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Clonal variation in the individual theaflavin levels and their impact on astringency and sensory evaluations

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HPLC analysis of Kenya clonal black tea liquors revealed the presence of four major theaflavins in the following order: theaflavin > theaflavin-3-gallate > theaflavin-3,3'-digallate > theaflavin-3'gallate. The total amounts and the ratios of the individual theaflavins varied with clones resulting in variations in the astringency of the teas as measured by theaflavin digallate equivalent. Theaflavin digallate and theaflavin digallate equivalent showed a better relationship with sensory evaluation than did total (Flavognost) theaflavins.

INTRODUCTION

Black teas manufactured from the young tender shoots of *Camellia sinensis* (L) O. Kuntze are valued for their plain and/or aroma quality parameters. In the tea trade, the African black teas are classified as plain to medium flavours. Such black teas sell for their plain quality parameters, i.e. theaflavins, thearubigins and caffeine. Theaflavins contribute to the astringency (briskness) and brightness while thearubigins contribute to the colour and thickness (mouth-feel) and caffeine is responsible for the stimulatory effects of black tea. Successful relationships have been demonstrated between the total theaflavins levels of Central African black teas and sensory evaluations or prices (Hilton & Ellis, 1972; Hilton & Palmer-Jones, 1975; Cloughley, 1981, 1983; Ellis & Cloughley, 1981). Such relationships were positive but less successful for Kenya black teas (Owuor *et al.*, 1986). The success obtained in the regressions between prices and total (Flavognost) theaflavins for Central African black teas led to the suggestion that total theaflavins level is the objective quality parameter (Davis, 1983) which may be used as a standard in black teas (Ellis & Cloughley, 1981; Davis, 1983). This suggestion was opposed by producers whose total theaflavins levels show little relationship with sensory evaluations (Othieno & Owuor, 1984) as it was argued there may be other more important black tea quality parameters. Indeed, some Kenyan black teas subsequently showed better relationships between aroma tea quality and sensory valuation (Owuor *et al.*, 1988; Owuor, 1992).

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A comparison between the total theaflavins levels of Kenyan and Central Africa black teas showed that Kenyan black teas had very high levels (Owuor *et al.*, 1986). The lack of significant relationship noted was therefore attributed to the total theaflavins levels probably being too high and above the threshold limit, making consumers look for other parameters in Kenya black teas (Owuor *et al.*, 1986). Recently, McDowell *et al.* (1991) demonstrated the presence of four theaflavins in black teas and also showed that the pattern of the individual theaflavins varied with country of origin. Earlier Sanderson *et al.* (1976) had worked out the astringencies of the individual theaflavins. Theaflavin-3,3'-digallate (TFDG) was shown to be 6·4 times, and theaflavin monogallate (TFMG) 2·22 times more astringent than theaflavin (TF). These observations suggest that the significant regressions previously observed (Hilton & Ellis, 1972; Cloughley, 1981, 1983; Ellis & Cloughley, 1981; Hilton & Palmer-Jones, 1992) could in part be due to the prescence of more astringent theaflavins in black teas from Central Africa (McDowell *et al.*, 1991). This study was undertaken to assess if there is a better relationship between any individual theaflavin and sensory evaluation, and to establish if there is a critical theaflavin for black tea quality.

In an attempt to overcome the differentials in the contribution of the individual theaflavins to the astringency of black teas, Thanaraj and Seshadri (1990) developed a normalising equation based on the astringency of TFDG by using the astringency factors earlier developed by Sanderson *et al.* (1976). This equation

$$\text{TF}(\%) \quad \frac{\text{TDFG equivalent of total}}{} = (A/6{\cdot}4 + B/2{\cdot}22 + C)/100$$

(where TF is total theaflavins content; *A,B,C* denote the percentage of TF, TFMG and TFDG respectively), does not seem to estimate the astringency factor correctly. In this paper an assessment is made of the impact of using an improved astringency normalising factor (Owuor & McDowell, 1994) for the different theaflavins on the relationship between theaflavins and sensory evaluation presented.

Based on sensory evaluation data, clone 6/8 has been used as a quality standard in propagations studies in Kenya (Owuor *et al.*, 1987). It is not well-understood how the total theaflavins and/or theaflavin digallate equivalent levels of clone 6/8 compare with those of some widely used clones in Kenya. This study also compares the total theaflavins and TFDG equivalent of total TF levels (Owuor & McDowell, 1994) of clone 6/8 and those of other clones widely used. Because of the known sensory evaluation data of clone 6/8, it has been used in plant breeding studies in efforts to produce planting materials of improved quality. In one such study clone 6/8 was open-pollinated and produced seedling plants which were cloned. This study also compares the total TF and TFDG equivalent of total 6/8 and its progenies which have been shown to have suitable yields.

