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Influence of region of production on clonal black tea chemical characteristics

Analytical Methods

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Abstract

Production of black tea from the same vegetatively propagated (VP) cultivars, in Kenya and Malawi, shows variations in both chemical composition and quality. Whereas it is possible to produce black teas with similar total theaflavins and individual theaflavins, brightness and total colour levels, black teas from Kenya generally have higher thearubigins, total volatile flavour compounds and flavour index. The black tea fermentation process is much faster in Malawi compared to that in Kenya, as evidenced by faster production of plain black tea chemical parameters, especially theaflavins. Consequently, in Malawi the maximum amount of theaflavins formation takes a shorter fermentation duration than in Kenya. Given ample fermentation duration, fermentation in Kenya produces a similar amount of theaflavins. This makes it necessary to optimise fermentation time, in different geographical regions even when the same cultivar is being processed. The other plain black tea quality parameters (thearubigins, brightness and total colour) were higher in black tea which was processed in Kenya than those processed in Malawi. However, the pattern in the changes in the individual theaflavins or theaflavins digallate equivalent followed that of total (Flavognost) theaflavins, suggesting that the flavan-3-ols patterns in tea leaf might not have been affected by the geographical area of production. The total volatile flavour compounds (VFC), Group I and II VFC and the flavour index were higher in black teas processed in Kenya, further demonstrating the fact that high grown Kenyan teas are more flavoury. In both Kenya and Malawi black teas, aroma quality declined with a long duration of fermentation. Short fermentation time is therefore a method of producing more aromatic black teas. The variations in black tea quality between Malawi and Kenya were possibly due to difference in environmental conditions, leading to different shoot growth rates and biochemical composition in the shoots. $© 2007 Elsevier Ltd. All rights reserved.$

Keywords: Geographical area of production; Same clones; Chemical composition; Quality; Kenya; Malawi

1. Introduction

Tea beverages (from Camellia sinensis L.O. Kuntze) have been claimed to be the most widely consumed drinks after water. Due to the large demand, commercial production of the plant has been reported from as far north as 49 N, Outer Carpathians, in the former Soviet Union to as far south as 33° S, Natal, South Africa ([Shoubo, 1989\)](#page-8-0) and from altitudes ranging from sea level in Japan, to as high as 2700 m above mean sea level in Kenya and Rwanda. The plant is adaptable to environments with large climatic variations. These variations in environment and growing conditions are thought to cause variations in tea quality. Many studies have compared the chemical composition of black teas produced in different countries. These studies demonstrated that the composition of the volatile flavour compounds in black tea [\(Wicremasinghe, Wick, & Yama](#page-8-0)[nishi, 1973; Yamanishi et al., 1968a, Yamanishi, Wicrema](#page-8-0)[singhe, & Perera, 1968b](#page-8-0)), black tea aroma [\(Aisaka,](#page-7-0) [Kosuge, & Yamanishi, 1978; Owuor & Obanda, 1996](#page-7-0)) and black tea plain quality parameters ([Owuor, Horita,](#page-8-0) [Tsushida, & Murai, 1986b\)](#page-8-0) vary with geographical area

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of production. However, it is also known that the chemical and quality variations occur due to the variation in the genetic make-up of the plants, even when they are grown under similar conditions in one environment [\(Magoma,](#page-8-0) [Wachira, Obanda, Imbuga, & Agong, 2000; Owuor, Tsush](#page-8-0)[ida, Horita, & Murai, 1988; Owuor & Obanda, 1995](#page-8-0)). We report one experiment where the two cultivars were grown in two different environments and manufactured under identical conditions.

Part of the difficulty in making a valid comparison in the quality of tea from different parts of the world is the ability to source plants of the same genetic make-up, grown in different environments. In the 1980s, the Tea Research Foundation of Central Africa (TRFCA) and the Tea Research Foundation of Kenya (TRFK) exchanged a few vegetatively propagated (VP) cultivars, for test under different regions. The TRFCA provided cultivar SFS 150 and TRFK cultivar 6/8. These cultivars had been demonstrated in the areas they were produced to have good desirable attributes. TRFCA cultivar SFS 150 exhibits good low temperature growth, a high degree of drought tolerance and a high yield potential but only makes an acceptable average black tea with quality inferior to the high quality cultivars SFS 204, PC 108 and PC 168. TRFK cultivar 6/ 8 makes good quality black tea but succumbs to drought. This has now provided an opportunity in which both clones are grown under two widely varying tea growing geographical regions. A study was initiated to determine if there are quality variations in the black teas produced by the two cultivars grown in Malawi and in Kenya.

One noted difference in the processing of black tea in Malawi and Kenya is fermentation time. In Malawi fermentation is normally done for shorter periods, ranging from 30 to 75 min, to maximise the amounts of theaflavins [\(Cloughley, 1979\)](#page-7-0). In Kenya, however, fermentation time is normally 70 to 140 min ([Owuor & Reeves, 1986](#page-8-0)). It was not established in these studies whether the noted difference in optimal fermentation times was due to the geographical area of production or variations in the genetic constitution of the cultivars used in the studies.

