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Caffeine Contents of Clonal Tea; Seasonal Variations and Effects of Plucking Standards Under Kenyan Conditions

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ABSTRACT

Clones of tea have different contents of caffeine. The tender younger leaves of tea bushes contain higher caffeine contents than older leaves. Leaf stalks accumulate low levels of caffeine. Fine plucking standards ensure high caffeine content in brewed tea. Seasonal fluctuations in two leaves and a bud were found to differ by 24% to 30% caffeine content, depending on clones. Effects of clonal differences and seasonal fluctuations are discussed in the light of international tea standards and quality characteristics of brewed teas.

INTRODUCTION

Tea is made from the young tender shoots of *Camellia sinensis* (L.) O. Kuntze. Due to its refreshing and mild stimulant effects, it is one of the most widely drunk non-alcoholic beverages. The mild stimulant effect of tea is due to the presence of caffeine (1,3,5-trimethylxanthine) and two other isomeric dimethylxanthines: theophylline and theobromine, the dimethylxanthines being present in tea in only small amounts (Cloughley, 1982). These xanthines are known to be central nervous system stimulants (Stagg & Mullin, 1975). The important rôle that caffeine has in black tea quality characteristics has been acknowledged by several workers. Although without defining its rôle, Hilton & Ellis (1972) acknowledged its contribution to tea quality. Bhatia (1963), Mullin *et al.* (1969) and Deb &

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Ullah (1968) have asserted that caffeine contributes towards the briskness of black tea. Caffeine has been shown to complex with the polyphenols in tea, mainly theaflavins (Roberts, 1962; Collier *et al.*, 1972). This complex modifies the taste characteristic of both caffeine and theaflavins (Mullin *et al.*, 1969) making the tea taste brisker (Sanderson *et al.*, 1976). A quality characteristic which contributes positively during valuation of tea is its ability to form a coloured precipitate, or 'cream', when its infusion is cooled (Roberts, 1962; Smith, 1968). The extent of 'cream' formation is largely dependent on the amounts of caffeine present in tea.

In Malawi, Cloughley (1982) has shown that the caffeine content of tea is influenced by seasonal, genetic, agronomic and cultural factors. There are large variations between seasonal changes in Kenya and those in Malawi. Also, some agronomic and cultural practices in Kenya are different from those in Malawi. Clonal teas are genetically different. The manner in which these factors will affect caffeine levels in Kenyan teas is not known.

In the process of producing an International Tea Standard, by the International Standards Organization (ISO) and a Minimum Export Tea Standard (by UNCTAD) it has been recognised that there should be chemicals whose limits are set in the standards or which are merely used as markers to ensure that products sold as tea are real teas. Caffeine and theaflavins have been suggested as the components of black tea to serve this rôle. Recently, issues have been raised on the question of clinical importance of caffeine in beverages (Stagg & Mullin, 1975; Graham, 1978; Greden, 1979). It is with the foregoing in mind that studies were initiated to establish factors which affect the levels of caffeine in Kenyan teas.

MATERIALS AND METHODS

Plant materials used in this study were obtained from the Botany Department's multiplication plots of the Tea Research Foundation of Kenya, planted in the fields and spaced at 121·9 cm × 61·0 cm rectangularly, in 1967 at an altitude of 2178 m above mean sea level and latitude 0°22′ S. All the plots received 150 kg N per hectare per year in the form of NPKS 25:5:5:5 compound fertilizer. The leaf was harvested before 8 am every time since it is known that there are diurnal changes in various chemicals in tea (Hilton *et al.*, 1973). These bushes were normally

plucked every 12 to 14 days. To determine the distribution of caffeine in leaves of different ages, samples were obtained from clones 6/8, 31/8, 31/11, 12/12, 12/56, 12/19, 12/2 and 8/112 on the 31st January, 1984. Samples to show seasonal variation and effects of plucking standard on the caffeine contents of harvested leaves were plucked on the 15th of every month. Plucking standards were two leaves and a bud, three leaves and a bud, four leaves and a bud and five leaves and a bud.

The samples were sorted and the drying operation was begun before 9 am on the day of harvest in an oven at 100 ± 3 °C, for 10–12 h. The dried samples were ground using a coffee grinder then subjected to caffeine analysis.

CAFFEINE DETERMINATION

A modified method of Iwasa (1975) was used to determine the amount of caffeine in the samples.

The dried and ground sample (0·5 g) was weighed in a small dish. The sample was wetted using 5 ml of 5 % Na_2CO_3 and allowed to stand for 1 h. The sample was then placed in a thimble made from filter paper, then subjected to Soxhlet extraction using 200 ml of chloroform in a round bottomed flask for 2·5–3 h. Chloroform was then distilled off using a water bath; after adding 40 ml of water, the sample was boiled to completely remove chloroform. The solution was poured into a conical flask and 2·5 ml of 20 % $CuSO_4$ solution and 2·5 ml of 0·1 N NaOH solution were added. After adding 60 ml of water, the solution was filtered. Twenty-five millilitres of filtrate were poured into a separating funnel and extracted twice with 30 ml and 20 ml of chloroform. The chloroform was evaporated in a 100-ml flask in a water bath below 9 °C. Selenium Kjeldahl catalyst (0·1 g) and 3 ml of conc. H_2SO_4 were added and the nitrogen content determined by the Kjeldahl method. Caffeine was calculated as N × 3·464. Each analysis was done in triplicate. This method of analysis has been shown to have only an error of 0·54 % (Iwasa, 1975).

