.

## **Technical Note**

# Geographical Variation of Theaflavins, Thearubigins and Caffeine in Kenyan Clonal Black Teas

## ABSTRACT

Black tea clones have different contents of theaflavins, thearubigins and caffeine. The amounts vary with localities and the patterns of variations change from clone to clone. There was no significant relationship between altitude and theaflavins (except in clones 6/8, 31/8 and 31/11), or thearubigins or caffeine. Tea breeding programmes for high quality need to evaluate the clones in areas of intended release, as chemical composition—and hence quality—vary with localities.

## INTRODUCTION

The beverage, tea, made from the young tender shoots of *Camellia sinensis* (L.) O. Kuntze, is the most widely consumed non-alcoholic drink. The quality of tea has been known to vary with climatic and geographical areas of production (Hilton & Palmer-Jones, 1975). Recently, we demonstrated the variation in theaflavins (TF) contents of Kenyan and Malawi teas (Owuor *et al.*, 1986). Other previous studies have also compared teas which were grown under diverse climatic and geographical conditions. Yamanishi *et al.* (1968), for example, compared the quality of Dimbula, Uva, Nilgiris, Darjeeling and Benihomore teas. Hence, it has been assumed that large climatic and geographical variations are necessary for noticeable chemical composition and quality changes. Again, none of the previous studies has compared teas of the same clones produced under different climatic conditions. In Kenya, tea is grown in different locations at high altitudes

Food Chemistry 0308-8146/87/\$03.50 © Elsevier Applied Science Publishers Ltd, England, 1987. Printed in Great Britain

between 1500 and 2700 m. Apart from the variation in rainfall distribution in the east and west of the Rift Valley, other climatic changes are minimal. Recently, Mwakha (1985b) demonstrated that the tea shoot growth rate is inversely proportional to altitude in Western Kenya. Whereas the development of shoot to pluckable level takes 48 to 56 days in Malawi (Fordham, 1972) and 81 days in North Eastern India (Wight & Barua, 1955), similar development takes 60 to 90 days in Kenya (Magambo, 1983; Mwakha, 1985a, b). The variations in growth rates have been shown to affect quality of black tea (Hilton *et al.*, 1973). This study was therefore undertaken to show if there are variations of quality of Kenya black teas with growth rate and hence altitude (Mwakha, 1985b).

It is also known among farmers that tea quality varies with localities. Such variations have been attributed to different growth rates arising from changes in altitude (Mwakha, 1985b), average air temperature (Squire, 1978), rainfall (Carr, 1977), sunshine hours (Portsmouth & Rajiah, 1957), tea genotype (Fordham, 1972; Magambo & Omolo, 1982) and hormones (Raman, 1982). In Kenya, the quality changes arising from these factors have qualitatively been assessed by organoleptic evaluations. Despite the noted organoleptic variations, breeding work is still centralized at one point (Tea Research Foundation of Kenya) with an altitude of 2178 m above mean sea level. Although growth rate differences (Mwakha, 1985b) and yield differences (Njuguna, 1983) have been recorded for the same clones planted in different locations, chemical compositions have been assumed to stay constant. This study was therefore undertaken to quantify the changes occurring in TF, thearubigins (TR) and caffeine in clonal teas due to changes in geographical areas of production. These three components are known to be necessary for high quality of tea (Biswas & Sarkar, 1971; Hilton & Ellis, 1972; Hilton & Palmer-Jones, 1975; Owuor et al., 1986).

## MATERIALS AND METHODS

#### Tea leaves and manufacture

Tea shoots used in the study were obtained from clonal plots of commercial tea-producing companies in Kenya and the Tea Research Foundation of Kenya clonal field trials. The altitude varied from 1969 to 2260 m above mean sea level. All the teas were obtained within a radius of about 300 km. Plucking standard conformed to the normal commercial practice, i.e. about 90% good leaf, mainly two leaves and a bud with minor amounts of three leaves and a bud.