MATERIALS AND METHODS

Clonal leaf used in manufacture of the black tea was obtained from two clonal filed trials (CFT) of the Botany Department (Tea Research Foundation of Kenya) at an altitude of 2180 m amsl and latitude 0°22' south. Clones which were progenies of clone 6/8 were produced by open-pollination using clone 6/8 as the mother and were planted in 1979. The plants were receiving 100 kg N (as NPKS 25 : 5 : 5 : 5) per year applied in a single dose. Plucking conformed to normal commercial practice of mostly (>70%) two leaves and a bud plus minor amounts of three leaves and a bud and some loose leaf.

From each clone was plucked 1200 g green leaf which was then withered to achieve 70% physical wither in 14 h (Owuor *et al.*, 1987). The leaf was macerated using the crush, tear and curl (CTC) method and fermented at ambient temperature of 22–24°C for 90 min. The fermented leaf 'dhool' was fired using a miniature fluid bed drier and subjected to theaflavins analyses and sensory evaluation without sorting. Total theaflavins were determined by the Flavognost method (Hilton, 1973) as follows.

A tea infusion was made with 375 g (ml) of boiling water, preferably added from an overhead boiler into a tared flask, and 9 g of tea. The flask was shaken for 10 min, the infusion filtered rough cotton wool, and 10 ml pipetted into 10 ml of isobutylmethylketone; or 4-methylpentan-2-one (IBMK). The mixture was shaken for 10 min and allowed to stand until the layers separated. Two millilitres of the upper layer were pipetted into a test tube followed by 4 ml ethanol and 2 ml Flavognost reagent (2 g diphenylboric acid-2-ethyl ester dissolved in 100 ml ethanol). The contents were mixed and colour allowed to develop for 15 min. The optical density (OD) at 625 nm was read against an IBMK/ethanol (1 : 1 v/v) blank. The percentage of matter (DM) in the sample is determined by oven-drying.

$$\text{Theaflavin } (\mu\text{mol/g}) = \text{OD}_{625} \times \frac{47{\cdot}9}{\left(\frac{\text{DM}}{100}\right)}$$

The ratios of individual theaflavins were determined by HPLC (Steinhaus & Engelhardt, 1989; McDowell *et al.*, 1991). For HPLC analysis, liquors were prepared by adding 4 g of black tea to 195 ml deionised water that had just reached the boil and shaking for 10 min in a 475 ml capacity Thermos flask. Clean liquors were obtained by filtration through cotton wool. The hot liquors were cooled to room temperature by placing the flasks containing the liquors under a cold water tap (1–3 min). The liquor was diluted (1 : 1) with double-distilled water prior to HPLC analyses. The analyses were done using a Cecil Series 1000 HPLC with a 20 μl sample loop and a Hypersil 5μ ODS Column (25 cm × 4·6 mm). The UV monitor was set at 375 nm and results recorded and analysed using a JC600 Cecil data system. Solvent A was 1% aqueous acetic acid and solvent B was acetonitrile. A linear gradient from 8–31% solvent B over 60 min with a flow rate of 1·5 ml/min was used (McDowell *et al.*, 1991). The total amount of theaflavins (Hilton, 1973) was allocated to the individual theaflavins according to the ratios determined by HPLC (McDowell *et al.*, 1991) since the molar absorption coefficients of the four theaflavins are similar at 375 nm (Steinhaus & Engelhardt, 1989). Sensory valuation was done by professional black-tea tasters and scores were based on the sums of assessment of briskness, brightness, colour of infusion, thickness, flavour, colour and overall quality in a scale of 0–10 for each attribute.

RESULTS AND DISCUSSION

Using the Sanderson *et al.* (1976) astringency factors for the individual theaflavins, an improved TFDG equivalent was developed to normalise the contributions of the individual theaflavins towards astringency and hence quality of black tea (Owuor & McDowell, 1994). As theaflavin-3,3'-digallate is 6·4 and theaflavin monogallates are 2·22 times more astringent than theaflavin the improved equation was developed as

$$\text{TF}(\%) = \text{TFDG equivalent of total} = (A/6{\cdot}4 + B \times 2{\cdot}22/6{\cdot}4 + \text{C})/100$$

where *A,B,C* denote percent TF, TFMG and TFDG, respectively.

$$\text{TF } (\mu\text{mols/g}) = \text{TFDG equivalent of total} = \text{TF}/6{\cdot}4 + \text{TFMG} \times 2{\cdot}22/6{\cdot}4 + \text{TFDG}$$

since TFDG converts to TFDG equivalent as TFMG × 2·22/6·4 not as TFMG×2·22 as suggested by Thanaraj and Seshadri (1990).