Analysis of variance was done on the different levels of plucking and different clones using a split plot design. Clones were the main treatments and plucking standards were sub-treatments. The data were collected over a 12-month period and each month was used as a replicate in the analysis since the seasonal variations were not large.

TABLE 1
Distribution of Caffeine (Given as % dry weight) in Different Parts of Harvestable Tea Leaves

Leaf	*Clones*								
	6/8	*31/8*	*31/11*	*12/12*	*12/56*	*12/19*	*12/2*	*8/112*	*Mean*
Bud	6·43	6·90	6·88	7·92	6·73	6·96	6·15	5·70	6·33
	(0·19)[a]	(0·24)	(0·05)	(0·12)	(0·10)	(0·03)	(0·05)	(0·06)	(1·43)
First leaf	3·63	4·54	4·90	5·99	5·49	4·87	4·20	3·86	4·6
	(0·13)	(0·03)	(0·05)	(0·15)	(0·02)	(0·05)	(0·09)	(0·08)	(0·80)
Second leaf	3·09	3·45	3·59	4·20	4·19	3·64	3·01	3·60	3·60
	(0·28)	(0·05)	(0·23)	(0·09)	(0·04)	(0·03)	(0·01)	(0·01)	(0·44)
Third leaf	2·82	2·97	3·36	3·60	3·36	2·65	2·47	3·46	3·09
	(0·04)	(0·08)	(0·20)	(0·20)	(0·03)	(0·05)	(0·05)	(0·06)	(0·42)
Fourth leaf	2·29	3·07	2·51	2·96	2·67	2·53	2·42	3·22	2·71
	(0·04)	(0·13)	(0·08)	(0·09)	(0·12)	(0·03)	(0·03)	(0·02)	(0·34)
Mature leaf	2·4	3·16	2·74	2·63	3·24	2·73	2·31	2·79	2·75
	(0·10)	(0·26)	(0·04)	(0·07)	(0·12)	(0·03)	(0·01)	(0·09)	(0·34)
Leaf stalk	1·63	2·09	2·29	2·66	2·96	1·52	1·40	1·35	1·99
	(0·14)	(0·09)	(0·04)	(0·10)	(0·06)	(0·10)	(0·09)	(0·03)	(0·61)
Two leaves and a bud	3·15	4·50	4·17	5·08	4·09	4·13	4·13	4·18	4·18
	(0·08)	(0·04)	(0·12)	(0·06)	(0·03)	(0·08)	(0·04)	(0·06)	(0·53)

[a] Numbers in parentheses are the standard deviations.

RESULTS AND DISCUSSION

Cloughley (1982) has shown that theobromine and theophylline are in tea only in very minute concentrations. Their presence therefore contributes negligibly when the determination of caffeine is based upon the determination of total purine nitrogen.

The distribution of caffeine in the harvestable tea shoots, partitioned into different parts, is presented in Table 1. For each clone the terminal bud had the highest content of caffeine.

As the leaf matures, the amount of caffeine goes down. Under Kenyan conditions, the second leaf has about 50 % of the content in the bud on a dry weight basis. Under Malawi's conditions, this level was achieved in the third leaf (Cloughley, 1982). For leaves of the same age, each clone of tea has a different caffeine content. Apart from explaining in part why clones have different quality characteristics, this also demonstrates the fact that each clone of tea has a different ability to produce these purine bases. The Tea Research Foundation of Kenya recommends a plucking policy of two leaves plus a bud for the highest quality of brewed tea. The data on caffeine content in two and a bud is presented in the bottom row of Table 1. The data were obtained by harvesting two leaves and a bud and analysing them intact, since taking the average of bud plus first leaf and second leaf will not give a true indication as the leaves have different weights and distribution (Cloughley, 1982). It is also noted that the stalk had a low accumulation of caffeine.

Mean clonal and plucking standards effects on caffeine distribution in young tea leaves are presented in Table 2.

Although the data were taken over a year, for each clone, the seasonal variations were narrow. This is illustrated in Fig. 1 using two leaves and a bud of four different clones. The difference between the highest level and the lowest level as a percentage of the highest level was 24·8 % for clone 6/8, 30 % for clone 12/12, 24·6 % for clone 31/8 and 29·8 % for clone 31/11 (Table 1). Even though there were fluctuations, the changes under Kenyan conditions were not as large as those found under Malawi conditions (Cloughley, 1982) where over 60 % variations were noted in the course of the year. The Malawi (Cloughley, 1982) data showed wide variation, possibly due to the wide seasonal variation of environmental conditions, especially temperatures. Under Kenya tea-growing conditions the seasonal mean temperature variations are not large (Fig. 1).