For every clone and in all locations, manufacturing was replicated three

225

times, each sample using 1200 g from green leaf. All the green leaves were withered for 16 to 18 h to lose 28% to 30% of the original weight. The teas were macerated by the 'Crush, Tear and Curl' method and subsequently fermented in closed plates for 2 h (Owuor & Reeves, 1986). All the teas were manufactured between 7 and 11 am; thus, apart from minimal daily temperature differences occurring within these hours, all manufacturing conditions were similar. The marginal differences in fermentation rates due to changes in temperature (Cloughley, 1980) between 7 and 11 am were overcome by randomization. The manufactured teas were then subjected to analysis without sorting. Randomized complete block design was used to do statistical analysis for the results of the analyses of the different clones. Each location was considered a treatment.

## Chernical analysis

TF analysis was done by the Flavognost method (Hilton, 1974) while TR analysis followed the method of Roberts & Smith (1963). Caffeine was determined by HPLC (Owuor *et al.*, 1987).

#### RESULTS

Kenyan teas are classified as plain to medium flavoury in the tea trade. For such teas, quality is thought to be determined by the amounts of TF (Owuor *et al.*, 1986 and references therein), TR (Biswas & Sarkar, 1971, 1973) and caffeine (Hilton & Ellis, 1972; Mullin *et al.*, 1969). The variations of these compounds in the teas are shown in Tables 1, 2 and 3.

The high TF contents noted (Table 1) explain the general notion that Kenyan teas have high briskness and brightness (Owuor & Reeves, 1986). TF showed wide variations (statistically different in most cases up to  $P \le 0.001$ ) with geographical areas of production (Table 1) for all clones. Also there were linear increases of TF with altitude significant at  $P \le 0.05$  for clones 6/8 and 31/8, and  $P \le 0.01$  for clone 31/11.

TR contents, which are responsible for colour and thickness (Owuor & Reeves, 1986) of tea, also changed with both geographical locations. Geographical areas of production caused changes in TR in all clones, which were significant in most cases up to  $P \le 0.001$  (Table 2). There were no significant changes in TR contents with altitudes for all clones.

Caffeine data are presented in Table 3. In all clones different geographical areas of growing tea produced varying amounts of caffeine contents, significant up to  $P \le 0.001$  in many cases. No significant relationships were shown between caffeine and altitude.

Source	Altitudeª	Clones					
		6/8	31/11	31/8	TN 14-3	Ejulu	
Karirana Tea Estates (Limuru) <sup>b</sup>	2 260	32.0	28.6	28.6	28.3	25.0	
Tea Research Foundation (Kericho) <sup>c</sup>	2178	28.1	25.2	24.1	26.2		
Sitoi Tea Estate (Nandi Hills) <sup>e</sup>	2 060	32.3	25.6	25.9	30.2		
Kangaita Tea Farm (Mt Kenya) <sup>b</sup>	2 0 5 6	31.2		26.3	30.1	-	
Kagochi Tea Farm (Mt Kenya) <sup>b</sup>	1 998	29.9			28.2		
Changoi Tea Estate (Kericho) <sup>c</sup>	1 830	20.4	18.9	22.5	26.9	20.3	
Kaimosi Tea Estate (Kaimosi) <sup>e</sup>	1 830	22.0	21.9	24.1	27.1	24.6	
Sotik Tea Ćompany (Sotik) <sup>c</sup>	1 769	27.0	—		33.7		
CV (%)		3.31	1.83	1.35	1.58	2.82	
LSD, $P = 0.05$		1.62	0.83	0.62	0.29	1.49	
0.01		2.24	1.21	0.88	0.41	2.47	
0.001		3.12	1.81	1.27	0.57	4.62	
r <sup>d</sup>		0.73*	0.92**	0.75*	-0.32	0.57	

TABLE 1Changes in Theaflavin Contents ( $\mu$ moles g<sup>-1</sup>) with Geographical Area of Production in<br/>Clonal Teas

Geographical area is given in parentheses.

<sup>a</sup> In metres above mean sea level.

<sup>b</sup> East of Rift Valley.