Table 1. Changes in the theaflavins[a] (μmol) and sensory evaluation of black teas

Clone/ seedling stock	Total theaflavins	Theaflavin	Theaflavin-3-gallate	Theaflavin-3'-gallate	Theaflavin-3,3'-digallate	Theaflavin-digallate equivalent	Sensory evaluation
6/8	33·7	15·3	8·79	3·75	5·88	12·6	46
7/3	31·7	14·4	8·77	3·69	4·87	11·4	41
31/8	31·6	13·0	8·50	4·20	5·84	12·3	42
54/40	31·6	11·1	8·78	4·66	7·12	13·5	46
141/153	31·0	11·6	8·95	4·82	5·66	12·2	45
303/231	32·9	13·8	9·18	4·61	5·26	12·2	44
303/608	28·3	12·8	7·65	3·46	4·34	10·2	38
303/662	34·0	17·2	8·66	3·61	4·52	11·5	40
303/999	33·1	15·6	9·08	3·85	4·57	11·5	42
ST 18	28·2	11·4	8·04	3·73	5·01	10·9	39
CV (%)	5·54	5·80	5·55	6·56	5·83	5·66	7·76
LSD							
($P \leq 0·05$)	2·04	0·92	0·56	0·31	0·36	0·78	5
0·01	2·73	1·23	0·75	0·41	0·48	1·04	NS
0·001	3·59	1·62	0·98	0·54	0·63	1·37	
r^b	0·53	−0·14	0·72*	0·73*	0·79*	0·92***	

[a]Determined by Flavognost method (Hilton, 1973) and the individual theaflavins were calculated from the Flavognost theaflavins and the HPLC peak ratios while sensory evaluation was based on briskness, brightness, colour, thickness, infusion, flavour and overall quality on a scale of 0 to 10 for each attribute.
[b]Correlating coefficient (r) values of linear regression analyses between different theaflavins and sensory evaluations.
*, *** significant at $P \leq 0·05$ and 0·001, respectively.

In the first set of black tea (Table 1) the total theaflavins, individual theaflavins and theaflavin digallate equivalents of total theaflavins levels of clone 6/8, four of its progenies, i.e. clones 303/231, 303/608, 303/662, and 303/999, were compared with those of widely used clones, i.e. 7/3, 31/8, 54/40 and 141/153, while seedling stock 18 (ST 18) was used as control. All the clones had higher ($P \leq 0·05$) total TF than ST 18. Of the progenies of 6/8, only 303/608 produced black teas with significantly lower total TF than 6/8 while, of the widely grown materials, clones 31/8 and 141/153 produced black teas with significantly lower total TF. Thus, on using total TF as an objective quality indicator (Ellis & Cloughley, 1981; Davis, 1983) and clone 6/8 as the standard, clones 303/608, 31/8 and 144/153 would produce inferior plain black tea under Kericho tea growing conditions. But all the clonal materials except clone 303/608 produced plain black teas with superior ($P \leq 0·05$) total theaflavins than seedling tea. Thus where total TF is the quality parameter, these clones should be grown in preference to seedling stock for quality. When clone 6/8 was used as the standard, clones 31/8 and 141/153 produced inferior plain black teas as measured by total TF. However, when the TFDG equivalent of total TF is used as the quality criterion, clone 7/3 and all progenies of clone 6/8 except clone 303/231 were equivalent ($P \leq 0·05$) to ST 18. Thus, their production would lead to inferior plain black teas. Indeed black teas produced from the four progenies of 6/8 were inferior ($P \leq 0·05$) compared to that produced by their mother. However, the widely produced clones 31/8, 54/40 and 141/153 produced plain black teas with the same ($P \leq 0·05$) TFDG equivalent as clone 6/8. The data here demonstrate that the use of total TF or TFDG equivalent can lead to different conclusions and the use of either should be dependent on which one best relates to sensory evaluation and/or prices. Clone 6/8, some of its progenies like clone 303/365, 303/571, 303/687 and 303/865 produced black teas with significantly ($P \leq 0·05$) higher total TF than ST 18. All progenies of clone 6/8 except 303/679,303/745 and 303/868 produced black teas with TFDG equivalent levels similar to clone 6/8. Again, it was noted that, depending on the quality parameter used, different clones could be selected.