Previous studies demonstrated that for Central African black teas, there exists a good linear relationship between total theaflavin levels and sensory evaluations and/or prices ([Cloughley, 1980, 1983; Hilton & Palmer-Jones,](#page-7-0) [1975; Hilton & Ellis, 1972; Hilton, Palmer-Jones, & Ellis,](#page-7-0) [1973\)](#page-7-0). Although such a relationship was also positive for Kenyan black teas ([Owuor, Reeves, & Wanyoko, 1986a\)](#page-8-0), it was not significant. This was despite the fact that black teas from both regions are classified as plain to medium flavoury in the international tea trade. The difference in response has been puzzling and attributed to many factors. In Kenya, for example, it has been argued that the black teas have high and probably above optimal or threshold levels of theaflavins ([Owuor et al., 1986a,](#page-8-0) [Owuor, Othieno, & Reeves, 1987a](#page-8-0)). Consequently, other quality parameters like aroma were believed to be more important and obscured the contribution of theaflavins.

More recently, a better relationship was shown between the normalised theaflavin levels and sensory evaluation [\(Owuor & Obanda, 1995\)](#page-8-0). Despite the earlier noted differences in the relationship between total theaflavins and sensory evaluation in tea from Kenya ([Owuor et al.,](#page-8-0) [1986a, 1987a](#page-8-0)) and Central Africa ([Cloughley, 1980,](#page-7-0) [1983; Hilton & Palmer-Jones, 1975; Hilton & Ellis,](#page-7-0) [1972; Hilton et al., 1973\)](#page-7-0), with the use of the normalised factor (theaflavin digallate equivalent) there was good relationship with sensory evaluation for both Central African (Malawi) and Kenya teas ([Owuor et al., 2006\)](#page-8-0). However, it was observed that for Central African black teas, the presence of high levels of simple (non-gallated) flavan-3-ols was key to the production of black teas with high sensory evaluation (Wright et al., 2000; [Owuor](#page-8-0) [et al., 2006\)](#page-8-0), while for Kenyan black teas, high levels of flavan-3-ol gallate esters had the dominant role [\(Owuor](#page-8-0) [et al., 2006\)](#page-8-0). [Magoma et al. \(2000\)](#page-8-0) showed that the composition of flavan-3-ols in tea leaves is unique to cultivars. The objective of this study was to compare the chemical composition of black teas of similar VP cultivars produced under different environments.

2. Materials and methods

The leaf used for production of black tea in this study was obtained from vegetatively propagated (VP) cultivar tea fields at the TRFCA, Mulanje, Malawi, (altitude 650 m amsl, latitude 16° 05' S, longitude 35 $^{\circ}$ 37' E) and TRFK, Kericho, Kenya, (altitude 2180 m amsl, latitude 0° 22' S, longitude 35 $^{\circ}$ 21' E). The plants were grown under recommended agronomic practices.

The cultivars were plucked both at the Tea Research Foundation of Central Africa and Tea Research Foundation of Kenya and processed by the miniature CTC method at the respective institutions. Fermentation was varied at 30, 50, 70, 90 and 110 min at 28–30 C before firing. The processing was done on three different occasions and each manufacture was used as a replicate. The unsorted black teas were subjected to chemical analysis. The total theaflavins were analysed by the Flavognost method of [Hilton](#page-7-0) [\(1973\)](#page-7-0) while thearubigins, brightness and total colour were determined by the method of [Roberts and Smith \(1963\).](#page-8-0)

From one replicate, the individual ratios of the theaflavins were determined in duplicate by HPLC ([Bailey, McDo](#page-7-0)[well, & Nurstein, 1990; Steinhaus & Englehardt, 1989\)](#page-7-0) as outlined previously [\(Owuor & Obanda, 1997\)](#page-8-0). Using theaflavin ratios obtained from HPLC data and the Flavognost (total) theaflavin data for the same replicate, the amounts of individual theaflavins were calculated since the molar absorption coefficients of the four theaflavins are similar at 365 nm ([Steinhaus & Englehardt, 1989\)](#page-8-0). The astringency of the black teas was estimated using the modified theaflavins digallate equivalent factor ([Owuor & Obanda, 1995](#page-8-0)).

Simultaneous steam distillation–extraction (SDE water/ diethyl ether) was used to extract the volatile flavour compounds (VFC) ([Likens & Nickerson, 1964\)](#page-8-0) using cumene as

an internal standard. The dried (anhydrous $Na₂SO₄$), ether–VFC mixture was concentrated to about 100 µl and the concentrate subjected to gas chromatography (GC) and gas chromatography–mass spectrometry (GC–MS) analysis under the conditions of ([Baruah, Hazakira, Mah](#page-7-0)[anta, Horita, & Murai, 1986\)](#page-7-0). GC analysis was done on a Varian 3300 GC equipped with flame ionisation detector, while GC–MS analysis was done on a Hitachi model 663 GC coupled with a Hitachi M .80A and M.003 mass spectrometer. Peak identification was done by comparing retention times with those of authentic samples and then confirmed by GC–MS. The quantities of the compounds are expressed as the ratio of the area of the peak to that of the internal standard. In the determination $15 \mu g$ cumene as internal standard was used per gm of dry black tea.

The classification of the VFC into Group I and II was based on their odour characteristics [\(Owuor et al., 1986b,](#page-8-0) [Owuor, Takeo, Horita, Tsushida, & Murai, 1987b, 1987c,](#page-8-0) [1988; Owuor, 1992; Robinson & Owuor, 1992](#page-8-0) and references therein) and the retention times of the compounds during gas chromatographic analysis ([Yamanishi et al.,](#page-8-0) [1968a, 1968b; Wicremasinghe et al., 1973\)](#page-8-0). The VFC imparting green grassy, undesirable aroma or eluting before linalool were classified into Group I VFC and those imparting sweet, flowery aroma or eluting after linalool were group into Group II VFC. The ratio of Group II: Group I VFC (flavour index) was used to quantify black tea aroma quality [\(Owuor et al., 1986b, 1987b, Owuor,](#page-8-0) [Othieno, Horita, Tsushida, & Murai, 1987c, 1988; Robin](#page-8-0)[son & Owuor, 1992; Owuor, 1992](#page-8-0)).