<sup>c</sup> West of Rift Valley.

<sup>*d*</sup> Correlation coefficient of linear regression analysis between altitude and TF contents: \*Significant  $P \le 0.05$ ; \*\*Significant  $P \le 0.01$ .

## DISCUSSION

Although tea growth rate in Kenya is affected by altitude (Mwakha, 1985b) and quality, as measured by TF content, is related to growth rate in Malawi (Hilton & Palmer-Jones, 1973) for clones studied, only clones 6/8, 31/8 and 31/11 showed significant relationship between TF content and altitude. However, it had been demonstrated that TF content alone is not a reliable criterion for quality measurement as other factors seem to be important in Kenya (Owuor *et al.*, 1986).

Source	Altitude <sup>a</sup>	Clone					
		6/8	31/11	31/8	TN 14-3	Ejulu	
Karirana Tea Estate <sup>b</sup>	2 260	19.0	17.1	16.3	19.1	18.9	
Tea Research Foundation <sup>c</sup>	2178	15.0	14.3	15.4	16.0	~	
Sitoi Tea Estate <sup>c</sup>	2 060	16.2	13.3	13.5	15.7		
Kangaita Tea Farm <sup>b</sup>	2 0 5 6	18.9	_	16.3	19.1	-	
Kagochi Tea Farm <sup>b</sup>	1 998	17.8			18.0		
Changoi Tea Estate <sup>c</sup>	1830	15.5	15.9	15.9	15.7	15.8	
Kaimosi Tea Estate <sup>c</sup>	1830	16.2	14.2	14.9	16.2	18·7	
Sotik Tea Company <sup>c</sup>	1 769	17.8			18.0		
CV (%)		1.53	1.20	1.56	1.26	1.20	
LSD, $P = 0.05$		0.46	0.34	0.43	0.38	0.34	
0.01		0.63	0.49	0.62	0.53	0.49	
0.001		0.88	0.74	0.90	0.73	0.74	
r <sup>d</sup>		0.23	0.27	0.20	0.29	0.53	

 TABLE 2

 Variation in Thearubigin Contents (% dry weight) in Clonal Teas with Geographical Areas of Production

See Table 1 for superscripts  $a^{-c}$  and geographical regions.

<sup>d</sup> Correlation coefficient of linear regression analysis between altitude and TR contents.

#### TABLE 3

Effect of Geographical Areas of Production on the Caffeine Contents (% dry weight) in Clonal Teas

Source	Altitude <sup>a</sup>	Clone						
		6/8	31/11	31/8	TN 14-3	Ejulu		
Karirana Tea Estate <sup>b</sup>	2 260	3.47	3.82	4.56	3.83	3.37		
Tea Research Foundation <sup>c</sup>	2178	3.02	3.23	3.82	3.19			
Sitoi Tea Estate <sup>c</sup>	2 0 6 0	3.04	3.55	3.71	3.10			
Kangaita Tea Farm <sup>b</sup>	2 0 5 6	3.36	_	4.50	4.10			
Kagochi Tea Farm <sup>b</sup>	1 998	3.39			4.02			
Changoi Tea Estate <sup>c</sup>	1 830	2.77	2.69	3.05	3.35	3.58		
Kaimosi Tea Estate <sup>c</sup>	1 830	3.42	3.91	4.19	4.17	4.25		
Sotik Tea Company <sup>c</sup>	1 769	3.39	<u></u>		4.11			
CV (%)		2.38	1.49	1.92	2.28	2.64		
$\overline{\text{LSD}, P = 0.05}$		0.13	0.10	0.14	0.15	0.22		
0.01		0.19	0.14	0.20	0.21	0.37		
0.001		0.26	0.21	0.29	0.29	0.69		
r <sup>d</sup>		0.09	0.30	0.52	-0.34	-0.69		

See Table 1 for superscripts  $a^{-c}$  and geographical regions.

<sup>d</sup> Correlation coefficient of linear regression analysis between altitude and caffeine contents.