Clones have different abilities to produce caffeine. This was

TABLE 2
Mean Clonal (July, 1984–June, 1985) and Plucking Standards Effects on Caffeine Contents (%) of Tea

Clone	*Plucking standard*				
	Two leaves + bud	*Three leaves + bud*	*Four leaves + bud*	*Five leaves + bus*	*Mean of clones*
6/8	3·57	2·88	2·48	2·26	2·79
12/12	4·88	4·09	3·43	3·08	3·87
31/8	5·04	4·26	3· 1	3·31	4·08
31/11	4·22	3·73	3·21	2·97	3·58
Mean of plucking standard	4·88	3·74	3·21	2·91	
	CV (%) Clones		= 2·43		
	Plucking standard		= 5·16		
	LSD, $P =$	0·05	0·01	0·001	
	Clones mean	0·15	0·20	0·26	
	Plucking standard mean	0·08	0·10	0·13	
	Clones × plucking standards	0·15	0·20	0·26	

demonstrated by the highly statistically significant difference ($P \leq 0{\cdot}001$) between caffeine contents of different clones (Table 2).

Similarly, at different levels of plucking, caffeine contents of tea vary. Fine plucking (two leaves and a bud) ensures high caffeine contents in tea. The differences between the standards of plucking, Table 2, were very highly significant ($P \leq 0{\cdot}001$).

There were significant interactions between clones and plucking standard ($P \leq 0{\cdot}001$) (Table 2). For every clone, the coarser plucking standard resulted in low caffeine contents. Also, at every plucking standard level, clones with the abilities to have high caffeine contents maintained these levels and clones with abilities to have low caffeine contents continued to have the low levels. Caffeine distribution therefore consistently varies between clones and standards of plucking.

These results have some important implications. For caffeine contents of tea to be accepted as part of an international standard it must be borne in mind that, where tea growing is almost uniform throughout the year,

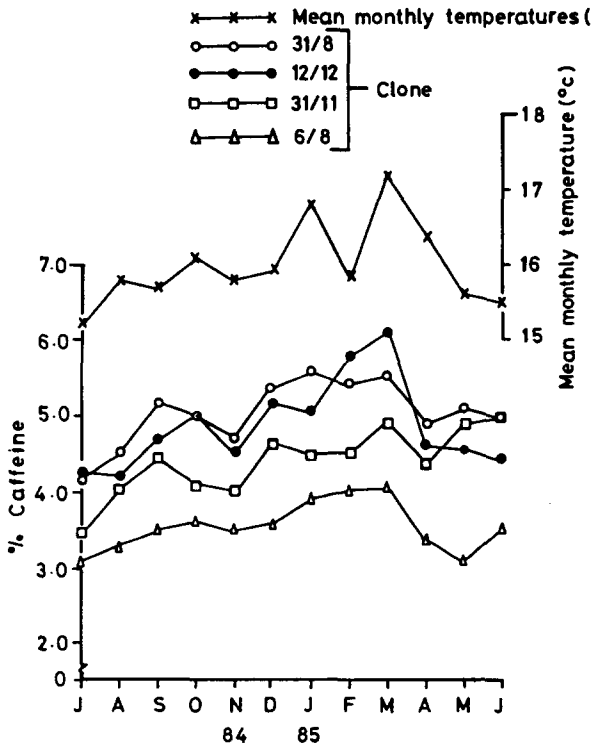

Fig. 1. Monthly clonal caffeine contents in two and a bud and mean temperatures (July, 1984–June, 1985)

there will be little seasonal variation in caffeine contents. If data from these countries were to be used to set the lower limit for caffeine contents in tea, then countries with growing conditions including winters (Cloughley, 1982) will be more likely to suffer as their teas will not conform to standards during the cold seasons.

Although it has been noted that coarse plucking results in teas with low caffeine contents (Table 2 and Cloughley, 1982), some clones have the ability to synthesise higher amounts of caffeine than others. Including caffeine contents of tea as a parameter in International Tea Standards and/or Minimum Export Tea Standards as a means of ensuring proper plucking standard would fail to detect coarsely plucked teas from caffeine-rich clones. Also, data from Malawi (Cloughley, 1982) and data presented here have shown that climatic conditions under which tea is grown affect the abilities of tea to manufacture caffeine. Since there are these variations, caffeine level in tea is not a good marker to detect

adulteration, because caffeine-rich tea may be adulterated and yet give a reasonable caffeine content. The only rôle caffeine content (in combination with other chemical components of tea) can serve is that of a marker to ensure that the product is authentic.

In general, teas which are finely plucked normally fetch higher prices than teas which are coarsely plucked. Higher levels of caffeine in finely plucked teas in part help explain why they are dearer. A plucking policy which is strictly two leaves and a bud would therefore lead to higher quality tea if manufacturing procedure is properly followed under Kenyan growing conditions.

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