3. Results and discussion

This study presents for the first time a comparison of the chemical quality parameters of black tea from the same clone processed under identical conditions grown under different environments. Despite the plants of each VP cultivar being of the same genetic make-up, there were noted differences in chemical composition due to geographical growing zones (Tables 1–6). There are several differences in the growing conditions in Central Africa (Malawi) and East Africa (Kenya). [Herd and Squire \(1976\)](#page-7-0) had shown that it takes only 42 days for shoots to develop from bud break to mature two- or three- leaves and a bud, in Malawi. In Kenya, such shoot development takes 70–120 days depending on the season [\(Mwakha, 1985a, 1985b\)](#page-8-0). Factors which affect the growth rate of the tea plant normally lead to variations in chemical composition and quality of black tea ([Robertson, 1983; Owuor, 1995\)](#page-8-0). Generally, factors which tend to lead to higher tea production have negative effects on black tea quality. It is noted that although the overall production of tea per unit area in Malawi and Kenya are about the same ([Anon, 1995\)](#page-7-0), 80% of the Malawi tea is produced in only five months between December and April ([Cloughley, 1983\)](#page-7-0). This is unlike in Kenya where tea production is more evenly distributed throughout the year with only relatively minor peak periods between April and May and October and November. The growth of tea in Malawi, in their main growing season, is therefore faster. The variations noted in the chemical composition of the same cultivars grown in the different regions are therefore attributable to

Table 1

Impact of geographical area of production on fermentation and quality of cultivar 6/8

Parameter	Source	Fermentation time (min)	Mean source				
		30	50	70	90	110	
Theaflavins (umoles/g)	Kenya	12.9	19.7	24.9	26.1	27.2	22.2
	Malawi	18.0	22.6	25.4	24.3	21.5	22.4
	Mean	15.5	21.2	25.2	25.2	24.4	
	$C.V.$ (%)			12.9			
	LSD ($P < 0.05$)	2.95			NS		
	Interactions			4.17			
	Kenya	11.3	13.5	13.7	14.3	15.5	13.6
	Malawi	8.03	8.64	9.11	9.32	9.21	8.86
	Mean time	9.67	11.1	11.4	11.8	12.4	
	$C.V.$ (%)			12.2			
	LSD ($P < 0.05$)			2.19			1.38
Thearubigins $(\%)$ Total colour $(\%)$ Brightness $(\%)$	Kenya	3.07	4.17	4.86	5.29	5.66	4.61
	Malawi	3.29	3.97	4.59	4.93	5.12	4.38
	Mean time	3.18	4.07	4.73	5.11	5.39	
	$C.V.$ (%)			6.68			
	LSD ($P < 0.05$)			0.48			NS
	Kenya	31.7	32.9	30.9	27.1	25.8	29.6
	Malawi	29.7	30.6	28.3	25.1	22.5	27.2
	Mean time	30.7	31.7	29.6	26.1	24.2	
	$C.V.$ (%)			23.9			
	LSD ($P < 0.05$)			NS			NS

Parameter	Source		Fermentation time (min)						
		30	50	70	90	110			
Theaflavins (μ moles/g)	Kenya	12.9	18.9	21.6	23.1	22.2	19.9		
	Malawi	15.0	22.1	21.8	21.3	21.2	20.3		
	Mean time	13.9	20.5	21.7	22.2	21.7			
	$C.V.$ (%)			8.69					
	LSD ($P < 0.05$)			1.78			NS		
	Interactions			2.52					
Thearubigins $(\%)$ Total colour $(\%)$ Brightness $(\%)$	Kenya	9.61	11.5	11.7	13.0	13.0	11.8		
	Malawi	6.97	7.83	7.89	8.56	8.87	8.02		
	Mean time	8.29	9.65	9.81	10.8	10.9			
	$C.V.$ (%)			5.08					
	LSD $(P < 0.05)$			0.80			0.51		
	Kenya	2.51	3.48	3.89	4.47	4.53	3.77		
	Malawi	3.14	4.21	4.58	5.22	5.34	4.49		
	Mean time	2.83	3.84	4.23	4.84	4.93			
	$C.V.$ (%)			13.4					
	LSD ($P < 0.05$)			0.88			0.56		
	Kenya	34.3	32.9	32.7	31.0	29.6	32.1		
	Malawi	30.1	29.7	27.2	22.3	21.7	26.2		
	Mean time	32.2	31.3	29.9	26.6	25.7			
	$C.V.$ (%)			21.8					
	LSD ($P < 0.05$)			NS			$_{\rm NS}$		

Table 2 Impact of geographical area of production on fermentation and quality of cultivar SFS 150

Table 3

Comparison of the variations in the theaflavins levels of Cultivar SFS150 Duration grown in Kenya and Malawi due to fermentation time