There was no relationship between TR and caffeine content in all clones or TF, in clones Ejulu and TN 14-3 with altitude. Thus altitude is demonstrated not to be an overridingly important factor for the contents TF, TR and caffeine in Kenyan tea.

However, there were significant (up to  $P \le 0.001$ ) variations in the TF, TR and caffeine contents for all the clones studied due to localities (Tables 1, 2 and 3). Thus localities do affect the chemical composition of clones. It is known that average air temperature (Squire, 1978), rainfall (both distribution and total amounts) (Carr, 1977), sunshine hours and cloud cover (Portsmouth & Rajiah, 1957) affect tea growth and hence quality. Although rainfall distribution in the east and west of the Rift Valley in Kenya are not the same, samplings in the studies were done during March and April 1986, when long rains were just starting. Not all areas where the samplings were done receive equal amounts of rainfall. The variations in the average air temperatures, cloud cover and total amounts of rainfall in the different locations where the samplings were done help to explain the noted significant differences in clonal teas' chemical compositions with localities. Samplings from Changoi Tea Estate and Tea Research Foundation of Kenya, both in Kericho and about 30 km apart, were done at the same time. However, variations were noted in TF, TR and caffeine contents within the same clones (significant mostly at  $P \le 0.01$ ). Total rainfall and cloud cover differences can in part explain the noted variations. The differences in TF, TR and caffeine contents between Kaimosi and Changoi samples are in part due to differences in the agro-ecological factors above and not altitude.

Pluckings from Kagochi and Kangaita Tea Farms were also done at the same time. These farms are about 20 km apart, with 50-m altitude difference. Rainfall distributions and total amounts are normally very similar (although not exactly equal). Clones 6/8 and TN 14-3, sampled from these sites, did not show significant differences in caffeine contents. While TN 14-3 showed a significant ( $P \le 0.05$ ) difference in TF contents, clone 6/8 did not. However, both the clones showed significant ( $P \le 0.001$ ) TR differences. Data presented thus indicate that as long as small micro-ecological factors or geographical changes exist, chemical composition of tea—and hence quality— will change. In tea breeding for quality, it is thus important to have clonal field trials in areas of intended release before farmers are provided with planting materials.

#### ACKNOWLEDGEMENTS

Teas used in this study were kindly provided by Messrs George Williamson Kenya Limited (Changoi, Kaimosi and Karirana Estates), Kenya Tea Development Authority (Kagochi and Kangaita Tea Farms), Eastern Produce Africa Limited (Sitoi Estate), Sotik Tea Company and Tea Research Foundation of Kenya. A fellowship award from the Matsumae International Foundation to P.O.O. to undertake the study is appreciated. The authors thank Dr C. O. Othieno and Dr T. Takeo for encouragement. The paper is published with the permission of the Director, Tea Research Foundation of Kenya.