The clones (Tables 1 and 2) exhibited variations in their total theaflavins levels as had been noted previously (Owuor *et al.*, 1987). The individual theaflavins also varied from clone to clone. Using black tea of undermined source, McDowell *et al.* (1991) has shown that the pattern of the individual theaflavins follows the country of origin. In this study, despite the variations in the levels of individual TF with clones, their levels followed the same pattern, i.e. theaflavin > theaflavin-3-gallate > theaflavin-3,3'-gallate. Despite the observed order, the ratios of the individual theaflavins in different black teas varied. Consequently, the order of total theaflavins in different black teas was not the same as that of the theaflavin digallate equivalent of total theaflavins. Thus two clones could have equivalent total theaflavins but have different qualities due to variations in astringency caused by the individual theaflavins. The data further suggest that a clone could have low total theaflavins levels but high astringency provided the percentage of the more astringent theaflavin (i.e. theaflavin digallate) was high in its black teas.

Table 2. Variations in total theaflavin[a] (μmol/g) and individual theaflavin composition and sensory evaluation of clone 6/8 and its progenies

Clone/ seedling stock	Total theaflavins	Theaflavin	Theaflavin-3-gallate	Theaflavin-3'-gallate	Theaflavin-3,3'-digallate	Theaflavin-digallate equivalent	Sensory evaluation
6/8	32·3	15·4	8·43	3·77	4·68	11·3	51
303/365	31·6	15·0	8·14	4·04	4·38	11·0	41
303/571	30·3	12·3	9·05	4·01	4·98	11·4	47
303/678	29·9	11·9	8·90	3·75	5·37	11·6	46
303/679	29·0	13·4	8·19	3·09	4·36	10·4	43
303/745	25·0	10·9	6·72	3·41	4·03	9·24	39
303/791	28·7	11·7	8·53	3·47	4·96	11·0	49
303/815	28·5	10·6	9·27	3·86	4·73	11·0	44
303/839	26·4	8·79	8·29	3·52	5·79	11·3	52
303/868	29·3	13·4	7·93	3·60	4·38	10·5	46
303/978	28·0	11·4	7·88	3·82	4·93	10·8	48
ST 18	26·9	10·8	4·69	3·54	4·89	10·5	40
CV (%)	4·79	5·20	4·62	4·73	4·53	0·74	6·61
LSD							
$P \leq 0{\cdot}05$	2·34	1·07	0·64	0·29	0·30	0·74	5
0·01	3·80	1·46	0·88	0·40	0·49	0·80	7
0·001	4·22	1·96	1·18	0·54	0·66	1·17	9
r^b	0·27	−0·06	0·47	0·13	0·65*	0·67*	

[a,b]As in Table 1.
*Significant at $P \leq 0{\cdot}05$.

As in the past (Owuor *et al.*, 1986), positive but insignificant regressions were observed between total theaflavins and sensory evaluation for Kenyan black teas. The relationships between theaflavins-3,3'-digallate or theaflavin digallate equivalent of total theaflavins and sensory evaluations were better than those of total theaflavins, theaflavin and/or theaflavin mono-gallate and sensory evaluations (Tables 1 and 2). This suggested that the theaflavin-3,3-digallate or theaflavin digallate equivalent of total theaflavins levels were better quality indicators for Kenyan black teas than total theaflavins used in the previous studies (Hilton & Palmer-Jones, 1975; Cloughley, 1981, 1983; Owuor *et al.* 1986).

The results have other implications. For some black teas significant relationships between total theaflavin levels and sensory evaluation and/or prices have been demonstrated (Hilton & Palmer-Jones 1975; Cloughley, 1981, 1983) while for others the relationship was poor (Owuor *et al.*, 1986). Changes in the patterns of individual theaflavin ratios have also been observed to depend on the country origin (McDowell *et al.*, 1991). Results presented here also show a better relationship between sensory evaluation and theaflavin digallate or theaflavin digallate equivalent levels. It therefore seems likely that, for black teas produced in countries where the more astringent theaflavin digallate is dominant, there is a likelihood of getting a better relationship between total TF and sensory evaluation than in countries where the percentage of theaflavin digallate in black tea is low. This aspect needs confirmation in further studies. The regression data presented should, however, be treated with caution. Although better relationships were observed between sensory evaluation and theaflavin digallate or theaflavin diallate equivalent of total theaflavins, the relationships were not perfect. Thus there are other black tea quality parameters which are also important (Owuor *et al.*, 1986, 1988; Owuor, 1992). Although the observed relationships between sensory evaluation and analyses are important for the general quality of Kenyan black tea, high levels of the parameters relating to sensory evaluation may not necessarily imply better black tea pricing. Sometimes these prices are determined by factors outside tea quality like supply and demand, consumer preferences as indicated by country importing tea, etc. Also for good tea prices, consistent manufacture is mandatory. Thus, the factors relating to quality should be used more as indicators of production of good black tea which may be likely to attract good prices when quality overrides other factors and as an objective method of ensuring consistently good manufacture.

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