Location	Fermentation time (min)	Total TF (flavognost)	TF	$TF-3-G$	$TF-3'-G$	TFDG	TFDG equiv.
Malawi	30	14.2	4.87	3.70	2.29	2.94	5.92
	50	13.2	5.04	3.35	2.23	2.54	5.26
	70	12.4	4.74	3.28	2.04	2.33	4.92
	90	12.2	4.49	3.21	2.06	2.47	5.00
	110	12.1	4.47	3.18	2.04	2.29	4.80
Kenya	30	13.6	5.96	3.41	2.38	1.78	4.72
	50	14.1	6.31	3.45	2.55	1.79	4.86
	70	14.8	6.30	4.14	2.56	2.22	5.53
	90	15.2	5.96	3.92	2.49	2.37	5.52
	110	16.8	6.53	4.77	2.80	2.65	6.30

Table 4

Comparison of the variations in the theaflavins levels of cultivar 6/8 grown in Kenya and Malawi due to fermentation time

Location	Fermentation time (min)	Total TF (flavognost)	TF	$TF-3-G$	$TF-3'-G$	TFDG	TFDG equiv.
Malawi	30	18.2	8.45	4.29	3.11	2.33	6.22
	50	17.3	8.09	4.48	2.75	1.98	5.74
	70	17.3	7.79	4.51	2.79	2.21	5.97
	90	15.1	5.96	4.10	2.56	2.49	5.73
	110	15.3	5.87	4.19	2.64	2.59	5.88
Kenya	30	11.4	7.51	1.79	1.65	0.42	2.21
	50	13.8	8.41	2.51	2.03	0.83	3.72
	70	18.9	9.90	4.56	2.80	1.65	5.75
	90	17.8	9.16	4.53	2.52	1.56	5.44
	110	17.8	8.70	5.11	2.41	1.60	5.57

difference in environmental conditions leading to variations in growth rates.

Also the TRFCA in Malawi is located at 650 m amsl while the TRFK in Kenya is situated at 2180 m amsl. Earlier [Obaga, Squire, and Lang'at \(1988\)](#page-8-0) and [Squire,](#page-8-0) [Obaga, and Othieno \(1993\)](#page-8-0) had observed a decrease in growth rate of same cultivars of tea with rise in altitude, even within a radius of only 10 km, where the variations

Table 5 Response of cultivar 6/8 to fermentation times of Kenya and Malawi

Fermentation time (mins)	Kenya					Malawi					
	30	50	70	90	110	30	50	70	90	110	
2-Methyl butanal	0.15	0.15	0.14	0.17	0.19	0.13	0.16	0.14	0.14	0.13	
Pentanal	0.06	0.05	0.05	0.07	0.06	0.04	0.04	0.04	0.05	0.05	
Hexanal	0.23	0.27	0.33	0.32	0.38	0.11	0.12	0.13	0.14	0.14	
$E-3$ -Penten-2-one	0.04	0.08	0.07	0.08	0.10	0.03	0.03	0.04	0.03	0.05	
Z-2-Penten-3-ol	0.11	0.12	0.10	0.14	0.12	0.07	0.09	0.06	0.09	0.07	
Heptanal	0.03	0.02	0.03	0.04	0.03	0.02	0.02	0.02	0.02	0.02	
Z-3-Hexenal	0.08	0.13	0.11	0.13	0.12	0.05	0.06	0.08	0.06	0.06	
E -2-Hexenal	1.99	2.48	2.55	2.79	2.89	1.19	1.30	1.43	1.57	1.65	
n -Pentyl furan	t^a	t	0.01	0.01	0.02	t	t	t	t	t	
n -Pentanol	0.02	0.01	0.02	0.02	0.02	0.01	0.01	0.01	0.02	0.01	
3,6,6-Trimethylcyclohexanone	0.01	0.01	0.01	0.02	0.01	t	t	t	0.01	0.01	
Z-3-Penten-1-ol	0.07	0.07	0.06	0.08	0.06	0.04	0.05	0.03	0.05	0.03	
