other clones and was therefore expected to be ranked as low. This lack of precision in the prediction of quality by the phenol reagent method was evidence of the fact that the method determines all phenolic components present in the green leaf whereas the same components give rise to different organoleptic responses in black tea (Sanderson et al 1976; Ding et al 1992). Not all flavanols are consumed in fermentation reactions during black tea processing (Ding et al 1992; Kuhr and Engelhardt 1992). These residual unoxidised flavanols, which are colourless, contribute to the astringency of black tea (Sanderson et al 1976), and thus directly influence the sensory evaluation of the black teas (Ding et al 1992). Flavanol glycosides present in green tea leaf and black tea do contribute substantially to the colour of black tea liquors (McDowell et al 1990) and could also contribute to astringency though that has yet to be confirmed. The disparity between the expected ranking of black teas from their total polyphenol content in green leaf as determined by the phenol reagent method and the actual tasters’ ranking suggests that a second method capable of discriminating between the contributions of individual substances present in the green leaf could give a better correlation with tasters’ evaluation of the black teas produced.

Using the HPLC method the levels of individual flavanols present in the green leaf were shown to vary significantly among clones, demonstrating that flavanol composition was clonal dependent. Not all flavanols correlated positively with tasters’ ranking of black teas. (+)Catechin and (−)epicatechin had negative but non-significant correlations with tasters’ ranking of black teas suggesting that the two substances have minimal roles in black tea quality. (−)Epicatechin gallate and (−)epigallocatechin gallate are both dihydroxylated on the B-ring and the two compete for oxidation by polyphenol oxidase(s) to form different products. But unlike (−)epicatechin gallate, (−)epicatechin is not esterified with the trihydroxylated moiety of gallic acid. It is thus expected that the products arising from (−)epicatechin would be less astringent than those from (−)epicatechin gallate (Ding et al 1992). It is probable that these differences in chemical structure translate into differences in organoleptic responses, hence the tasters’ perception of (−)epicatechin and its enantiomer, (+)catechin, as being different from that of (−)epicatechin gallate, considering that not all flavanols are consumed in fermentation reactions during black tea processing (Ding et al 1992; Kuhr and Engelhardt 1992). (−)Epicatechin gallate and (−)epigallocatechin gallate had positive and significant correlations with tasters’ preferences of black teas. The flavanol composition of BB35 showed very high levels of (−)epicatechin gallate which, in the presence of equally high levels of (−)epigallocatechin gallate, would be expected to favour more the formation of theaflavin-3,3′-digallate. Owuor and Obanda (1995) have shown the correlation between tasters’ preferences and the levels of theaflavin-3,3′-digallate in black teas to be positive and significant. Indeed, Hilton and Palmer-Jones (1973) suggested a causal relationship between the concentration of an individual flavanol in green leaf and total theaflavin levels in Central Africa black teas. Total theaflavin levels have been demonstrated to have significant correlations with sensory and price evaluations of Central Africa black teas (Ellis and Cloughley 1981). From the general expectation that high levels of (−)epicatechin gallate and (−)epigallocatechin gallate in green leaf would promote overall astringency and hence quality of black tea, the tasters’ ranking of BB35 above clones with larger values for total polyphenol content appears rational. Efforts are currently being made to determine the levels of unoxidized flavanols and the theaflavin composition of the black teas discussed in this paper.

The ability to synthesis and accumulate caffeine in green leaf was clonal dependent. Indeed, Owuor and Chavanji (1986) have reported likewise. Although in the present work monthly variations of caffeine for each clone were not followed, it was expected that as tea growing in the West Rift Valley of Kenya is almost uniform throughout the year, there will be little seasonal variation in caffeine content within individual clones (Owuor and Chavanji 1986). The positive and statistically significant correlation established between caffeine levels in the green leaf, two leaves and a bud portion, and tasters’ preferences of black teas is logical when it is considered that caffeine contributes towards the briskness of black tea (Bhatia 1963; Deb and Ullah 1968; Millin et al 1969). Caffeine will complex with polyphenols in tea, mainly theaflavins (Roberts 1962; Collier et al 1972) and the complex formed modifies positively the taste characteristics of both caffeine and theaflavins (Millin et al 1969; Sanderson et al 1976). Finally, the amount of caffeine present in tea positively influences the extent of ‘cream’ formation in cooled black teas (Roberts 1962; Smith 1968).

The results presented in this paper suggest that it may be possible to select for quality amongst high-yielding tea clones by determining the levels of flavanols, (−)epicatechin gallate and (−)epigallocatechin gallate, and caffeine in the pluckable green tea shoots.

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