#### REFERENCES

- Biswas, A. K. & Sarkar, A. R. (1971). Biological and chemical factors affecting the valuations of North East India plain teas. II. Statistical evaluation of the biochemical constituents and their effects on briskness, quality and cash valuations of black teas. J. Sci. Food Agric., 22, 191-5.
- Biswas, A. K. & Sarkar, A. R. (1973). Biological and chemical factors affecting North East India plain teas. III. Statistical evaluation of the biochemical constituents and their effects on colour, brightness and strength of black teas. J. Sci. Food Agric., 24, 1457–77.
- Carr, M. K. V. (1977). Response of seedling tea bushes and their clones to water stress. *Exp. Agric.*, 13, 317-24.
- Cloughley, J. B. (1980). The effect of fermentation temperature on the quality parameters and price evaluation of Central Africa black teas. J. Sci. Food Agric., **31**, 911–19.
- Fordham, R. (1972). Observation on the growth of shoots of tea (*Cammelia sinensis* L.) in Southern Malawi. J. Hort. Sci., 47, 221–9.
- Hilton, P. J. (1974). Tea. In: Encyclopedia of industrial chemical analysis, Vol. 18 (Snell, F. D. & Ettre, L. S. (Eds)), New York, John Wiley, 455-516.
- Hilton, P. J. & Ellis, R. T. (1972). Estimation of the market value of Central Africa tea by theaflavins analysis. J. Sci. Food Agric., 23, 227–32.
- Hilton, P. J. & Palmer-Jones, R. W. (1973). Relationship between flavanol composition of fresh tea shoots and the theaflavins content of manufactured tea. J. Sci. Food Agric., 24, 813-18.
- Hilton, P. J. & Palmer-Jones, R. W. (1975). Chemical assessment of the quality of tea and its relation to the market over an extended period. J. Sci. Food Agric., 26, 1681–7.
- Hilton, P. J., Palmer-Jones, R. W. & Ellis, R. T. (1973). Effect of season and nitrogen fertilizer upon the flavanol composition and tea making quality of fresh shoots of tea (*Camellia sinensis* L.) in Central Africa. J. Sci. Food Agric., 24, 819–26.
- Magambo, M. J. S. (1983). Growth of axillary buds to pluckable size after plucking the terminal shoot above them. Annual Report, Tea Research Foundation of Kenya.
- Magambo, M. J. S. & Omolo, J. G. (1982). Leaf growth in different tea clones. *Tea*, 3(2), 12–13.
- Mullin, D. J., Crispin, D. J. & Swaine, D. (1969). Non-volatile components of black tea and their contribution to the character of the beverage. J. Agric. Food Chem., 17, 717-22.

- Mwakha, E. (1985a). Tea shoot growth in the Kenya highlands. Tea, 6(1), 5-13.
- Mwakha, E. (1985b). Tea shoot growth at different altitudes in Western Kenya. *Tea*, **6**(2), 19-24.
- Njuguna, C. K. (1983). Kenya Tea Development Authority's observational clonal trials. *Tea*, **4**(1), 8–9.
- Owuor, P. O. & Reeves, S. G. (1986). Optimising fermentation time in tea processing. Food Chem., 21, 195–203.
- Owuor, P. O., Reeves, S. G. & Wanyoko, J. K. (1986). Correlation of theaflavins content and valuation of Kenyan black teas. J. Sci. Food Agric., 37, 507-13.
- Owuor, P. O., Obanda, M. A., Othieno, C. O., Tsushida, T., Horita, H. & Murai, T. (1987). Changes in chemical composition and quality of black tea due to plucking standards. *Agric. Biol. Chem.* (In press).
- Portsmouth, G. B. & Rajiah, E. S. (1957). Factors affecting shoot production in tea (*Camellia sinensis*) when grown as a plantation crop. III. The time factor and new shoot production. *Tea Quarterly*, 28, 21–9.
- Raman, K. (1982). Effect of 'Miraculan' on yield of four tea clones during drought. *Planters Chronicle*, **77**(11), 349–51.
- Roberts, E. A. H. & Smith, R. F. (1963). Phenolic substances of manufactured tea. IX. Spectrophotometric evaluation of tea liquors. J. Sci. Food Agric., 14, 689–700.
- Squire, G. R. (1978). Stomatal behavior of tea (*Camellia sinensis*) in relation to environment. J. Appl. Ecol., 15, 287-310.
- White, W. & Barua, D. W. (1955). The nature of dormancy in the tea. J. Expt. Bot., 6, 1–5.
- Yamanishi, T., Kobayashi, A., Nakamura, H., Uchida, H., Mori, S., Ohsawa, K. & Sasakura, S. (1968). Flavour of black tea: Comparison of various types of black teas. Agric. Biol. Chem., 33, 379–86.

## Philip O. Owuor, A. Martin Obanda

Tea Research Foundation of Kenya, PO Box 820, Kericho, Kenya

#### Tojiro Tsushida, Hiroshi Horita & Toshinobu Murai

National Research Institute of Tea, Kanaya, Haibara, Shizuoka 428, Japan

(Received 8 October 1986; revised version received 16 February 1987; accepted 5 March 1987)