n -Hexanol	0.03	0.02	0.03	0.02	0.02	0.01	t	t	t	0.01	
Z-3-Hexen-1-ol	0.09	0.10	0.09	0.10	0.09	0.03	0.02	0.02	0.01	0.01	
Nonanal	0.03	0.04	0.05	0.04	0.05	0.02	0.02	0.02	0.02	0.02	
$E-2$ -Hexen-1-ol	0.04	0.05	0.05	0.05	0.06	0.03	0.02	0.01	0.03	0.02	
E,Z-2,4-Heptadienal	$\mathsf t$	t	0.01	t	0.01	t	$\mathbf t$	t	$\mathbf t$	t	
$E,E-2,4$ -Heptadienal	0.05	0.06	0.06	0.07	0.09	0.03	0.03	0.03	0.03	0.02	
Sum of Group 1 VFC	2.98	3.66	3.77	4.15	4.32	1.81	1.97	2.06	2.27	2.30	
Linalool oxide I	0.07	0.08	0.07	0.06	0.07	0.03	0.03	0.02	0.02	0.02	
Linalool oxide II	0.20	0.23	0.22	0.20	0.22	0.06	0.08	0.05	0.07	0.06	
Bezaldehyde	0.03	0.05	0.03	0.04	0.04	0.04	0.05	0.05	0.06	0.06	
Linalool	0.99	1.10	1.12	1.06	1.03	0.29	0.20	0.19	0.18	0.17	
Alpha-Cedrene	0.35	0.35	0.36	0.45	0.56	0.12	0.15	0.18	0.19	0.20	
Beta-Cedrene	0.05	0.04	0.04	0.06	0.08	0.01	0.01	0.02	0.02	0.03	
3,7-Dimethyloctatrienol	0.03	0.03	0.03	0.03	0.04	0.01	$\mathbf t$	0.01	0.01	\mathfrak{t}	
Beta-Cyclocitral	0.04	0.04	0.04	0.04	0.05	0.02	0.03	0.04	0.03	0.02	
Phenyl acetaldehyde	0.32	0.44	0.53	0.57	0.56	0.54	0.59	0.87	0.90	1.00	
Neral	0.09	0.06	0.07	0.04	0.05	0.02	0.02	0.02	0.02	0.01	
Alpha-Terpineol	0.05	0.05	0.06	0.05	0.06	0.02	0.02	0.02	0.02	0.02	
Linalool oxide III	0.02	0.02	0.01	0.01	0.03	0.01	\mathbf{t}	$\mathbf t$	$\mathbf t$	\mathbf{t}	
Linalool oxide IV	0.02	0.02	0.02	0.03	0.03	0.02	t	t	t	t	
Methyl salicylate	0.35	0.41	0.43	0.40	0.41	0.14	0.06	0.09	0.08	0.05	
Nerol	0.04	0.05	0.05	0.05	0.05	0.02	0.02	0.02	0.02	0.02	
Geraniol	1.51	1.74	1.67	1.47	1.47	0.51	0.40	0.34	0.37	0.38	
Benzyl alcohol	0.04	0.03	0.02	0.05	0.05	0.03	0.05	0.02	0.02	0.02	
2-Phenyl ethanol	0.57	0.61	0.66	0.80	0.64	0.36	0.34	0.27	0.37	0.32	
Beta-Ionone	0.13	0.13	0.13	0.18	0.15	0.09	0.09	0.06	0.08	0.08	
Epoxy-beta-Ionone	0.20	0.21	0.24	0.28	0.24	0.13	0.14	0.10	0.13	0.12	
Nerolidol	0.11	0.12	0.12	0.13	0.14	0.06	0.07	0.06	0.06	0.04	
Cedrol	0.17	0.10	0.11	0.14	0.21	0.07	0.12	0.11	0.12	0.14	
Bovolide	0.07	0.07	0.06	0.07	0.08	0.04	0.05	0.04	0.05	0.08	
Methyl palmitate	0.04	0.02	0.05	0.04	0.04	0.01	0.02	0.02	0.03	0.03	
Trimethylpentadecan-2-one	0.19	0.15	0.13	0.16	0.13	0.08	0.09	0.07	0.07	0.05	
E-Geranic acid	0.41	0.42	0.46	0.40	0.53	0.59	0.08	0.08	0.07	0.06	
Sum of group II VFC	6.10	6.61	6.67	6.94	7.02	2.67	2.71	2.74	2.98	2.90	
Flavour index (group II/I)	2.05	1.81	1.78	1.67	1.63	1.48	1.38	1.33	1.31	1.26	
Terpene index		0.40	0.40	0.41	0.40	0.40	0.41	0.39	0.39	0.39	

 a t = trace.

in environmental conditions are considered minimal. Under those conditions, there was slower growth at higher altitude leading to yield decline. The produced black teas however showed an increase in quality with rise in altitude [\(Owuor,](#page-8-0) [Obaga, & Othieno, 1990\)](#page-8-0). One factor that can cause quality difference for black teas produced in TRFCA and TRFK is the large variation in altitudes.

Generally, the African black teas are classified as plain to medium flavoury in the tea markets. Such teas are sold

for their theaflavins, thearubigins, brightness and total colour. The effects of growing the tea cultivars in different geographical regions on the plain black tea quality parameters are presented in [Tables 1 and 2](#page-2-0). One noted difference in the processing of black tea in Malawi and in Kenya is the difference in fermentation times. In Malawi, the general ambient temperatures are high, averaging between 25 and 30 °C. In Kenya, especially in Kericho, the ambient temperature are relatively low, averaging between $20-25$ °C. To

Table 6 Response of cultivar SFS 150 to fermentation times of Kenya and Malawi

Fermentation time (mins)	Kenya					Malawi					
	30	50	70	90	110	30	50	70	90	110	
2-Methyl butanal	0.13	0.12	0.20	0.20	0.17	0.11	0.12	0.15	0.15	0.15	
Pentanal	0.05	0.05	0.06	0.04	0.05	0.03	0.04	0.04	0.06	0.05	
Hexanal	0.28	0.36	0.45	0.45	0.57	0.14	0.15	0.15	0.17	0.19	
$E-3$ -Penten-2-one	0.04	0.05	0.06	0.07	0.08	0.05	0.05	0.04	0.06	0.06	
Z-2-Penten-3-ol	0.12	0.10	0.12	0.12	0.11	0.08	0.09	0.08	0.09	0.07	
Heptanal	0.03	0.02	0.03	0.03	0.04	0.02	0.02	0.02	0.02	0.02	
Z-3-Hexenal	0.08	0.10	0.09	0.10	0.10	0.06	0.07	0.09	0.09	0.09	
$E-2$ -Hexenal	1.87	2.03	2.56	2.41	2.77	2.09	2.29	2.33	2.56	2.63	
n-Pentyl furan	0.02	0.02	0.02	0.03	0.03	0.01	0.01	0.01	0.01	0.01	
n -Pentanol	0.03	0.02	0.04	0.02	0.04	0.01	0.01	0.01	0.01	0.01	
3,6,6-Trimethylcyclohexanone	0.01	0.01	0.01	0.02	0.01	$t^{\rm a}$	\mathbf{t}	$\mathbf t$	$\mathbf t$	0.01	
Z-3-Penten-1-ol	0.07	0.05	0.06	0.06	0.05	0.05	0.07	0.05	0.04	0.04	
n -Hexanol	0.03	0.02	0.02	0.03	0.03	0.01	$\mathbf t$	0.01	\mathbf{t}	0.01	
Z-3-Hexen-1-ol	0.07	0.07	0.08	0.09	0.06	0.04	0.03	0.02	0.02	0.02	
Nonanal	0.04	0.03	0.04	0.16	0.06	0.03	0.03	0.03	0.04	0.03	
E-2-Hexen-1-ol	0.04	0.04	0.05	0.06	0.05	0.03	0.03	0.03	0.03	0.03	
E , Z-2, 4-Heptadienal	0.02	0.01	0.01	0.01	0.01	t	t	t	$\mathbf t$	t	
$E, E-2, 4$ -Heptadienal	0.09	0.07	0.08	0.09	0.08	0.02	0.04	0.03	0.03	0.02	
Sum of group 1 VFC	3.02	3.17	3.98	3.99	4.31	2.78	3.05	3.09	3.38	3.44	
Linalool oxide I	0.27	0.18	0.22	0.20	0.17	0.08	0.08	0.09	0.08	0.06	
Linalool oxide II	0.89	0.63	0.77	0.66	0.58	0.34	0.30	0.29	0.25	0.20	
Benzaldehyde	0.05	0.05	0.07	0.07	0.07	0.06	0.06	0.08	0.07	0.08	
Linalool	1.66	1.28	1.49	1.49	1.39	0.85	0.69	0.61	0.54	0.46	
Alpha-Cedrene	0.32	0.23	0.28	0.35	0.40	0.12	0.08	0.18	0.19	0.18	
Beta-Cedrene	0.03	0.03	0.03	0.03	0.05	0.01	0.01	0.01	0.03	0.02	
3,7-Dimethyloctatrienol	0.04	0.03	0.04	0.04	0.04	0.01	0.02	0.02	0.02	0.02	
Beta-Cyclocitral	0.04	0.03	0.04	0.05	0.04	0.02	0.02	0.03	0.03	0.03	
Phenyl acetaldehyde	0.26	0.30	0.34	0.38	0.35	0.62	0.69	0.74	0.90	0.99	
Neral	0.04	0.04	0.03	0.04	0.06	0.01	0.02	0.03	0.02	0.02	
Alpha-Terpineol	0.08	0.06	0.07	0.08	0.07	0.03	0.05	0.05	0.06	0.05	
Linalool oxide III	0.03	0.03	0.02	0.03	0.02	0.02	0.02	0.01	\mathbf{t}	0.02	
Linalool oxide IV	0.06	0.05	0.06	0.07	0.07	0.02	0.02	0.02	0.02	0.02	
Methyl salicylate	0.22	0.17	0.20	0.22	0.20	0.07	0.14	0.12	0.10	0.08	
Nerol	0.04	0.03	0.04	0.04	0.03	0.02	0.03	0.02	0.02	0.02	
Geraniol	0.17	0.16	0.16	0.15	0.14	0.10	0.09	0.08	0.07	0.06	
Benzyl alcohol	0.04	0.04	0.04	0.03	0.04	0.02	0.04	0.03	0.02	0.07	
2-Phenyl ethanol	0.40	0.39	0.42	0.48	0.36	0.57	0.54	0.44	0.42	0.31	
Beta-Ionone	0.09	0.10	0.10	0.12	0.12	0.12	0.13	0.10	0.09	0.09	
Epoxy-beta-Ionone	0.15	0.14	0.15	0.16	0.13	0.17	0.19	0.16	0.14	0.14	
Nerolidol	0.16	0.13	0.14	0.16	0.14	0.08	0.10	0.11	0.11	0.10	
Cedrol	0.11	0.07	0.13	0.11	0.11	0.24	0.21	0.10	0.16	0.15	
Bovolide	0.06	0.05	0.08	0.05	0.06	0.08	0.08	0.06	0.07	0.07	
Methyl palmitate	0.05	0.03	0.04	0.03	0.04	0.02	0.02	0.01	0.02	0.02	
Trimethylpentadecan-2-one	0.07	0.07	0.11	0.09	0.10	0.11	0.14	0.11	0.08	0.10	
E-Geranic acid	0.01	0.01	0.02	0.02	0.02	0.01	0.01	0.01	0.01	0.01	
Sum of group II VFC	5.34	4.33	5.09	5.11	4.80	3.80	3.78	3.51	3.35	3.39	
Flavour index (group II/I)	1.77	1.37	1.28	1.29	1.11	1.36	1.24	1.14	0.99	0.99	
Terpene index	0.94	0.93	0.93	0.94	0.93	0.92	0.92	0.92	0.92	0.92	

 $^{\mathrm{a}}$ t = trace.

overcome the problem of difference in fermentation temperatures causing the variations in quality, fermentation was done in environmentally controlled units in Kenya set between 28–30 °C. Fermentation times were varied to establish if the differences in optimum fermentation times [\(Cloughley, 1980; Owuor & Reeves, 1986\)](#page-7-0) were due to variations in geographical area of production or the cultivars used. The results are presented in [Tables 1 and 2](#page-2-0) for plain tea quality parameters and [Tables 5 and 6](#page-4-0) for the VFC for cultivars 6/8 and SFS 150, respectively.

Despite fermenting at the same temperature, development of theaflavins in Malawi was much faster than in Kenya for both cultivars ([Tables 1 and 2\)](#page-2-0). Theaflavin development during fermentation had been suggested as a method for estimating optimal fermentation time [\(Cloughley, 1980\)](#page-7-0), especially for Central African black teas, where high levels of theaflavins in black tea lead to higher tea prices ([Ellis & Cloughley, 1981; Davies, 1983\)](#page-7-0). Thus, judged by theaflavins levels, fermentation in Malawi should be shorter than fermentation in Kenya for

production of market desired black teas. Given ample fermentation duration, the theaflavins levels of Kenyan black teas reach similar levels as those of Malawi. It is therefore necessary that in a given area of production, optimal fermentation durations are developed for various cultivars to maximise the theaflavin production. In fact, the breeding programme at TRFCA has been requested to give equal importance to relatively slow fermenters which would otherwise have been discarded [\(Anon, 2005\)](#page-7-0), now that it has been realised that these slow fermenters are potentially high quality plant materials, if fermented at their optimum fermentation time.

For cultivar 6/8 in Kenya, theaflavins (TF), thearubigins (TR) and total colour (TC) increased with increase in fermentation time, while brightness declined after 50 min whereas in Malawi brightness, TF and TR declined after 50, 70 and 90 min, respectively [\(Table 1](#page-2-0)), implying that TC and brightness respond similarly in the two countries while both TF and TR show differential response between countries. For the slow fermenting average quality cultivar SFS 150, both TR and TC steadily increased with fermentation time while brightness and TF decreased after 30 and 50 min, respectively, in Malawi whereas in Kenya only TC steadily increased up to 110 min but TF and TR decreased after 90 min and brightness after 30 min, implying that both cultivars have similar optimum fermentation time for development of maximum levels of TC and brightness in both regions. Long fermentation times produced more coloury black teas at the expense of brightness. In addition, maximum total colour in cultivar 6/8 was attained at the same fermentation time in both regions, while the other three quality parameters (except TR) require shorter duration in Malawi than in Kenya. However, whether a similar trend would exist with fast fermenting high quality TRFCA cultivars such as SFS 204, PC 108 and PC 168 is a matter of speculation and would therefore warrant further investigation. Indeed, these results suggest the need for further experimentation involving more cultivars from both Kenya and Malawi.

The variations in thearubigins were, however, more dramatic. In both regions and cultivars, there were increases in thearubigins levels with increase in fermentation durations. This is similar to what had been observed in previous studies [\(Owuor, Orchard, & McDowell, 1994\)](#page-8-0). However, the thearubigins levels in black tea produced in Kenya were significantly higher than those in Malawi black teas. Since the thearubigins are thought to contribute to the total colour in black tea, it was expected that the results would mimic those of total colour. The cause of this significant difference cannot be explicitly explained. However, it had been observed that the thearubigins fraction used in the assay also contains high amount of flavonol glycosides ([McDowell, Bailey, & Howard, 1990\)](#page-8-0). The presence of the flavonol glycoside makes the [Roberts and Smith](#page-8-0) [\(1963\)](#page-8-0) method of thearubigins assay, used in the study, overstate the value of thearubigins since it does not discriminate between the flavonol glycosides and thearubigins in tea ([Bailey et al., 1990, Bailey, Nurstein, & McDowell,](#page-7-0) [1991; McDowell et al., 1990](#page-7-0)). A more extensive HPLC study is necessary to determine if the noted differences persist or if flavonol glycoside levels in tea vary with geographical region.

Although theaflavins are useful plain black tea quality parameters and a good relationship had been obtained between the levels in black tea and prices or/and sensory evaluation for Malawi black teas [\(Cloughley, 1980; Ellis](#page-7-0) [& Cloughley, 1981; Hilton & Palmer-Jones, 1975; Hilton](#page-7-0) [& Ellis, 1972](#page-7-0)), such relationships were positive but less significant for Kenya black teas ([Owuor et al., 1986a\)](#page-8-0). More recently, the relationship between the theaflavins and sensory evaluation for Kenya tea was improved by normalising the contribution of the individual theaflavins to the taste of tea [\(Owuor & Obanda, 1995](#page-8-0)). Indeed, by normalising the contribution of the individual theaflavins, a significant relationship has been demonstrated for both Kenya and Malawi black teas [\(Owuor et al., 2006](#page-8-0)). A comparison of the individual theaflavins and normalised theaflavin factor (theaflavin digallate equivalent) in black tea produced by cultivars 6/8 and SFS 150 in both Malawi and Kenya are presented in [Tables 3 and 4](#page-3-0). The changes in patterns of the individual theaflavins and theaflavin digallate equivalent occurred in the same way as the Flavognost (total) theaflavins. It is therefore apparent that the pattern of catechin (flavan-3-ols) ratios in the cultivars might not have been affected by the geographical area of production. Indeed, [Magoma et al. \(2000\)](#page-8-0) showed that the flavan-3-ol patterns are unique to tea cultivars. However the actual ratios vary with growing weather conditions, with the epigallocatechin gallate dominating under fast growing conditions ([Robertson, 1983\)](#page-8-0), while the total catechins could vary as reflected in the thearubigins levels.

The concept of black tea quality can be broadly divided into the sensations of sight (colour), taste and aroma. The non-volatile components are responsible for taste, with some of these compounds also being responsible for the colour. However, the volatile components comprise aroma. Although many aroma compounds are primary tea plant metabolites, many VFC in tea are secondary metabolites derived from carotenes, amino acids, unsaturated fatty acids plus other lipids and terpene glycosides during tea processing ([Robinson & Owuor, 1992\)](#page-8-0). The composition and concentrations of the VFC plays a vital role in the valuation and/or pricing of black tea ([Owuor, 1992; Owuor](#page-8-0) [et al., 1988; Wicremasinghe et al., 1973; Yamanishi et al.,](#page-8-0) [1968a, 1968b](#page-8-0)). Generally the compounds are classified into two groups. The Group I VFC comprise those compounds which, although are characteristic of black tea aroma, are deleterious to quality as they impart a green and grassy smell to black tea, while the Group II VFC have a desirable, sweet flowery smell [\(Owuor, 1992; Owuor et al.,](#page-8-0) [1988; Robinson & Owuor, 1992; Wicremasinghe et al.,](#page-8-0) [1973; Yamanishi et al., 1968a, 1968b](#page-8-0) and references therein). The ratio of Group II to Group I, flavour index has been used to classify teas in the order of their flavour

quality [\(Owuor, 1992; Owuor et al., 1986b, 1987c, 1988,](#page-8-0) [1990; Robinson & Owuor, 1992; Wicremasinghe et al.,](#page-8-0) [1973; Yamanishi et al., 1968a, 1968b](#page-8-0)). The composition of the volatile flavour compounds in cultivars 6/8 and SFS 150 produced in Kenya and Malawi are presented in [Tables 5 and 6.](#page-4-0) For every given fermentation time, the total VFC, the sum of Group I and II VFC were higher for cultivar 6/8 produced in Kenya than Malawi. Although the same trend was observed in SFS 150, the difference was less pronounced, especially in the sum of Group I VFC. However, the better measure of the aroma quality is the flavour index. In both cultivars and regions, the flavour index decreased with long fermentation durations. Short fermentation duration is therefore one method of producing more aromatic black teas.

There were also variations in the flavour index with regions. Generally the black teas produced in Malawi had lower flavour index [\(Tables 5 and 6\)](#page-4-0). This is probably due to the environmental conditions of growth. Due to low altitude and higher temperatures, the growth rate is faster in Malawi than in Kenya. Factors which increase growth rates, e.g. decrease in altitude [\(Mahanta, Baruah, Owuor,](#page-8-0) [& Murai, 1988; Owuor et al., 1990\)](#page-8-0), high rates of nitrogen fertilizers [\(Owuor et al., 1987c](#page-8-0)) and pruning [\(Owuor &](#page-8-0) [Lang'at, 1988](#page-8-0)), reduce black tea aroma. The higher flavour index for black teas produced in Kenya than those produced in Malawi, explain the noted observations in tea trade that Malawi black teas are generally plainer than Kenyan black teas. In tea trade the high grown Kenya clonal teas are classified as medium flavoury black teas while Malawi black teas are classified as plain.

Terpene index as a ratio of gas chromatographic peak area of linalool plus its derivatives to the sum of gas chromatographic peak areas linalool plus its derivatives and geraniol together with its derivatives, had been shown to be specific to cultivars and is a chemotaxonomic method of classifying/characterising cultivars [\(Takeo, 1981, 1983\)](#page-8-0). In earlier studies, it was demonstrated that terpene index is stable to withering process, nitrogenous fertilizer rates and regions of production within Kenya [\(Owuor et al.,](#page-8-0) [1987b](#page-8-0)), altitude, pruning, maceration method and grading [\(Owuor, 1989\)](#page-8-0). However, there was variation in the terpene index with age of the harvested shoots ([Owuor et al.,](#page-8-0) [1987b](#page-8-0)). Other factors which may cause the variations in terpene index to limit its use as a chemotaxonomic tool remain unidentified. The data on effects of geographical area of production and fermentation times on the terpene indices of cultivars 6/8 and SFS 150 are also presented in [Tables 5 and 6](#page-4-0), showing that the terpene index of a cultivar does not vary with where it is grown or with fermentation duration. Being a ratio, it has a limitation that it can have only values from 0 to 1. It is therefore possible that several unrelated tea cultivars may have same terpene index [\(Wachira, 1996\)](#page-8-0). Thus, despite its limitations, it is a good method of identifying cultivars.

Several countries have sought or attempted to import vegetatively propagated (VP) cultivar teas, for planting,

to boost the quality of their teas. In such cases it is assumed the quality of the cultivars in the areas of selection would be maintained. The results presented here demonstrate that there are variations in black tea quality of vegetatively propagated cultivars tea due to geographical areas of production. Thus importation of cultivars to replicate quality at the selection site cannot be assumed. The imported cultivars should therefore undergo further quality tests in new areas to determine optimum processing conditions if they are imported to ensure quality. Despite lack of obvious gain in quality with importation, there are advantages in countries exchanging tea planting materials. Recently, it was observed that despite the large extent in which tea is grown in different countries, most countries are operating on a very narrow genetic base [\(Wachira, Waugh, Hackett,](#page-8-0) [& Powell, 1995](#page-8-0)). The only way for expanding the genetic base and adequately be buffered against possible environmental hazards is through exchange of planting materials.

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